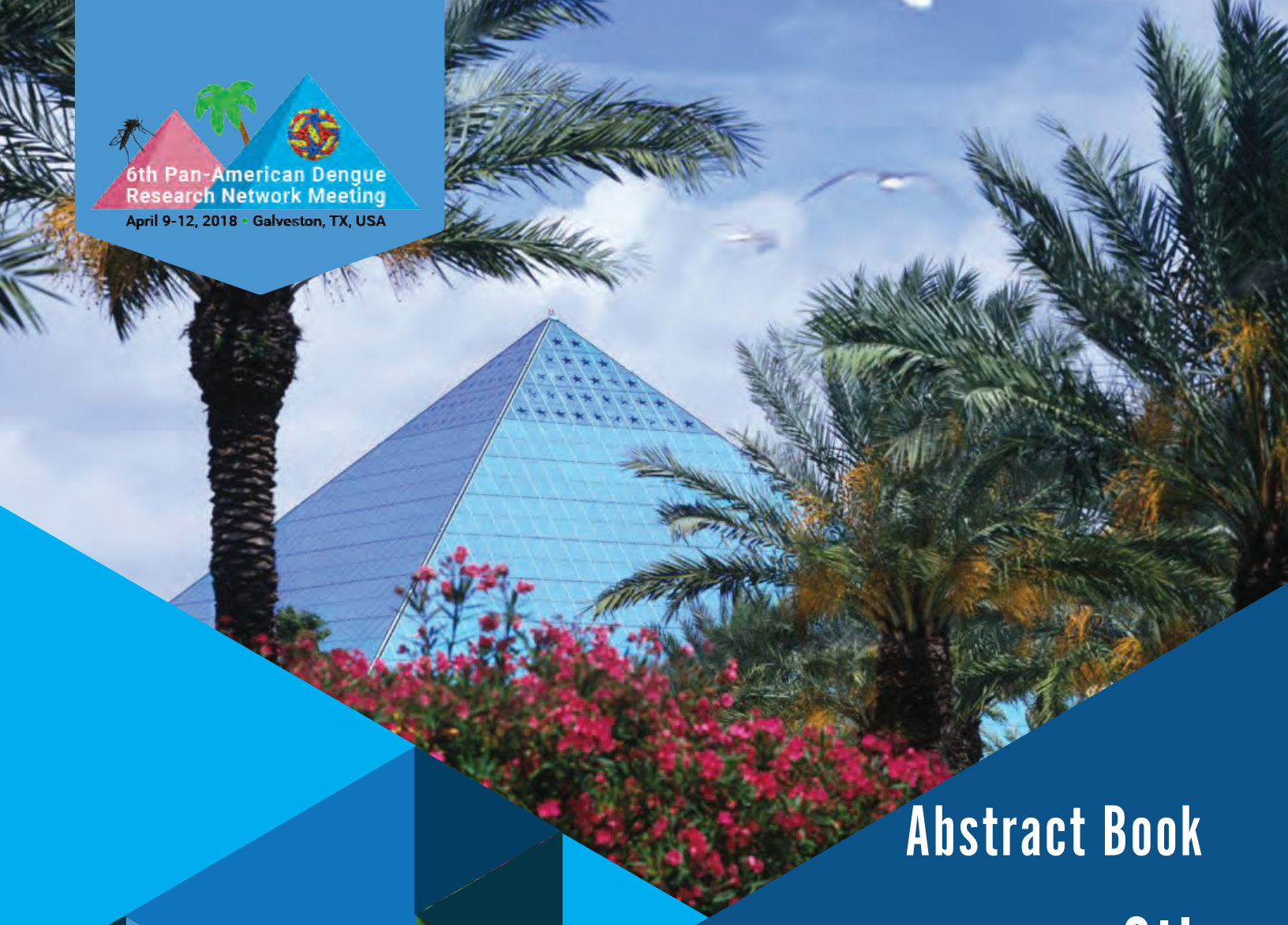

Abstract Book

6th Pan-American Dengue Research Network Meeting

April 9-12
Galveston,
TX, USA

WELCOME TO THE 6TH PANDENGUE NET MEETING IN GALVESTON, TX!

Thanks to all of you for participating in the 6th PanDengue Net meeting!

It is an honor that The PanDengue Net executive committee selected Galveston and The University of Texas Medical Branch (UTMB) to host this meeting. The Gulf Coast of Texas and specifically Galveston has had a rich history of circulation of arboviruses, such as dengue and yellow fever, and development of vector control programs to prevent their impact on human public health. Since 1994, UTMB has built an infectious disease program that is internationally recognized as a leader in emerging and arboviral diseases.

This meeting will give the opportunity to professionals who work in different areas of arboviral infections, such as dengue, chikungunya, Zika, Mayaro, yellow fever as well as other emerging and neglected arboviruses, to present their latest research and establish collaborations that will further enhance their visibility and scientific vistas. We also hope that this meeting will offer the opportunity to all trainees to not only network with established investigators but also to exchange ideas and share their vision about their work.

Thanks to the members of the Executive and Scientific Committees and all volunteers for their effort and passion in the organization of this Meeting.

We hope you will enjoy the meeting and our Texas hospitality!

Nikos Vasilakis, PhD
Professor and Vice Chair for Research
Department of Pathology
Center for Biodefense and Emerging Infectious Diseases
Center for Tropical Diseases
Institute for Human Infections and Immunity
University of Texas Medical Branch

Scott Weaver, PhD
John Sealy Distinguished University Chair in Human Infections and Immunity;
Director, Institute for Human Infections and Immunity;
Scientific Director, Galveston National Laboratory,
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Pei-Yong Shi
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Irene Bosch
Ernesto Marques
Jaime Torres
Jorge Munoz

Vector Biology/Ecology/Vector Control
Amy Morrison
George Dimopoulos
Nikos Vasilikis
Luciano Moreira
Roberto Barrera

AGENDA

	Monday 4/09/18 PM	Tuesday 4/10/18 AM	Tuesday 4/10/18 AM
AM	**Registration Monday to Wednesday 8:00 am-6:00 pm Thursday 8:00 am- 12:00 pm**	**Moderators: Mauricio Nogueira/Isabel Rodriguez Expo A**	**Moderators: Berlin Londono/ Amy Morrison Expo B**
		Epi/Phylogenetics (concurrent) **Nuno Faria** [8:30 - 8:50 + 5 Q&A] **Myrna Bonaldo** [8:55 - 9:15 + 5 Q&A] *Genomic and structural features of the Yellow Fever virus from the ongoing Brazilian outbreak* **Mauricio Nogueira** [9:20 - 9:40 + 5 Q&A] The interplay of Zika and other Flavivirus in an endemic area Short talk 1 **Drumond, Betania** Yellow fever epizootics in urban areas: Brazil, 2016/2017 [9:40 - 9:50 + 5 Q&A] Short talk 2 **Cruz, Cristopher** THE PHYLOGEOGRAPHY AND PHYLODYNAMICS OF DENV-3 IN PERU	**Vector Biology (concurrent)** **Connor McMeniman** [8:30 - 8:50 + 5 Q&A] Identification of Chemosensory Circuitry Orchestrating Aedes aegypti Attraction to Humans **Berlin Londono** [8:55 - 9:15 + 5 Q&A] Mosquito saliva: Where are we in terms of tracking vector-host interactions? **Bradley White** [9:20 - 9:40 + 5 Q&A] The Debug Project at Verily: Scaling the Sterile Insect Technique **Short talk - 1 Granada, YE** [9:40 - 9:50 + 5 Q&A] Aedes Aegypti Resistance is associated with mutations in sodium channel **Short talk - 2 Hughes G** [9:55 - 10:05 + 5 Q&A] Microbiota and Zika virus interactions in field-collected Aedes aegypti
		Coffee Break 10:10 - 10:30	**Coffee Break 10:10 - 10:30**
		Moderators: Mauricio Nogueira/Isabel Rodriguez	**Moderators: Berlin Londono/ Amy Morrison**
		Modeling/Burden (concurrent) **Isabel Rodriguez-Barraquer** [10:30 - 10:50 + 5 Q&A] *Towards refining the global dengue map: insight from seroprevalence and case data* **Zulma Cucunuba** [10:55 - 11:15 + 5 Q&A] Combining different information sources to estimate the burden of Chikungunya epidemic, the case of Colombia **Eugenia Corrales** [11:20 - 11:40 + 5 Q&A] Neotropical Bats that Co-habit with Humans Function as Dead-End Hosts for Dengue Virus. **Short talk 1 - Sequetin, Mariana** - Yellow fever in São Paulo State: a threat in peri-urban centers? [11:45 - 11:55 + 5 Q&A] **Short talk 2- Coello-Escoto, Ana** - A pipeline for measuring antigenic relationships among diverse, low-passage dengue viruses [12:00 - 12:10 + 5 Q&A]	**Vector Ecology/Control (concurrent)** **Luciano Moreira** [10:30 - 10:50 + 5 Q&A] City-wide deployment of Wolbachia in Brazil as an arbovirus control strategy **Pablo Manrique** [10:55 - 11:15 + 5 Q&A] New Tools for the Control of Aedes aegypti and challenges for control programs **Julien F. Pompon** [11:20 - 11:40 + 5 Q&A] How non-coding flaviviral RNA enhances mosquito transmission **Short talk - 1 Ortiz, M** [11:45 - 11:55 + 5 Q&A] Integrated Vector Control Management After Two Hurricanes in Puerto Rico **Short talk - 2 Astete, H** .[12:00 - 12:10 + 5 Q&A] COMPARISON of the efficacy of the bg sentinel and suna traps for capture of Aedes aegypti mosquitoes in Peru
		Lunch: 12:20 am - 1:30 pm	
PM	6-9 Meet & Greet	**Moderators: Eva Harris and Ana Sesma Expo A**	
		Epi/Immunology DENV-ZIKV **Leah Katzelnick** [1:30 - 1:50 + 5 Q&A] Effect of pre-existing antibodies to DENV and ZIKV on subsequent DENV and ZIKV antibody titers, infection, and disease in **Mauro Teixeira** [1:55 - 2:15 + 5 Q&A] The role of glutamate on Zika-induced neuronal damage **Alex Sette** [2:20 - 2:40 + 5 Q&A] CD8 T cell immunity to Zika virus in humans is shaped by prior DENV exposure and associated with an IFN gamma/cytotoxic signature **Short talk - 1: Delgado** et al. Cross-reactive immune responses upon sequential dengue and Zika virus infection [2:45 - 2:55 + 5 Q&A] **Short talk 2 Haller** et al., MAYV emergence in a CHIKV endemic world: Is MAYV a new a threat? - 3:00 - 3:10 + 5 Q&A]	
	Hotel Convention Center Expo 6:30 Call to order - Nikos Vasilakis 6:35 General welcome to Galveston and introduction to UTMB - Danny Jacobs 6:45 Acknowledgments, credits and last minute announcements to participants - Nikos Vasilakis, Carlos Sariol and Andrea Gamarnik 7:00 Introduction of Keynote Speaker - Robert Tesh 7:10 Keynote Lecture - **Tom Monath** "Yellow Fever: Creature from the Black Lagoon" 8:00 Reception	**Coffee Break 3:15 - 3:45**	
		Moderators: Eva Harris and Ana Sesma Expo A	
		Immunology **Ernesto Marques** [3:45 - 4:05 + 5 Q&A] Zika and Dengue antibody interactions: implications for diagnostics and vaccines **Irene Ramos** [4:10 - 4:30 + 5 Q&A] Immune profiling of dengue virus infections in human immune cells **Eng-Eong Ooi** [4:35 - 4:55 + 5 Q&A] Insights into determinants of symptomatic flaviviral infection **Short talk - 1 Tan** et al. -Guey Chuen Perng-, Functional analysis of PBMC in dengue endemic and non-endemic regions [5:00 - 5:10 + 5 Q&A] **Short talk - 2 Ramjag** et al., Characterization of Immune Responses to Selected Arboviruses and determination of seroprevalence in Trinidad [5:15 - 5:25 + 5 Q&A]	
		Break [4 - 5 pm]	
		POSTER SESSION 5 - 7 pm (Poster Session will run from Tuesday 8:00 am to Thursday noon)	

AGENDA

	Wednesday 4/11/18 AM	Thursday 4/12/18
AM	**Moderators: Andrea Gamarnik/ MarianoGarcia-Blanco Expo A**	**Moderators: Pei Yong Shi/ Albert Ko Expo A**
	Virology **Rosa Maria del Angel** [8:30 - 8:50 + 5 Q&A] NS3 and NS5 from dengue and Zika modulate nuclear citoplasmic transport and innate immune response by targeting nuclear proteins **Stacy HORNER** [8:55 - 9:15 + 5 Q&A] Defining how the RNA modification m6A regulates Flaviviridae virus infection **Cybele Garcia** [9:20 - 9:40 + 5 Q&A] Role of PML in the intrinsic immunity against flaviviruses **Short talk - 1 Campos**, Ribosomal stalk proteins are required for DENV translation elongation [9:40 - 9:50 + 5 Q&A] **Short talk 2- De Borba** RNA structure duplication in the DENV genome: Functional redundancy or host specificity2 [9:55 - 10:05 + 5 Q&A]	**Diagnostics/Prognostics** **Eva Harris** [8:30 - 8:50 + 5 Q&A] Development and evaluation of serological methods for Zika diagnosis and surveillance and use in studies of seroprevalence and long-term immunity **Irene Bosch** [8:55 - 9:15 + 5 Q&A] A low cost methodology to detect specific exposure to Zika virus **Susan Wong** [9:20 - 9:40 + 5 Q&A] The flavivirus multiplex microsphere immunoassay for recent and past infection. **Short talk - 1 Makeda** et al Predictive biomarkers for severe dengue [9:40 - 9:50 + 5 Q&A] **Short talk - 2 Medina** 1 MAC-ELISA that can differenbtiate DENV and ZIKV infections / Why VLP could achieve such specificity? [9:55 - 10:05 + 5 Q&A]
	Coffee Break 10:10 - 10:30	**Coffee Break 10:10 - 10:30 Expo A**
	Moderators: Andrea Gamarnik/ MarianoGarcia-Blanco Expo A **Virology/Antivirals** **Andrea da Poian** [10:30 - 10:50 + 5 Q&A] Anaplerotic role of glucose in the oxidation of endogenous fatty acids during dengue virus infection **Subash Vasudevan** [10:30 - 10:45 + 5 Q&A] Dengue antiviral therapeutics in the time of Dengvexia and Eliminate Dengue **Bruno Canard** [10:55 - 11:15 + 5 Q&A] Flavivirus NS5: one protein, several activities, and many functions **Short talk - 1 Shin** et al, A combined genetic/proteomic approach identifies functional host factors associated with flavivirus RNA [11:45 - 11:55 + 5 Q&A] **Short talk - 2 Sacramento** et al, Sofosbuvir as an antiviral against Zika virus [12:00 - 12:10 + 5 Q&A]	**Vaccines** **Pei-Yong Shi** [10:30 - 10:55 + 5 Q&A] A single-dose live-attenuated Zika vaccine **Short talk - 1 Swanstrom** et al., [11:00 - 11:10 + 5 Q&A] Mapping the target epitopes of the type specific antibody responses induced by a live-attenuated dengue vaccine **Round-Table** (15 each, 10 Q/A) **Benedetta Ghezzi** [11:40 - 11:55 +10 Q&A] Takeda's dengue vaccine: a program update **Hansi Dean** [11:15 - 11:30 + 10 Q&A) Comprehensive analysis of the immune response elicited by Takeda's tetravalent dengue vaccine **Jonhatan Smith** [12:05 - 12:20 + 10 Q&A) Virus–Like Particle (VLP) Vaccines for Chikungunya and Zika
	Lunch: 12:20 am - 1:30 pm	**Lunch: 12:30 am - 1:30 pm**
PM	**Moderators: Carlos Sariol & Ernesto Marques Expo A** **Pathogenesis** **Mike Diamond** [1:30 - 1:50 + 5 Q&A] Host Immunity to Zika Virus Infection **Pereira Lenore** [1:55 - 2:15 + 5 Q&A] Replication of Zika virus in first-trimester human placentas, mechanisms of viral pathogenesis and host cell defense **Carlos Sariol** [2:20 - 2:40 + 5 Q&A] Time: Key factor for DENV and ZIKV interactions. **Short talk - 1 Kumar** et al Zika virus replication and persistence in human Sertoli cells [2:45 - 2:55 + 5 Q&A] **Short talk - 2 Costa** et al Annexin A1 as a novel pro-resolving molecule against DENV infection [3:00 - 3:10 + 5 Q&A]	**Bob Tesh Symposium (12:15-4 pm) Expo B** **Historical overview**; 12:15 - 12:30 **Scott O'Neill** [12:30 - 12:55 + 5 Q&A] **Sandra Laurence Lopez Verges** [1:00 - 1:25 + 5 Q&A] **Mike Diamond**, WUSL [1:30 - 1:55 + 5 Q&A] **Amy Morrison**, UCD [2:00 - 2:25 + 5 Q&A] **Tom Monath**, NewLink [2:30 - 2:55 + 5 Q&A] **Mariano Garcia-Blanco** [3:00 - 3:25 + 5 Q&A] **Scott Weaver** [3:30 - 3:55 + 5 Q&A]
	Coffee Break 3:15 - 3:45	
	Moderators: Albert Ko and Betania Drumond Expo A **Clinical** **Karin Nielsen-Saines** [3:45 - 4:05 + 5 Q&A] Mother to Child Transmission of Zika virus and infant outcomes following in utero exposure **James Sejvar** [4:10 - 4:30 + 5 Q&A] Arboviruses and Guillain-Barre syndrome - Lessons From the Zika Virus Outbreak **Stephen Thomas** [4:35 - 4:55 + 5 Q&A] Developing a Zika Vaccine: the Knowns and Unknowns **Short talk - 1 Collins** et al ZIKV pregnant women serology study [5:00 - 5:10 + 5 Q&A] **Short talk - 2 Drumond, B,** et al. Yellow fever: clinical, virological and immunological aspects [5:15 - 5:25 + 5 Q&A]	
	Break [4 - 5 pm]	
	POSTER SESSION 5 - 7 pm **(Poster Session will run from Tuesday 8:00 am to Thursday noon)**	

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Invited speakers

Tuesday 4/10

Epi/Phylogenetics
Nuno Faria, UK
Myrna Bonaldo, Brazil
Mauricio Nogueira, Brazil

Modeling/Burden
Isabel Rodriguez-Barraquer, US
Zulma Cucunuba, UK
Victor Mauricio Herrera, Colombia

Epi/Immunology DENV-ZIKV
Leah Katzelnick, US
Mauricio Nogueira, Brazil
Alex Sette, US

Immunology
Ernesto Marques US/Brazil
Irene Ramos, US
Eng-Eong Ooi, Singapur

Vector Biology
Connor McMeniman, US
Berlin Londono, US
Bradley White, US

Vector Ecology/Control
Luciano Moreira, Brazil
Pablo Manrique, Mexico
Juliet F Pompon, Singapur

Wednesday 4/11/18

Virology
Rosa Maria del Angel, Mexico
Stacy Horner, US
Cybele Garcia, Argentina

Virology/Antivirals
Andrea da Poian, Brazil
Subash Vasudevan, Singapur
Bruno Canard, France

Pathogenesis
Mike Diamond, US
Pereira Lenore, US
Carlos Sariol, Puerto Rico

Clinical
Karin Nielsen-Saines, US
James Sejvar, US
Stephen Thomas, US

Thursday 4/11/18

Diagnostics/Prognostics
Eva Harris, US
Irene Bosch, US
Susan Wong, CDC, US

Vaccines
Pei-Yong Shi, US

Round-Table
Hansi Dean, Takeda
Benedetta Ghezzi Takeda
Jonathan Smith, US

Epi/Phylogenetics

Myrna C. Bonaldo
mbonaldo@ioc.fiocruz.br

Lab. Biologia Molecular de Flavivírus, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil

Genomic and structural features of the Yellow Fever virus from the ongoing Brazilian outbreak

Since late 2016, yellow fever (YF) outbreak of unprecedented sanitary severity and ecological catastrophe has been recorded in Southeastern Brazil. The first determination of the whole YF virus genomes from two naturally infected howler-monkeys (*Alouatta clamitans*) revealed that the current YF virus belongs to the modern lineage (sub-lineage 1E) of South American genotype I. Furthermore, ongoing YF viral genome displays a new signature of nine amino acid alterations (Bonaldo *et al*., Mem Inst Oswaldo Cruz, 2017). We extended the study to infected mosquitos, humans and non-human primates samples of 2016-2018 epidemic in Rio de Janeiro State. Our data confirm that viral strains associated with the most severe YF epidemic in South America in the last seventy years display unique amino acid substitutions mainly located in highly conserved positions at non-structural proteins NS3 and NS5. The study also supports that YFV spread southward into Rio de Janeiro state following different sylvatic dispersion routes that converged in the border of the Great Metropolitan area comprising nearly 12 million inhabitants. Our original results can help public health authorities to guide the surveillance, prophylaxis and control measures to face such severe epidemiological situation.

Mauricio Nogueira

The interplay of Zika and other Flavivirus in an endemic area

In this presentation, we will show the interplay between Zika and other flavivirus such as Dengue and Yellow Fever in a endemic area with large DENV circulation and Yellow Fever vaccination. We will present and discuss the role of previous flaviviruses immunity in both general population and pregnant women infection. We will also discuss the magnitude of the Zika outbreak in a naive population and the potential role of/impact in NHP of the region.

Modeling/Burden

Isabel Rodríguez-Barraquer

Division of HIV, ID and Global Medicine, Department of Medicine, University of California San Francisco, San Francisco, CA.

Towards refining the global dengue map: insight from seroprevalence and case data

Good understanding of the transmission dynamics of infectious diseases is important to design effective control strategies. In particular, estimates of the force of infection (the per capita hazard of infection), and of the basic reproductive number (R_0) are critical because they give insight into the level of control that is required to reduce incidence and eventually block transmission. In this talk, we will present a modeling

framework that we have been developing to refine the global dengue map. By combining serological and age-specific case data, we have produced detailed updated maps of the transmission hazard and R_0 of dengue in several countries including Thailand, Brazil, Mexico and Colombia. Furthermore, by combining these estimates with a geostatistical machine-learning models based on environmental convariates, we have produced a high-resolution map of global dengue force of

Zulma Cucunubá

Combining different information sources to estimate the burden of Chikungunya epidemic, the case of Colombia'

The methodological approach combine the use of passive and active surveillance data and administrative clinic data. Firstly, by using weekly surveillance data at subnational level we have estimated the Reproductive numbers and transmission patterns of CHIK. Secondly, based on a household-based cross-sectional sero-survey in four major cities of we have estimated attack rates CHIK and principal risk factors and compared them to incidence data. Thirsty, through administrative data we have estimated the costs of illness of this disease to the health system.

Victor Mauricio Herrera

Red AEDES, Bucaramanga, Colombia

Population based studies in a dengue endemic area in Colombia: Opportunities to characterize the burden and understand the natural history of emerging infectious diseases

In this presentation, we will describe our experience conducting population-based studies to characterize transmission and natural history of arboviruses in a dengue endemic area. Cohort studies such as ours in Piedecuesta, Colombia, combining serological and clinical surveillance provide ideal scenarios to estimate the burden of arboviral infections, in terms of incidence and complications. They also provide an opportunity to determine risk factors for infection and prognostic factors of progression to severe forms. In addition, by establishing strategies of active follow-up, such as regular telephonic contact to assess any febrile syndrome, the Piedecuesta cohort provided a unique opportunity to detect the transmission of emerging infectious diseases such as Zika during the 2015-2016 epidemic in Colombia, and to understand its natural history.

Epi/Immunology DENV-ZIKV

Leah Katzelnick

Affiliation: Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, USA

Title: Effect of pre-existing antibodies to DENV and ZIKV on subsequent DENV and ZIKV antibody titers, infection, and disease in a Nicaraguan pediatric cohort

The majority of infections with dengue viruses 1-4 (DENV1-4) and Zika virus (ZIKV) are inapparent, but 20-25% result in symptomatic disease and a small fraction result in severe manifestations including Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS) and for Zika, Guillain-Barré Syndrome and congenital birth defects. Secondary DENV infections are a known risk factor for DHF/DSS, but the specific relationships between the level of pre-existing antibodies to DENV or ZIKV and subsequent protection against or risk of disease remains obscure. We used multiple statistical approaches to study the relationship between anti-DENV and anti-ZIKV binding antibodies and subsequent antibody response and disease risk in the Nicaraguan Pediatric Dengue Cohort Study (2004-present, n=~3,700). We found that risk of DHF/DSS was highest within a narrow range of pre-existing anti-DENV antibody titers, while protection from symptomatic dengue disease was observed in those with high pre-existing antibody titers. We are performing similar analyses to understand the effect of pre-existing immunity induced by DENV on the risk of subsequent ZIKV infection and Zika disease, as well as the impact of the Zika epidemic on anti-DENV antibodies in the cohort. These results have implications for studies of dengue and Zika pathogenesis and for vaccine development, because enhancement, not just lack of protection, is a concern.

Alessandro Sette
alex@lji.org

CD8 T cell immunity to Zika virus in humans is shaped by prior DENV exposure and associated with an IFN gamma/cytotoxic signature

We show that memory T cell responses elicited by prior infection with DENV recognize ZIKV-derived peptides and that DENV pre-exposure influences the timing, magnitude and quality of ZIKV T cell response. Additionally, we show that ZIKV-specific responses target different proteins than DENV-specific responses. Finally, we will present recent data analyzing the transcriptomic profiles of ZIKV-specific CD8 T cells.

Immunology

Ernesto T. A. Marques[1, 2]

1) Department of Infectious Diseases and Microbiology, University of Pittsburgh, Center for Vaccine Research, Pittsburgh, PA, USA. 2) Department of Virology, Instituto Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, Pernambuco, Brazil.

Zika and Dengue antibody interactions: implications for diagnostics and vaccines.

Summary: Dengue and Zika viruses are simultaneously endemic in several regions. It is known that anti-flavivirus antibodies cross react. The effects of cross-reactions between Dengue and anti-Zika antibodies are not fully understood. It has been postulated that Zika and dengue cross-reactive antibodies can modify the clinical presentations, leading to protection or to more severe disease depending on the circumstances. We will present evidences supporting the effects of Zika and dengue interactions, its implications for dengue and Zika vaccines and the development of specific diagnostic tests.

Irene Bosch

MIT
Institute for Medical Engineering and Science
Building E25-406, 45 Carleton Street, Cambridge, MA 02139

Title: A low cost methodology to detect specific exposure to Zika virus

ZIKV virus (ZIKV) was responsible for the explosive 2015–2017 epidemic in the Americas. ZIKV infection during pregnancy is linked to devastating birth defects and associated anomalies, designated congenital ZIKV syndrome (1) whereas in adults, ZIKV infection has been associated with Guillain Barré syndrome (2). Flaviviruses are enveloped RNA viruses containing an aprox. 11-kb positive-stranded RNA genome that encodes three structural and seven nonstructural proteins. Cells infected by flaviviruses secrete nonstructural protein 1 (NS1). Antibody responses generated in response to flavivirus infections are notoriously cross-reactive, representing a significant obstacle for the specific diagnosis of infection using serological assays. Conventional ELISAs using traditional viral antigen E (Envelope protein) have been found to poorly differentiate among flavivirus infections. This is especially problematic with antibodies to ZIKV and dengue virus (DENV), which co-circulate in the Americas. Neutralization assays can measure virus-specific neutralizing antibodies; however, specificity is affected by the production of cross-reactive neutralizing antibodies. After secondary DENV infections their is more challenge to accurately identification of ZIKV infections, therefore it has made very challenging to determine the burden and rate of asymptomatic infections, define the incidence of congenital ZIKV syndrome among infected women, and identify neurologic complications associated with ZIKV infection. We propose a low cost, simple ELISA test that can distinguish dengue and ZIKV infections by measuring the amounts of antibodies against NS1 protein in serum of the mothers and children born during the ZIKV epidemics. We also show the a point of care device performance using patient samples to detect active infection of dengue, Zika or Chikungunya as a commercial point of care device fabricated a very low cost.

Eng Eong
engeong.ooi@duke-nus.edu.sg

University of Duke

Insights into determinants of symptomatic flaviviral infection
I will share unpublished findings from a case of dengue in an immunosuppressed host as well as immuno-metabolomic analysis of healthy volunteers who received the live attenuated yellow fever vaccine that collectively provide new insights into the determinants of symptomatic infection.

Vector Biology

Conor J. McMeniman, Ph.D.
cmcmeni1@jhu.edu

Johns Hopkins University, Baltimore, MD 21205, USA

Assistant Professor
Johns Hopkins Malaria Research Institute
Department of Molecular Microbiology & Immunology

Identification of Chemosensory Circuitry Orchestrating Aedes aegypti Attraction to Humans

The primary vector of dengue and Zika viruses, *Aedes aegypti*, is a highly anthropophilic mosquito species that blood feeds preferentially and frequently on humans. To orient towards us, *Ae. aegypti* is largely thought to rely on its exquisitely tuned sense of smell to pick up on volatile chemicals emanating from our skin and breath. We are employing genome-engineering and two-photon imaging in olfactory centers of the mosquito brain to identify the signature components of human scent that attract *Ae. aegypti* towards humans. Applied implications will be discussed.

Berlin Londono

Assistant Professor
Department of Entomology
Kansas State University

Mosquito saliva: Where are we in terms of tracking vector-host interactions?

The hallmark entry site for dengue virus (DENV) into the human hosts is the skin. Here, Aedes mosquitoes inject the virus along with their saliva while blood feeding. This saliva has potent immune-modulatory molecules able to induce antibody production. Our preliminary work shows that these antibodies may block the activity of the saliva modifying virus infection success. In addition, extensive research shows that the level of anti-saliva antibodies in human serum are directly correlated to the

intensity of exposure to mosquito bites and risk of disease. In this presentation, we will discuss the role of anti-saliva antibodies in transmission dynamics from the vertebrate to the arthropod and vice versa.

Bradley White

The Debug Project at Verily: Scaling the Sterile Insect Technique

Sterile insect technique has been used to suppress and, in some cases, even locally eliminate agricultural pests but has not been successfully applied to insect vectors of human disease. The primary obstacle to successful SIT in the Dengue vector Aedes aegypti has been difficulty in mass production and delivery of competitive, male-only sterile mosquitoes. In this talk, I'll review the progress we have made in scaling Aedes aegypti SIT by combining entomological and molecular techniques with hardware and software engineering.

Vector Ecology/Control

LUCIANO A. MOREIRA

FIOCRUZ/ Instituto de Pesquisas René Rachou

City-wide deployment of Wolbachia in Brazil as an arbovirus control strategy

We will present the current status of the city-wide deployment of Wolbachia in two cities: Rio de Janeiro and Niterói, Brazil as an arbovirus (dengue, Zika and chikungunya) strategy. Activities related to the large-scale operations as well as challenges will be discussed.

Pablo Manrique
pablo_manrique2000@hotmail.com

New Tools for the Control of Aedes aegypti and challenges for control programs

The last decade has produced several reviews and studies showing that vector control methods can have important entomological outcomes. The evidence of some innovative methods and tools with potential, are still in process. Current evaluation and results of methods such as IRS, House Screening and Wolbachia performed in Mexico will be presented.

Douglas E Norris
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Johns Hopkins Bloomberg School of Public Health
Department of Molecular Microbiology and Immunology
Johns Hopkins Malaria Research Institute

How can big data from robotic smart traps revolutionize mosquito biology

Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, 21205.
Using traps for surveillance of vector and pathogen populations is an important component of many vector management programs, however this technology has remained largely unchanged over many years. Project PREMONITION has developed a robotic “smart” mosquito trap that captures live mosquitoes individually for mosquito surveillance, pathogen discovery and assessment of diversity. The PREMONITION trap uses infrared sensors and algorithms to identify flying insects by wing beat frequency, capturing only target mosquito species and reducing non-targeted captures. In addition to recording putative species identification, additional parameters including precise time of capture, temperature, humidity and ambient light, are recorded, effectively providing foraging activity data throughout the collection period and association with key abiotic factors. Due to a unique design, each specimen is tagged with the data it produced, enabling new bioinformatics analyses and providing incomparable insight into mosquito biology and ecology.

Virology

Rosa Maria Del Angel Nunez
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NS3 and NS5 from dengue and Zika modulate nuclear citoplasmic transport and innate immune response by targeting nuclear proteins.

Considering the important roles of NS5 during DENV replication, and its presence in the nucleus of infected cells, we isolated and identified nuclear cellular proteins that interact with NS5. Among others, the heterogonous nuclear ribonucleoprotein F (hnRNPF) and the DEAD-box RNA helicase DDX5 were identified as NS5 binding proteins. Although both proteins have a nuclear localization in mock-infected cells, they were relocated to the cytoplasm in DENV infected cells. Since the main regulator of the nuclear-cytoplasmic transport is the nuclear pore complex (NPC), the integrity and function of this structure was evaluated during infection with DENV and ZIKV. Our results indicate that some of the components of the NPC are cleaved/degraded during infection by the protease activity of NS2B-NS3, causing a reduction in the cytoplasmic transport of mRNAs. On the other hand, NS5 protein is involved in the relocation of some nuclear proteins to the cytoplasm. This relocation induces important changes in immune response. In summary, we describe novel functions for NS3 and NS5 proteins in a) the modulation of nuclear-cytoplasmic transport and in b) the inhibition of interferon response by targeting nuclear proteins.

Stacy Horner

Center for RNA Biology**,** Department of Molecular Genetics and Microbiology
Duke University Medical Center

Defining how the RNA modification m6A regulates *Flaviviridae* virus infection

This talk will describe our recent work on the molecular mechanisms of how the dynamic RNA modification *N6*-methyladenosine (m^6A) regulates infection by positive-sense stranded RNA viruses of the *Flaviviridae* family. We have found that m^6A marks the positive-sense stranded RNA genome of viruses in the *Flaviviridae* family, including HCV, ZIKV, dengue virus, yellow fever virus, and West Nile virus. In addition, we have found that m^6A regulates their replicative life cycles. Interestingly, *Flaviviridae* infection also induces broad changes in the m^6A "epitranscriptome" of host mRNAs. Taken together, this work reveals that m^6A is a conserved regulatory mark on the RNA genomes of viruses with the *Flaviviridae* family, and it suggests that m^6A provides an additional layer of gene regulation to the virus-host interactions that regulate infection.

Cybele García
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Universidad de Buenos Aires, Argentina

Role of PML in the intrinsic immunity against flaviviruses

Intrinsic immunity is mediated by constitutively expressed cellular proteins that can block virus replication immediately. While the triggering of the innate immune response through interferon signaling requires at least a few hours, intrinsic immunity effectors are present even before the first host-pathogen interaction. Thus, intrinsic immunity is the first cellular immune response against a virus. Our goal is to characterize effectors that mediate intrinsic and innate immune responses.

Promyelocytic leukemia protein (PML) is one of the many known proteins to be involved in intrinsic immunity against viruses. PML is the key organizer of subnuclear structures called Nuclear Bodies (PML-NBs). Different DNA as well as RNA viruses encode proteins that interact and disrupt PML-NBs to overcome PML-mediated antiviral response.

The present work is about the antiviral role of PML against the flavivirus dengue (DENV) in cell culture. Using PML-silencing and overexpression studies, we demonstrated that PML has antiviral activity against the four different DENV serotypes. Moreover, by using classic molecular biology techniques, as well as confocal microscopy and advanced fluorescence microscopy techniques, we showed that DENV NS5 protein interacts specifically with the PML-IV isoform and disrupts PML-NBs to neutralize PML antiviral effect. In conclusion, this work provides the first evidence to support the role of PML as a restriction factor against DENV. Understanding both innate and intrinsic immunity effectors is of great importance to identify potential targets for the development of broad-range antiviral drugs.

Virology/Antivirals

Andrea T. Da Poian

Instituto de Bioquímica Médica Leopoldo de Meis
Universidade Federal do Rio de Janeiro

Anaplerotic role of glucose in the oxidation of endogenous fatty acids during dengue virus infection

Mitochondrial bioenergetics of DENV-infected hepatic cells was studied. Our results showed that infection favors the cellular capacity of metabolizing glucose, impairing the normal metabolic flexibility that allows the oxidative machinery to switch among the main energetic substrates. However, instead of being used as an energy source, glucose performs an anaplerotic role in the oxidation of endogenous fatty acids, which become the main energetic substrate during infection and were shown to be essential for virus replication. We also found that infected cells were much less susceptible to the Crabtree effect, the glucose-mediated inhibition of mitochondrial oxygen consumption, suggesting that infection favors cellular respiration by increasing ADP availability.

Subhash VASUDEVAN Ph.D.
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Emerging Infectious Diseases Program - Duke-NUS Medical School Singapore

“Dengue antiviral therapeutics in the time of Dengvexia and Eliminate Dengue”

Despite the advances in understanding the 3D structures of dengue viral non structural proteins, the drug discovery and development efforts against the viral enzymatic targets is lagging behind with several challenges. At the same time the dengue vaccine development and dengue biological vector control program efforts have started to make headway. Some of the risks and opportunities for dengue antiviral drug discovery and development will be discussed.

Bruno Canard
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Flavivirus NS5: one protein, several activities, and many functions

I will describe the current knowledge on NS5 structures regarding the MTase, the Polymerase, and the way they talk to each other through their protein-protein interface. I will present the current knowledge on the mechanisms of both enzymes, as well as the substrate specificities during the Flavivirus life cycle. For the MTase, I will discuss how a guanosine could be N7-methylated, then a 2’-O methyl added to the first transcribed adenosine, as well as how internal methylation may occur, and its potential roles. For the polymerase, I will present which residues in conserved motifs A-G may participate in RNA synthesis fidelity, with connection to the current knowledge on Picornavirus and HCV polymerases, in the context of the design of antiviral drugs.

Pathogenesis

Michael Diamond
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The Herbert S. Gasser Professor
Departments of Medicine, Molecular Microbiology, Pathology & Immunology
Associate Director, The Andrew M. and Jane M. Bursky Center for Human Immunology and Immunotherapy Programs
Washington University School of Medicine

Host Immunity to Zika Virus Infection
I will discuss new data on how the route of inoculation (intravaginal versus subcutaneous) and hormonal stage impacts Zika infection in female mice, and how we have used genetic strategies to develop a fully immunocompetent mouse model of Zika virus infection and pathogenesis

Lenore Pereira, PhD

Department of Cell and Tissue Biology, School of Dentistry, University of California San Francisco, San Francisco, CA, USA

Replication of Zika virus in first-trimester human placentas, mechanisms of viral pathogenesis and host cell defense

Zika virus (ZIKV), a *Flavivirus*, responsible for the recent American pandemic, causes a spectrum of congenital malformations rarely seen with other neurotropic viruses. How ZIKV disseminates from maternal circulation to the fetus is poorly understood. For almost two decades, we have investigated the biology of CMV infection in developing human placentas and deciphered mechanisms that result in pathology observed in placentas from newborns with congenital disease. We recently completed studies of ZIKV infection in cells isolated from human placentas and amniochorionic membranes and reported patterns of viral proteins in explants of chorionic villi from first-trimester placentas (Tabata, *et al.* Cell Host & Microbe, 2016; Tabata, *et. al.* Journal of Infectious Diseases, 2017). Comparisons of ZIKV infection between prototype and pathogenic strains revealed impaired replication and functional defects in infected cells. Host cell factors were identified that modulate viral replication and could prolong viremia. Our studies suggest strategies to strengthen natural protection and reduce virus transmission at the uterine-placental interface.

Carlos A. Sariol

Caribbean Primate Research Center, Department of Microbiology and Medical Zoology, Department of Medicine, University of Puerto Rico, Medical Sciences Campus.

Key factor for DENV and ZIKV interaction.

It has been proven that time interval between consecutive DENV infections infection is critical in determining disease severity. Previous studies from human populations suggest that the window of cross-protection induced by a first infection with DENV against a second symptomatic infection is approximately 2 years. Nevertheless, longer intervals of time between infections result in an increase in the severity of dengue manifestations. Dengue and Zika (ZIKV) viruses are members of the Flaviviridae family and transmitted mainly through the same vector, the Aedes aegypty mosquitoes. This provides the perfect vehicle to affect previously endemic Dengue virus (DENV) areas with ZIKV1. In addition, ZIKV and DENV share a homology in the amino acid sequence of at least 50%, and it has been demonstrated that ZIKV undergoes Antibody-Dependent Enhancement (ADE) in vitro in response to previously generated antibodies from other flaviviruses including dengue.
However, there is gap understanding the role of the interval of time between a primary DENV and a secondary ZIKV infection in the pathogenesis of ZIKV.
In this work, we exposed to ZIKV three cohorts of rhesus macaques previously infected with DENV at different period of time. A third flavivirus-naive cohort was included as a control group. We assessed the outcome of ZIKV viremia, clinical parameters and the cellular and humoral immune response and compared the result among all cohorts. For the first time, we are showing in vivo in non-human primates, that time interval between the two infections are critical for the ZIKV infection outcome. Our results have a tremendous impact to understand the complex interplay among flavivirus, for the prediction of ZIKV outcomes in a setting of previous DENV immunity and for vaccines design and immunization schedules.

Clinical

Karin Nielsen-Saines
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Professor of Clinical Pediatrics
Division of Pediatric Infectious Diseases
David Geffen School of Medicine at UCLA
Director, Center for Brazilian Studies

Mother to Child Transmission of Zika virus and infant outcomes following *in utero* exposure

In this presentation, we will provide a review of the epidemiology and pathogenesis of Zika virus mother to child transmission and summarize data on pregnancy and infant outcomes following antenatal ZIKV exposure during the 2015-16 epidemic in Rio de Janeiro, Brazil.

James J. Sejvar
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Division of High-Consequence Pathogens and Pathology
National Center for Emerging and Zoonotic Diseases, Centers for Disease Control and Prevention

Arboviruses and Guillain-Barre syndrome -Lessons From the Zika Virus Outbreak.

Description: Guillain-Barre syndrome (GBS) is an uncommon peripheral nerve disorder. GBS is due to an immune response to an antecedent antigenic stimulus which leads to formation of autoreactive antibodies and / or T cells which go on to attack self-proteins on peripheral nerves, leading to the characteristic limb weakness, cranial nerve palsies, and in severe cases, neuromuscular respiratory failure. Approximately 70% of GBS patients report an infectious illness (respiratory or gastrointestinal symptoms) in the weeks before weakness onset. GBS incidence increases with increasing age, with peak incidence in ages 50 and above. Many different pathological agents, including various bacteria and viruses, have been temporally associated with GBS, but with rare exceptions (Campylobacter jejuni), a causal association between infection and GBS is absent. Beginning in 2013, outbreaks of Zika virus have been strongly associated with a much greater-than-expected increase in incidence of GBS, well above the expected 1.2 cases / 100,000 population / year as determined by meta-analyses. This talk will explore the evidence for ZIKV as a cause of GBS, and highlight evidence for other arboviruses and their role in GBS.

Stephen Thomas
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Developing a Zika Vaccine: the Knowns and Unknowns

The current state of the art in Zika vaccine development with a focus on reviewing the results of human trials will be reviewed. Animal data will be discussed in the context of proposing potential immune correlates of protection and risk. Finally, remaining gaps in understanding these topics together with ideas of how these are impacting clinical development plans will be reviewed.

Diagnostics/Prognostics

Eva Harris

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley

Development and evaluation of serological methods for Zika diagnosis and surveillance and use in studies of seroprevalence and long-term immunity

Diagnosis and surveillance of Zika in the Americas have been challenging due to similar clinical manifestations and extensive antibody cross-reactivity with endemic flaviviral diseases, such as dengue. We developed several new serological assays and evaluated four serological and two real-time RT-PCR methods in acute, early convalescent, and late convalescent samples from the same individuals in a long-term pediatric cohort study in Nicaragua. We then used the Zika NS1 blockade-of-binding (BOB) ELISA to perform seroprevalence studies and spatial analyses of anti-ZIKV antibodies in both pediatric and adult cohorts in Nicaragua. The Zika NS1 BOB assay was also used for seroprevalence and serological studies in a number of other countries in both Latin America and Asia. Analysis of anti-DENV and anti-ZIKV neutralizing antibodies in longitudinal samples collected months to years after DENV and ZIKV infection in Nicaragua will also be presented. Together, these studies provide critical tools and information about DENV and ZIKV infection and immunity in regions where the viruses co-circulate.

Irene Bosch

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A low cost methodology to detect specific exposure to Zika virus

ZIKV virus (ZIKV) was responsible for the explosive 2015–2017 epidemic in the Americas. ZIKV infection during pregnancy is linked to devastating birth defects and associated anomalies, designated congenital ZIKV syndrome (1) whereas in adults, ZIKV infection has been associated with Guillain Barré syndrome (2). Flaviviruses are enveloped RNA viruses containing an aprox. 11-kb positive-stranded RNA genome that encodes three structural and seven nonstructural proteins. Cells infected by flaviviruses secrete nonstructural protein 1 (NS1). Antibody responses generated in response to flavivirus infections are notoriously cross-reactive, representing a significant obstacle for the specific diagnosis of infection using serological assays. Conventional ELISAs using traditional viral antigen E (Envelope protein) have been found to poorly differentiate among flavivirus infections. This is especially problematic with antibodies to ZIKV and dengue virus (DENV), which co-circulate in the Americas. Neutralization assays can measure virus-specific neutralizing antibodies; however, specificity is affected by the production of cross-reactive neutralizing antibodies. After secondary DENV infections their is more challenge to

accurately identification of ZIKV infections, therefore it has made very challenging to determine the burden and rate of asymptomatic infections, define the incidence of congenital ZIKV syndrome among infected women, and identify neurologic complications associated with ZIKV infection. We propose a low cost, simple ELISA test that can distinguish dengue and ZIKV infections by measuring the amounts of antibodies against NS1 protein in serum of the mothers and children born during the ZIKV epidemics. We also show the a point of care device performance using patient samples to detect active infection of dengue, Zika or Chikungunya as a comercial point of care device fabricated a very low cost.

Wong, Susan J.

Wadsworth Center, New York State Department of Health, Albany, NY, USA

The flavivirus multiplex microsphere immunoassay for recent and past infection.

In 2016 our laboratory tested 10,274 sera from travelers at high risk of Zika and dengue infections. More than 40% of our travelers had evidence of previous flavivirus infection. An urgent need exists for serologic tests that can accurately identify recent Zika infection beyond the window of RT-PCR and IgM serology. Also needed is an assay that can be used to identify flavivirus naïve and flavivirus experienced study subjects for vaccine trials in dengue endemic areas. We developed a multiplex microsphere immunoassay measuring total antibodies to recombinant Zika envelope, Zika NS1, DENV1 NS1, DENV1 NS1, DENV3 NS1 and DENV4 NS1 proteins. This assay discriminated evidence of infection at some time by flavivirus, by Zika, by dengue, or by both Zika and dengue. It is important to determine the recency of Zika infection and if it happened during a current pregnancy. We performed a multiplex IgG avidity assay using an 8M urea incubation step to measure the Zika and dengue avidity indices in patients with antibodies to both viruses. Results are available within about 4 hours on sera from 40 patients. This test strategy has a relatively high throughput and quick turn-around time compared to IgM ELISAs followed by virus neutralization which may miss Zika infection if beyond 3 months since exposure. Our test algorithm has provided rapid results in pregnant women, newborns, Guillaine Barre patients, and monkeys. Detection of Zika infection in pregnant women can be enhanced by use of tests that measure IgG to nonstructural proteins. In assessing primary dengue infection, measurement of antibodies to NS1 of all four dengue serotypes is important. In early acute infection, antibodies most often develop to the highly cross-reactive envelope protein and then to the nonstructural proteins. In about 10% of the time, antibodies will develop to NS1 before they are detected to envelope protein. In primary dengue infection, the early samples have only antibodies to the NS1 of one serotype. In secondary dengue infections, the antibodies bind to NS1 of all four dengue serotypes.
Funding by New York State Department of Health

Vaccines

Pei-Yong Shi

University of Texas Medical Branch, Galveston, Texas, USA

A single-dose live-attenuated Zika vaccine

Summary: Zika virus (ZIKV) infection during pregnancy can result in devastating congenital abnormities or fetal demise. The infection can also cause Guillain-Barré syndrome in adults. The development of a safe and efficacious vaccine is a public health priority. This presentation summarizes the current status of Zika vaccine development. In addition, I will present the preclinical safety and efficacy results of a single-dose live-attenuated vaccine. The attenuated vaccine contains a deletion in the 3'UTR region of the viral genome. A single-dose immunization of this vaccine prevents ZIKV infection in non-human primates, diminishes mouse *in utero* transmission, and protects male reproductive damage. The vaccine also showed an excellent safety profile in mouse and non-human primates. These results suggest that further development of this single-dose live-attenuated vaccine is warranted for humans.

Round-Table
Hansi Dean

COMPREHENSIVE ANALYSIS OF THE IMMUNE RESPONSE ELICITED BY TAKEDA'S TETRAVALENT DENGUE VACCINE

Dean, Hansi, Egan, Michael, Wallace, Derek

Takeda Vaccines, Inc., Cambridge, MA; Takeda Pharmaceuticals International AG, Zürich, Switzerland

Funding of the research: Takeda Vaccines

Dengue viruses are re-emerging pathogens with a global disease burden of approximately 100 million infections annually. Takeda Vaccines has developed a live attenuated tetravalent dengue vaccine (TDV). TDV is based on a recombinant attenuated dengue serotype 2 virus (TDV-2). For dengue serotypes 1, 3, and 4, the pre-membrane (prM) and envelope (E) genes replace the corresponding TDV-2 genes in the attenuated backbone to form chimeric viruses (TDV-1, TDV-3, and TDV-4). The safety and efficacy of TDV is currently being evaluated in a double-blinded phase III controlled study being conducted in eight dengue-endemic countries in Latin America and Asia.

Dengue vaccine development has been hampered by an incomplete understanding of the host immune response following vaccination and the lack of clear immune correlates of protection. To address these issues, we have taken a systematic approach towards detailed characterization of humoral, cellular and innate immune responses elicited by TDV. Data will be presented on magnitude, type-specific, cross-reactive and epitope-specific neutralizing antibody responses, magnitude, functionality and specificity

of T cell responses, innate immune responses and characterization of anti-NS1 antibody responses following vaccination. Recognizing that non-neutralizing antibody responses may contribute to vaccine efficacy, progress on assays capable of quantitating the magnitude and avidity/affinity of the total dengue-specific antibody response, ADCC and other functional activities will be presented.

Currently, clinical samples collected pre- and post-vaccination at early and late timepoints from children and adults participating in phase 2 randomised double-blind trials conducted in dengue-endemic countries are being used to fully characterize the innate, humoral and cellular immune response following vaccination with TDV. We will present our progress on the comprehensive analysis of the immune response elicited by TDV, and opportunities to compare immune responses elicited by tetravalent vaccination with immune responses following natural infection. Development of a panel of immunological assays may be applied to case-controlled analysis of breakthrough dengue infections in on-going efficacy trials to identify immune correlates or surrogates of protection against dengue.

Benedetta Ghezzi
Takeda

Title Takeda's dengue vaccine: a program update

Briefly, we will describe our dengue vaccine development program including our current and planned clinical trials and ongoing work to characterise the immune response to our vaccine.

Jonathan Smith
VLP development teams at PaxVax and the Vaccine Research Center, NIH

Virus–Like Particle (VLP) Vaccines for Chikungunya and Zika

Summary: A VLP vaccine for chikungunya has been assessed in Phase 1 and Phase 2 clinical trials, and has been shown to be well tolerated and highly immunogenic. An additional phase 2 trial is underway to optimize dose and to assess the potential advantages of an alum formulation. VLP vaccines for Zika have been constructed using native and mutated sequences, and have been assessed for their relative immunogenicity and efficacy in preclinical animal studies. A combination chikungunya and Zika VLP vaccine has also been shown to induce high levels of neutralizing antibody to both viruses without interference.

Abstracts Selected for Oral Presentations

Epi/Phylogenetics

Short talk 1: Drumond, Betania - YELLOW FEVER EPIZOOTICS IN URBAN AREAS: BRAZIL, 2016/2017, Brazil

Short talk 2: Cruz, Cristopher - THE PHYLOGEOGRAPHY AND PHYLODYNAMICS OF DENV-3 IN PERU, Peru

Short talk 1: Sequetin, Mariana - Yellow fever in São Paulo State: a threat in peri-urban centers?, Brazil

Short talk 2: Coello-Escoto, Ana - A pipeline for measuring antigenic relationships among diverse, low-passage dengue viruses, US

Epi/Immunology

Short talk 1 : Delgado et al. Cross-reactive immune responses upon sequential dengue and Zika virus infection. US,

Short talk 2 : Haller et al., MAYV emergence in a CHIKV endemic world: Is MAYV a new a threat? US

Immunology

Short talk 1 : Guey Chuen Perng, Functional analysis of PBMC in dengue endemic and non-endemic regions (Taiwan)
Short talk 2: Ramjag Anushka et al, Characterization of Immune Responses to Selected Arboviruses and determination of seroprevalence in Trinidad (BSRI US and Trinidad)

Virology/Antiviral – Pathogenesis

Virology

Short talk 1: Campos, et al., Ribosomal stalk proteins are required for DENV translation elongation, US

Short talk 2: De Borba et al., RNA structure duplication in the DENV genome: Functional redundancy or host specificity, Argentina

Virology/Antiviral

Short talk 1 : Shin et al., A combined genetic/proteomic approach identifies functional host factors associated with flavivirus RNA, US

Short talk 2 : Sacramento et al., Sofosbuvir as an antiviral against Zika virus, Brazil

Pathogenesis

Short talk 1 : Kumar et al., Zika virus replication and persistence in human Sertoli cells, Canada

Short talk 2 : Costa et al., Annexin A1 as a novel pro-resolving molecule against DENV infection, Brazil

Vaccines:

Short talk: Swanstrom et al., Mapping the target epitopes of the type specific antibody responses induced by a live-attenuated University of North Carolina, Chapel Hill, US

Clinical/ Diagnostic

Clinical

Short talk 1: Collins ZIKV pregnant women serology study Nicaragua/US.
Short talk 2: Rezende I, et al. Yellow fever: clinical, virological and immunological aspects, Brazil

Diagnostic

Short talk 1: Makeda Predictive biomarkers for severe dengue US
Short talk 2: Medina 1 MAC-ELISA that can differenbtiate DENV and ZIKV infections / Why VLP could achieve such specificity? CDC, Puerto Rico

Vector Biology/Ecology/Control

Vector Biology

Short talk 1 Pompon J How non-coding flaviviral RNA enhances mosquito transmission. US
Short talk 2 Hughes G Microbiota and Zika virus interactions in field-collected Aedes aegypti. US

Ecology/Control

Short talk 1 Ortiz, M Integrated Vector Control Management After Two Hurricanes in Puerto. Puerto Rico
Short talk 2 Astete H COMPARISON of the efficacy of the bg sentinel and suna traps for capture of Aedes aegypti mosquitoes in Peru. Peru

Epi/Phylogenetics

Yellow fever epizootics in urban areas: Brazil, 2016/2017

Sacchetto, Lívia[1]; Rezende, Izabela Maurício[1]; Mello, Érica Munhoz[2]; Arruda, Matheus Soares[1]; Costa, Thais Aalkifeles[1]; Cruz Ana Luísa Ccampos[1], Prado, Alaine Isabela A[1]; Stump, Rodolfo GAV[1]; Massara, Rodrigo L[1]; Pascoal, Ana MO[1], Perini, Fernando[1]; Santos, Fabrício[1]; Paglia, Adriano P[1]; Teixeira, Érika P[3], Barreto, Cecília[3]; Vilela, Daniel AR[3]; Matos, Laerciana SS[3], Alves, Pedro Agusto[4], Kroon, Erna G[1]; Trindade, Giliane de Souza[1], Thoisy, Benoit[5]; Drumond, Betânia Paiva[1]

[1]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
[2]Centro de Controle de Zoonoses, Belo Horizonte, Minas Gerais, Brazil.
[3]Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis/Centro de Triagem de animais silvestres, Belo Horizonte, Minas Gerais, Brazil.
[4]Instituto René Rachou- Fiocruz, Belo Horizonte, Minas Gerais, Brazil
[5]Institut Pasteur, Cayenne, French Guiana.

In 2016/2017, Brazil faced the largest epidemics and epizootics of yellow fever (YF) in recent years. They were first detected in Minas Gerais (MG) state and then spread to other regions. A total of 1696 human cases and 162 deaths was reported. The epizootics had some uniqueness, being widespread in MG, and confirmed in 121 and suspected in 351 municipalities. We received, 395 non-human primates (NHPs) carcasses to be identified, processed and analyzed. Total viral RNA was extracted from liver and used for one-step real time PCR with specific primers and probe for YFV. From 157 samples analyzed so far, 64 (40.7%) were positive. YFV was detected in *Alouatta* sp. (29.6), *Callicebus* sp. (26.5) and *Callithrix* sp. (43.7), in rural (48.4%), periurban (3.1%), and urban areas (40.7%). It is important to note that YFV was detected in 11 *Callithrix* sp., which are currently found in forest fragments in urban area of Brazil. NHPs infected with YFV were from 10 out of 12 regions of the state, especially from Metropolitan, South/Southeast and Zona da Mata regions. Phylogenetic analysis, based on envelope gene, indicated that the YFV strains clustered within Genotype South America-I. Bayesian analysis indicated that the median substitution nucleotide rate of YFV from NHP was 5.9 X 10^{-4} and the most recent common ancestor was estimated to exist in 2014 (95%BCI = 2013-15). This indicate that virus might have been silently circulating previously to the outbreak in MG, in 2016/2017, contributing to the wide dissemination of YFV throughout the state. Positive animals have been collected up to July 2017, indicating a prolonged circulation of the virus in MG, including in urban areas, what could be a risk to the reurbanization of YF. Although there is a vaccine against YF, it is not recommended for children under 6 months, pregnant women, and immunesuppressed persons. In that way, a possible reurbanization of YF could be a threat to those people. Further studies are goingo to be conducted to investigate de dynamics of YFV maintenance/circulation in urban areas in Brazil. *Funding of research*: FAPEMIG (APQ01574-17), CNPq, CAPES, ERDF from European Commission, and Institut Pasteur de La Guyane.

The phylogeography and phylodynamics of denv-3 in Peru

Cristhopher Cruz[1], Milena Alba[1], Christopher Mores[1], Amy Morrison[1,2], Tom W. Scott[2], Simon Pollett[3], Mariana Leguia[1]
1) U.S. Naval Medical Research Unit No. 6, Lima, Peru; 2) University of California, Davis, California; 3) WRAIR Viral Diseases Branch, Silver Spring, Maryland

Dengue virus continues to cause outbreaks in Latin America despite ongoing efforts to control the spread of the disease and its vector. Several phylogenetic studies have documented the evolution of dengue virus serotype 3 (DENV-3) in Latin America reporting the circulation of different genotypes. However, Peruvian strains have not been thoroughly analyzed and several questions about introduction, distribution and emergence remain. These are relevant for the surveillance and control of DENV-3 in Peru. To address these questions, we sequenced the complete genomes of 213 DENV-3 viruses derived from naturally infected individuals in Peru and neighboring countries (Bolivia, Ecuador, Paraguay). The sequences generated in this study were compared to additional sequences deposited in global sequence databases. Phylogenetic analyses revealed circulation of three distinct sub-lineages of DENV-3 genotype III in Peru, with some co-circulation on a national scale. Peru experienced two distinct introductions via Ecuador (2000-2001), and a third introduction via Colombia (2015). Within Peru, viral traffic results show inter-department transmission between coastal and eastern parts of Peru. Additional analyses performed in Iquitos, the largest Amazonian city in the country, suggest a single introduction and persistence, rather than a mixed population of multiple introductions from other locations within Peru. Bolivia, Brazil, and Paraguay strains do not cluster with Peruvian DENV-3 isolates, suggesting a lack of evidence to support importations from the east despite evidence of ample DENV-3 viral traffic between the three countries. The results strengthen our knowledge about introduction and evolution of DENVs in Peru, reinforcing the need to establish robust surveillance programs for the introduction or emergence of new DENV genotypes.
Funding of research: 800000.82000.25GB.B0016, US Dept. of Defense Armed Forces Health Surveillance Center (AFHSC), Global Emerging Infections Surveillance and Response Systems (GEIS)

A pipeline for measuring antigenic relationships among diverse, low-passage dengue viruses

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Dengue is a disease of global concern caused by any of the four antigenically related dengue viruses (DENV1-4). Antigenic differences between sequential infecting DENV strains may be important to individual risk and population level transmission of specific viral types. We have developed a pipeline for measuring antigenic relationships among global DENV1-4 strains as well as DENV1-4 isolated from patients in Bangkok, Thailand from 1994-2006. In order to work with diverse, low-passage strains, we optimized multiple assay parameters of the plaque reduction neutralization test (PRNT), including use of C6/36 (*Aedes albopictus*) cells on 96-well plates. We significantly reduced the 'edge effect' (when fewer plaques are observed in wells in the edge as compared to the centers of each plate), under conditions of higher humidity (90% vs. 80%), higher volume (200µl vs. 100µl), and fewer days until starting the neutralization assay (1 vs. 4 days). To increase the magnitude of $PRNT_{50}$ titers, we assessed multiple incubation times of the virus-serum mix (30, 60, 90, and 120min) and found that by 90min the $PRNT_{50}$ titer doubled from the 30min titer for DENV-positive antisera, without increasing titers in DENV-negative antisera. Finally, to standardize the immunostaining process, we tested different concentrations of 2H2 1° and peroxidase conjugated 2° antibodies and found concentrations at which nearly all (n=176) DENV strains could be reliably stained. To increase the speed and consistency of PRNT data analysis, we developed Viridot (Viridot package), a program for R with a user interface in shiny that counts viral plaques of a variety of phenotypes, estimates $PRNT_{50}$ titers, and performs other calculations for the PRNT. Finally, $PRNT_{50}$ titers were evaluated using antigenic cartography, a method to quantify and visualize antigenic relationships among viruses based on the ability of individual sera to neutralize individual viruses. Resulting antigenic maps of these diverse DENV strains reveal that genotype, and to a lesser degree, time, lineage, and amino acid position in the envelope protein, are associated with antigenic differences. Future research should expand on existing antigenic maps and incorporate antigenic measurements into models to measure the effect of antigenic differences on risk of dengue disease.

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Cross-Reactive Immune Responses Upon Sequential Dengue and Zika Virus Infection

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The high level of DENV seroprevalence in areas where ZIKV is circulating have raised concerns on the risk of increased ZIKV disease severity for patients with a history of previous DENV infection. Recent studies have shown that anti-DENV pre-existing antibodies may enhance ZIKV infection and increase disease severity. However, little has been shown about the ability of these antibodies to neutralize ZIKV in the same context and regarding the T cell epitopes that are specific or shared between both of these viruses. Given these facts, to determine the role of DENV pre-immunity in ZIKV infection, the aim of this study was to analyze the T and B cell responses against ZIKV. Using PBMC from blood donors with previous history of DENV/ZIKV or ZIKV infection, we have identified ZIKV epitopes by screening T-cell responses against overlapping peptides spanning the ZIKV proteome by IFN-gamma enzyme-linked immunospot analysis. Furthermore, plasma samples were analyzed to quantify the neutralizing and enhancing activities of antibodies against DENV and ZIKV infections using a flow cytometry-based assay. Our results show that the ZIKV non-structural proteins NS1, NS3 and NS5 contain most of the immunodominant peptides that induce a strong T-cell response. Interestingly, in donors with a history of DENV infection, specific peptides were also identified as DENV CD8+ T-cell epitopes and the strongest T-cell responses observed in these donors correspond to sequences with a high level of amino acid identity with the four serotypes of DENV. These results strongly support the activation of cross-reactive T-cells in this context. Additionally, plasma samples from ZIKV-infected donors exhibited neutralizing activity only against ZIKV, and one donor showed enhancing activity for DENV4 infection. The highest neutralizing activity against ZIKV infection was observed in samples from donors with previous DENV and ZIKV infection, strongly suggesting the induction of cross-reacting antibodies induced upon sequential DENV and ZIKV infection. These data have crucial implications for future ZIKV and DENV vaccines and provide new opportunities to study the role of subsets of DENV- or ZIKV-specific T cells in the induction of broadly neutralizing antibodies in the context of sequential flavivirus infections, which could modulate disease severity.

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MAYV emergence in a CHIKV endemic world: Is MAYV a new a threat?

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Mayaro virus (MAYV) is an arthralgic alphavirus in the family *Togaviridae*. MAYV is transmitted primarily between arboreal mosquitoes belonging to the genera Haemagogus and non-human primate hosts. MAYV causes an acute febrile illness similar to chikungunya virus (CHIKV), an evolutionary relative in the Semliki Forest complex. MAYV infection results in fever, arthralgia, rash, and occasionally persistent joint pain that can last up to one year post infection. MAYV emergence is typically sporadic with Brazil and Peru being the most affected. Human cases have been reported from several countries in Central and northern South America, including Brazil, French Guiana, Haiti, Peru and Venezuela. Recent outbreaks in Peru and Venezuela, and an atypical case from Haiti suggest the virus remains of public health concern throughout the Americas.
Previous studies provide strong evidence of cross-protection among alphaviruses, so we hypothesized that previous exposure to chikungunya virus or vaccines against CHIKV may affect the outcome of MAYV disease and/or limit the emergence of this virus in humans. To assess the extent of cross-protection between CHIKV and MAYV, we pre-exposed immunocompetent and immunocompromised (IFNARa/b$^{-/-}$) mice to an Asian American lineage of CHIKV, two CHIKV vaccines, and a recently developed live-attenuated MAYV vaccine, and subsequently challenged each with MAYV. Mice were monitored for disease and bled to measure serum viremias and antibody titers. We observed cross-protection against MAYV for mice pre-exposed to CHIKV, similar to protection provided by the MAYV vaccine, and moderate but significantly reduced cross-protection from CHIKV vaccinated animals. Prior infection with ZIKV and VEEV provided no protection against MAYV disease or viremia. Studies to elucidate the mechanism underlying the observed cross-protection suggest that both humoural and cellular immunity are responsible. Specifically, cross-neutralizing antibodies produced following CHIKV infection play the primary role in diminishing viremia and disease, but T-cells further influence this reduction. Continued research on the mechanism underlying the observed cross protection will determine the extent to which prior CHIKV vaccination or infection may affect the re-emergence of this virus in the Americas.

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Immunology

Functional analysis of PBMC in dengue endemic and non-endemic regions

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Dengue is one of the most troublesome mosquito-borne human viral diseases in the world. The disease mainly affects tropical and subtropical temperate zone, where dengue is endemic since the disease is year round and high percentage of populations are infected by the dengue virus (DENV) based upon the serological studies. In non-dengue endemic country, such as Taiwan, where very low percentage of dengue antibody can be detected. Pilot study demonstrated that stem cells in peripheral blood mononuclear cells (PBMC) are much more permissive to DENV infection. In order to further understand the potential effects of PBMC in dengue endemic zones, we collected blood from more than 200 migrant workers landed in Taiwan on the very first day from four countries, Thailand, Indonesia, Vietnam, and Philippines, where dengue is endemic. The sero-prevalence of DENV infections was performed with commercial ELISA kits. The levels of stem cells in PBMC were analyzed by FACS. Sorted phagocytic cells were exposed to DENV briefly and subjected to RNA microarray analysis. These results were compared with blood collected from local residents. Results revealed that over 90% of migrant workers were infected by DENV based upon serological assay, only 15% of local residents were positive. The levels of stem cells in PBMC were significantly higher in migrant workers. RNA array data suggested that phagocytic cells were more anergy to DENV, and more toward to signaling pathways for adaptive immune cells upon stimulation in dengue endemic subjects. In contrast, the phagocytic cells were very robust response to DENV and more toward to homeostasis events upon exposed to DENV in local individuals. The cumulative results suggest that immune cells are constantly being challenged by DENV and more tolerant to DENV infection in dengue endemic countries, implying a new research avenue on development of vaccine or antiviral drugs.

Characterization of Immune Responses to Selected Arboviruses and determination of seroprevalence in Trinidad

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We aim to identify and determine the seroprevalence of arboviral infections in Trinidad, and to characterize immune responses against them, in order to identify antibodies that may be useful in the development of novel diagnostic tests, therapeutics and vaccines. Serum, plasma and PBMCs were collected from individuals presenting with acute undifferentiated fevers at a major hospital. Samples were collected within two weeks of onset of symptoms, then at four months and one year post-onset. Using RT-PCR and / or serological assays, Zika virus (ZIKV) and Chikungunya virus (CHIKV) infections were identified in individuals with and without evidence of prior Dengue virus (DENV) infection. B-cell isolation will be carried out and clones screened for the presence of antibodies that inhibit entry or viral release. An anti-budding assay based on a vaccine strain of CHIKV is currently being optimized for screening for the latter in CHIKV experienced individuals. Using Immune Repertoire Capture technology (IRCTM), we characterized plasmablasts from an individual with acute ZIKV infection and previous exposure to DENV. We observed evolution of clonal families of antibodies and diverse patterns of neutralization, even within an individual lineage. Further analysis of plasmablasts from primary and secondary flavivirus infections are underway in order to determine the extent to which ‘original antigenic sin’ from previous DENV infections shapes the humoral response to ZIKV infection, and the impact this may have on neutralizing and enhancing antibodies. In addition to studies based on symptomatic individuals, a serosurvey for ZIKV and CHIKV is currently underway using a cohort of normal, healthy women presenting at antenatal clinics.

Ribosomal stalk proteins are required for dengue translation elongation

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The Flavivirus genus contains several arthropod-borne viruses that pose global health threats, including Dengue virus (DENV). To understand how these viruses infect human cells, we previously conducted genome-scale RNAi screens for several flaviviruses and identified two ribosomal proteins that were crucial host factors required for translation of the DENV genome: RPLP1 and RPLP2 (RPLP1/2). These proteins form a stable heterodimer that attach to the ribosome through binding another ribosomal protein, RPLP0, forming a structure termed the ribosomal stalk. The stalk is thought to function by recruitment of elongation factors to the ribosome, but evidence from our group and others show it is not broadly required for cellular translation. This suggests the hypothesis that RPLP1/2 are accessory ribosomal proteins that function to promote translation of specific cellular mRNAs sharing undefined features with the DENV genome. To address this hypothesis, and identify the defect in DENV translation associated with RPLP1/2 depletion, we performed ribosome profiling. This technique allows quantitative mapping of ribosomes across the transcriptome under different experimental conditions. Treatment of cell lysates with RNAse degrades mRNA that is unprotected by translating ribosomes, allowing for deep sequencing of ribosome-bound fragments. We infected A549 cells for 2.5 hours with DENV under control or RPLP1/2-depleted conditions, and then processed samples for ribosome profiling. We observed that overall ribosome occupancy is altered in the viral open reading frame with RPLP1/2 knockdown, consistent with a role for RPLP1/2 in promoting translation elongation. Several prominent ribosome stalling sites were evident in DENV RNA, many of which were enhanced by RPLP1/2, most notably in the envelope protein coding sequence. We also observed that RPLP1/2 depletion resulted in altered ribosome density in mRNAs encoding other ribosomal proteins and transmembrane domain-containing proteins. We are currently identifying cis-acting features in the DENV genome and cellular mRNAs that are responsible for changes in ribosome density due to RPLP1/2 depletion. This work increases our knowledge on DENV translation regulation and sheds light on the function of RPLP1/2 in translation of specific cellular RNAs.

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RNA structure duplication in the DENV genome: functional redundancy or host specificity

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The dengue virus (DENV) genome contains a single open reading frame flanked by two highly structured untranslated regions (5' and 3'UTRs). The 3'UTR bears two pairs of duplicated RNA structures: two stem-loops (SLs) and two dumbbells (DBs), both involved in highly stable structures, including tertiary interactions like pseudoknots (PK). We have previously shown that the two SLs have different functions in vertebrates and invertebrate hosts. In this regard, a host adaptable SL structure has been identified, which was associated to the generation of different species of sfRNAs. Interestingly, DENV isolated from infected *Aedes aegypti* and *Aedes albopictus*, showed positive selection of mutations in one of the two DB structures. In order to evaluate the relevance of this selection, we introduced the identified mutations in the context of an infectious clone and assessed viral replication in mosquito and human cells. The phenotype of the viruses obtained indicated that mutations that destabilize the PK of DB2 increase the ability of the virus to replicate only in mosquito cells, explaining the natural selection of those mutations in mosquitoes, and providing evidence of different roles of this DB structure in the two hosts. Mechanistic studies designed to evaluate the function of the identified RNA elements supported the idea that the PK interaction of DB2 competes with genome cyclization, reducing RNA synthesis. Thus, disruption of the PK may be responsible for enhancement of viral replication in mosquito. Our data support an antagonistic role of the two DBs, while the DB1 has been involved in promoting genome cyclization (de Borba, *et al.* J. Virol., 2015), elements of DB2 compete with this RNA cyclization. In summary, our studies indicate that each structure of the two pairs, SLs and DBs, play different functions during viral replication. In addition, each of these RNA elements displays specific roles in the two hosts.

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A combined genetic/proteomic approach identifies functional host factors associated with flavivirus RNA

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Abstract
Dengue virus and Zika virus are flaviviruses that cause severe human disease in many countries worldwide. A thorough understanding of the cellular factors and pathways required by these viruses for efficient replication may lead to novel therapeutic options. Functional genetic screens using CRISPR and haploid genetics found defined ER-localized multi-protein complexes as critical for dengue and Zika virus infection including oligosaccharyltransferase (OST complex). Our previous study suggests that some of the ER-localized proteins are important for the formation of a functional replication complex, that consists of viral RNA, the viral non-structural proteins and host proteins anchored to the ER-membrane. To gain further insights in the composition of the viral RNA replication complex, we used CHIRP-MS (Comprehensive identification of RNA binding proteins by mass spectrometry). This unbiased technique relying on in vivo crosslinking followed by oligonucleotide-based pull down, allowed us to pinpoint host proteins that are physically associated to flaviviral RNA during infection. Several of the top-scoring hits in the functional genetic screen including OST subunits were also identified as viral RNA binding proteins reinforcing the notion that they may serve as structural components of the viral replication complex. Further intersection of the RNA binding data with the functional genetic screens, which we now have extended to all 4 serotypes of dengue virus as well as 3 different Zika strains, pointed to several new RNA binding proteins (RBPs). Using this intersection, we nominated a few host RBPs without known roles in the viral life cycle. We show, through genetic knockouts and directly assessing their RNA interactomes, the functional importance of these RBPs for the dengue and Zika virus infection cycles.

Sofosbuvir as an antiviral against Zika

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Introduction: Zika virus (ZIKV) is a member of the *Flaviviridae* family which causes congenital malformations and neurological disorders in adults. Thus, antiviral interventions are an urgent necessity. Sofosbuvir is clinically approved against HCV and targets the protein that is most conserved among the members of the *Flaviviridae* family, the viral RNA polymerase.
Objective: To evaluate pre-clinically the anti-ZIKV activity of sofosbuvir.
Material and Methods: In silico, cell-free, -based and in vivo assays were used. Sequencing of the virus genome was conducted. Short- and long-term neuromotor and cognitive assays were performed.
Results: Sofosbuvir inhibits ZIKV RNA polymerase, targeting conserved amino acid residues. Sofosbuvir inhibited ZIKV replication in human hepatoma (Huh-7) cells, neuroblastoma (SH-Sy5y) cells, iPS-derived neural stem cells (NSC) and brain organoids. Besides acting directing on the viral RNA polymerase, we found that sofosbuvir induced A-to-G mutations in the viral genome. In neonatal Swiss mice, sofosbuvir reduced acute levels of ZIKV from 60 to 90% in blood plasma, spleen, kidney, and brain. Early treatment with sofosbuvir doubled the percentage and time of survival of ZIKV-infected animals. Sofosbuvir also prevented the acute neuromotor impairment triggered by ZIKV. In the long-term behavioural analysis of ZIKV-associated sequelae, sofosbuvir prevented loss of hippocampal- and amygdala-dependent memory.
Discussion/conclusion: Our results indicate that sofosbuvir inhibits ZIKV replication, which is consistent with the prospective necessity of antiviral drugs to treat ZIKV-infected individuals.
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Zika virus replication and persistence in human Sertoli cells

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Introduction: Zika virus is an arbovirus associated with microcephaly in newborns and Guillain–Barré syndrome in adults. As well as transmission by mosquitoes, the virus can be sexually transmitted in humans where it can persist in semen for several months after initial infection. However, very little is known regarding the cell type that support virus replication and persistence in human testes.
Objectives: The objectives of this study were to: i) Examine the replication and persistence of Zika virus in two predominant cell types in testis, Sertoli cells and Leydig cells; ii) Determine the changes in gene expression profile of Sertoli cells following Zika infection and; iii) Study the effect of differentially expressed genes on viral infection.
Materials and methods: Primary human Sertoli and Leydig cells were infected with Zika virus strains MR766 (African lineage) and strain PRVABC59 (Asian lineage). Viral replication and titer were measured by quantitative real-time PCR and plaque assay respectively while the percentage of infection was determined by FACS analyses.
Results and discussion: We observed that Sertoli but not Leydig cells supported high levels of Zika virus infection that were in fact comparable to permissive continuous cell lines. Despite this, Sertoli cells were highly resistant to virus-induced apoptosis and induction of antiviral genes was muted. Viral entry was dependent on the TAM family receptor Axl. Zika virus readily established persistent infection in Sertoli cells with virus shedding observed for >6-weeks. The transcriptional profile of Sertoli cells was dramatically affected by virus infection with basic fibroblast growth factor (FGF2) being the most upregulated mRNA. Further analyses revealed that this cytokine enhances Zika virus replication and supports viral persistence in Sertoli cells. In summary, our findings provide key insights into mechanisms regulating Zika virus replication and persistence in the male reproductive tract. As such, these studies may aid in developing strategies to minimize sexual transmission of this pathogen.
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Annexin-A1 as a Novel Pro-resolving Molecule Against Dengue virus Infection

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Dengue is a mosquito-borne disease caused by one of four serotypes of *Dengue virus* (DENV1-4). Severe dengue is characterized by excessive inflammation, thrombocytopenia, hemoconcentration and increased vascular permeability. Understanding endogenous control points and molecules within the excessive inflammatory response may give new view on disease pathogenesis and novel therapies. Recent studies have shown that Annexin-A1 (AnxA1), a molecule that signals via FPR2 receptor, exert central roles in the regulation of resolution responses. In the absence of this molecule, the resolution of inflammatory responses is delayed or inadequate. Our aim was to identify potential pro-resolving mediators associated with resolution of DENV outcomes. MENTHODS: Anx-A1 levels from plasma of DENV-infected individuals (dengue with or without warning signs and severe dengue) or heath controls or plasma from control and DENV-infected wild type (WT) mice infected by DENV were evaluated by ELISA assays. WT, AnxA1$^{-/-}$ and FPR2$^{-/-}$ mice were infected by DENV-2 (05K3295 strain) and disease parameters (platelets numbers, % of hematocrit and vascular permeability) were evaluated at 24, 48 and 72 hours after DENV infection. *In vitro*, infection of bone marrow derived macrophages (BMDM) from WT and Anx-A1 mice was performed and Anx-A1 expression evaluated by western blot and viral loads by plaque assay. Our results reveal reduced Anx-A1 levels in serum of DENV-infected patients or mice infected by DENV in comparison to health individuals or mice controls. Interestingly, Anx-A1 levels were even further reduced in patients with severe dengue in comparison to dengue without warning signs. In mice, DENV infection of AnxA1$^{-/-}$ and FPR2$^{-/-}$ yields a stronger infective reaction with more pronounced thrombocytopenia, hemoconcentration and plasma extravasation, leading to enhanced liver tissue damage. Treating WT or Anx-A1$^{-/-}$ DENV-infected mice with Ac_{2-26}, an AnxA1-active N-terminal peptide, resulted in protection on vascular inflammation and organ damage. Additionaly, Anx-A1 expression was elevated on BMDM infected by DENV and elevated viral loads were recovered from BMDMs supernatant from AnxA1$^{-/-}$ and FPR2$^{-/-}$ mice in comparison to WT littermates. Finally, Ac_{2-26} treatment to BMDMs from AnxA1$^{-/-}$ mice or A129 infected by DENV (viremia model) resulted in reduction of viral loads. Therefore, this study provides proof-of-concept that resolution biology is relevant to the regulation of host responses against DENV infections and suggests new ways for definition of pathogenesis and therapeutic approaches. Funding: Instituto Nacional de Ciência e Tecnologia em Dengue e Interação Microrganismo-Hospedeiro – APQ-03606-17 (INCT-dengue). Fundação de Amparo a Pesquisa de MG (FAPEMIG). Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível superior (CAPES).

Mapping the target epitopes of the type specific antibody responses induced by a live-attenuated Dengue vaccine

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Dengue virus is the most common arthropod borne virus and threatens about one-third of the global human population. The LATV TV003, a live-attenuated vaccine comprised of components directed to each of the four serotypes of Dengue virus (DEV1-DEN4), has been shown in a human challenge model to completely protect all vaccinated subjects from infection following challenge with DENV-2. While the tetravalent vaccine induces type specific antibodies directed against each individual serotype component, the strain specific epitope specific antibody response elicited following tetravalent vaccination remains unknown. Using homotypic and heterotypic depletions in combination with epitope-transplanted rDENVs, we map the portion of the polyclonal immune response directed against type-specific neutralizing epitopes. Importantly, the type-specific neutralizing response to DENV1 is partially directed towards the hMAb 1F4 EDI/II hinge region epitope while the DENV2 antibody response tracks strongly with the 2D22 EDIII quaternary and/or EDIII linear epitope. In addition, the DENV3 5J7 and DENV4 126/131 epitope specific responses were shown to partially track with the EDI/II hinge region of each virus in most individuals. By comparing the antigenicity of each individual component to the tetravalent mix, our data support the hypothesis that the LATV tetravalent vaccine elicits strong type specific responses against each component of the vaccine.
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Seroepidemiology of pregnant women and adverse outcomes during Zika emergence in Nicaragua

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The Zika epidemic has underscored the importance of tracking emerging infectious diseases, particularly in at risk populations such as pregnant women; however, expeditious implementation of surveillance systems is a major challenge. In León, Nicaragua, we established a cohort of women pregnant during 2016 (n=253) with the following objectives: 1) Monitor for adverse fetal outcomes associated with Zika infection 2) Determine the rates and risks for vertical Zika transmission in maternal infection 3) Establish cost-effective methods for surveillance of emerging pathogens in pregnant women that could be readily implemented in resource-limited settings. Our interim analysis is presented here (n=123). We leveraged the existing public health infrastructure for prenatal care to follow women from presentation through birth. We then utilize a reverse chronology testing algorithm to serologically screen samples for evidence of recent and remote Zika infection, finding the vast majority of women in this region (98%) have IgG reactivity in a Zika antigen capture ELISA. A very high degree of correlation between IgG results using cord blood or maternal peripheral blood (98% concordant, R^2=0.814, $p<0.0001$) was observed, indicating that cord blood can effectively be used for serologic studies on pregnant women for clinical, research, or epidemiologic purposes without having to perform venipuncture on the mother. We next used a 96 well, high throughput screen for Zika-neutralizing antibodies, defining positive as an estimated FRNT50 values of 800 or greater (64% positive). On a subset of samples, full neutralization testing and NS1 BOB assay were used as confirmatory tests, with 78% of screen-positive results validated by one or both tests. Taken together, these data indicate high rates of Zika infection (approximately 50% of this cohort). Adverse outcomes occurred in 7% of pregnancies; however, rates did not differ by Zika FRNT50 screen positive (5/73=7%) vs negative (3/41=7%). Our study is limited by incomplete testing for alternative cause of congenital anomalies. Analysis of the remaining subjects is ongoing and will determine if risk for adverse pregnancy outcomes segregates by our screening method or other serologic variables. This attractive approach to efficiently gather seroepidemiologic information could be applied to other outbreak settings.

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Yellow fever: clinical, virological and immunological aspects

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In 2016/2017, Minas Gerais (MG), Brazil faced the largest epidemics and epizootics of yellow fever (YF) in recent years. The pattern of the epidemics in MG fit the expected one during YF epidemics, apart from the huge affected geographical area and high numbers of notified (1696) and confirmed (475) cases in humans and a total of 162 confirmed deaths. We follow up 96 patients admitted to a tertiary hospital and YF was confirmed in 74 patients (by serological, molecular or viral isolation). Different biochemical, virological and immunological parameters were analyzed. Viral load detected in sera during infection course ranged from 10e7 to 10e1 genomes copies/mL of sera, with a decrease in viral load after seroconversion. Viral genome was detected by PCR, and confirmed by sequencing in urine, of at least two patients. Nineteen patients evolved to death and increasing levels of AST (above 1000U/L) in 72 hours of clinical follow-up was as a risk factor to death (odds ratio = 26.15). Lower levels of IL10 and higher levels of TNF, CXCL8 and CCL5 were observed in patients who died, when compared to patients who recovered. Vaccination coverage was very low, especially among positive patients living in rural areas. A total of 29 had no history of YF vaccination and 22 were vaccinated in the beginning of 2017, during the outbreak. From the 74 confirmed cases, 33 had received at least one dose of the YF vaccine. We investigated the source of infection in 20 patients (4 deaths and 16 recoveries) who were vaccinated previously (15 days to 1 month) to the onset of the symptoms. All strains grouped within the Genotype I clade from South America, indicating a infection with wild type virus. Complete genomes from three patients were determined and nine amino acid substitutions were unique to YFV strains from the 2016/2017 outbreak. Further molecular and biological characterization of these strains will be performed. Moreover, vaccination campaigns should be intensified especially among persons living in rural areas in Brazil. The results indicate that AST and other immunological parameters could be used as predictors of yellow fever severity. *Financial support*: FAPEMIG (APQ01574-17), CNPq (449458/2014-8), CAPES.

Predicting the development of severe dengue by an eight-gene set

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Introduction: There is an urgent need to identify biomarkers predictive of severe dengue. Single cohort transcriptomic studies have not yielded generalizable results or a parsimony gene set predictive of severe dengue. We hypothesized that integrating gene expression data from heterogeneous patient populations with dengue infection would yield a conserved gene set predictive of severe dengue. Methods: Ten dengue gene expression datasets were identified in publically available microarray repositories. A novel integrated multi-cohort platform was used to detect differentially expressed gene transcripts between uncomplicated and severe dengue patients and to validate the identified putative signature in silico and prospectively in a new cohort of 35 patients in Colombia. Dengue diagnosis was made based on a positive NS1 antigen and/or anti-NS1 IgM antibody and confirmed by RT-PCR and IgG avidity measurements. The expression level of the individual genes was measured via microfluidic qRT-PCR assays in peripheral blood samples. Results: Using the multi-cohort analysis to analyze 446 blood samples of dengue patients from seven gene expression datasets we identified an eight-gene set that predicts the development of severe dengue. We validated the diagnostic power of this gene set to distinguish dengue with or without warning signs from severe dengue in three independent datasets composed of 84 samples with a global area under the curve (AUC) 0.80 [95% CI 0.68-0.88]. Moreover, we prospectively validated this eight-gene set in a new cohort composed of 35 dengue patients and 20 healthy controls from Colombia with an AUC of 0.80 [95% CI 0.69-0.91]. The predictive power of the eight-gene set was not modulated by age, genetic background or viral strain. The severity scores measured in patients with severe dengue progressively declined during the course of infection and were higher than the scores measured in six Zika patients. Conclusion: Our findings indicate that the eight-gene set predicts the development of severe dengue prior to its onset and suggest that dengue infection itself triggers this host response. These findings may provide new insights into the pathogenesis of severe dengue and have implications for the development of prognostic molecular assays to identify patients at risk for severe dengue.

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A MAC-ELISA that can differentiate dengue and Zika virus infections

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Zika virus (ZIKV) is a mosquito-borne flavivirus that recently caused a pandemic and has been associated with congenital birth defects and adverse neurological outcomes. The arrival of Zika virus (ZIKV) to dengue DENV endemic areas came along with diagnostic challenges in serology. The current ELISA assays cannot simultaneously distinguish these viruses. Furthermore, cross-reactive antibodies produced in DENV and ZIKV infections make the PRNT unreliable for diagnostic purposes as most dengue endemic countries experienced ZIKV mainly as a secondary flavivirus infection. The goal of this study was to develop a MAC-ELISA that could simultaneously discriminate between DENV and ZIKV infections during the convalescent phase during which molecular diagnosis is no longer reliable. A ZIKV/DENV MAC-ELISA was developed using non-infectious virus-like particles (VLPs) as antigen to detect the presence of ZIKV or DENV anti-premembrane/envelope IgM. We analyzed over 400 well-characterized acute and convalescent cases of ZIKV, DENV, and non-flavivirus febrile illness from our established Sentinel Enhanced Dengue Surveillance System (SEDSS) in Ponce, Puerto Rico. Infections were identified with a diagnostic algorithm that utilized the ratio of the average OD450 reacting to ZIKV antigen/average OD450 reacting to DENV antigen to determine ZIKV positive cases. This was followed by calculating the average OD450 to DENV/the average OD450 to the normal antigen to determine DENV positive cases. Specimens that did not react above the cutoff value for DENV were considered negative for both viruses. The developed ZIKV/DENV duo MAC-ELISA displayed a 100% sensitivity and 100% specificity for ZIKV, and 100% sensitivity and 100% specificity for DENV compared to our in-house ZIKA MAC-ELISA and InBios DENV Detect IgM, respectively. A novel approach to differentiate DENV and ZIKV infections serologically was developed. Our ZIKV/DENV duo MAC-ELISA displayed sensitivity that was equivalent to both ZIKV and DENV stand-alone assays. The assay specificity was high enough that it can potentially replace the highly laborious PRNT for confirmation of ZIKV or DENV IgM detection.

How non-coding flaviviral RNA enhances mosquito transmission

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Abstract:
Dengue viruses produce a non-coding subgenomic flaviviral RNA (sfRNA) that we identified as the first viral enhancer of mosquito transmission. We determined that two sfRNA substitutions, identified in epidemic isolates, increase sfRNA quantity in salivary glands. Higher sfRNA quantity then inhibits components of the antiviral Toll pathway and increases virus production in salivary glands and infection rate of saliva, a proxy for transmission. To characterize the mechanism of action, we hypothesized that alteration of mosquito immunity results from sfRNA interactions with proteins. Using RNA affinity chromatography in mosquito cells coupled with quantitative MS, we identified 14 sfRNA-interacting proteins and characterized their antiviral functions *in vivo* in *Aedes aegypti*. Our results discovered the antiviral property of the sfRNA-interacting Staufen. Staufen contains four dsRNA binding domains (dsRBD) and is known to mediate RNA decay. We sought to determine whether sfRNA-Staufen interaction impacted dengue virus in salivary glands, as supported by higher expression of *Staufen* mRNA in salivary glands than in midgut. After oral infection, we observed that dsRNA-mediated depletion of Staufen increased virus genomic copies and sfRNA in salivary glands, but also in midgut. Using inoculation to bypass the midgut stage, we determined that Staufen antiviral function took place in the salivary glands. Altogether, our study characterized a viral strategy to enhance mosquito transmission and epidemic potential that involves sfRNA-mediated immune inhibition and is located in salivary glands, a tissue relevant for transmission. Identification of viral determinant of transmission and new antiviral factors will help design novel vector control approaches.

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Microbiota and Zika virus interactions in field-collected Aedes aegypti.

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The microbiome profoundly influences many aspects of the mosquito biology, such as reproduction, immunity and vector competence. While our understanding of tripartite interactions is expanding, most of our knowledge comes from experiments conducted with lab-reared mosquitoes, which have a different microbiome compared to their field counterparts. Furthermore, we have a poor understanding how variation of the microbiome between individuals in the field affects vector competence. Here we aimed to determine if specific microbiota from field caught *Aedes aegypti* influence Zika virus (ZIKV) infection and dissemination rates. To do this, host seeking female *Ae. aegypti* were caught in the field from three sites in Texas and offered a ZIKV infected blood meal. At 10 days post infection, mosquitoes were assessed for viral infection and titer as well as microbiome composition by 16S rRNA amplicon sequencing and total bacterial load by qPCR. In parallel, these experiment were also replicated with lab-reared mosquitoes with the inclusion of a blood fed treatment without virus enabling us to determine how ZIKV infection altered the microbiome. In general, the microbiome community structure of ZIKV infected and uninfected mosquitoes were similar. Furthermore, no changes were seen in total microbiome load between groups. However, specific bacterial taxa were differentially abundant between infected and uninfected mosquitoes. In lab-reared mosquitoes, ZIKV infection altered the microbiome diversity and total bacterial load in a strain specific manner. We disscuss these results in the context of how the microbiome could cause variation in vector competence, in addition to the potential for developing novel microbial control strategies to reduce mosquito-borne disease.

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Integrated vector control management after two hurricanes

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The Puerto Rico Brain Trust for Tropical Disease Research and Prevention, a program of the Puerto Rico Science, Technology, and Research Trust (PRSTR), held a meeting with local and international health experts to discuss the implementation of a long-term solution to control arboviral diseases in the Island. The conclusion of the meeting was a white paper describing the need to establish an integrated vector control program in Puerto Rico to monitor and control the mosquito *Aedes aegypti*, the vector in the Island for dengue, Zika, and chikungunya. As a result, in September 2016, the Puerto Rico Vector Control Unit (PRVCU) was established through a cooperative agreement between the Centers for Disease Control and Prevention (CDC), and the Puerto Rico Science, Technology, and Research Trust. PRVCU follows an integrated vector management (IVM) strategy, combining vector surveillance and monitoring, vector control operations, and island-wide community mobilization programs. During the first year, PRVCU focused on implementing vector surveillance program, creating innovative information systems, and boosting community engagement through citizen mobilization and education programs. The surveillance program started on three municipalities in the metropolitan area (Bayamón, Carolina, and San Juan) using ovitraps. Preliminary surveillance data showed between 77% to 92% positive presence of *Ae. aegypti* oviposition activity in a three week period. Eggs collected will be tested for resistance to several commonly used EPA approved pesticides. Initial results showed resistance to pyrethroids pesticides. After hurricanes Irma and María, PRVCU worked in liaison with Puerto Rico Department of Health, CDC, and Department of Defense to conduct surveillance and control activities across the Island. In addition, PRVCU launched a media campaign with three key messages about appropriate management of personal water reservoirs, water removal from hurricane debris, and personal protection. The community mobilization program started house to house interventions, distribution of educational material and repellents, and educational activities across the whole Island. Up to date, PRVCU has impacted 68 out of 78 municipalities on Island.

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COMPARISON of the efficacy of the bg sentinel and suna traps for capture of Aedes aegypti mosquitoes

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Aedes aegypti is the primary vector of dengue, chikungunya, and other viruses. *Aedes*-borne virus prevention and control programs have traditionally relied on household surveys, primarily of immature stages to access transmission risk. Fixed traps have numerous advantages over house to house surveys, as they collect adult stages and sampling can be standardized in space and time. The BG sentinel trap uses color contrast and a chemical lure to attract mosquitoes. The SUNA trap was developed for *Anophelines* in Africa and utilizes MB5 as a lure. Our objective was to evaluate both traps, with different combinations of lures and attractants for *Ae. aegypti*. Two traps (SUNA, BG), two lures (BG, MB5, no lure), and CO_2 (with or without) for a total of 9 treatments were compared in an abandoned lumberyard, heavily infested with natural populations of *Ae. aegypti*, in the city of Iquitos, Peru. Five SUNA and four BG traps were placed in covered locations a minimum of 100m apart. Traps were run from 0600-1800 and positions were rotated each day for 9 consecutive days; this process was repeated 2 additional times (3 replicates). A total of 10,654 mosquitoes comprised of 9 species were collected during the experiment. *Ae. aegypti* was the most abundant species (N=6,534, 61%). The average number of *Ae. aegypti* captured per trap night in BG (23) and SUNA (30) was not statistically different (p=0.60). Traps using the BG lure captured significantly more *Ae. aegypti* than no lure (LSmeans p=0.013) but this difference was not significant when compared to the MB5 lure. CO_2 had the highest impact on *Ae. aegypti* counts (p<0.0001); traps with CO_2 averaged 34 *Ae. aegypti* per trap-night compared to 14 *Ae. aegypti* per trap-night without CO_2. Our study demonstrated that the SUNA trap was as effective as the BG sentinel at capturing adult *Ae. aegypti* mosquitos. CO_2 significantly improved collection efficacy and appeared more attractive than lures. Both traps provide practical alternatives to labor intensive household aspirator collections that are subject to significant collector bias and could contribute to entomological surveillance and vector control evaluations.

Abstracts for Poster Presentation

Epi / Phylogenetics

Emergence of Chikungunya in Rio de Janeiro/Brazil years before surveillance detection

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Whereas there are abundant data concerning the prevalence and phylogenetic history of dengue (DENV) and Zika (ZIKV) viruses in Brazil, Chikungunya virus (CHIKV), another clinically important arbovirus, has received less attention. Here, we investigated individuals with a presumptive diagnosis of arbovirus infection who presented within five days of illness onset to sentinel health care units or Quinta D'Or Hospital, a private and general hospital in the city of Rio de Janeiro, during the interval March 2016 through July 2017. Serum or plasma samples were screened for DENV, ZIKV and CHIKV by RT-qPCR. RNA samples with the lowest ct values for CHIKV, which were negative for DENV and ZIKV, were selected for Virome Capture Sequencing Platform for Vertebrate Viruses (VirCapSeq-VERT) analysis. To investigate the origins of CHIKV in Rio de Janeiro, the newly generated genomes obtained were analyzed by using a relaxed lognormal molecular clock model in a Bayesian inference. Here, we show that CHIKV is the most prevalent known arbovirus circulating in Rio de Janeiro. The East-Central-South-African (ECSA) genotype of CHIKV has been circulating in the Northeastern Region of Brazil since 2010 and likely reached Rio de Janeiro, in the Southeastern Region of Brazil, in 2012. The observation that the ECSA genotype of CHIKV was circulating undetected for at least four years underscores the need for improvements in molecular methods for viral surveillance.

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Google Trends as chikungunya nowcast tool in seven american countries/territories

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Introduction: During a chikungunya epidemic it is often difficult to maintain individual case epidemiological surveillance, and often there is a significant delay in the flow of information due to the large number of cases. This information is important for disease control and patient care planning.
Objective: The objective of this study was to evaluate the accuracy of Google Trends as a tool for the nowcast of chikungunya cases with reference to reported cases.
Methods: Data were obtained from Brazil, Colombia, Costa Rica, Guadeloupe, Guatemala, Martinique and Dominican Republic on national official websites. Correlation analyzes were performed between the number of reported cases weekly and the Google Trends search indicator (we will call it GTI-ChikV), weekly lags of -2 to +2 were used between the two sets of data. We also analyzed the peak week of reported cases of chikungunya and GTI-ChikV. An exploratory analysis of these results was made considering the following predictors: total population, population density, Human Development Index, GINI Index, GDP per Capita, absolute number of cases, incidence coefficient and cases per kilometer-square.
Results: The Pearson's correlation between weekly chikungunya and GTI-Chik cases was 0.78, 0.94, 0.93, 0.65, 0.69, 0.92 and 0.89 (median = 0.89) respectively for Brazil, Colombia, Costa Rica, Guadeloupe, Guatemala , Martinique and the Dominican Republic. The difference in weeks between the peak of reported cases and the peak of cases by GTI-ChikV (peak reported - peak GTI-ChikV = peak difference) was respectively: -4.0, -6, -6, 1.0, 1 (median = 0). There was no significant correlation between the R values and the selected predictors. There was a negative correlation between the peak difference modulus and the absolute number of cases (Rho = -0.77; p> 0.05).
Discussion: There was a strong correlation between the monthly number of chikungunya cases reported and the GTI-ChikV (median = 0.89). In addition, 4 of the 7 countries reported the peak of reported cases and the GTI-ChikV peak between 0 and 1 week of difference, the difference between the peaks was smaller the greater the number of cases. GTI-ChikV can be a useful tool for epidemiological surveillance, nowcast of chikungunya cases, and health planning.

Yellow fever in São Paulo State: a threat in peri-urban centers?

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Yellow Fever virus (YFV) is a single positive RNA virus that belongs to family *Flaviviridae*, genus *Flavivirus.* In humans, yellow fever may vary from inapparent to a fatal disease. In Brazil, the virus is maintained by a sylvatic transmission cycle involving non-human primates (NHP) and forest canopy-dwelling mosquitoes, mainly *Haemagogus* spp. Urban yellow fever, transmitted by *Aedes aegypti*, was erradicated in 1942. This is a descriptive study encompassing epizootic events occurred between July 2016 and August 2017, in São Paulo State, Brazil. Fresh tissues from NHP found dead were sent to Adolfo Lutz Institute, São Paulo, for YFV laboratory surveillance using RT-qPCR. A total of 935 NHP were sent during this period from several cities, being 175 *Alouatta* sp. 547 *Callithrix* sp., 8 *Callicebus* sp., 21 *Cebus* sp., 30 *Sapajus* sp., 3 *Leontopithecus rosalia* and 1 *Ateles* sp. 155 animals were not identified. A total of 163 (17,4%) were positive for YFV in 33 (5,11%) cities, with some of the animals found in the city center or near them, revealing the existence of YFV in urban areas in the countryside. Until the beginning of 2017, only cities within the vaccination recommended area showed viral circulation. However, in the beginning of 2017, positive NHP were found in cities where human population was naïve, putting millions under risk. YF cases were distributed throughout the year, with most cases occurring in November 2016 and April 2017. Virus was isolated from 3 NHP samples and from an *Haemagogus leucocelaenus* collected in Ribeirão Preto, a city with 600.000 inhabitants where a fatal human case occured. Phylogenetic analysis using Maximum-likelihood clustered samples obtained from a NHP and from a mosquito with Brazilian and other South American isolates, showing a temporal association, as we found more similarity with NHP isolates from Venezuela from the 2000`s outbreak. However, for a better evaluation of viral dissemination in South America, more complete genomes must be obtained. Laboratory detection of positive NHP triggered a rapid mass vaccination in new areas, and any human urban-associated case was detected in Brazil so far.

Development of a dengue virus sequencing assay for genomic surveillance

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The recent increase in global transmission of the 4 dengue virus (DENV) serotypes has been implicated with the increase in virus genomic diversity especially in regions of high incidence, endemic transmission, or were previously unexposed to DENV. This expansion has driven the divergence of genotypes and other sub-genotypic lineages, which have been frequently associated with epidemic potential and more severe clinical manifestations. Knowledge of dengue virus diversity and transmission in the Americas is limited, largely affected by the lack of genomic surveillance and substantial underrepresentation in the public genome repositories. In order to address this deficiency, we collaborated with the Pan American Health Organization (PAHO) to develop the Dengue Genomic Surveillance Project in the Americas (ViGenDA) to incite the systematic study of DENV genomic diversity integrated to molecular and epidemiological surveillance. Thereupon, we developed a partial genome sequencing assay capable of sequencing directly from clinical specimens and is readily adaptable to any dengue molecular surveillance laboratory. Serotype-specific oligonucleotides were designed to detect contemporary genotypes with public health relevance. The CDC Dengue Virus E Gene Sequencing Assay is serotype-specific and targets the envelope (E) glycoprotein gene, which is amplified by a single RT-PCR protocol and sequenced with a bi-directional terminator dye Sanger method. Consensus sequence of the complete E gene is assembled with a minimum 2x coverage achieved through this assay. To determine if the assay can amplify and sequence global strains of DENV, we tested a panel of 25 DENV strains isolated from diverse global regions representing a variety of genotypes within the 4 serotypes. We obtained longer than E gene contigs of sequence with ≥ 2x coverage from all strains except for a sylvatic isolate whose target sequence varies significantly from the rest of the contemporary strains and only partial sequence was obtained. To date, this assay has been transferred to over 14 laboratories, partners of the network of arbovirus laboratories of the Americas (RELDA) through a series of laboratory workshops. More than 4 participating laboratories currently implement this assay as part of their molecular epidemiological surveillance and efforts are underway to harmonize dengue genomic surveillance across the Americas.

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Excess mortality in Guadeloupe and Martinique during chikungunya epidemic, 2014

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Introduction: In some chikugunya epidemics, deaths are not fully captured by the traditional surveillance system, based on case reports and death reports. This phenomenon has already been described in other situations.
Objective: This is a time series study to evaluate the excess of mortality associated with epidemic of chikungunya virus (CHIKV) in Guadeloupe and Martinique, Antilles, 2014.
Methods: The estimated population (total 784,097 inhabitants) and mortality data by sex and age were accessed at the Institut National de la Statistique et des Études Économiques. Epidemiological data on CHIKV (cases, hospitalizations, and deaths) were obtained in the official epidemiological reports of the Cellule de Institut de Veille Sanitaire. The excess of deaths for each month in 2014 and 2015 was the difference between the expected and observed deaths by age groups, considering the 99% confidence interval threshold. We calculated the excess of deaths by age group and age adjusted mortality rates.
Results: There was an excess of 639 deaths, it was higher among the elderly, but also occurred among young adults. There was a strong correlation between monthly excess of deaths and reported cases of chikungunya ($R=0.81$, $p<0.005$), also with a 1-month lag ($R = 0.87$, $p <0.001$), and between monthly rates of hospitalization for CHIKV and the excess of deaths with a delay of 1 month ($R = 0.87$, $p <0.0005$).The peak of the epidemic occurred in the month with the highest mortality, returning to normal soon after the end of the CHIKV epidemic. No excess deaths were observed in 2015.
Discussion: The overall mortality estimated by this method(639 deaths) was about 4 times greater than that obtained through deaths certificates(160 deaths). The calculation of excess deaths is a statistical tool that can contribute to a better evaluation of the impact of chikungunya on mortality in different age groups.

Excess deaths associated with chikungunya epidemic of 2014 in Jamaica

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Introduction: Although some studies have demonstrated the potential for lethality, chikungunya has been considered a virus of high morbidity but of low fatality. This may be due to the difficulty of establishing the relationship between the diagnosis of the case and death. This phenomenon has already been described for influenza and the respiratory syncytial virus.
Objective: The objective of this study was to evaluate the excess of deaths associated with the chikungunya epidemic in Jamaica.
Methods: We assessed the excess of all causes of mortality by age groups during the chikungunya epidemics in Jamaica, 2014. Excess mortality was estimated by subtracting deaths observed in 2014 from that expected based on the average mortality rate of 2012-2013, with confidence interval of 99%.
Results: Overall mortality 91.9 / 100,000 population, 2,499 additional deaths than expected coincided with the peak of the epidemic, there was a strong correlation between the monthly incidence and the excess of deaths (Spearman Rho = 0.939; $p < 0.005$). No other significant epidemiological phenomenon occurred on that island that could explain this increase in mortality.
Discussion: This study suggests that mortality associated with chikungunya is underestimated in Jamaica, as has already been the case in other countries. The excess mortality found in Jamaica was 2499 deaths, more than 12 times the total deaths reported by all the countries of the Americas in the year 2014 for PAHO (194 deaths). The concepts that this virus has low fatality need to be reviewed by public health authorities. The excess of deaths could be a strategic tool for the epidemiological surveillance of chikungunya as it has already been used in influenza and respiratory syncytial.

MULTIPLE ARBOVIRUSES PRESENT DURING MEXICAN URBAN OUTBREAKS

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In recent years, chikungunya and Zika viruses rapidly spread throughout the Americas. As dengue virus serotypes 1-4 have been cocirculating in Mexico for the past decade, the arrival of these newly emerging arboviruses represented a significant public health challenge, particularly in terms of diagnostics. In this work, we summarize and compare the results of cross-sectional sampling studies performed in 3 major Mexican cities and 2 studies conducted in a rural community during arbovirus outbreaks. In 2015, a sampling study (n=95) in the coastal city of Veracruz found 43% of samples positive for chikungunya RNA and 2% positive for dengue RNA. In 2016, a similar study in Monterrey (n=70) showed a mixed outbreak with 20% positive for Zika RNA, 4% positive for chikungunya and 1.4% for dengue; while in Merida (n=20) 50% were positive for Zika RNA and 5% positive for dengue virus RNA. In contrast, in a rural community of Oaxaca in 2015 with a population of 4800 inhabitants, a sampling survey found (n=52) that 11.5% samples were positive for chikungunya with no other arbovirus detected and in 2016, a larger study (n=145) in the same community showed 8.3% positive for Zika virus RNA, but no other arboviruses were detected during these outbreaks. We can speculate that the rural nature of the community reduced the risk of exposure to other arboviruses. Nevertheless, these results should be considered, particularly in urban areas, as multiple arboviruses appear to be present in these outbreaks, and that due to logistics and local guidelines only a limited number of cases actually get confirmed in the laboratory.

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Category 3-Epi-Phylogenetics-Modeling-Burden.

EVOLUTIONARY HISTORY AND SPATIO-TEMPORAL DYNAMIC OF DENGUE VIRUS SEROTYPES FROM COLOMBIA.

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Introduction - All four dengue virus serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) are co-circulating in Colombia since 2001. Much remains unknown about evolution and the spread of viral populations. **Objective -** In this study we prompted us to investigate the origin, evolution, and dispersion dynamics of all four DENV serotypes from Colombia. **Materials and Methods -** Sequence analysis were conducted using newly (n= 186) and available (GenBank; n= 275) envelope gene sequences from Colombian viruses (185 DENV-1; 80 DENV-2; 154 DENV-3; 42 DENV-4) sampled in the 80´s and the period 1998-2017. The uncorrelated lognormal relaxed-clock and non-parametric *Skygrid* model were used. A Bayes factor test (BF > 9) was used to identify well-supported migration pathways using SpreaD3 v0.9.6 software. All analyses were implemented in BEAST v1.8.3 software, five independent MCMC were used with at least 100 million generations each. **Results -** From each virus serotype, a unique genotype was recovered. DENV-1, genotype V: all strains sampled in the period 1998-2017 formed a unique linage originated from one introduction around 1993 (95% BCI: 1992-1995), most likely from Venezuela (PP=1.00). DENV-2, genotype Asian/American: strains sampled in the 1990s and thenceforth formed a lineage that originated from an introduction around 1987, most likely from the Lesser Antilles (PP= 0.93). DENV-3, genotype II: strains sampled in the period 2001-2017 formed two lineages that co-circulated, which were originated from introductions around 1995 and 1993 most likely from the Greater Antilles (PP=0.81) and Venezuela (PP=1.00), respectively. DENV-4, genotype II: strains sampled in 1998-2017 formed two groups originated from an introduction around 1993 from Venezuela (PP=0.65); viruses sampled in the northeast region grouped with viruses from Venezuela and Brazil, while those from the southwestern region grouped with viruses from Ecuador and Peru. For all four serotypes, the Caribbean region was relevant for their introductions; for DENV-2 and -3, it was Central America; and for DENV4, was South America. Demographic reconstruction suggested a steady increase in diversity over time. **Conclusion-** The results could contribute to a better understanding regarding spread and diversity of dengue serotypes in Colombia, which could be useful for supporting the dengue control program.

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GENETIC STRUCTURE AND DIVERSITY OF DENGUE VIRUS SEROTYPE-1 FROM COLOMBIA

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Introduction- All four dengue virus (DENV) serotypes are present in Colombia with alternative predominance of each one. The DENV-1 genotype V represents from 4% to 85% of all circulating viruses, depending upon the year. How the serotype predominance was linked with its genetic diversity help to understand the virus transmission pattern.

Objective - The purpose of the present study was to estimate the degree of genetic differentiation among populations of DENV-1 from Colombia sampled in periods of distinct predominance.

Materials and Methods- One-hundred one full-length E-gene sequences were obtained from DENV-1 strains sampled in the northwestern Colombian region, which were combined with 69 E-sequences (GenBank) from Colombian strains sampled in other regions of the country. *Genetic population* structure and temporal stability were investigated through Analysis of Molecular Variance (AMOVA) and *pairwise Fst* statistics using the Arlequin 3.5 software. NJ trees were build *using* population *pairwise Fst* data. Haplotype networks were generated under criterion MJ (Median-Joining) using the Network 5.0.01 software.

Results- A unique DENV-1 lineage was recovered**.** The viral population exhibited high haplotype (h=0.99) and low nucleotide (π=0,013) diversity. Three (A-C) haplogroups were recovered (FST=0.34; $p < 0.0001$). HA, contains the oldest isolates (1985-2006) and some recent (2008-2016) isolates; HB, contains some old (1996-2010); and HC contains the most recent isolates (2008-2016). Also, three temporary *distinct* populations were recovered ($p < 0.0002$, FST:0.21-0.133): isolates from the first (1998-1999) and second (2008-2016) periods of the virus serotype predominance fall into group I and II, respectively; and isolates sampled in 2000-2007, when the serotype was less predominant, fall into group III.

Conclusion- High genetic diversity should reduce extinction risk in the DENV-1 population as it could provide the evolutionary potential to adapt to changing environmental conditions. Thus, maintaining of the virus transmission is expected.

This study was carried out thanks to financial support from Colombian Government: Fondo de Ciencia, Tecnología e Innovación del Sistema General de Regalías (BPIN 2013000100011; agreement 5246 signed between Universidad Industrial de Santander and Gobernación de Santander, August 11, 2013), and Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas (Grant # 1102-04-13042). The study was also funded by Sanofi Pasteur.

DENV-1 E PROTEIN: COMPARISION AMONG COLOMBIAN AND CYD-TDV VACCINE STRAINS

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Introduction-DENV-1 is one of the most prevalent dengue serotype in Colombia. Clinical trials found the recent licensed vaccine CYD-TDV (Dengvaxia) to be modestly effective *against DENV1*. Much remains unknown about genetic differences between the vaccine strain and its homologs strains circulating in Colombia.

Objective-In this study we prompted us to perform analysis of the level of sequence differentiation between CYD-TDV vaccine DENV-1 strain and wild-type DENV-1 from Colombia at epitope locations targeted by virus neutralising human monoclonal antibodies (mAbs).

Materials and Methods-Data set included full-length nucleotide sequences of DENV-1 E protein from viruses (n=102) sampled in Colombia in the period 1998-2016, the vaccine strain (DENV-1:CYD-TDV, Genbank # KX239894), and representative strains of all five genotypes. The amino acid sequence were subjected to multiple sequence alignment (MSA) to recognize the conserved residues using MEGA-7 program. A phylogenetic tree was constructed by Maxima likelihood method. We used ExPASy tool for structure prediction, and modeling 3D structure using the representative PDB structure 3J2P. For predicting lineal B-cell epitopes, we used *the BepiPred* method.

Results-The DENV-1 E gene phylogeny revealed that the vaccine strain belongs to genotype I and local viruses belong to genotype V. Positions of amino acid non-identity were dispersed across the E protein and do not cluster to any particular structural domain**.** In domain III, three substitutions (S-T, A-T and T-S) were identified at positions 339, 369 and 397, which contain B-cell epitopes; and in domain II, three substitutions (A-E, E-K, K-E) were identified at position 195 (fusion peptide), 202 and 203. The analysis identified eighteen and sixteen antigenic zones (5-27 amino acids) in the vaccine strain and local viruses, respectively, of which nine presented positions of amino acid non-identity. The E protein 3D structures showed no significant differences (RMSD < 0.3 Å).

Conclusion-Data provide insights into the characteristics of DENV-1 viruses circulating in Colombia relative to its homolog CYD-TDV strain, which can serve as a baseline for future research about factors implicated in the vaccine efficacy.

This study was carried out thanks to financial support from Colombian Government: Fondo de Ciencia, Tecnología e Innovación del Sistema General de Regalías (BPIN 2013000100011; agreement 5246 signed between Universidad Industrial de Santander and Gobernación de Santander, August 11, 2013), and Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas (Grant # 1102-04-13042). The study was also funded by Sanofi Pasteur.

MONITORING DENV DURING INTRODUCTION OF ZIKAV AND CHIKV

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Introduction-Chikungunya virus (CHIKV) and Zika virus (ZIKAV) were introduced to dengue virus (DENV) endemic areas of Colombia in 2014-2015. The concomitant presence of the new arbovirus could lead to a difficult for monitoring the dynamics of DENV serotypes. This is because their similar clinical symptoms and the potential for ZIKAV cross-reactivity with DENV.
Objective-In this study, we prompted us to assess the effect of CHIKV and ZIKAV introductions on the DENV virological surveillance.
Materials & Methods-Dengue suspected patients were included during periods of absence and presence of CHIKV and ZIKAV. Acute sera were tested by virus isolation in C6/36 or Vero cells, and virus identification was done by RT-PCR. The isolated viruses were genotyped using phylogenetic analysis of full-length E-gene sequences.
Results-Decrease of both the virus isolation rate in C6/36 cells and proportion of DENV isolates were observed. In absence of ZIKAV and CHKV: the isolation rate (IR) was 18.0 (59 isolates from 319 sera), 100% of isolates were DENV. In the period of CHIKV introduction (2014-2015): the IR was 5.3 (95 isolates from 1800 sera), 97% DENV and 3% ZIKAV. In the period of ZIKAV introduction (2016 - 2017): the IR was 4.0 (58 isolates from 1473 sera), 66% DENV and 34% ZIKAV. From 42 sera collected in 2014-2015 and tested in Vero cells, 4 (virus isolation rate of 9.5) CHKV strains were isolated, while none from 188 sera collected in 2016. From all DENV isolates, 74% resulted serotype 1 and 2 and the remaining serotype 3 and 4. The genotypes identified were as follows: DENV-1, V; DENV-2, Asian/American; DENV-3, III; DENV-4, II; CHIKV, Asian; ZIKAV, Asian.
Conclusion- Our results emphasize the role of the laboratory in the early detection of new arbovirus transmission and provide information important for routine DENV, ZIKAV and CHKV virological surveillance.

This study was carried out thanks to financial support from Colombian Government: Fondo de Ciencia, Tecnología e Innovación del Sistema General de Regalías (BPIN 2013000100011; agreement 5246 signed between Universidad Industrial de Santander and Gobernación de Santander, August 11, 2013), and Fondo Nacional de Financiamiento para la Ciencia, la Tecnología y la Innovación, Francisco José de Caldas (Grant # 1102-04-13042). The study was also funded by Sanofi Pasteur.

Simple protocol for population (Sanger) sequencing for Zika virus genomic regions

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Introduction. A number of Zika virus (ZIKV) sequences were obtained using Next-generation sequencing (NGS), a methodology widely applied in genetic diversity studies and virome discovery. However Sanger method is still a robust, affordable, rapid and specific tool to obtain valuable sequences.
Objective. The aim of this study was to develop a simple and robust Sanger sequencing protocol targeting ZIKV relevant genetic regions, as envelope protein and nonstructural protein 5 (NS5). In addition, phylogenetic analysis of the ZIKV strains obtained using the present protocol and their comparison with previously published NGS sequences were also carried out.
Methods. Six Vero cells isolates from serum and one urine sample were available to develop the procedure. Primer sets were designed in order to conduct a nested RT-PCR and a Sanger sequencing protocols. Bayesian analysis was used to infer phylogenetic relationships.
Results. Seven complete ZIKV envelope protein (1,571 kb) and six partial NS5 (0,798 Kb) were obtained using the protocol, with no amplification of NS5 gene from urine sample. Two NS5 sequences presented ambiguities at positions 495 and 196. Nucleotide analysis of a Sanger sequence and consensus sequence of previously NGS study revealed 100% identity. ZIKV strains described here clustered within the Asian lineage.
Conclusions. The present study provided a simple and low-cost Sanger protocol to sequence relevant genes of the ZIKV genome. The identity of Sanger generated sequences with published consensus NGS support the use of Sanger method for ZIKV population studies. The regions evaluated were able to provide robust phylogenetic signals and may be used to conduct molecular epidemiological studies and monitor viral evolution.

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Zika virus phylogenetic relationship between puerto rico and other american countries

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Introduction. Zika virus (ZIKV) is an arthropod-born virus (arbovirus) in the *Flaviviridae* family, that is transmitted to humans primarily via the bite of an infected *Aedes* species mosquito. In Puerto Rico, the first locally transmitted infection was reported in December 2015. To date, approximately 39,000 confirmed cases have been reported of which approximately 4,000 were pregnant women. Much attention has been given to the link of ZIKV infection and birth defects. However, ZIKV evolution and its association to pregnancy outcomes/birth defects remains unknown.
Objective. To analyze the genetic diversity and phylogenetic relationships of Puerto Rico ZIKV and those from other parts of the Americas.
Materials and Methods. We analyzed two hundred one complete genome sequences, which were obtained from the Virus Pathogen Resources Database. Maximum-likelihood analysis was performed to observe phylogenetic relationships between PR sequences and sixteen American countries. Bayesian analysis was used to estimate the timescale of the epidemic, demographic growth patterns and gene flow.
Results. The Maximum-likelihood analysis confirmed that the Puerto Rico, Colombia and United States isolates clustered together. The Bayesian tree obtained by using the MacClade program for the evolutional direction of the ZIKA virus, showed a bi-directional gene flow between Puerto Rico and the United States. In addition, a possible gene flow was observed from Puerto Rico to Colombia.
Conclusion. Understanding the ZIKV population will allow us to identify mutations with functional implications for ZIKV pathogenesis. Further studies will focus on sequencing the virus directly from samples collected as part of our ongoing surveillance.
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Household-Based Cluster Investigations during a DENV-2 Outbreak—American Samoa

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Introduction: The United States territory of American Samoa recently experienced outbreaks of multiple viruses transmitted by *Aedes* species mosquitoes, including chikungunya virus (CHIKV) in 2014, dengue virus type-3 (DENV-3) in 2015, and Zika virus (ZIKV) in 2016. ZIKV transmission was waning but ongoing when an outbreak of DENV-2 was detected in November, 2016. As of December, 2017, the ongoing DENV-2 outbreak involved 420 laboratory-positive cases (7.6 per 1,000 population). We conducted household-based cluster investigations to identify individual and behavioral risk factors for DENV infection as well as characteristics associated with reporting symptomatic infection.

Methods: Patients reported as PCR- or NS1-positive dengue cases were visited within 30 days of their reported date of illness onset. All individuals residing within 50 meters of index case-patients' households were invited to participate in the investigation. Questionnaires were administered to heads of household and participating household members to collect data on household characteristics, demographics, individual behaviors, and acute febrile illness within the past 90 days. Serum specimens were collected to identify patients with current and recent infection by RT-PCR and IgM ELISA for detection of nucleic acid and antibodies against DENVs, ZIKV, and CHIKV. A novel microimmunoassay will be used to detect antibodies against the ZIKV NS1 protein, to identify individuals with historic ZIKV infection.

Results: Among 22 household-based cluster investigations conducted, surveys and serum specimens were collected from 252 participants. Of participants, 56% were female, and median age was 32.5 years (range: 1–94 years). Sixty-nine (27.5%) participants reported febrile illness during the prior three months, for which 30 (43.5%) sought medical care. All serum specimens are currently pending diagnostic testing to identify factors associated with current or recent DENV infection, and to determine the proportion of symptomatic-to-asymptomatic DENV infections. Potential associations between this ratio and historic ZIKV infection will be explored.

Conclusions: This outbreak provided an opportunity to investigate population-specific risk factors for DENV infection and development of disease. Diagnostic testing is underway to determine if historic infection with ZIKV was associated with protection from infection or increased odds of reporting symptomatic DENV infection.

Urticaria and antihistaminic consumption as indicator of Zika outbreak magnitude.

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In Iquitos, the largest city of the Peruvian Amazon, with a population of ~ 600000, the first case of Zika fever was reported on May 2016. It was an acute febrile patient and the outbreak is still going on. Not only the fever but most importantly a pruritic maculopapular rash was the most common sign. When the Zika outbreak started, fever was the common key criteria in arbovirus surveillance being performed in Iquitos that added a few laboratory issues (low viremia, budget restrictions) that led to an underestimation of this outbreak. The sudden appearance of a rash made some patients to seek medical attention and receive antihistaminic medication. We aim to use this consumption as a surrogate to determine the magnitude of the Zika outbreak in 2016 in Iquitos. We compared the incidence of confirmed Zika fever cases, the incidence of acute urticaria (because clorphenamin maleat is also indicated) and the amount of clorphenamin maleat consumption by months during June to December 2015 and 2016 in the Ministry of Health (MoH) primary care facilities.We considered that 1 syrup, 10 pills or 2 ampoules were used for each person. 273 laboratory confirmed cases of Zika fever cases in 2016 with a peak in October but 0 cases in 2015. A total of 858,094 and 510,060 units of clorphenamin maleat (pill, syrup and ampoule) were used in 2016 and 2015 respectively; representing a 62% increase, although the diagnoses of urticaria presented a reduction of 15%. The differences according to the drug presentations were 17696, 308418 and 21920 for ampoule, pills and syrup respectively representing 8848, 30841 and 21920 people in each group. We considered the next biases: these data considered only the most common prescribed antihistaminic, is only from the MoH excluding formal workers health insurance (EsSalud) and private sector. Also, some patients would have gone more than once to the health facility. The increase of antihistaminic usage was not due to urticaria diagnoses which decreased along this period, but It could be attributed to Zika virus infection, representing an estimated of more than 60000 persons in these facilities.

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Category: 3-Epi-Phylogenetics-Modeling-Burden

Molecular and Clinical Characterization of Chikungunya Virus Infections in Mexico

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Introduction: Even though clinical features of Chikungunya fever in Mexican population have been described before, there is no detailed information. The existence of different Chikungunya viruses (CHIKV) circulating in Chiapas and if there are associated clinical features is unknown.

Objective: The aim of this study was to make a full description of the clinical features in Chikungunya-infected patients, and the molecular epidemiology of CHIKV.

Methods: We studied patients who sought medical assistance in Tapachula, Chiapas, Mexico, from June through July 2015. Infection was confirmed by rRT-PCR. After patient's written informed consent, blood was withdrawn and clinical manifestations were recorded. Viruses were isolated and E1 gene was sequenced. Phylogeny reconstruction was inferred using maximum likelihood approach.

Results: We studied 52 patients with confirmed CHIKV infection. They were more likely to have wrist, metacarpophalangeal and knee arthralgia. Confirmed patients had higher pain than CHIKV negative patients and needed help to fulfill daily activities. Two combinations of clinical features were obtained to differentiate between Chikungunya fever and acute undifferentiated febrile illness. After 14 months post-onset of symptoms, pain continued on the four studied patients and the knee was the mostly affected joint. Ten CHIKV were isolated and belong to the Asian lineage. Seven of the viruses were not identical to the formerly reported. Patients infected with the divergent CHIKV strains showed a broad spectrum of clinical manifestations.

Conclusion: We defined the complete clinical features of Chikungunya fever in 52 confirmed patients from Southeastern Mexico. These findings can help to distinguish between CHIKF from other febrile diseases. We found co-circulation of CHIKV strains in the state of Chiapas. Different CHIKV strains were associated with different clinical features.

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Genetic Variability of Chikungunya Virus in Southeastern Mexico

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Introduction: Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes Chikungunya fever. The existence of different CHIKV viruses co-circulating in Mexico is unknown.

Objective: The aim was to identify the genetic variability of CHIKV in Southeast Mexico.

Methods: We studied patients who sought medical assistance in Tapachula, Chiapas, Mexico, from June through July 2015. Infection was confirmed by rRT-PCR. After patient's written informed consent, blood was withdrawn and clinical manifestations were recorded. Viruses were isolated and E1 gene was sequenced. Phylogeny reconstruction was inferred using maximum likelihood approach and maximum clade credibility.

Results: We studied 20 patients where CHIKV's E1 gene was sequenced. The 20 sequences grouped in the Asian lineage, specifically with the Caribbean strains. We found 14 synonymous substitutions and two non-synonymous substitutions. The non-synonymous substitutions occurred in the E1 protein, V4A and A342V.

We found seven different CHIKV strains circulating in the state of Chiapas. Six sequences were identical to the formerly reported CHIKV strain in Chiapas, 2014. Five sequences formed a separate monophyletic clade joining with two sequences reported in Yucatan state. In addition, four minor clades were formed. Two of them involved strains identified in Yucatan. The remaining two clades were composed of Chiapas' strains. These clades had a bootstrap support greater than 61 and posterior probability greater than 0.96. Three sequences were not identical to the former reported but did not group on a monophyletic clade.

Interestingly, the isolated viruses from two patients (father and son) that shared dwelling, and sought medical attention on the same day were different. The virus isolated from the father was from one clade and the virus isolated from the son was a virus that didn't group on a clade. The virus isolated from the son had the non-synonymous mutation V4A. Regarding clinical manifestations, the father reported mild arthralgia at the elbows. In contrast, the son reported intense generalized arthralgia.

Conclusion: We identified co-circulation of CHIKV strains in Tapachula, Chiapas. Greater studies are needed to associate the severity of disease and CHIKV variants.

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SAINT LOUIS ENCEPHALITIS VIRUS CIRCULATING IN TWO BRAZILIAN CITIES

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Saint Louis Encephalitis (SLEV) is a *Flavivirus* that belongs to the *Flaviviridae* family; it was identified in 1933, during an outbreak of encephalitis in Saint Louis, Missouri. SLEV was isolated for the first time in Brazil in the State of Pará during 1970s. The virus was isolated again in 2004 in the state of São Paulo and was detected in a dengue outbreak in São José do Rio Preto, SP in 2006. The virus was also detected in other parts of the country in the years that followed. The present study reports the circulation of SLEV in two cities from different Brazilian states. We analyzed human and mosquito samples from Sinop, Mato Grosso and Araraquara, São Paulo. Serum samples from patients presenting dengue symptoms and mosquitoes pools were collected between 2011 and 2016. Viral RNA was extracted with different methods: mosquito pools with an in-house method and human serum with a commercial kit. Serum samples and mosquito pools were tested for the presence *Flavivirus* and *Alphavirus* by RT-PCR. Positive samples were sequenced in ABI377 automated sequencer. Sequences were aligned using BLAST. Three serum samples and one mosquito pool from Sinop, as well as one serum sample from Araraquara were positive for SLEV. Sequencing confirmed our PCR results. Phylogenetic analysis using part of the NS5 gene produced a cladrogram that was very similar to previous phylogenetic studies with SLEV. Sequences from Sinop and Araraquara were allocated in the same clade. The sequence that is intimately related to ours is from Pará. This result indicates that SLEV is spreading throughout the country over the years. Our samples were collected in settings where major outbreaks caused by dengue, zika and chikungunya viruses were occurring. In such epidemiological scenarios, other viruses such as SLEV may be underreported or reported as dengue due to similarity of initial symptoms.
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Coinfection as a risk factor for dengue severity

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Introduction: Acute febrile illnesses (AFI) caused by arboviruses and influenza are difficult to differentiate clinically. Even more so, coinfections are difficult to identify and require thorough diagnostic testing. Identifying patients with coinfection could help determine if coinfections causes increased severity and guide clinical management.

Objective: Evaluate the association of dengue virus (DENV) and respiratory virus coinfection with patient demographic characteristics, signs, symptoms, and disease severity.

Materials and Methods: Using data from the Sentinel Enhanced Dengue Surveillance System in Puerto Rico from May 2012–May 2017, we compared patients with confirmed DENV monoinfection to patients with confirmed coinfection of DENV and respiratory viruses including influenza, adenovirus, parainfluenza, respiratory syncytial virus (RSV), human coronavirus, and human metapneumovirus. All viruses were identified by detection of nucleic acid by polymerase chain reaction. We evaluated if coinfection was associated with demographics, signs and symptoms, or disease severity (i.e. hospitalization, admission to intensive care unit, life-saving interventions, plasma leakage, hemorrhage, and severe organ involvement).

Results: Among 18,075 patients enrolled in SEDSS, 719 (4.0%) had DENV infection, among whom 34 (4.7%) had coinfection. The most common co-infecting respiratory viruses were adenovirus (18%), influenza A (15%), and metapneumovirus (15%). While sex was not associated with coinfection, patients with coinfection were significantly younger (median=11 years; range 0–74) than those with monoinfection (median=15 years; range 0–77) (p=0.003). Compared to patients with monoinfection, a larger proportion of patients with coinfection had runny noses (25.6% vs. 48.5%, respectively; p=0.004), liver enlargement >2 centimeters (1.2% vs. 8.8%; p=0.005), and among women unexpected vaginal bleeding (3.9% vs. 28.6%; p=0.003). Although patients with monoinfections were more frequently hospitalized (37%) compared to patients with coinfections (26%), this difference was not significant (p=0.22). Among 261 hospitalized patients, administration of oxygen was more common among those with coinfection versus monoinfection (22% vs. 2%; p<0.001). No other severity measures differed between these two groups.

Conclusions: AFI patients with DENV and respiratory virus coinfections more frequently presented with runny nose, hepatomegaly, and menorrhagia. Among hospitalized patients, oxygen was administered more frequently to coinfected patients. This information can assist clinicians in identifying potential DENV coinfections and guide clinical management.

Funding of research: (Arial, 11 points, justified, cite only the project number and funding organisation).

VIRAL ETIOLOGY OF PEDIATRIC ACUTE FEBRILE ILLNESS IN PONCE, PUERTO RICO.

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Introduction: Acute febrile illness (AFI) is an important cause of pediatric hospital admission, with significant associated morbidity and mortality in some age groups. Causes of AFIs such as dengue, influenza, chikungunya, and Zika virus disease are common throughout the tropics and in Puerto Rico. Knowledge of the most common locally etiologic agents of AFI is necessary to guide clinical management and direct prevention efforts.
Objective: To describe the viral etiology of acute febrile illness and report differences by age group in pediatric patients recruited through a sentinel enhanced AFI surveillance system (SEDSS).
Materials and methods: We analyzed data for pediatric patients (≤18 years old) enrolled in SEDSS during May 7, 2012 through May 6, 2017 at Saint Luke's Episcopal Hospital in Ponce and Guayama, Puerto Rico. Patients presenting to the Emergency Department with fever onset ≤7 days were eligible to enroll. We present the prevalence of different viral etiologies by age groups 0-3 months, 4-23 months, 2-4 years, and ≥5 years, and assess differences using Chi square test.
Results: 10,838 patients are included in the analysis. A viral pathogen was found in 47%. There were significant differences by age group in the main pathogens identified. Respiratory syncytial virus (RSV) was identified in 9%, 7%, 5% and 1% ($p<0.001$) in the 0-3 months, 4-23 months, 2-4 years, ≥5 years age groups, respectively. Dengue was identified in 2%, 2%, 2%, and 12% ($p<0.001$) respectively. Chikungunya was identified in 7%, 5%, 6%, and 9% ($p<0.005$) respectively. Zika was identified in 0.3%, 2%, 3%, and 7% ($p<0.001$) respectively. Influenza was identified in 8%, 6%, 12%, and 16% ($p<0.001$) respectively. The frequency of Zika virus increased with age as the RSV frequency decreased.
Discussion: Causes of fever can be difficult to distinguish clinically and point of care laboratory services may be limited in some low-resource areas. Knowing the local epidemiology of AFI and recognizing viral infections as a common etiology in children may help to reduce the use of unnecessary antibiotics and invasive studies. In the context of Puerto Rico, arboviruses should be suspected and studied in febrile children, especially those 5 years of age and older.
Funding of research: Doesn't apply.

DENGUE COHORT IN YUCATAN, MEXICO: BASELINE AND FIRST YEAR FOLLOW-UP.

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Dengue is the most prevalent mosquito-borne viral disease of humans and is caused by the four serotypes of dengue virus. To estimate the incidence of dengue and other arboviruses, we analyzed the baseline and first year follow-up of a prospective school-based cohort study and their families in three cities in Yucatan, Mexico. Through enhanced surveillance activities, acute febrile illnesses in the participants were detected and yearly blood samples were collected to evaluate dengue infection incidence. A Cox model was fitted to identify hazard ratios of arboviral infections in the first year of follow-up of the cohort.
The incidence of dengue symptomatic infections observed during the first year of follow-up (2015 - 2016) was 3.5 cases per 1,000 person-years (95\% CI: 1.9, 5.9). The incidence of dengue infections was 33.9 infections per 1,000 person-years (95\% CI: 31.7, 48.0). The majority of dengue infections and seroconversions were observed in the younger age groups (≤ 9 years old). Other arboviruses were circulating in Yucatan during the study period. The incidence of symptomatic chikungunya infections was 8.6 per 1,000 person-years (95% CI: 5.8, 12.3) and the incidence of symptomatic Zika infections was 2.3 per 1,000 person-years (95% CI: 0.9, 4.5). Our model shows that having a dengue infection during the first year of follow-up was significantly associated with being female, living in Ticul or Progreso, and being dengue naïve at baseline. Age was not significantly associated with the outcome; it was confounded by prior immunity to dengue that increases with age. This is the first report of this cohort and provides incidence estimates of the three arboviruses co-circulating in all age groups. This study provides important information for understanding dengue and other arboviruses epidemiology and informing public health policies.

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Phylogeography of dengue in Colombia 2000-2017

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Introduction. Dengue is endemic in Colombia. All four serotypes circulate and peak incidence was observed in 1998, 2002, 2010 and 2014. The molecular evolution and viral population dynamics of those epidemics have not been studied in detail.
Aims. To describe the geographic origin, the routes of dispersion and the dynamics of circulation of DENV serotypes in Colombia during 2000-2017.
Methods. More than 250 not previously analyzed sequences of E-gene of the four serotypes of DENV were aligned and analyzed by phylogenetic, phylogeographic and phylodynamic methods; the results were compared with the epidemiological behavior of the disease.
Results. DENV genotypes circulating in Colombia correspond to those previously described in South America. Several lineages within each genotype co-circulated for varying periods of time with occasional lineage replacements. A high frequency of exchange of strains between Colombia and Venezuela was inferred from the data; import and export of strains from or to Central America, the Caribbean and other countries of South America were also detected at low frecuency. The population dynamics of these lineages, as can be inferred from the diversity of the sequences, did not correlate with the incidence of the disease.
Conclusions. A hyperendemic pattern was observed with oscillations in the population size of each serotype. DENV circulating in Colombia and Venezuela behaves as a single endemic population.
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Zika virus infection in Honduras, 2016-2017

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Introduction: Zika virus (ZIKV) infection has affected significantly affect Latin America in 2015-2016. However, most studies have been reported from Brazil and Colombia, but there is lack in Central America. We analyzed the incidence, incidence rates and evolution of cases at national, departments and municipalities of Honduras during 2016-2017, since the epidemics begun on January 2016. **Methods:** Observational study in which the incidence of ZIKV infection, in Honduras, 2016-217, was estimated, based on data from national epidemiological surveillance, obtaining the number of cases for each department and municipality, 2016-2017 (by epidemiological weeks, EW), from EW1-2016 to EW24-2017. Data used for this study are constituted from confirmed cases. Data proceed from 298 primary municipal notification units, collected at 18 departments, centralized in Tegucigalpa (Capital District, CD). Cases of Zika were clinically and laboratory confirmed (RT-PCR). **Results:** From January 1, 2016 to June 17, 2017, a total of 32,405 of Zika were reported (cumulated rate of 368.5 cases/100,000 pop). From them, 1% were confirmed by RT-PCR. The highest peak was reach on the EW 6°, 2016 (2,559 cases; 29.34 cases/100,000pop), followed them, after a decrease to 93 cases (1.07 cases/100,000pop) (EW-12°, 2016), of a second at EW 24°, 2016 (988 cases, 11.33 cases/100,000pop). The department with the highest number of cases was Cortés (13,128 cases, 2,832.9 cases/100,000 pop), however, Francisco Morazán department (which includes the CD) had the highest incidence rate (3,213.6 cases/100,000 pop, 10,587 cases). From the total cases of Francisco Morazán, 99.25% occurred at the capital city, Tegucigalpa. So far, 4 cases of congenital Zika syndrome (CZS) have been confirmed. **Discussion:** Pattern and evolution of Zika in Honduras has been like those that occurred for chikungunya in 2015, that we analyzed and published (J Public Health Infect 2016), affecting predominantly the central and capital area of the country, reaching also high incidences there >2,000 cases/100,000 pop (2%). Studies using geographical information systems, to map its epidemiology, as well on the clinical aspects linked to, such as the CZS and the associated Guillain-Barre syndrome, are necessary in this country, given the extend of the epidemics and the potentially associated long-term consequences.

Knowledge about Yellow Fever in medical students from Colombia, 2017

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Introduction: Recent epidemics of Yellow Fever (YF) in Africa (Angola), with imported cases in Asia (China), as well in the Americas (Brazil), have raised concern of the reemergence of this flaviviral disease, in addition to other pathogens in this family (eg. Dengue and Zika). In this epidemiological scenario, assessing knowledge and perceptions in medical students about epidemiology, symptoms and transmission of YF from Colombia would be highly relevant. **Methods:** An observational cross-sectional study was performed among assistants who attended a national medical students symposium on YF, May 2017, Villa-de-Leyva, Colombia. Attendees who agreed to be part (convenience sample), filled out a questionnaire (based on information from WHO about YF) about basic knowledge on the epidemiology, symptoms and prevention of disease (five questions), before and after the meeting. **Results:** A total of 280 questionnaires were applied. The mean age of participants was 21.02 year-old (±3.6; range 17–42, 55.0% female, 45% male), 53.9% belonging to 5° and upper semesters (range 6.1% at 1° to 11° 1.1%). Correct knowledge about YF transmission was 62.8% at baseline, increasing to 87.9% (χ^2=22.167; p<0.001), for symptoms was 68.3% and 80.2%, respectively (χ^2=7.681; p=0.053), incubation period was 28.0% and 77.6%, respectively (χ^2=67.509; p<0.001), preventive measures and usefulness of YF vaccine 64.0% and 86.2%, respectively (χ^2=21.151; p<0.001), and about case fatality rate during toxic phase of disease 40.9% and 55.2% respectively (χ^2=11.72; p=0.008). **Discussion:** Although relatively wide scale YF vaccination has been applied, a growing number of outbreaks have been documented in several African countries in the last decade, but also recently in the Americas, particularly in Brazil, with the concern of expansion to other countries in the region. During 2016-2017, other countries, including Colombia, Ecuador, French Guiana, Peru, Bolivia and Suriname have reported suspected and confirmed yellow fever cases. In Brazil, since July 2017, São Paulo state has reported 37 suspected YF cases, of which one fatal case was confirmed. This enhance the need for preparedness to YF, including education, as was provided by the assessed training done in Villa de Leyva, Colombia, where the baseline knowledge was relatively low and the intervention significantly increased it.

Bibliometric assessment of the global scientific production on Sindbis virus

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Introduction: Sindbis virus (SINV) is an arbovirus (genus *Alphavirus*) widely distributed in Africa, Asia-Pacific and Europe, causing infections by 6 genotypes. As other emerging zoonotic arbovirus (such as Usutu, Kyasanur, Orthobunyavirus), its potential arrival to Latin America is of concern. Although all of this, there is no bibliometric studies assessing advances in its research. **Methods**: Bibliometric studies at 7 databases: Web of Sciences (WoS)®, Scopus®, GoPubMED, SciELO, Google Scholar, LILACS and ScienceDirect®, assessing the global scientific production on SINV, measuring the number of article per countries, productive institutions, number of original articles, published historically and during the last 5 years (2012-2016), productive authors, citations and H index, among other indicators. **Results**: Those most productive countries in WoS (N=771): USA (337[43.7%]), France (53[6.9%]) and UK (53[6.9%]). At Scopus (N=3486): USA (1751[50.23%]), Germany (160[4.59%]) and Japan (150[4.3%]). At GoPubMed (N=2898): USA (353[12.18%]), Japan (38[1.31%]) and UK (33[1.13%]); production during 2012-2016 at WoS: 684 articles; at Scopus: 420 articles; GoPubMed: 296 articles. Countries with higher H-index (Scopus): USA (112, 68,419 citations), France (42, 4,313 citations), Germany (41, 5,075 citations), Japan (37, 5,512 citations), UK (34, 3,629 citations). Most productive cities on the topic: New York, USA (31 articles), Helsinki, Finland (18) and Beijing, China (17). **Discussion:** As in other bibliometric studies, scientific production by USA and their groups is predominant. In areas such as Latin America, investigation on SINV should began, particularly related to preparedness, medical education, epidemiology and transmission. At the same time, is necessary to increase its global research and initiate also this in Latin America, a suitable region where other recent arboviruses have emerged, such as was the case of chikungunya and Zika.

Dengue burden: Colombia and Venezuela, two sides of a coin

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Introduction: Dengue represents a major public health problem in South America generating a high burden of disease and elevated economic cost.

Objective and Methods: A comparative analysis was performed on the impact and cost of dengue in Venezuela (VEN) and Colombia (COL), over a period of ten and three consecutive years, respectively. Statistical non-parametric Pearson's Chi^2 test and Yates' correction for continuity were applied when indicated.

Results/Discussion: Average disability-adjusted life years (DALYs) lost in non-epidemic years was similar (69.22 per 10^6 population in VEN and 83.88 in COL). In contrast, the DALYs lost in epidemic years was 8.3 folds larger in COL as compared to VEN (1,198.73 per 10^6 population and 145.23, respectively; $p < 0.00001$). Such difference may be explained in part by the wider increase in dengue incidence seen during epidemic years in COL (3.41 fold) in contrast to VEN (2.1 fold), but more significantly by a sharp rise of cases of DF hospitalized (2.09 fold; $p < 0.00001$) and cases of DHF hospitalized in ICUs (7.04 fold; $p < 0.00001$) during epidemic years, a pattern not observed in VEN.

The average total cost of dengue management in COL ($32,645,068), including vector control activities, was markedly lower than that of VEN ($80.718.978), as were also the individual costs of patients for any form of the illness ($402.79 vs. $1.105.28). Such differences are explained by major disparities in the organization of the health care system in both countries. In COL, where about 96% of the population's health care is covered under the General System of Health and Social Security, the cost of each case was very similar to that of the public health sector in VEN ($479,88), but much lower than the private sector ($1.730,68). Of note, the contribution of each component to the overall cost, i.e. direct medical, direct non-medical, and indirect costs, was comparable.

Conclusion: Our results show that closely-related dengue endemic countries with analogous levels of incidence and disease burden, may nonetheless exhibit marked differences not only in the management of its various clinical forms, but also in cost, which are mostly influenced by organizational characteristic of their health systems.

Urticaria and antihistaminic consumption as indicator of Zika outbreak magnitude.

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In Iquitos, the largest city of the Peruvian Amazon, with a population of ~ 600000, the first case of Zika fever was reported on May 2016. It was an acute febrile patient and the outbreak is still going on. Not only the fever but most importantly a pruritic maculopapular rash was the most common sign. When the Zika outbreak started, fever was the common key criteria in arbovirus surveillance being performed in Iquitos that added a few laboratory issues (low viremia, budget restrictions) that led to an underestimation of this outbreak. The sudden appearance of a rash made some patients to seek medical attention and receive antihistaminic medication. We aim to use this consumption as a surrogate to determine the magnitude of the Zika outbreak in 2016 in Iquitos. We compared the incidence of confirmed Zika fever cases, the incidence of acute urticaria (because clorphenamin maleat is also indicated) and the amount of clorphenamin maleat consumption by months during June to December 2015 and 2016 in the Ministry of Health (MoH) primary care facilities.We considered that 1 syrup, 10 pills or 2 ampoules were used for each person. 273 laboratory confirmed cases of Zika fever cases in 2016 with a peak in October but 0 cases in 2015. A total of 858,094 and 510,060 units of clorphenamin maleat (pill, syrup and ampoule) were used in 2016 and 2015 respectively; representing a 62% increase, although the diagnoses of urticaria presented a reduction of 15%. The differences according to the drug presentations were 17696, 308418 and 21920 for ampoule, pills and syrup respectively representing 8848, 30841 and 21920 people in each group. We considered the next biases: these data considered only the most common prescribed antihistaminic, is only from the MoH excluding formal workers health insurance (EsSalud) and private sector. Also, some patients would have gone more than once to the health facility. The increase of antihistaminic usage was not due to urticaria diagnoses which decreased along this period, but It could be attributed to Zika virus infection, representing an estimated of more than 60000 persons in these facilities.

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Category: 3-Epi-Phylogenetics-Modeling-Burden

NOVEL MOLECULAR SIGNATURES OF CHIKUNGUNYA VIRUS IN PUERTO RICO

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Objective: Chikungunya virus (CHIKV) is an arthropod-borne *Alphavirus* transmitted to humans primarily via *Aedes* mosquitoes. In Puerto Rico (PR), the first locally transmitted infections were reported in May 2014. Although the virus in PR is related to the Asian/American lineage, many autochthonous cases have emerged recently in the Caribbean region, raising the question of how will CHIKV evolve and adapt in PR. Given the role of the envelope glycoprotein (E1) in viral evolution and transmission, we analyzed the genetic diversity and phylogenetic relationships between PR E1 gene sequences and those from other parts of the world. **Materials and Methods:** To analyze the overall genetic variation, 772 E1 gene nucleotide sequences were obtained from the Virus Pathogen Resources Database. Maximum-likelihood analysis was performed to observe phylogenetic relationships between PR sequences and forty-eight countries from around the world. **Results:** Analysis of E1 gene identified variations at four nucleotide positions, which include synonymous and non-synonymous mutations. In addition, two nonsynonymous amino acid changes, E1T207M and E1S120L, are unique in PR CHIKV sequences, and E1T155I was found to be shared between PR (n=3) and Colombia (n=1) strains. **Conclusions:** The analysis of E1 gene reveals new molecular signatures in PR CHIKV sequences, one of which was also found in Colombia. While studies have shown possible relationships between E1T98A and E1A226V with viral adaptation and spread, no PR sequence contained these vector-adaptive mutations. Thus, constant monitoring of the virus remains an important factor to establish control strategies to monitor viral spread.

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Parallel epidemiological behavior of main arboviruses in Latin America

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Over the past 30 years Dengue has been considered a public health priority in Latin America and the Caribbean (LAC). With the introduction and explosive expansion of Chikungunya in 2013-14 and Zika in 2015-16, the co-circulation of these three arboviruses represents a major public health challenge for the region. To better understand the transmission dynamics of these arboviruses, we analyzed the epidemiological data of LAC countries with the highest incidence rate of Zika, Dengue and Chikungunya during 2010-2017. Dengue has been the predominant arboviral infection in the region with recurrent outbreaks produced by any of the four serotypes. During 2013, LAC had an epidemic year of Dengue with approximately 2.3 million cases and an incidence of 455.9 per 100,000 population. During the same year, Chikungunya emerged in LAC inflicting more than 1,300,000 suspected and confirmed cases in the region, with a total of 184 deaths and causing important acute and chronic disabilities that had a substantial impact on the quality of life of the population. Zika followed two years later with an explosive outbreak initiating in Brazil. During 2016, 48 countries and territories in the Americas confirmed autochthonous, vector-borne transmission of Zika virus with reportedly multiple introductions in Honduras, Colombia, Mexico, Puerto Rico, and other Caribbean islands. Although Dengue continues to represent the highest disease burden among the arboviruses described, Zika infections generated public health alarm as a cause of severe neurological symptoms and microcephaly in fetuses. Brazil has had the highest number of reported Zika cases worldwide (more than 200,000 by 24 December 2016) and the highest number of cases associated with microcephaly and other birth defects (2,366 confirmed by 31 December 2016). For poorly understood reasons during 2017 there was a parallel decrease in Dengue, Chikungunya and Zika cases compared to 2016. However, the LAC epidemiology dynamics of arboviruses during 2010–17 exhibit the potential to sustain their circulation. Zika transmission may follow similar patterns to Dengue and Chikungunya, generating large, sporadic outbreaks with a high degree of under-reporting.
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Climate variability and Chikungunya epidemiology, Coffee-Triangle region of Colombia, 2015-2016

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Introduction: It has been suggested that emerging arboviral diseases, such as chikungunya and Zika, have been at least partially driven not just by migration and social factors, but also by climate change and variability. In the case of Latin America in 2014 occurred the introduction of the Asian genotype which led to the subsequent epidemics in the region, including Colombia. The, we were interested in assess the influence of climate in an *Aedes*-endemic region of Colombia where CHIK was introduced in early 2015, the Coffee-Triangle region (constituted by 53 municipalities), regard the epidemiology of CHIK during 2015-2016 epidemics. **Methods:** Epidemiological surveillance data (weekly cases) were collected, and incidence rates were calculated. Poisson regression models were used to assess the influence of the macroclimatic variable ONI (Oscillation Niño Index) on the CHIK incidence rate, adjusting by year and week. Monthly satellite images for total rainfall and surface temperature were obtained from the Tropical Rainfall Measuring Mission (1 month – TRMM) imagery database from NASA Earth Observations (NEO, NASA, USA) (http://neo.sci.gsfc.nasa.gov/) and were analyzed with Google Earth® software. **Results:** A total of 15,785 cases of CHIK were identified. Quindio department concentrated 6,404 of them (1,045.17 cases/100,000 pop). La Virginia, Risaralda department, was the municipality with the highest cumulated incidence, 3,770 cases/100,000 pop). At nonlinear regressions, significant associations were found with ONI ($p<0.05$), at 35 out of 53 municipalities of the region, being higher at: Genova ($r^2=0.4986$), Belen de Umbría ($r^2=0.4902$), Dosquebradas ($r^2=0.4270$), Pereira ($r^2=0.3926$) Calarca ($r^2=0.3478$), Santuario ($r^2=0.3259$) and La Tebaida ($r^2=0.3253$). For the whole region was $r^2=0.2834$. **Discussion:** El Niño significantly affected the incidence of chikungunya in the region. This association with climate change and variability should be considered in the elements influencing disease epidemiology and pathogens emergence. In addition, predictive models should be developed further with more available data from disease surveillance. This information should also be considered for studies relating to climate change and vector control.

Social representations of Zika Virus Disease patients, Pereira, Colombia, 2016-2017

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Introduction: Despite Zika epidemics in Latin America (2015-2016), affecting significantly from morbidity and disability associated to the congenital Zika syndrome and Guillain-Barre syndrome, most of the research has been quantitative. So far, no qualitative studies have been published, especially related to an integrative social vision of health education and prevention. **Methods:** A descriptive qualitative study for the appraisal of social representations was done. This was based on the theory of Serge Moscovici. The method was Grounded Theory. Interviewed population corresponded to 3 adult patients (2 males [50 and 47 y-old] and 1 female [23 y-old], all from low socioeconomical status, without universitary level) affected by Zika virus disease (RT-PCR confirmed) during the epidemics (2016), from the prioritized, according risk, area of Cuba, Pereira city, Risaralda, Colombia, epidemic and later endemic for Zika. They were selected by convenience sampling, the interview was an open, semi-structured, in-depth, with a previous script, for its application. **Results:** The main core derived from the analysis was the “lack of information”, particularly derived from the need of more health education, continuous, periodically, and perform by expert health care workers (HCW) trained in Zika and VBD. Despite that, they perceived Zika as dangerous disease and harmful pathogen especially for the pregnant women. However, they have no clarity between the different arboviral diseases from Zika, such as the case of dengue and chikungunya. Although, some preventive practices that they referred at the households, such as avoid collections of water and have information about vector transmission, there is a misinformation about specific practices for prevention of Zika and other VBD, such as boiling water, use of self-prescribed antibiotics, among others. Finally, they referred that information about diagnosis and confirmation was not properly provided by the HCW to them. **Discussion:** Current findings have significant implications, given the lack of preventive information, as well specifically on Zika and other VBD, particularly provided by HCW. This is consistent with a health system that have been traditionally focused on treatment more than prevention. This raise the need for more community prevention with better and evidenced-based practices, especially considering future emerging diseases.

Impact of chikungunya on the epidemiology of arthropathies, Colombia, 2014-2015

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Introduction: Chikungunya (CHIK) virus can lead to multiple rheumatological and non-rheumatological chronic consequences, including the pCHIK chronic inflammatory rheumatism (pCHIK-CIR). However, this last is no necessarily often screened and diagnosed, even in CHIK-endemic areas. The, we assessed if after the introduction and circulation of CHIK the attention and diagnoses of arthropathies was increased in Colombia. **Methods:** An ecological study was conducted using data from the nationwide Colombian health information system RIPS (Individual Record of Health Services Provision), assessing the incidence of arthropaties (M00.0-M25.9) and from surveillance for CHIK, during the period 2014-2015. CHIK was introduced in late 2014 to Colombia. An analysis of the trend of arthropaties for the previous period (2012-2014, preCHIK) was done. Linear regression models were run between CHIK (independent variable) and arthropaties (dependent variables) ($p<0.05$), using licensed Stata IC 14®. **Results:** From January 2012 to August 2014 (preCHIK), there were between 9,200 to 17,000 month cases of arthropaties (288-308 cases/100,000 pop). At the epidemics beginning, 2014, the report of arthropaties increased in 126%, with rates ratios 1.24 times higher (358.15 cases/100,000 poop), then, decreasing mildly to 2015. At the regression models, between CHIK and arthropaties for 2014-2015, there was a strong significant association ($r^2=0.9761$; $p=0.0037$). Juvenile arthritis was the diagnosis with highest increase, 36.8% from 2012-2013 to 2014-2015. **Discussion:** Accurate population impact of CHIK on rheumatological and chronic diseases is still to be better defined. It would be possible that present findings reflect diagnostics where if patients are *de novo* assessed would be serologically positive for CHIK, using IgG, which would be still positively high up to 36 months post-infection. Nationwide and population based studies of pCHIK-CIR are required. Extensive studies assessing all the rheumatological conditions that would be yield after infection are also needed. Finally, pCHIK-CIR should be included in the surveillance and notification, however, is not yet a specific condition at the International Classification of Diseases (ICD-10), something expectable to change in the upcoming ICD-11, as proposed by our group to the World Health Organization.

Climate variability and Dengue epidemiology, Coffee-Triangle region, Colombia, 2007-2013

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Introduction: Despite emerging arboviral diseases, such as chikungunya and Zika, dengue continues to be the most important viral vector-borne disease in the world, particularly in Asia and Latin America, and is significantly affected by climate variability. The influence of climate in an endemic region of Colombia. The Coffee-Triangle region (constituted by 53 municipalities), from 2007 to 2013, was assessed. **Methods:** Epidemiological surveillance data (weekly cases) were collected, and incidence rates were calculated. Poisson regression models were used to assess the influence of the macroclimatic variable ONI (Oscillation Niño Index) on the dengue incidence rate, adjusting by year and week. Monthly satellite images for total rainfall and surface temperature were obtained from the Tropical Rainfall Measuring Mission (1 month – TRMM) imagery database from NASA Earth Observations (NEO, NASA, USA) (http://neo.sci.gsfc.nasa.gov/) and were analyzed with Google Earth® software. **Results:** Monthly variation of dengue rates was: 0 to 684.90 cases/100,000 hab (Montenegro, Quindio, 2010). At nonlinear regressions, significant associations were found with ONI ($p<0.0001$), at municipalities of Quindío department: Calarcá ($r^2=0.7177$), Armenia ($r^2=0.6287$), La Tebaida ($r^2=0.6084$), Montenegro ($r^2=0.4945$) y Quimbaya ($r^2=0.4725$); Caldas deparment: Chinchiná ($r^2=0.6834$) and La Dorada ($r^2=0.6140$); Risaralda: Pereira ($r^2=0.6245$), Dosquebradas ($r^2=0.5678$) and La Virginia ($r^2=0.5654$). **Discussion:** El Niño significantly affected the incidence of dengue in the region. This association with climate change and variability should be considered in the elements influencing disease epidemiology. In addition, predictive models should be developed further with more available data from disease surveillance. This information should also be considered for studies relating to climate change and vector control.

pCHIK-CIR: An ethnographic vision of the social impact of disease

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Introduction: Chikungunya virus (CHIKV), an alphavirus, is the causative agent of the CHIKV fever, an *Aedes*-borne disease that can lead to long-term sequelae in around half of patients, the post-CHIKV-chronic inflammatory rheumatism (pCHIK-CIR). This consequence can have also relevant social impacts, but few qualitative studies have explored this issue. **Methods:** Qualitative research methods consisted of 26 open and in-depth interviews in patients serologically diagnosed with CHIKV infection (IgM) during acute phase (<3 weeks) and follow-up for 2 years (chronic phase, pCHIK-CIR), in La Virginia municipality (endemic area), Risaralda, Colombia, during July-November 2017. The analysis was made with emphasis on the ethnographic method. Consolidated criteria for reporting qualitative research (COREQ) was applied. **Results:** It is evident that pCHIK-CIR affects routine performance and work of patients, in terms of efficacy as well continuity of daytime activities. Pain descriptions help to a better understanding of healthcare workers about the nature of social limitations of the patients. Patients with pCHIK-CIR, facing sharp physical limitations, tend to significantly modify their habits/life styles and creating “autotherapy” modalities. **Discussion:** Patients need to establish sudden changes to try to solve the pain-related problems, affecting their life style, working activities and their relationships with other people. Treatment effective affordability lead them to search for unconventional alternatives, to learn to live with pain. The social impact of pCHIK-CIR manifests from different dimensions in the following context: since reduction of work capability, as well as efficiency in the continuity of common activities, of low or high impact, even reaching to the modification of life style, all of it to mitigate joint pains. These findings imply that the impact of pCHIK-CIR is not limited to clinical and biological consequences, but also extends to complex aspects such as the social life.

Quality of life after 2-year of chikungunya infection in Colombia

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Introduction: Impact of post-chikungunya (CHIK) chronic inflammatory rheumatism (pCHIK-CIR) on Quality of Life (QoL) has been reported in some studies from La Reunion, France, India and Colombia. In this country our group published its consequence after 1-year of follow-up. **Methods:** In a cohort study among 62 cases serologically diagnosed in La Virginia, Risaralda, Colombia, followed-up by 2-years, demographic and clinical characteristics were collected at baseline. QoL status by 36-item short-form health survey (SF-36) at 1-year and 2-years were assessed and compared. pCHIK-CIR cases were identified according to validated criteria (WHO/PAHO, 2015). Those with other arbovirosis during follow-up were excluded. **Results:** Of the total CHIK-infected subjects in this cohort, 43 (69.4%) reported persistent rheumatological symptoms (pCHIK-CIR). All dimensions of SF36 as well as physical and mental component summaries were impaired in pCHIK-CIR+ compared to pCHIK-CIR- subjects. Differences in median scores between both groups, pCHIK-CIR- with 83.2% and pCHIK-CIR+ with 51.4%, were statistically significant ($p<0.0001$). In addition, in six dimensions, differences were also significant ($p<0.05$) (physical functioning [89.5%/62.1%], role physical [89.5%/39.0%], bodily pain [88.2%/44.4%], general health [77.7%/51.4%], vitality [79.5%/50.6%] and health transition [68.4%/40.7%]). When compared evolution from 1-year to 2-year, the more prominent reduction was found in health transition from 50.9% to 40.7%, as well bodily pain from 51.6% to 44.0%. Global median scores reduced from 54.2% to 51.4%. **Discussion:** Despite possible cohort attrition bias, the comparability of pCHIK-CIR+/- subjects allows the confirmation of a long-term impact of CHIK infection with less chance of returning to a previous health status. We observed sharp reductions in QoL not only during active pCHIK-CIR+ associated illness but also for several months and now more than 2 years after infection compared to healthy normal subjects that reached clinical recovery. This has implications for developing intervention programmes in countries with high risk of CHIK outbreaks but also to consider the long-term impact of CHIK infection in a significant proportion of infected patients, even more considering that in countries such as Colombia, after the 2014-2015 epidemics (with estimations of 3 million cases), transmission still occurs with >1,000 new cases in 2017.

Baseline risk associated with 1-2-Years pCHIK-CIR, Risaralda, Colombia, 2015-2017

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Introduction: Although there has been an increasing interest in studies assessing the progression to chronic phase of chikungunya (CHIK) disease, such as the post-CHIK chronic inflammatory rheumatism (pCHIK-CIR), yet there is a lack of studies the baseline risk factors influencing such progression. **Methods:** In February-June 2015 a group of 283 patients were diagnosed with CHIK and were enrolled in follow-up. From them, 152 (53.7%) developed pCHIK-CIR after 12 weeks. After 2-year of follow-up, we analyzed demographic and clinical baseline risk factors that were associated with the persistence of pCHIK-CIR after 2 years. Incidence according exposition (relative risk, RR) and proportions (χ^2) with their respective 95% confidence intervals (95%CI), were calculated using Stata-IC 14.0® licensed, p significant <0.05. Those with other arboviroses during follow-up were excluded. **Results:** A total of 171 patients were assessed and valid for analyses after 1 year, 61.98% have pCHIK-CIR+ at that point; 62 patients were assessed and valid for analyses after 2 years, 43 (69.4%) corresponding to those that progressed to the development of pCHIK-CIR+ and 19 (30.6%) that were free of disease after 2 years (pCHIK-CIR-). Arthralgias of elbow, wrists, MCP, knees and ankles were all present in >59% at baseline. Patients with female sex developed 1-year-pCHIK-CIR in 69.4% compared to 50.8% in males (RR=2.198; 95%CI 1.151-4.202) and at 2-year-pCHIK-CIR in 79.1% compared to 47.4% in males (RR=1.669; 95%CI 1.014-2.747), living in urban area 76.5% compared to 36.4% in rural area (RR=2.705; 95%CI 1.389-5.267), none or just primary education 86.7% vs 53.1% secondary and universitary (RR=1.631; 95%CI 1.145-2.325). Baseline myalgia was 83.8% in those that progressed to 1-year-pCHIK-CIR, compared to 56.8% (RR=3.927; 95%CI 1.535-10.048). History of self-reported depression was higher in those that developed pCHIK-CIR+ (p=0.062), live in a lower socieconomical level (p=0.240), and age ≥45 y-old (p=0.078). **Discussion:** More studies are required, with larger size but also long-term assessment, in order to better define the risk factors. In areas that still present transmission and new cases of CHIK, such factors should be considered in order to enhance early interventions as well mitigation of the chronic consequences of CHIK infection.

Disease Burden and Costs Attributable to Zika in Colombia, 2016

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Introduction: Zika virus (ZIKV) disease have emerged in the Americas causing significant morbidity in 48 countries. In addition, it is expected to be a considerable cause of disability and economic burden especially in the region developing countries, given its subacute and chronic sequelae, particularly its Guillain-Barre syndrome and the Congenital Zika Syndrome (CZS, including microcephaly). In this setting, there is a lack of studies assessing ZIKV burden and costs in Latin America. **Methods:** We estimated incidence rates for ZIKV during the 2016 outbreak in Colombia using surveillance epidemiological data provided by the Colombian National Institute of Health (that include RT-PCR diagnosed cases), as well using demographic data from the National Administrative Department of Statistics (DANE) (calculated as cases/100,000 pop). The burden of disease was estimated through Disability Adjusted Life Years (DALYs) lost (according 2004 WHO methodology) and the costs (direct and indirect) were estimated based on the national recommendations for ZIKV acute and chronic phase attention. **Results:** There was a total of 106,659 cases (9.2% confirmed by RT-PCR), with incidence rates ranging from 0 to 1,490.3 cases/100,000 population (San Andres islands) in different departments. All departments were affected. An estimate was made of total DALYs of 6.44 years lost /100,000 population (lower limit of the 95%CI). The 2016 outbreak estimated costs were at least US$ 23.9 million (COP$ 71,914,802,929) (currency exchange for Dec. 31, 2016) (lower limit of the 95%CI), which were 67.2% due to diagnosis and management of suspected and confirmed cases of CZS. **Discussion:** Our estimates raise concerns about the effects of continued ZIKV spread in Colombia and other Latin-American countries. The lack of effective transmission control for this disease and potential for spread means that there will be significant acute and chronic disability and related costs in the short and long term for Latin American health care systems. In addition is important to mention that in 2017 ZIKV is still transmitted with 1,765 cases up to November 18 (248 among pregnant women).

Diversity & Impact of Flaviviruses Carried by Mosquitoes of the Aedes Genus in Botswana

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Botswana, in particular the North West district of Botswana boasts many destinations such as the Okavango Delta, Moremi Game Reserve, Maun, Kasane, Linyati, Selinda and Kwando that are popular with tourists. These areas are surrounded by lakes, channels, rivers, swamps, lagoons and reed beds making them ideal breeding habitats for mosquitoes. While mosquitoes are known natural vectors of viruses and parasitic protozoa of medical significance, most attention has been given to malaria, a non-viral infection. Malaria is the only mosquito-borne infection that is routinely tested for in health facilities in north-western Botswana. The recent incidence of new Aedes-borne diseases such as Zika, increased invasion of vectors into novel environments, as well as Botswana's reliance on tourism, all increase the likelihood of the introduction of new vector species and pathogen complexes. Consequently, it is important that we routinely investigate if there are any new infectious threats to people that live in or visit areas such as Botswana's North West district. The prospective work will focus on Dengue viruses, Yellow Fever virus and Zika virus.

RISK OF TRANSFUSION-TRANSMITTED DENGUE: STUDY OF BLOOD DONORS DURING AN OUTBREAK IN BRAZIL

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Since the majority of dengue infections are asymptomatic, an infected individual may present and be eligible for blood donation, which may lead to the transfusional transmission of this agent. However, there is a large discrepancy between the number of cases of viremic blood donors observed and the paucity of reports of transfusional dengue in the literature.
In Barretos/SP, there was a large outbreak of dengue in the first four months of 2013, with an incidence of 3,735/100,000 inhabitants. In this city is located the Barretos Cancer Hospital (HCB), where it operates the blood bank responsible for the transfusional medicine of the institution and other health services on the region, performing on average, 1,090 transfusions per month.
In order to study the possible risk of transfusional transmission of dengue, we tested 1,822 samples of serum or plasma from all blood donors of the HCB collected from March to April of 2013, peak of dengue season in Brazil, with the aim to estimate the incidence and prevalence of markers of dengue exposure and determine the prevalence of dengue serotypes in cases of viremic donors.
The detection of dengue RNA was performed by RT-qPCR in the QuantStudio5 PCR System (ThermoFischer™) using primers and probe that detect all viral serotypes. Positive samples were submitted to another qPCR to determine the serotypes with primers and probes specific for each dengue serotype. The serologic tests for detection of IgM and IgG antibodies anti-DENV used were CaptureDxSelect ™ Dengue Virus IgG and Dengue Virus IgM CaptureDxSelect ™ (Focus Diagnostics).
We found a rate of 0.38% DENV RNA positive samples, all of them genotyped as DENV-4 and a seroprevalence of 51.32% of IgG and 16.85% of IgM. This high incidence observed reflects the incidence in the general population during the outbreak and indicates a high risk of transfusional transmission of dengue.

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Virology / Antiviral – Pathogenesis

Contribution of Dengue NS1:T164S mutation in disease severity and transmission

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Abstract: Dengue virus (DENV) causes severe and sudden human epidemics around the world and the contribution of viral factors affecting transmission and increased severity is poorly understood. Recently it was shown that vascular leakage, the hallmark of severe dengue disease, can be triggered through Toll-like receptor 4 pathway by the secreted form of dengue non-structural protein 1 (sNS1). We show through reverse genetics introduction of a single site-specific mutation T164S of NS1 protein – observed frequently in DENV-2 epidemics in the Americas – that it can lead to early suppression of virus production and increased sNS1 in human cell-line, *ex-vivo* human tissue, and AG129 mice, even though identical levels of RNA replication was detected. Compared to the parent DENV-2 clinical isolate, we observed enhanced mosquito infectivity of the NS1:T164S mutant virus suggesting increased potential for epidemic transmission. Nanostring-based probing of 268 inflammation-associated human genes revealed prominent up-regulation of complement-mediated genes that can induce vascular leakage. AG129 mice infected with NS1:T164S mutant virus displayed early severe symptoms and caused higher lethality coupled with enhanced complement activation and inflammation than WT-infected mice. This study demonstrates that NS1 mutation-driven clade changes leading to increased disease severity observed in dengue epidemics in Cuba and the Americas can indeed be recapitulated in cellular and mouse model of infection. The single NS1:T164S change that was introduced into a relatively benign DENV2 infectious clone from a clinical isolate from Singapore belonging to the “cosmopolitan” genotype IV acquired the phenotype of the Cuban/American clade within genotype III.

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Impact of serological cross-reactivity on ZIKV/DENV pathogenesis in mice

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Zika virus (ZIKV) has spread rapidly into regions where other flaviviruses, such as dengue virus (DENV), are endemic. Antibody-dependent enhancement (ADE) has been implicated in more severe forms of flavivirus disease, especially in patients who have experienced secondary heterotypic DENV infections. To date, though the potential for ADE by serological cross-reaction between ZIKV and DENV has been clearly shown *in vitro*, it still remains controversial whether pre-existing immunity to ZIKV or DENV alters the course of ZIKV/DENV infection *in vivo*. Here we investigated the effects of serological cross-reactivity on ZIKV or DENV pathogenesis in AG129 mice (deficient in IFNα/β and γ receptors) or A129 mice (deficient in IFNα/β receptor).

ZIKV- or DENV-immune serum was collected from mice at 4-8 weeks after infection, and injected in naïve mice one day prior to ZIKV/DENV infection. Mice infected with ZIKV or DENV were also used for the sequential ZIKV/DENV infection at 8 weeks post-infection. Furthermore UV-inactivated ZIKV or DENV with 3x boosters were used to immunise mice and followed by challenge with lethal inoculum of ZIKV or DENV. Mouse mortality, kinetics of viremia and tissue viral load were examined through the course of infection.

Inoculation of ZIKV-immune serum enhanced dengue disease severity, whereas DENV-immune serum did not alter ZIKV pathogenesis in mice. ZIKV-infected mice were completely protected from sequential lethal DENV infection accompanied with 2-log viremia suppression. DENV-infected mice were also protected from lethal ZIKV infection, however the viremia at early stage of infection was only 2-fold reduced compared with primary ZIKV infection. These results indicate that DENV pathogenesis was readily affected by ZIKV immunity, whereas DENV immunity had less impact on ZIKV pathogenesis in mice. However, immunization with inactivated DENV significantly increased viremia when the mice were challenged with ZIKV, suggesting that ZIKV infection can be enhanced by DENV immunity under certain conditions in vivo. Overall these results should be taken into consideration when designing ZIKV or DENV vaccines.

RELATIONSHIP BETWEEN DENGUE VIRUS INFECTION AND THE LEVELS OF EICOSANOIDS

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The clinical course of dengue is influenced by multiple factors. In this context, the inflammatory process and its regulation play a major role in dengue prognosis. For instance, an exacerbated inflammatory response can trigger systemic tissue injury and disturb the endothelial permeability. The eicosanoid molecules have considerable influence upon the regulation of many inflammatory mechanisms involved in the pathogenesis of several diseases. However, few studies have analysed the role of eicosanoids during Dengue virus infection. In order to quantify molecules involved in the cyclooxygenase (COX) and lipooxygenase (LOX) pathways during dengue, blood samples were collected from patients with mild dengue and plasma levels of thromboxane A_2 (TXA_2), prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) and also mRNA levels of thromboxane A2 synthase (*TXA_2S)*, prostaglandin E_2 synthase (*PGE_2S)*, leukotriene A4 hydrolase (*LTA_4H)*, cyclooxygenase-2 (*COX-2)* and 5-lipoxygenase (*5-LOX)* were analysed. Moreover, as lipid bodies (LB) are organelles engaged in the synthesis of eicosanoids, we measured their levels in peripheral blood leukocytes and also the percentage of vacuolated monocytes in the blood circulation of DENV-infected individuals, as well as the DENV load. From 40 volunteers enrolled in this study, 12 healthy volunteers were negative in RT-qPCR, ELISA NS1 and also ELISA IgM tests. The remaining 28 volunteers presented dengue symptoms and were positive at least in one of the three tests used (17 were positive using RT-qPCR, 20 were positive using ELISA NS1 and 16 were positive in ELISA IgM). Due to the wide variation of days of symptoms reported by the volunteers (1-12 days of symptoms), they were divided into two groups according to the presence and absence of IgM. Our results showed that DENV infection increases the levels of TXA_2 in IgM-positive individuals as well as the amount of LB in monocytes collected from IgM-negative individuals, which is related to viral replication, suggesting that the balance TXA_2 and IgM levels play a protective role against the development of the severe symptoms of dengue such as vascular leakage.

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Dengue ADE: Knowns and Unknowns

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What have we learned concerning the pathogenesis of human dengue infections?

Knowns: A holistic model of dengue pathogenesis is emerging. Intrinsic antibody dependent enhancement (iADE) results in logarithmic increases in virion production when infectious dengue immune complexes partially disable innate cellular defenses of target Fc-receptor bearing cells. Ultimately, disease severity is proportional to an aggregate mass of dengue-infected tissue macrophages, the largest number being in spleen. Peak dengue vascular permeability syndrome (DVPS) (thrombocytopenia, altered hemostasis, activated complement, elevated liver enzymes and vascular permeability) occurs during defervesence, a time when CD 8+ and CD 4+ T cells are actively killing virus-infected cells. The nature and role of human T cell responses has been clarified. T cells are directed at epitopes found predominantly on NS 3 and NS 5 dengue non-structural proteins do not worsen heterotypic infection DVPS but contribute to prevention of secondary infections. Based upon in vitro and animal model data, DVPS is the direct result of circulating secreted dengue NS1, a molecular analog of bacterial endotoxin polysaccharide. Dengue NS1 activates toll-receptor 4 on human myeloid cells releasing TNF-a, IL-6, IFN-b, IL-1b, and IL-12, producing a “cytokine storm” that is an effect of NS1 activity and not the cause of DVPS. NS1 may also control month to month increase in severity of heterotypic dengue infections. A dengue 2 T164S NS1 mutation accompanied this phenomenon in Cuba in 1997. In a human cell-line, *ex-vivo* human tissue, and AG129 mice this mutation resulted in early suppression of virus production and increased production of sNS1. A human resistance gene has been identified. Black Cubans with proven reduced incidence of DVPS during secondary dengue infections had lower expression of OSB PL10 gene compared with Europeans. This gene interacts in the LXR/RXR activation pathway integrating lipid metabolism and immune functions and is a key player in dengue virus entrance into cells, replication and cytokine production.

Unknowns: Why do dengue antibody complexes produce in vivo ADE while complexes with non-dengue flavivirus antibodies do not? Of 12 possible sequential dengue infections, dengue 1 then 2 or dengue 1 then 3 produce severe disease, but others less so.

mice, DENV infection of AnxA1$^{-/-}$ and FPR2$^{-/-}$ yields a stronger infective reaction with more pronounced thrombocytopenia, hemoconcentration and plasma extravasation, leading to enhanced liver tissue damage. Treating WT or Anx-A1$^{-/-}$ DENV-infected mice with Ac_{2-26}, an AnxA1-active N-terminal peptide, resulted in protection on vascular inflammation and organ damage. Additionaly, Anx-A1 expression was elevated on BMDM infected by DENV and elevated viral loads were recovered from BMDMs supernatant from AnxA1$^{-/-}$ and FPR2$^{-/-}$ mice in comparison to WT littermates. Finally, Ac_{2-26} treatment to BMDMs from AnxA1$^{-/-}$ mice resulted in reduction of viral loads. Therefore, this study provides proof-of-concept that resolution biology is relevant to the regulation of host responses against DENV infections and suggests new ways for definition of pathogenesis and therapeutic approaches.

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PLATELET FUNCTION IS INHIBITED DURING INFECTION WITH DENV IN VITRO.

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There are many very important events that condition the progression to severe dengue, these include cytokine storm and a profound thrombocytopenia, still, the molecular mechanisms underlying these events are not fully understood. Increasing evidence supports the fact that a significant dysfunction in platelets, mostly in patients with secondary infections, causes an inhibition of platelet aggregation that might be determined by self-reactive antibodies produced in prior infections. To determine whether the infection with DENV affects the primary hemostasis of platelets *in vitro*, we evaluated aggregation induced by thrombin and ADP agonists following treatment with DENV. We found that DENV is capable of producing a reduction of 15% in platelet aggregation when these were induced with a thrombin agonist and a reduction of 57% when platelets were induced with an ADP agonist. Surprisingly the previous results were observed after purified DENV particles were incubated with a platelet suspension for just two hours, which suggests that DENV particles have a direct effect over platelet aggregation that is independent of viral replication and self-reactive antibodies. These changes in platelet aggregation may be correlated with the clinical manifestations observed during severe dengue, such as thrombocytopenia and hemorrhages. Further studies are needed to demonstrate if this phenomenon could be induced by the interaction of platelet with receptors for DENV particles at early times post-infection (post-interaction). This report clearly shows the inhibition of platelet aggregation mediated by platelet-virus interactions.

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Dengue fatal cases present virus specifc HMGB1 response in peripheral organs

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Introduction: Dengue disease is an acute viral illness caused by. dengue virus (DENV) that can progress to hemorrhagic stages leading to about 20000 deaths every year worldwide. Despite many clinical investigations regarding dengue, the immunopathogenic process by which infected patients evolve to the severe forms is not fully understood. Apart from differences in virulence and the antibody cross reactivity that can potentially augment virus replication, imbalanced cellular immunity is also seen as a major concern in the establishment of severe dengue. Despite its epidemiological relevance, there are many knowledge gaps concerning dengue pathogenesis, especially with regards to the circumstances that drive a mild clinical course to a severe disease. Objective: In this work, we investigated the participation of high mobility group box 1 (HMGB1), an important modulator of inflammation, in dengue fatal cases. Material and Methods: Histopathological and ultrastructural analyses revealed that liver, lung and heart post-mortem samples were marked by tissue abnormalities, such as necrosis and apoptotic cell death. Results: These observations go in line with an HMGB1-mediated response and raised concerns regarding the participation of this cytokine in promoting/perpetuating inflammation in severe dengue. Further experiments of immunohistochemistry (IHC) showed increased expression of cytoplasmic HMGB1 in dengue-extracted tissues when compared to non-dengue controls. Co-staining of DENV RNA and HMGB1 in the host cell cytoplasm, as found by *in situ* hybridization and IHC, confirmed the virus specific induction of the HMGB1-mediated response in these peripheral tissues. Discussion/Conclusion: This report brings the first *in-*

Dengue virus infection induces mitochondrial dysfunction in C6/36 insect cells.

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INTRODUCTION: Viruses regulate mitochondrial function and dynamics to promote viral proliferation. Dengue virus (DENV) infection induces mitochondrial fusion in Huh-7 hepatic cells while induces mitochondrial fission in A549 lung cells, and both phenotypes facilitated virus propagation. These results suggest that DENV infection causes different alterations in mitochondria depending of the cell type. Given that DENV has two hosts with different metabolism and immune response, we investigate the mitochondrial functions and dynamics during DENV infection in C6/36 insect cells derived from *Aedes albopictus.*
METHODOLOGY: To evaluate mitochondrial functions during the first 48 hours post-infection, we used direct and indirect methods to measure oxygen consumption, ATP production, ROS production, and mitochondrial transmembrane potential. To determine mitochondria dynamics, we used fluorescence and electronic microscopy.
RESULTS an DISCUSSION: Our data showed that DENV infection induces oxidative stress without falling cell survival. Also, promotes enhancement in ATP production but not cause changes in mitochondrial respiration of C6/36 infected cells compared with the mock infected cells. Furthermore, we offer the first evidence that DENV infection induces translocation of mitofusins to mitochondria, and elongation of these organelles in insect cells. However, additional studies are needed to elucidate the mechanism that DENV use to mediate mitochondrial dysfunction and regulate cell apoptosis.

Highlighted Poster

K48-linked polyubiquitination of dengue NS1 protein inhibits its interaction with NS4B

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Abstract

Dengue virus (DENV) is a member of the Flaviviridae family, which is transmitted to mammalian species through arthropods, and causes dengue fever or severe dengue fever in humans. The DENV genome encodes for multiple nonstructural (NS) proteins including NS1. NS1 plays an essential role in replication by interacting with other viral proteins including NS4B, however how these interactions are regulated during virus infection is not known. By using bioinformatics, mass spectrometry analysis, and co-immunoprecipitation assays, here we show that DENV-NS1 is ubiquitinated on multiples lysine residues during DENV infection, including K189, a lysine residue previously shown to be important for efficient DENV replication. Data from in vitro and cell culture experiments indicate that dengue NS1 undergoes modification with K48-linked polyubiquitin chains, which usually target proteins to the proteasome for degradation. Furthermore, ubiquitinated NS1 was detected in lysates as well as in supernatants of human and mosquito infected cells. Ubiquitin deconjugation of NS1 using the deubiquitinase OTU resulted in increased interaction with the viral protein NS4B suggesting that ubiquitinated NS1 has reduced affinity for NS4B. In support of these data, a K189R mutation on NS1, which abrogates ubiquitination on amino acid residue 189 of NS1, also increased NS1-NS4B interactions. Our work describes a new mechanism of regulation of NS1-NS4B interactions and suggests that ubiquitination of NS1 may affect DENV replication.

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Investigation of arboviruses in Aedes aegypti and albopictus in Brazil

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Introduction: Arboviruses are diseases caused by viruses and transmitted by arthropods. Originally, they occur in the wild cycle, but many of them have spread and adapted to the urban areas and are currently responsible for major epidemics, being dengue fever, chikungunya fever and zika fever the most documented in Brazil in the last four years. The viruses that cause these three arboviruses, *Dengue virus* (DENV), *Zika virus* (ZIKV) and *Chikungunya virus* (CHIKV), are transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes. In the State of Maranhão, these two species are occurring and there are records of arbovirus cases in several municipalities, besides not having diagnostic laboratories for arboviruses in the region. In this context, the main objective of this study was to investigate the infection by DENV, ZIKV and CHIKV in *Ae. aegypti* and *Ae. albopictus* of the State of Maranhão. Metodology: For this, mosquitoes were captured using Nasci aspirator, followed by identification and formation of pools, extraction of viral RNA and real-time RT-PCR – Trioplex CDC, the positives were confirmed by standard gold tests used un the country's laboratory network. Results and Discussion: A total of 3,166 arbovirus vectors arthropods were captured, 348 *Ae. aegypti* and 12 *Ae. albopictus*. In relation to the two species, a total of 162 pools of mosquitoes were formed and, of these, three presented positivity, being: AR849404 (*Ae. aegypti* ♀ detected with CHIKV), AR849486 and AR849487 (both *Ae. aegypti* ♀ detected with DENV2). Conclusion: These data reinforce the information of the Ministry of Health about the circulation of arboviruses in the interior of Maranhão and proves the performance of *Ae. aegypti* as responsible for transmission in these areas.

Found for Research: IEC/SVS/MS; FAPEMA/MA

Cordia curassavica from Colombia: natural source in therapeutics for dengue

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Introduction: There are no specific drugs for dengue treatment despite extensive research in synthetic inhibitors. Medicinal plants are rich source of new componds in therapeutics, appropriated species for research and discovering new alternative therapeutics for dengue are required. **Aim:** In this work, he urged us to identify Colombian medicinal plants that produce essential oils with an inhibitory effect on the replication of DENV and the cytokines / chemokines involved in the pathogenesis of dengue. **Materials and Methods**: EO were obtained from ten medicinal plants cultivated in Colombia by using microwave-assisted *hydrodistillation. Samples were analized in diferents* cell-based assay to identify the strongest essential oils active on dengue. The EO more active was analized by gas chromatography–mass spectrometry. **Results:** We found that the *Cordia curassavica* (Jacquin) Roemer & J.A.Schultes essential oil showed the most potential anti-dengue: low cytotoxic profile (MTT: CC_{50} > 100 µg / mL); reduced the cytopathic effect > 50% (Image J software); reduced the replication of the four serotypes (ELISA-NS1, ELISA-*in-situ*) to IC_{50} <30 µg / mL with SI > 5; did not show sensitizing effect (THP-1 / IL-8: 276 ± 105.1 *versus* 142 ± 4.2 pg / mL); and reduced levels of TNF-α (87%), IL-8 (67%), and INF-γ (47%) in culture supernatants of LPS-stimulated PBMC (peripheral blood mononuclear cells. The main constituents of the essential oil were: *trans*-β-caryophyllene (21.4%), germacrene D (17.8%) and α-copaene (16.5%). The EO of *Piper marginatum* Jacquin and *Baccharis trinervis* Persoon showed moderate antiviral activity and the other plants showed no activity. **Conclusion:** The *Cordia curassavica* essential oil could serve as start point for development of new prophylactic/therapeutic alternatives for dengue treatment.

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HISTONE-DEACETYLASE INHIBITION BY VALPROIC-ACID DOWNREGULATES CYTOKINE EXPRESSION IN DENGUE-INFECTED MACROPHAGES

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Introduction. Natural infection with dengue virus (DENV) induces an increase in the production of cytokines, which play an important role in disease pathogenesis. Despite numerous scientific studies, there are still no commercially available disease-specific therapeutics. Previous evidence shows that inhibiting histone deacetylase enzymes (HDACs) regulates the immune response in several inflammatory disease models. **Objective**. The aim of the current study was to evaluate the effect of HDAC inhibition in the production of inflammatory cytokines in human monocyte-derived macrophages infected with DENV serotype 2 (DENV-2). **Methods**. Human monocyte-derived macrophages (MDMs) were treated with the well-known HDAC inhibitor trichostatin A (TSA) or valproic acid (VPA) before or after infection with DENV-2, and the inflammatory cytokine content in culture supernatants was quantified by flow cytometry. Also, cytokine gene transcription was evaluated by real time RT-PCR. **Results**. We found that infected MDMs secreted IL-8, IL-1β, IL-6, TNF-alpha and IL-10, but not IL-12. Strikingly, treatment of infected cells with VPA had a differential and concentration-dependent effect on the production of specific cytokines without eliciting significant changes in cell viability. VPA treatment before infection reduced 63% and 93% the TNF-alpha and IL-6 secretion and post-infection treatment caused also an 87.3% and 86.6% reduction in these cytokines. Similar results were obtained after IL-8, IL-10 and IL-1β quantification. Both pre- and post-infection treatment also significantly reduced the transcription rates of cytokine genes measured by real time PCR. **Conclusion**. These results suggest that HDAC inhibition during DENV-2 infection could exert an important regulatory effect in the production of inflammatory cytokines, representing a significant advance in the design of novel therapeutic dengue treatments.

DC-SIGN and TLR3 polymorphism are associated with Chikungunya infection and Disease.

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Introduction. DC-SIGN and TLR3 are pathogen recognition receptors involved in phagocytosis and antiviral response by recognizing pathogenic glycoprotein and double stranded RNA, respectively.
Objective of the study. In this study we have investigated if single nucleotide polymorphisms (SNP), in genes encoding DC-SIGN and TLR3 are associated with Chikungunya infection and disease.
Material and Methods. A total of 290 individuals were investigated and include 145 subjects with clinical diagnosis of Chikungunya disease confirmed by RT-PCR (cases) and 145 asymptomatic subjects (control) matched with cases by age, gender and time frame of exposure. Cases and controls were enrolled in the city of León after the introduction of Chikungunya in Nicaragua (July 2014 and December 2015). Following DNA purification from blood (QIAamp DNA Mini Kit), TaqMan® Genotyping Assays (Thermo Fisher Scientific) were used to identify SNPs in DC-SIGN (rs4804803) and TLR3 (rs3775291). Chikungunya IgG antibodies were examined by ELISA (Euroinmune) to determine Chikungunya infection, either symptomatic or asymptomatic.
Results. This study show that the frequency of the DC-SIGN heterozygous genotype was significantly higher in cases than in controls (OR = 4.9, P<0.001, 22/145 vs 5/145). Similarly, the frequency of the TLR3 mutant genotype was significantly higher in cases than in controls (OR = 2.7, P<0.05, 19/145 vs 7/145). Furthermore, the current study show that while the frequency of the DC-SIGN heterozygous genotype was significantly higher in IgG-seropositive subject than in seronegative (OR = 2.8, P<0.05, 21/164 vs 6/123), the frequency of the TLR3 mutant genotype did not differ significantly between IgG groups. SNPs in TNF and a deletion in CCR5 did not show association.
Conclusion. This study indicate that DC-SIGN and TLR3 may play important roles in Chikungunya disease and DC-SIGN G allele may facilitate infection, but, functional studies of these mutations are required to establish any conclusion.
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Disulfide bond formation as an antiviral target against chikungunya virus

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Chikungunya virus (CHIKV) is a mosquito-borne virus in the family Togaviridae genus *Alphavirus*, which causes a debilitating and often chronic arthritis. It has been shown that alphaviruses require conserved cysteine residues for proper assembly and function of the E1/E2 heterodimer spike proteins, and it has been suggested that host protein oxidative-folding enzymes may aid in disulfide bond formation. Therefore, compounds which inhibit host thioredoxins and regulatory enzymes may exert antiviral effects against chikungunya virus (CHIKV) and other enveloped viruses by interfering with envelope protein folding and progeny virion infectivity. To establish the potential for targeting oxidative folding pathways as an antiviral strategy, time-course cytotoxicity curves and dose-response curves against CHIKV, Venezuelan equine encephalitis virus (VEEV), and zika virus (ZIKV) were established in HEK293 cells for the protein disulfide isomerase (PDI) inhibitors 16F16 and PACMA31, thioredoxin-reductase (TRX-R) inhibitor auranofin, and endoplasmic reticulum oxidoreductin inhibitor EN460, and therapeutic indices calculated (CC_{50}/EC_{log50}). Further, genome:plaque-forming unit (PFU) assays together with cell-cell fusion assays were used to assess the effect of 16F16 and auranofin on progeny virion function. Finally, an *in-vivo* study using the C57BL/6 model of CHIKV infection was used to evaluate the efficacy of PACMA31 and auranofin. Auranofin significantly inhibited CHIKV, VEEV, and ZIKV replication with TIs between 1.45-104.5, 16F16 inhibited all three viruses with TIs between 0.69-12.2, and PACMA31 and EN460 both inhibited CHIKV replication, but at ECI_{log50} >10 and TIs between 1-20.6. 16F16 treatment significantly increased the genome:PFU ratio and left intracellular CHIKV RNA unaffected, while auranofin treatment also significantly increased the genome:PFU ratio but decreased intracellular CHIKV RNA; similarly, treatment with 16F16 resulted in decreased cell-cell fusion events while auranofin resulted in a significant decrease in overall infected cells. Finally, auranofin treatment significantly reduced footpad swelling in CHIKV-infected mice. These studies demonstrate the potential for targeting oxidative folding pathways as an antiviral strategy: inhibiting PDI with 16F16 appeared to decrease viral replication by causing production of defective particles, while inhibiting TRX-R may have resulted in expedited cell-death processes in infected cells.

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Increased activity of indoleamine 2,3-dioxygenase and T regulatory cell frequencies during dengue infection

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Dengue virus (DENV) is a member of the family *Flaviviridae* and there are four distinct serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. Indoleamine2,3–dioxygenase (IDO) is an enzyme that initiates the degradation of tryptophan as well as differentiation of T cells into regulatory T cell phenotype. The role of IDO in DENV infection is still unclear. In this context, we evaluated the role of IDO during DENV infection through its measurement in plasma samples of patients from 2013 outbreak. Additionally, clinical features, cytokine profile and the CD4+CD25+Foxp3+ frequency were evaluated. Patients were classified in the following groups: 158 (70, 2%) Dengue Fever without warning signs (DFwoWS), 65 (28,9%) Dengue Fever with warning signs (DFwWS) and 2 (0,9%) severe dengue. Our results showed increased IDO activity, and lower levels of tryptophan in infected patients. Quantification of cytokines indicated: i) increased levels of cytokines (TNF-α, IL-6 and IL-10) in DFwWS and DFwoWS patients compared to healthy individuals. ii) Increased levels of IL-10 in DFwWS/severe dengue compared with DFwoWS. Correlation analysis showed that IL-10 circulating levels were inversely correlated with platelets counts and directly associated with AST levels. In addition, IDO activity was inversely correlated with AST and ALT levels. Finally, we observe an increase in the frequency of CD4+CD25+Foxp3+ cells in infected patients that were directly associated with IDO activity. Our results suggest that IDO plays an important role in the antiviral response during DENV infection and probably in the induction of cells with regulatory phenotype.

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Characterization of cell death parameters in HIV patients during Dengue acute disease

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Dengue is known as the most important arboviruses in the world. Currently, approximately 40 million people are infected with HIV. In this context, both diseases HIV are considered major public health problems and dysregulation of apoptosis are seen as important factors in the depletion of T cells and disease progression. Little is known about the immunological mechanisms that could explain the mildest form of Dengue in DENV and HIV coinfected patients. We aim to investigate the functional role of lymphocytes subsets in the clinical manifestations and cell death during coinfection with DENV and HIV in order to understand the mechanisms involved in susceptibility to severity of both diseases. The study was carried out in Brazil from 2010 to 2011. Dengue patients were classified according to the parameters established for the WHO in 2009. Immunologic assessments by specific death markers and multiple labeling of PBMCs were performed by flow cytometry. Analysis of apoptosis–related proteins expression profile was performed using PBMC lysates. $CD4^+$ T-cell counts and the frequencies of CD4 T-cell subset were diminished in HIV infection, more expressively in coinfected patients. We also demonstrate in coinfected patients, that the proportion of $CD4^+$ and $CD8^+$ T cells expressing CD95 was higher than in healthy individuals. Interesting, high frequency of CD4 T cells expressing low levels of Bcl-2 were found in coinfected patients compared to monoinfected groups. Despite T-cell apoptosis was increased in DENV and HIV infection, this was not affected by coinfection even in HIV patients receiving antiretroviral treatment. The analysis of apoptosis-related protein expression profile showed that not only molecules with pro but also those with anti-apoptotic functions are over expressed suggesting that survival mechanisms could be protecting cells against apoptosis caused by viral, immune, oxidative and/or genotoxic stresses in both groups of patients. Our findings suggest an up-regulation mechanism of death markers expression on T-cells in HIV/DENV coinfection, contributing to depletion of CD4 and CD8 T-cells in peripheral blood. Further studies will be of great interest to elucidate the effects of Dengue on treated HIV infected patients and could generate new tools for prevention and treatment of both infections.
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INTRAHOST DIVERSITY OF DENV-2 IN PATIENTS WITH DIFFERENT CLINICAL OUTCOMES

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Dengue fever (DF) can be caused by any of the four antigenically related but genetically distinct dengue virus (DENV) serotypes. According to disease severity, DF is classified into classic dengue with or without warning signs (ws) and severe dengue. DENV-2 has been circulating in the state of Rio de Janeiro since 1990. In 2008, a DENV-2 outbreak was associated with increased disease severity and mortality. Viral factors contributing to severe pathogenesis remain poorly characterized. Therefore, we analyzed DENV-2 intrahost genetic diversity in 21 serum samples of patients diagnosed as classic dengue (43%), classic dengue with ws (33%) and severe dengue (24%) in the state of Rio de Janeiro between 2007 and 2011, in order to determine its possible correlation with disease severity. Full-length viral genomes were deep sequenced using an amplicon-free approach, on the Illumina NextSeq500 sequencing system. Single nucleotide variants (SNV) and their frequencies were determined for each sample. Viral intrahost population structure was characterized by richness, nucleotide diversity and complexity, but no obvious difference was observed among samples clinically classified ($p>0,05$). Variability along viral genome was increased in the 5´untranslating region and the capsid-coding gene, independently to patient´s clinic. It remained stable in the rest of the genome only in severe cases. Noteworthy, severe cases presented a mean frequency for SNVs of 13% (while classic cases with or without ws, 5%) ($p<0,05$), as well as a lower proportion of non-synonymous SNV (NS-SNV) across the genome (61%) compared to classic cases with or without ws (78%). Moreover, they seem subjected to a strong purifying selection (mean dN/dS 0,28), while it approaches neutrality in classic cases with or without ws (mean dN/dS 0,83 and 1,03 respectively) ($p<0,05$). Finally, 248 of the 1362 NS-SNVs reported for the whole dataset, were found repetitively among the samples (from 8 to 60%). However, 200/248 were absent in severe cases, suggesting they may not be involved in severe pathogenesis of DENV-2. Results generated in this study contribute to the understanding of the viral dynamics of DENV-2 and highlight the need for further investigation to elucidate the role of each of these minority mutations in the evolution of severe disease by DENV.
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POLYHISTIDINE-TAG POSITION TRIGGERS DIFFERENTIATED EXPRESSION AND FUNCIONALITY OF RECOMBINANT DENV2-NS3 PROTEIN

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Introduction: Several recombinant strategies have been conducted to obtain the DENV2-NS3 protein biologically active. NS3 protein plays a relevant role on DENV replication. It has been identified as one of dengue proteins target for antiviral development and for inducing a CD8+ T-cells immune response.
Objective of the study: The goal of this work is to design an effective strategy to obtain high levels of a recombinant NS3 protein functionally active.
Materials and methods: Two genetic constructions based on N- and C- terminal position of 6xHistidine tag in the NS3 protein (N-NS3 and C-NS3) were developed. The NS3 expression conditions were assessed in three E.coli strains: BL21(DE3), M15[pREP4] and XL1-Blue. Factors such as temperature, induction time, IPTG concentration, and growth medium conditions were optimized for NS3 expression. The integrity and purity of the NS3 protein was monitored by SDS-PAGE and Western Blot, using anti-NS3 protease polyclonal antibody and anti-His monoclonal antibody. The NS3 activity was determined by protease assay.
Results and Discussion: The highest expression of the NS3 protein was reached into BL21(DE3) cells, regardless of polyhistidine-tag position in the recombinant protein. However, after cellular disruption differences in the location of both 6xHistidine-tag NS3 proteins were showed. The C-NS3 protein was detected in the soluble fraction whereas the N-NS3 was found insoluble forming inclusion bodies. Upon purification process, NS3 proteins were recognized by both polyclonal and monoclonal antibodies. Despite the solubility of the C-NS3 protein it was functionally inactive, whereas the refolded N-NS3 protein exhibited protease activity. Even though polyhistidine-tag fusion partner improve the expression levels of the NS3 protein, the solubility detected in the C-NS3 is queried. We hypothesized that C-NS3 could form a soluble structure in which the polyhistidine-tag interact with protein active sites, blocking the access of substrates to these.
Conclusions: The recombinant strategy through denaturing-refolding process for NS3 protein production shown to be useful a126nd efficient for obtaining an active DENV2-NS3 protein, which could be used as recombinant antigen for the design of serological tests, the screening of antiviral compounds and dengue vaccine formulation.

Highlighted Poster

Characterize the properties of chikungunya virus particle derived from ex vivo and in vitro culture system

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Chikungunya virus (CHIKV) belongs to Alphavirus genus of Togaviridae family. CHIKV was identified in Africa in 1953 and has recently reemerged, causing explosive outbreak and reaching 5 continents. The cryo-EM structure of CHIKV has been published in 2013 and showed that CHIKV is icosahedral with T = 4 symmetry arrangement. The outer surface of mature CHIKV particles is composed of 80 spikes, each spike is formed by three copies of an E1–E2 heterodimer. E1 protein contains fusion loop, and E2 proteins carries putative receptor binding site and protects fusion loop before activation. Although a lot of studies on CHIKV, there are no effective vaccine and medicine against CHIKV. The currently production of CHIKV vaccine is based on *in vitro* culture system. However, in 2015, our cooperator, Prof. Perng and his team in National Cheng Kung University, found that dengue virus (DENV) presented different morphologies *in vitro* and in vivo. And *ex vivo* DENV which derived from human primary cell shares the same features with in vivo DENV. Hence, we suspected that CHIKV, which is also transmitted by Aede mosquito, may has the same phenomenon.

Here, CHIKV attenuated vaccine strain 181/25 clone was used to infect vero cell line and human primary cells isolated form bone marrow to present the *in vitro* and *ex vivo* culture system. Cryo-EM and immunogold labeling were used to characterize the CHIKV. The features of our cryo-EM *in vitro* CHIKV structure agreed with the features of CHIKV structure published in 2013. On the other hand, cryo-EM images showed that *ex vivo* CHIKV was wrapped by membrane, called CHIKV vesicle here. Immunogold labeling and affinity grid confirmed that the vesicle-like particle was CHIKV. Even though we couldn't solve the CHIKV vesicle 3D structure using single particle reconstruction approach due to the heterogeneity of the *ex vivo* CHIKV production, the TEM images analyses showed that CHIKV presented different morphologies derived from *in vitro* and *ex vivo* culture system. Our finding here may provide the hints for antiviral candidates and effective vaccine development.

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Characterization of Infectious Dengue Microparticles in Plasma of Acute Patients

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Microvesicles (MVs) are lipid bilayer-covered cell fragments shedding from cell surface or internal endosome membrane, contain RNAs, mRNAs and functional cellular proteins and can mediate cell-to-cell communication. The sizes for MVs are dynamic, ranging from 30nm to 1μm. HIV and HCV have been shown to explore the unique characteristics of MVs to eschew immune surveillance and disseminate within the body. Dengue virus (DENV), a positive sense single-stranded RNA virus, is one of the most important mosquito-borne human viral diseases globally. Despite high viremia in dengue patient, the identity of the dynamic density and physical appearance of dengue particles has not been solved until recently. The infectious dengue particles are various in size and appear as microvesicles surrounding with human host membrane. However, the biological functions and properties of these dengue vesicles have not been investigated. Dengue microparticles in acute dengue plasma were fractionated and isolated via centrifugation with different forces and gradient, the morphology and biological properties from each fraction were studied. Results showed that higher copies of viral RNA and lower number of viral plaques in microparticles fraction under 100,000g were observed, in contrast, lower copies of RNA with higher viral plaques was seen in single viral particle fraction above 100,000g. The formation of comic tail plaques, suggesting better efficiency of infection, was much higher in microparticles than that of single particles. Furthermore, during short term infection, much more viral RNA was detected within DNEV-microparticles co-cultured cells, indicating a higher entry efficiency of DENV-microparticles. Electron microscopy (EM) data revealed that many viral particles “packaged” inside a membrane in microparticles were observed. Our results indicated that DENV may circulate as MVs in dengue patients avoiding human immune surveillance to enhance transmission efficiency and cause severe dengue symptoms.

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Therapeutic Targeting of the RNA dependent RNA polymerase of Multiple Arboviruses

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Climate change, rising populations, and increases in travel, have led to the global spread of a broad array of arboviruses. Arboviruses including Dengue (DENV), Usutu (USUV), Zika (ZIKV), Chikungunya (CHIKV), Venezuelan Encephalitis (VEE), Eastern Encephalitis (EEV) and Western Encephalitis (WEEV) viruses are an increasing threat to health and economic stability of the world's population. For arboviruses, there are few vaccines available and we are currently unable to predict the next global threat; as a result we are poorly prepared for the next arbovirus epidemic. These factors have created a need to develop "pan-antivirals" that are broadly protective against arboviruses. We hypothesized that antivirals targeting the RNA dependent RNA polymerase (RdRp) (NS5-flaviviruses, nsP4-alphaviruses) would be broadly protective against infection. To challenge this hypothesis we targeted the RdRp using EIDD-1931 (β-D-N(4)-hydroxycytidine) an established compound with known activity against Hepatitis C virus. We demonstrated that EIDD-1931 was highly effective against multiple arboviruses with a half maximum effective concentration of 1.3μM CHIKV-LR, 1.8μM VEE-TRD, 1.1μM EEEV, 2.1μM WEEV, 20.7μM USUV, 8.7μM ZIKV-PRVABC59 in vitro. Using CHIKV as the representative arbovirus, we completed a series of experiments to dissect the mechanism of action. A concern with antivirals is the possibility of rapid development of resistance, to test this we used suboptimal doses of EIDD-1931 to select for CHIKV resistant mutants. After twenty passages we selected resistant isolates with a broad range of EC_{50} concentrations (1.5mM to 15mM). This range of resistance to β-D-N(4)-hydroxycytidine indicates that this nucleoside analogue most likely drives these RNA viruses into error catastrophe. We are currently using a CHIKV infectious clone to identify the mutation/s that lead to resistance. To determine if we could improve the efficacy and stability of EIDD-1931 we evaluated multiple structurally related hydroxycytidine compounds that become nucleoside analogues after phosphorylation to a 5'-triphosphate by host cell kinases. The results of these studies indicate that multiple modification improve the stability and some modifications improve the efficacy relative to EIDD-1931 against CHIKV. Our current in vitro results and preliminary in vivo results indicate that hydroxycytidine compounds like EIDD-1931 could be used to treat a range of arbovirus infections. Funding: NIAID contract HHSN272201500008C; NIAID K22 AI 104794-01, SLU Presidential Research Funds to AKP and JDB.

Repression of NF-kB signaling by Zika virus NS5

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Zika virus (ZIKV) has emerged as an important pathogen in the past several years, and a strong correlation between microcephaly and other neurological abnormalities in neonates born to mothers infected during pregnancy has been established. An increase in other neurological conditions in adults, such as Guillain-Barre syndrome, has also been observed in infected populations. The innate immune response is a cascade of events initiated upon pathogen infection that is critical for protecting cells from infection, limiting viral replication and pathogenesis, and contributing to the establishment of the adaptive immune response. Our research indicates that ZIKV infection represses the nuclear factor-kappa B (NF-kB) pathway, an important component of innate signaling, required for the optimal initiation of interferon regulated factor (IRF)-dependent transcription. Using NF-kB responsive reporter constructs as well as measurement of transcription of known NF-kB-responsive genes, we observed that transcriptional activation of target genes via NF-kB is inhibited in the presence of ZIKV. Notably, examination of canonical NF-kB signaling events (i.e., phosphorylation of IkB and nuclear translocation of NF-kB) is not impaired, suggesting that the inhibitory effect executed by ZIKV occurs downstream of these events, most likely in the nucleus. Additionally, using fibroblasts expressing inducible ZIKV proteins, we have identified the NF-kB-repressive factor as nonstructural protein 5 (NS5). Current research is focused on characterization of the region/motifs of NS5 effecting NF-kB inhibition and the mechanism of repression. This research demonstrates yet another function of flavivirus NS5 that contributes to replication and pathogenesis through regulation of the host innate response.

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Highlighted Poster

A Small-Molecule Oligosaccharyltransferase Inhibitor with Pan-flaviviral Activity

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Name of the presenter is underlined

The mosquito-borne flaviviruses include important human pathogens such as dengue, Zika, West Nile, and yellow fever viruses, which pose a serious threat for global health. Recent genetic screens identified endoplasmic reticulum (ER)-membrane multiprotein complexes, including the oligosaccharyltransferase (OST) complex, as critical flavivirus host factors. Here, we show that a chemical modulator of the OST complex termed NGI-1 has promising antiviral activity against flavivirus infections. We demonstrate that NGI-1 blocks viral RNA replication and that antiviral activity does not depend on inhibition of the N-glycosylation function of the OST. Viral mutants adapted to replicate in cells deficient of the OST complex showed resistance to NGI-1 treatment, reinforcing the on-target activity of NGI-1. Lastly, we show that NGI-1 also has strong antiviral activity in primary and disease-relevant cell types. This study provides an example for advancing from the identification of genetic determinants of infection to a host-directed antiviral compound with broad activity against flaviviruses.

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Platelet lipids in the pathogenesis of dengue.

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Dengue, an arthropod borne human disease is an acute viral infection with potential fatal complications. Majority of dengue patients normally recover after 2- 5 days of acute illness. In small proportions of patients, the acute febrile stage may be followed by a plasma leakage, thrombocytopenia and hemorrhage, indicating severe form. Presently methods are available to diagnose dengue viral infection, but there is no absolute means to monitor and early predict the severity of disease. The inability to early predict the progression of severity among normal dengue cases leads to large number of hospitalization and this puts hospitals under pressure. Identification of potential biomarkers and developing cost effective prognostic tools that predicts the development of dengue severity would stream-line the admission criteria and helps for effective disease management. It is reported that severe symptoms are largely due to the host immune responses rather than virus-induced cytopathology. Decreased number of platelets and depressed immune system shows that platelets are involved in the disease severity. Cellular lipids play an important role in several aspects of viral replication, but their implication in dengue is not known. Since platelet activation is regulated by various lipids, we studied the changes (if any) in platelet lipids among dengue cases. GC-MS analysis revealed a significant change in the levels of fatty acid esters among dengue cases. The study assessed the Platelet Sphingosine 1 Phosphate levels (S1P) in adult samples (21 dengue and 17 non dengue febrile illness cases) on day 1 and day 5 post admission by HPLC. Analysis showed a significant decrease in the S1P levels in dengue cases throughout the course of infection. We also found an alternation in S1P levels among severe dengue cases compared to non-severe cases. This shows that sphingolipids are involved in the pathogenesis of dengue. To decipher the exact role of sphingolipids, further detailed studies are required.

Highlighted Poster

Dengue Virus NS1 contributes to virus infection by enhancing virus attachment and entry.

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Dengue virus (DENV) is an arthropod borne human pathogen within the *Flaviviridae* family. Of the ten DENV viral proteins, NS1 remains the most elusive in terms of function. Currently NS1 has been linked to vascular permeability and disease progression, and secreted NS1 (sNS1) was recently suggested to have a role in infection of mosquitos. Furthermore previous work has shown that internalized NS1 can function in increasing endocytic activity in hepatocytes and correspondingly DENV infection, by an unknown mechanism. From an in-depth characterization of numerous NS1 variants we have now identified NS1 mutants which release equal numbers of virus particles as WT but are reduced in specific infectivity. These mutants secrete NS1 to near WT levels, yet the mutant NS1 appears to have a lower affinity for virus and cell interactions. Here we will show a role for NS1 in contributing to DENV infection through modulating virus attachment to cells and demonstrate that cell bound sNS1 is sufficient to increase a DENV infection. This is the first report of the flavivirus NS1 protein affecting virus infectivity, through direct modification of virus-cell interactions.

Highlighted Poster

IRF3-Dependent Innate Immune Stimulation by Small Molecules as an Antiviral Strategy Against Emerging Arboviruses

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The ongoing concurrent outbreaks of Zika, Chikungunya, and Dengue viruses in Latin America and the Caribbean highlight the need for development of broad-spectrum antiviral treatments. The type I interferon system has evolved in vertebrates to generate cellular and tissue responses that actively block replication of multiple known and potentially zoonotic viruses. As such its selective stimulation through pharmacologic agents may represent a novel therapeutic tactic for simultaneously impairing growth of multiple virus types and rendering host populations resistant to virus spread. Moreover, using pathway-specific compounds to explore the differential susceptibility of viruses of interest to innate immune processes represents a potentially powerful tool for understanding the molecular bases of virus-host interactions. In light of this we undertook a molecular screen to identify small molecules that stimulate the type I interferon response. This work identified many novel compounds that induce gene synthesis by way of the transcription factor interferon regulatory factor 3 (IRF3). These include interferon beta as well as many interferon-stimulated genes that confer direct antiviral activity. Analysis was then undertaken using a loss-of-function approach in cells from which key innate signaling molecules were deleted by CRISPR/Cas9 technology to identify crucial cellular protein targets. This revealed essentiality of IRF3 adaptor proteins TRIF and STING for innate immune activation by different compounds. Importantly, treatment of human cells with these molecules triggers interferon-associated responses that strongly inhibit replication of emerging flaviviruses such as Zika and Dengue but also emerging alphavirues including Chikungunya, Venezuelan equine encephalitis, Mayaro, O'nyong'nyong and Ross River viruses. This work also revealed important and discrepant biological roles for innate signaling proteins in replication of these viruses on human cells that may lead to identification of new therapeutic host targets. Ultimately synthetic innate immune activators such as these may serve multiple therapeutic purposes including direct antimicrobial responses and indirect facilitation of microbe-directed adaptive immunity.

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Monocytic cells respond to dengue virus activating NLRP10 molecule

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Human cells infected by dengue virus respond to it through numerous cellular sensors such as PRRs (Pattern Recongnition Receptors). Among these receptors, NLRs (NOD-like receptors) have not been studied in dengue viral infections, so far only NLRP3 response to virus in macrophage-like cells have been described in detail. In severe cases of dengue, an important deregulation in numerous cytokines occurs in patients. However, the origin of this high level of cytokines have not been completely elucidated. Clearly, NLRP3 is involved in the immune cellular response to dengue virus in macrophages, but how this inflammasome is regulated remains unknown. An important molecule that seems to regulate NLRP3 is NLRP10.

In this work, we examined the effect of agonists and antagonist of NLRP3 inflammasome over the expression of NLRP10 molecule in DENV-infected THP-1 macrophages-like cells. Following THP-1 cells differentiation, the expression of NLRP10 was assessed in different experimental groups, including one primed by LPS, this group showed a significant expression of NLRP10. The NLRP3 agonist, nigericin impacted negatively the expression of NLRP10. Meanwhile, DENV and NLRP3 antagonist produced a notorious increase in NLRP10. The activation or inhibition of NLRP3 inflammasome was validated indirectly by quantifying IL-1β cytokine. Contrary to what was expected NLRP3 do not negatively regulates NLRP3 inflammasome in DENV-infected macrophages.

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Isolation of dengue virus from a human in Ciudad Juarez, Mexico

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Dengue is considered the most important human arboviral disease worldwide. Annually, hundreds of thousands of human infections caused by dengue virus (DENV) occurs mainly in tropics areas where the vectors, *Aedes aegypti, and Aedes albopictus,* are distributed. An increase of dengue outbreaks in humans has been reported mainly in Mexican communities bordering the southeast of Texas since 1980. However, to date, there is not enough evidence of DENV in other USA-Mexican border communities even though the *Aedes aegypti* mosquito inhabits the entire border region. As part of surveillance for Arthropod-borne Viruses in mosquitoes in El Paso, Texas and Ciudad Juarez, Mexico, a blood sample of a female patient, that experienced undifferentiated fever and arthralgia, was collected in late October 2015 and inoculated into *Aedes albopictus* cells (C636). Dengue virus serotype 1 (DENV-1) was isolated in C636 cells and identified by Indirect immunofluorescence test and sequencing of the generic Flavivirus Reverse transcription polymerase chain reaction (RT-PCR) amplicon. Nucleotide sequencing of the premembrane and envelope genes grouped the DENV-1 human isolate in the Central America clade. This result suggests local transmission of DENV-1 in Ciudad Juarez, Mexico as the patient did not experience any travel history. Further epidemiological studies could assess the transmission of DENV in this USA-Mexican border community.

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SHORT-TERMED EXPOSURE TO DENGUE PROVIDES IMMUNITY TO ZIKA INFECTION

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Dengue virus (DENV) is perhaps the most medically important arbovirus; it's endemic in at least 100 countries throughout the world, including Puerto Rico, imposing a large public health burden. Dengue virus can be subgrouped into four serotypes; infection with any of the serotypes results in lifelong neutralizing immunity to the infecting serotype, but only a short-lived immunity to the others. The duration of protection between primary and secondary DENV infections remains debatable, with studies claiming heterotypic immunity can be protective for up to 6 months, and others reporting it varies from weeks to a year. Another arbovirus, Zika virus (ZIKV), is responsible for the current epidemic affecting Brazil, Central America and the Caribbean. This member of the *Flaviviridae* family is transmitted primarily through the bite of *Aedes spp*. mosquitoes, providing the perfect vehicle to affect previously endemic Dengue virus (DENV) areas with ZIKV. ZIKV and DENV share a homology in amino acid sequence of at least 50%, and it has been demonstrated that ZIKV undergoes ADE *in vitro* in response to previously generated antibodies from other flaviviruses. In our previous work, titled *Zika virus pathogenesis in rhesus macaques is unaffected by pre-existing immunity to Dengue virus*, we attempted to determine if DENV long-term protection against heterotypic DENV serotypes extends to ZIKV as well. For this study, we wished to consider a shorter interval of time based on the previous results. Three cohorts of rhesus macaques were selected; cohorts 1 (n=6) and 2 (n=4) had been exposed to DENV-2 one year and three months earlier respectively. A third flavivirus-naive cohort (cohort 3, n=6) was included as a control group. Implications of previous exposure to DENV at different periods of time in the ZIKV pathogenesis are discussed. Using PCR to measure viremia in serum, and neutralization assays to determine DENV nAbs neutralizing ability against ZIKV, our results show differences in the ZIKV infection outcome among all three cohorts. Cytokine and immune cells phenotyping will also be discussed.

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MEFLOQUINE AND CHLOROQUINE ANALOGS INHIBIT ZIKA VIRUS REPLICATION

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Zika virus (ZIKV), an emerging *Flavivirus*, was recently associated with severe neurological complications and congenital diseases. Therefore, development of antiviral drugs capable of inhibiting ZIKV replication is urgent. Chloroquine and mefloquine – antimalarial agents – have confirmed anti-ZIKV activity and, importantly, are considered safety molecules for use with pregnant women. However, chloroquine's anti-ZIKV potency is around 10 μM, suggesting that modifications to its structure could be promising for obtaining more effective anti-ZIKV therapies. So, the aim of this study was to synthesize and evaluate the ability of new chloroquine or mefloquine derivatives to inhibit ZIKV replication *in vitro*. For this, we firstly synthesized twenty chloroquine analogs by thermal and ultrasonic means. The ultrasonic procedures are simple, safe and with short reactions times – 30-180 s compared to 30-180 min reactions times for the thermal method. Among these derivatives, the chloroquine analog N-(2-((5-nitrofuran-2-yl)methylimino)ethyl)-7-chloroquinolin-4-amine (**40**) was the most potent, reducing ZIKV replication by 72% at 10 μM. Compound **40** exhibits an EC_{50} value of 0.8 ± 0.07 μM, compared to 12 ± 3.2 μM for chloroquine. Good activities were also obtained for other compounds, including those with aryl groups = phenyl, 4-fluorophenyl, 4-nitrophenyl, 2,6-dimethoxyphenyl, 3-pyridinyl and 5-nitrothien-2-yl. Importantly, the number, positions and types of substituents attached to the aromatic ring are critical factors for the biological activity. Moreover, we also obtained a series of mefloquine derivatives, by classical chemical reactions, and demonstrated that analogs N1-(2,8-Bis(trifluoromethyl)quinolin-4-yl) ethane-1,2-diamine (**3a**) and 2-((2,8-Bis(trifluoromethyl)quinolin-4-yl)amino)ethanol (**4**) were the most potent within this series, both with mean EC_{50} values of 0.8 μM. This represents a potency 5 times higher when compared to mefloquine ($EC_{50:}$ 3.6 ± 0.3). Additionally, the potency of compounds did not affect the cytotoxicity when compared to the reference compounds. In conclusion, the results indicate that these group of compounds are a good follow-up point for the potential discovery of new drugs against Zika disease.

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Viral Retinopathology in Zika Virus-Infected A129 Mice

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Zika virus (ZIKV) causes a variety of disease manifestations in patients. Although the most common disease, Zika fever, is a self-limiting mild febrile illness, less common but more severe outcomes have been described. One of these complications is characterized by damage to the eyes, particularly in infants, resulting in damage to which the full extent has not been fully understood. To begin to describe the pathogenesis associated with this infection, adult A129 mice deficient in the type-I interferon signaling pathway were used. Acute infection with the FSS13025 strain of ZIKV resulted in high titers in the eye and evidence of infection by immunohistochemistry in the activated Muller and retinal pigmentation epithelial (RPE) cells in the retina. Interestingly, the less pathogenic Mex1-7 strain caused a long-term infection in the Muller cells without cell death. Additionally, retinal infection was observed in 5-day-old C57Bl/6 mice but not adult mice, suggesting that the blood-retinal barrier is important in mice with intact innate immune systems. To confirm these findings, cell culture of established cell lines or *ex vivo* cultures of Muller and RPE cells were infected with ZIKV. RPE cells are capable of amplifying FSS13025 and Mex1-7 strains of ZIKV to high titers (>10^6 pfu/ml) with significant cytopathic effect. Muller cells, on the other hand, are capable of being infected without cytopathic effect but also without high titers in cell culture supernatant (<10^3 pfu/ml). Although mouse models are still being optimized for their use in pathogenesis studies, early work suggests that the eye is a target of ZIKV infection and these models may prove useful for screening of drugs and vaccines to prevent or treat ocular Zika disease.

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Zika immunity modulates Dengue-elicited immune response kinetics in rhesus macaques

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Zika virus (ZIKV) recent outbreak in Dengue (DENV) endemic regions has raised concerns about flavivirus cross-immunity, infection enhancement, and the implications of these in development of severe clinical manifestations. During the ongoing ZIKV outbreak, part of the population naïve to DENV such as newborns, DENV-naïve children and adults, and travelers from non-flavivirus endemic areas could be exposed to a ZIKV infection prior to DENV. After the outbreak, herd immunity will reduce ZIKV transmission and DENV will re-emerge and potentially infect the ZIKV-immune population. At the moment, there is little evidence that shows that ZIKV-immune serum can enhance DENV infection by antibody-dependent enhancement (ADE) *in vitro* and *in vivo*. Despite the available evidence, the dissected role of short and long-term ZIKV pre-existing immunity in a subsequent infection with DENV remains unclear. Our study aim to clarify these aspects by infecting 14 rhesus macaques with DENV-2. Cohort 1 (N=4) and 2 (N=6) were exposed to ZIKV 10 and 2 months, respectively, before DENV-2 infection, and cohort 3 (N=4) was naïve to ZIKV. Cohorts were clinically monitored and bled sequentially for serum and PBMCs samples collection up to 3 months after DENV infection. Preliminary results from 10 months ZIKV-immune and naïve cohorts show no significant differences in the magnitude of DENV viremia between cohorts, but a reduction of total viremia days was observed for the ZIKV-immune animals. The serological profile shows that ZIKV-immune animals produced a boost of cross-reactive antibodies after DENV infection, also resulting in higher titers of neutralizing antibodies against DENV and ZIKV than the naïve animals. Of interest, the levels of liver enzymes (AST and ALT) in ZIKV-immune animals, compared to their baseline levels, were higher than the naïve ones. This may be related to previous ZIKV-induced liver damage 10 months before DENV infection. These results provide insights that despite ZIKV-immunity enhance DENV *in vitro*, DENV pathogenesis can be unaffected by long-term ZIKV-immunity *in vivo* and the elicited immune response could be positively modulated for protection. Our findings contribute to the understanding of upcoming DENV dynamics in endemic areas where recently ZIKV was introduced.
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BRAIN IMMUNOPATHOLOGY OF DENV-2 INFECTED MICE AND FATAL CASES

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Dengue virus is not considered a non-neurotropic virus anymore, as in the last epidemics neurological manifestations are becoming more common, such as encephalopathy, encephalitis, immune-mediated syndromes (acute transverse myelitis, disseminated acute encephalomyelitis and Guillain-Barré syndrome), muscle dysfunction and neuro-ophthalmic disorders. To advance in this field, some animal models are being developed, even with the lack of an immunocompetent animal model for dengue mimicking the disease in humans. The objective of this work was to compare the findings of mouse model and fatal cases of dengue, with regard to histopathology, viral antigens and cytokines detections. BALB/c mice were infected with DENV-2 non-neuroadapted by intravenous route. Histology, ultrastructure and immunostain assay was performed in murine model and fatal cases to characterized histopathology aspects, virus antigens and cytokines detection. The infected animals did not exhibit symptoms, but the histopathological analysis of the brain and cerebellum revealed mainly inflammatory infiltrate, gliosis, haemorrhage and edema areas. Neuronal cells (microglia and astrocytes) were altered in morphology and quantity, suggesting their activation due to infection. *"In vitro"* experiment was performed to evaluate which neuronal cells were permissive to infection and to viral replication. Mixed cell neuronal culture was infected with DENV-2, and virus replication was assessed by NS3 protein detection, a non-structural protein, that were present in microglia, glial cells and neurons. "*In vivo*", virus replication occurred in endothelial

cells, microglia and neurons. Ultrastructural evaluation showed neurons, microglia and astrocytes with changes leading to apoptosis. In addition, $CD8^+$ cells and cytokines, such as TNF-α, IFN-γ and RANTES, which may be involved in neuropathogenesis of dengue, were also detected. Besides the animal model, fatal cases of infection of DENV-2 and DENV-3 were also analyzed, and revealed similar results. Histopathological analysis showed tissue damage; and neuronal cells were altered, different from the control. $CD8^+$ cells, viral antigens and cytokines were also detected. These findings may help to better understand the main tissues and cells of the central nervous system involved in the pathogenesis of dengue in correlations with studies of tissue samples from fatal cases, thus contributing significantly to the knowledge of the disease, and to new vaccine or therapeutic approaches against the disease.

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Zika Infection in Pregnant Rhesus Causes Placental Dysfunction and Immunopathology

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Introduction: Zika virus (ZIKV) infection during pregnancy leads to an increased risk of fetal growth restriction and fetal central nervous system malformations; outcomes broadly referred to as the Congenital Zika Syndrome (CZS).
Study Objective: To develop a Zika virus infection and disease model in pregnant rhesus macaques and determine the effect of ZIKV infection on placental and fetal outcomes.
Materials and Methods: We subcutaneously infected pregnant rhesus macaques with $ZIKV_{PRABC59}$ ($1x10^5$ ffu) at three different time points across gestation. We investigated the impact of persistent ZIKV infection on uteroplacental blood flow, pathology, and fetal development at 135 days gestational age.
Results: Despite seemingly normal fetal growth and persistent fetal-placenta-maternal infection, advanced non-invasive *in vivo* imaging studies reveal dramatic effects on placental oxygen reserve accompanied by significantly decreased oxygen permeability of the placental villi. The observation of abnormal oxygen transport within the placenta appears to be a consequence of uterine vasculitis and placental villous damage in ZIKV cases. In addition, we demonstrate a robust maternal-placental-fetal inflammatory response following ZIKV infection. At necropsy, we detected ZIKV RNA in placenta and a number of fetal tissues, and in the urine from all of the fetuses. Three out of four male fetuses displayed histological evidence of urogenital abnormalities and degeneration. In addition, we demonstrate a robust maternal-placental-fetal inflammatory response following ZIKV infection.
Conclusion: This clinically relevant translational model of ZIKV infection during pregnancy may facilitate future mechanistic studies and aid our understanding of the detrimental consequences of maternal ZIKV on neonatal outcomes.

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Highlighted Poster

FMRP represses ZIKV infection through blocking viral translation

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Zika is an important health problem due to materno-fetal transmission and association with congenital microcephaly. To date, several viral and cellular factors has been associated with ZIKV infection, however there is no data regarding the identity of host cell proteins that interact with ZIKV subgenomic flaviviral RNA (sfRNA). This stable RNA fragment, comprising most the 3′ UTR of the viral genome, accumulates during flavivirus infection due to incomplete degradation of viral RNA by cellular ribonucleases. Using RNA affinity chromatography and mass spectrometry, we identified FMRP (Fragile X Mental Retardation Protein) as a ZIKV sfRNA-binding protein. FMRP is a translational repressor and reduced levels of FMRP cause Fragile X syndrome. We validated interaction between FMRP and both the sfRNA and viral genome by RNA-immunoprecipitation and northern blot. Knockdown (KD) of FMRP resulted in elevated levels of viral yield and ZIKV infected cells but did not affect dengue virus, a related flavivirus, indicating that FMRP acts as a specific restriction factor for ZIKV. We evaluated translation of ZIKV RNA using an infectious reporter virus expressing NanoLuc luciferase. An increase of luciferase signal without a change in viral genome levels was observed in FMRP KD cells compared to control cells, suggesting that ZIKV translation is inhibited by FMRP. Together, these results suggest that FMRP represses ZIKV infection through blocking viral translation. We further tested the hypothesis that sfRNA, which accumulates to high levels during the course of infection, is capable of reversing ZIKV repression mediated by FMRP. For this purpose, we analyzed a ZIKV strain (ZIKV-10-del) that is compromised for sfRNA production. In control cells, we observed that ZIKV-10-del is deficient in replication compared to WT ZIKV. Importantly, in FMRP KD cells ZIKV-10-del infection was increased to similar levels as WT ZIKV, suggesting that sfRNA enhances ZIKV infection, at least in part, by antagonizing FMRP. In summary, our observations suggest that FMRP acts as a ZIKV restriction factor early in infection but this activity is restrained later in infection, likely through FMRP sequestration by the sfRNA.

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An evolutionary mutation enhances Zika virus evasion of host interferon induction

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Introduction: Virus-host interactions determine an infection outcome. The Asian lineage of Zika virus (ZIKV), responsible for the recent epidemics, has fixed a mutation in the NS1 gene after 2012 that enhances mosquito infection. **Materials and methods:** ZIKV pre-epidemic strain, Asian strain was used to screen the interferon-β production inhibition effects of ZIKV nonstructural proteins. Compared with the epidemic strains from Asian lineage, the epidemic strains isolated after 2012 have undergone an A188V mutation in the NS1 gene that enabled the protein to suppress interferon-β induction. This mutational effect was consistently observed in cell culture, ex vivo BMDCs, and A129 mouse model. **Results:** The NS1 A188V mutation confers NS1 to inhibit interferon-β induction. This mutation enables NS1 binding to TBK1 and reduces TBK1 phosphorylation. Engineering the mutation into a pre-epidemic ZIKV strain debilitated the virus for interferon-β induction; reversing the mutation in an epidemic ZIKV strain invigorated the virus for interferon-β induction; these mutational effects were lost in IRF3-knockout cells. Additionally, ZIKV NS2A, NS2B, NS4A, NS4B, and NS5 could also suppress interferon-β production through targeting distinct components of the RIG-I pathway; however, for these proteins, no antagonistic difference was observed among various ZIKV strains. Our results support the mechanism that ZIKV has accumulated mutation(s) that increases the ability to evade immune response and potentiates infection and epidemics.

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MAYARO VIRUS REQUIRES THE UBIQUITIN-PROTEASOME SYSTEM FOR EFFICIENT REPLICATION

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Mayaro virus (MAYV) is an emerging arbovirus that belong to *Alphavirus* genus. MAYV causes frequent outbreaks in human populations in South America and there is no treatment or vaccine for the control of this infection. As intracellular obligate parasites, the viruses have developed different strategies to replicate in host cells. One of them, consist in the manipulation of post-translational modifications, such as ubiquitination. The ubiquitin-proteasome system (UPS) regulates protein degradation, receptor trafficking, DNA repair, cell cycle progression, gene transcription, innate immunity, autophagy and viral infection. However, the role of UPS in the context of MAYV replication remains unknown. The aim of this study was to evaluate the role of UPS in the MAYV replication.

Western blot analysis was done to assess the blocking of proteasome activity in Vero-E6 or Hela cells treated with the proteasome inhibitors MG132 (10 μM) and Lactacystin (25 μM) using anti-ubiquitin lysine-48 antibody. Cell viability assays of Vero-E6 and Hela cells treated with MG132 and Lactacystin at different concentration during 24 and 48 hours was performed using the MTT method. Viral titer of supernatants from Vero-E6 or Hela cells infected with MAYV and treated with proteasome inhibitors or not during 24 hours was evaluated by plaque assay technique.

Our data show that both MG132 and Lactacystin promote an effective blockage of proteasome activity as expected, as we detected a significant accumulation of ubiquitinated proteins in treated cells with these compounds. Cell viability assays indicate that the proteasome inhibitors do not have apparent cytotoxicity effect at 24 hours after treatment. However, we observed a substantial reduction of cell viability at 48 hours after treatment with MG-132. Finally, we found that the treatment of infected cells with both MG132 and Lactacystin during 24 hours, reduces significantly the MAYV titer in comparison with control samples and this effect was in a dosage-dependent fashion.

Our results suggest that functional ubiquitin-proteasome system is necessary for an efficient MAYV replication and this pathway could be a pharmacological target in this viral infection. Future studies will determine in which part of the viral cycle this pathway is implicated.

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Cytokine/chemokine profile in monoinfected DENV, ZIKV, CHIKV and coinfected patients

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Introduction: Cytokines and chemokines induce inflammation in an attempt to promote viral clearance. However, in dengue, an exacerbated production of these inflammatory mediators may lead to pathogenesis through phenomena called a "cytokine storm". **Objective:** Characterizing the cytokine/chemokine profile in monoinfected DENV, ZIKV or CHIKV patients and in those DENV/ZIKV or ZIKV/CHIKV coinfected. **Material and Methods:** Molecular and serological diagnosis for DENV and CHIKV and molecular diagnosis for ZIKV were performed to confirm acute infection. We evaluated DENV (n=38), ZIKV (n=26), CHIKV (n=34), DENV/ZIKV (n=19), and ZIKV/CHIKV (n=38) patients during the outbreak of 2016 in Brazil. We had x healthy donors as controls. We quantified IP-10, RANTES, MCP-1, MIF, IL6, IL10, IFNγ, TNF and, IL15 by ELISA. Comparisons between groups were made using Mann-Whitney test and Principal Component Analysis (PCA). $P<0.05$ were considered significant. **Results:** Regardless of the virus infection, all patients had high levels of IP-10 and MCP 1 compared to healthy donors. Low levels of RANTES were seen in DENV/ZIKV patients compared to DENV patients or controls. While in DENV or ZIKV patients IL10 levels increased, in DENV/ZIKV patients IL10 decreased in relation to donors. Regarding pro-inflammatory cytokines, TNF was increased in all infected patients, while IL6 was increased in ZIKV, CHIKV and DENV/ZIKV patients compared to donors. High levels of IFNγ were detected only in DENV patients.

Interestingly, IL15 levels were decreased in ZIKV/CHIKV patients compared to DENV/ZIKV and DENV patients. The chemokines/cytokines levels were analyzed by PCA, which generated three groups of patients: (1). High producer of IP10, MCP1, IFNγ, IL10 that included CHIKV/ZIKV and DENV patients; (2). High producer of IL6 and IL15 that included ZIKV and DENV/ZIKV and (3). CHIKV a pattern of cytokines with high IL6 but low IL15. **Discussion/Conclusion:** We confirmed an intense inflammatory environment in all acutely infected patients regardless of the virus. However, there was no clear association between the cytokine/chemokine profiles with a specific group of patients. We emphasize the decrease in IL-15 levels in ZIKV/CHIKV, which could represent a loss in the activation of NK cells in this specific group, requiring further investigation.

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Antiviral activity of anisomycin against dengue and Zika viruses

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We investigated the antiviral activity of anisomycin, an alkaloid produced by *Streptomyces griseolus*, against dengue (DENV) and Zika (ZIKV) viruses. We first examined the effect of anisomycin on cell viability in Vero, A549, HepG2 and U937 cell lines using an MTS assay. The effect of non-cytotoxic concentrations of anisomycin on viral multiplication was analyzed using a virus yield inhibition assay. Anisomycin caused a dose dependent inhibition of viral production in Vero cells, with 50% effective concentration values of 23.2, 31.3, 24.8, 61.6 and 33.0 nM for DENV-1, DENV-2, DENV-3, DENV-4 and ZIKV, respectively. A 99.99% inhibition of DENV-2 yield was observed at the highest concentration of anisomycin (200 nM) even at a multiplicity of infection of 50 PFU/cell. The compound exhibited antiviral activity against clinical isolates of DENV and against DENV-2 grown in several different human cell lines. Both the kinetics of DENV-2 entry and uncoating were not affected by the compound. However, a strong inhibition of DENV-2 protein expression and viral RNA synthesis was demonstrated. The toxicity and antiviral activity of anisomycin was evaluated in a mouse model of ZIKV morbidity and mortality. Doses up to 100 mg/kg/d were well-tolerated. Mice were treated with anisomycin (4, 20, or 100 mg/kg/d) for 10 days beginning 4 h after ZIKV infection. Viremia on day 5, weight change between 0 and 21 day post-infection (dpi) and survival through 28 dpi were used as primary endpoints. The results obtained suggest a reverse dose response, since a more rapid mortality rate was observed associated with treatment with 100 mg/kg/d in comparison with untreated, infected mice, while animals treated with 4 mg/kg/d of anisomycin died significantly ($p<0.05$) later than the control group. In addition survival data correlated with viremia data since significant reduction in viral RNA was only observed after treatment with the lower dose of anisomycin.
In conclusion, anisomycin is a potent and selective *in vitro* inhibitor of DENV and ZIKV that impairs a post-entry step of the viral replicative cycle, and treatment with a low dose of anisomycin (4 mg/kg/d) appeared to provide some minimal benefit in a mouse model.

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Targeting the host: N-8′-(2′′-tetrahydrofuranyl)-octyl-deoxynojirimycin (2THO-DNJ) antiviral efficacy in dengue infection

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Introduction: Certain iminosugars with glucose stereochemistry have antiviral activity against particular enveloped viruses possessing *N*-linked glycoproteins. This antiviral activity is proposed to occur through competitive inhibition of endoplasmic reticulum α-glucosidases, leading to a reduction in functional viral glycoprotein folding and infectious virion production. Dengue virus (DENV) is susceptible to antiviral effects mediated by particular iminosugars.
Study objective: To characterise the antiviral activity of the iminosugar *N*-8'-2''-tetrahydrofuranyl-octyl-deoxynojirimycin (2THO-DNJ, UV-12) against DENV.
Methods: Primary human monocyte-derived macrophages from healthy donors were infected with DENV-2 (strain 16681), treated with 2THO-DNJ, and antiviral assays performed.
Results: 2THO-DNJ has potent antiviral activity against DENV, reducing the secretion of infectious virions. Free oligosaccharide analysis and isothermal titration calorimetry support α-glucosidase inhibition as the mechanism of action of 2THO-DNJ.
Discussion/conclusion: The antiviral effect of 2THO-DNJ is primarily mediated by a reduction in virion secretion rather than virion infectivity. In the future, the galactostereochemistry compound, 2THO-deoxygalactonojirimycin, which is not expected to inhibit α-glucosidases, will be utilised in antiviral efficacy studies to discriminate between sugar head and tail group effects.

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Antiviral effects of iminosugars on dengue-infected monocyte-derived dendritic cells

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Introduction
Dengue virus (DENV) infections range from asymptomatic illnesses to severe haemorrhagic disease and death. Iminosugars are monosaccharide mimics that competitively inhibit α-glucosidases in the host endoplasmic reticulum. *In vitro* studies have demonstrated antiviral effects of iminosugars against dengue in monocyte-derived macrophages. Dendritic cells (DC) are important targets for dengue infection and they play a significant role in the immune response against early dengue infection. Given the important role played by DC in innate immune defence against DENV, it is useful to investigate the antiviral effects of iminosugars in a DENV-infected DC model.

Objective
To evaluate the antiviral effects of the three deoxynojirimycin (DNJ) derivatives and a deoxygalactonojirimycin (DGJ) control in DENV-infected primary human monocyte-derived DC.

Materials and methods
N-(n-Nonyl)-deoxygalactonojirimycin (*N*N-DNJ), 8-tetrahydrofuranyl-octyl-DNJ (2ThO-DNJ/UV-12) and *N*- (8'-ethoxyoctyl)- deoxynojirimycin (E*O*O-DNJ) were used as DNJ derivatives. *N*-(n-Nonyl)-deoxygalactonojirimycin (*N*N-DGJ), which has galactose stereochemistry was used as the control. Immunofluorescence was used to detect % infection of DENV. Secretion of infectious virus was evaluated with plaque assays while total secreted virus was quantified with qRT-PCR.

Discussion and conclusion:
We demonstrated that iminosugar derivatives of DNJ but not DGJ elicit antiviral activity in DENV-infected monocyte- derived DC. Compounds *N*N-DNJ, E*O*O-DNJ and 2ThO-DNJ reduced the % of infected cells and inhibited the secretion of DENV in a dose dependent manner. In conclusion, we have demonstrated for the first time that the iminosugars *N*N-DNJ, E*O*O-DNJ and 2ThO-DNJ have antiviral effects on DENV-infected DC similar to those seen in DENV-infected macrophages.

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An NS5 mutation interacts epistatically with the 3'untranslated region of dengue virus for increased epidemiological fitness

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The 1994 dengue epidemic in Puerto Rico coincided with the replacement of a clade of dengue virus serotype 2 (DENV2) with another. We have previously found that the endemic PR1 clade of virus differed from the epidemic PR2B clade of DENV2 by several substitutions, three of which were in the 3' untranslated region (UTR) of the genome. These mutations resulted in increased formation of subgenomic flavivirus RNA (sfRNA) that bound TRIM25 to inhibit its E3 ligase activity necessary for amplified and sustained RIG-I signaling. While the mutations in the 3'UTR explains sfRNA formation, it does not explain the observed reduction of PR2B genomic RNA (gRNA) levels in infected cells, suggesting that mutations elsewhere in the genome compromises the 5'cap formation for increased exoribonuclease digestion of gRNA. Using ancestral state reconstruction and Bayesian Evolutionary Analysis by Sampling Trees (BEAST) analysis, we found that mutations in the NS5 (S269N, K375R, R514K, R596K and R891K) occurred after the 3'UTR substitutions and immediately preceded the 1994 outbreak. We constructed infectious clones that represent each of the 3 phylogenetic nodes of PR2B. Using site directed mutagenesis, we identified the S269N NS5 mutation at the linker region reduced genomic capping for increased exoribonuclease digestion of gRNA that accentuates sfRNA to gRNA ratios. Our findings suggest that epistatic interaction between the 3'UTR and NS5 linker is a contributory factor of PR2B's epidemiological fitness.

Dissecting Virus-Host Interactions Using System Approaches

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RNA viruses are the etiological agents of many recent outbreaks and epidemics of viral origin, and include endemic viruses such as dengue virus (DENV) and re-emerging ones such as Zika virus (ZIKV). Due to their high inherent mutation rate during replication, RNA viruses will remain persistent threats to human health. There are no effective antiviral therapeutics or vaccines against many of these viruses. While antiviral drug development has traditionally been directed at virus targets, host factors and pathways have now emerged as effective targets for antivirals, such as Maraviroc for HIV. Current methods to identify antivirals rely heavily on inefficient large-scale screens. We aim to take a more directed approach by first identifying host factors that interact with virus proteins during virus infection or and pathways that are perturbed by the virus proteins. We have applied this guided two-pronged systems approach to two closely related viruses: DENV and ZIKV. Through these proteomic and transcriptomic analyses, we have identified host proteins and pathways that are engaged by DENV and ZIKV proteins during infection. By comparing the results from both viruses, we have also uncovered common and unique host proteins and pathways between the two viruses. These proteins and pathways, and their biologic functions will be presented.

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Characterization of a murine model of non-lethal, symptomatic dengue disease

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The mosquito-borne disease dengue (DEN) is caused by four serologically- and genetically-related viruses, termed DENV-1 to DENV-4. Dengue is a self-limited febrile illness but can also lead to severe dengue which is life-threatening. Following infection, individuals usually develop homotypic immunity to the infecting serotype, but cross-protection is short-lived, and infection by the other three DENVs is a risk. Therefore, it is important to study the four DENVs. Historical setbacks due to the lack of human-like mouse models of dengue were partially remedied with characterization of lethal DENV-2 infection models in immunocompromised AG129 mice (deficient in IFN-α/β and IFN-γ receptors). Recently, our group has contributed additional AG129 mouse infection models using human isolates DENV-1 WP/74, DENV-3 C0360/94, and DENV-4 703-4. Thus, lethal dengue models exist for the four DENVs. Here we compare a non-lethal, disseminated model of DENV-3 using strain D83-144 to that of the lethal outcome following infection by strain C0360/94. Intraperitoneal inoculation of adult AG129 mice with strain D83-144 led to signs of dengue infection, such as increased pro-inflammatory cytokines, thrombocytopenia, hypoalbuminemia, lymphopenia, and systemic infection. Approximately 4-7 days after infection the mice recovered, and neutralizing antibodies were present one month after infection. However, infection by strain C0360/94 led to more severe features of dengue, including coagulopathy and lethal outcome. Molecular analysis revealed that the two Genotype II DENV-3 strains are closely related, with only 13 amino acid differences, including surface amino acid changes in the E protein, which may affect the virulence of DENV-3 strains in AG129 mice. Overall, this study characterizes infection by the low passage, non-mouse lethal strain D83-144 in AG129 mice and demonstrates that systemic D83-144 infection induces many features of human dengue. However, the lethal strain C0360/94 causes more severe dengue than D83-144. The results suggest that the AG129 mouse model has applications to investigate the role of factors associated with mild or severe disease.

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Tudor domain containing protein 3 (TDRD3) contributes to the assembly of infectious Dengue virus particles

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Abstract:
Viruses have evolved many strategies to hijack and use cellular factors for entry, RNA synthesis, and propagation. Understanding the roles/functions of these subverted host factors in virus propagation will help in development of novel antiviral strategies. Previous genome wide siRNA screens performed by our laboratory identified the 'Tudor domain containing protein 3' (TDRD3) as a potential host factor required for DENV and YFV infection. TDRD3 is one of the major effector molecules that recognizes and binds methylated arginines of target substrates and facilitates gene transcription. Knockdown of TDRD3 in two different cell lines (Huh7 & MCF7) resulted in a significant reduction of viral titers after DENV infection. Additionally, we showed that TDRD3 is required for efficient propagation of YFV and ZIKV, indicating that different members of flavivirus require TDRD3. Using recombinant DENV-2 virus engineered to express *Renilla* luciferase (RLUC) and ZIKV replicon system, we demonstrated that depletion of TDRD3 has no effect on translation or RNA synthesis; however virions secreted from TDRD3-depleted cells contained less viral RNA and were significantly less infectious. Together, these results suggest that TDRD3 plays an important role in flaviviral assembly.

Primary and secondary dengue infection leads to heart tissue compromise

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Dengue, considered the most important arthropod-borne viral disease, is transmitted by the bite of mosquitoes of the Aedes genre and caused by one of the 4 distinct serotypes of dengue virus. Over 500,000 people are hospitalized annually and around 2,5 million, living in endemic areas, are at risk of infection, which makes the development of a vaccine of the utmost importance. Therefore, studies aimed at the establishment of animal models are of great relevance for better understanding the disease as well as testing of vaccines and anti-viral drugs. This study purpose was investigating ultrastructural alterations caused by DENV primary or secondary infection in BALB/c mice heart. A group of BALB/c mice was infected with DENV-2 for primary infection studies. For secondary infection studies, another group was infected with DENV-1 and, 4 months later, reinfected with DENV-2. Uninfected mice were used as negative controls. The mice were anesthetized, euthanized and fixed by perfusion, with 4% paraformaldeyde in sodium phosphate buffer for 30 min, 72h.p.i. Samples were post-fixed by immersion in 2% glutaraldehyde in sodium cacodylate buffer and 1% buffered osmium tetroxide, dehydrated in acetone, embedded in epoxy resin, and polymerized at 60ºC during 3 days. Ultrathin sections were picked up onto copper grids and stained with uranyl acetate and lead citrate and observed at a Zeiss EM-900 transmission electron microscope. Our analyses showed involvement of heart in DENV infection. Primary and secondary infection studies of mice heart showed edema, endothelium activation characterized by presence of transport vesicles, free platelets and inflammatory cells in interstitium, mitochondria presenting rarefied cytoplasm, and disorganization of muscle fibers. These results point not only to BALB/c mice susceptibility to DENV infection, but also to the fact that, although it is not an often reported occurrence, dengue can lead to heart compromise.

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Category: Virology-Pathogenesis-Antivirals

PREVIOUS DENGUE INFECTION MODULE ZIKA IMMUNOLOGICAL PATTERNS AND CLINICAL OUTCOME

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Introduction: Zika virus is an emerging mosquito-borne flavivirus causing large outbreaks in populations previously exposed to dengue virus. It has been demonstrated that previous dengue immunity module the cellular immune response and the clinical development during secondary heterotypic infection, and the immune humoral and cellular cross-reactivity due to similarity between both viruses. However, the possible modulation of cellular immune response and in consequence in Zika clinical evolution and impacts in fetal damage remains to be investigated.

Objective: To explored the influence of previous dengue infection in the expression of cellular immunological mediators and clinical evolution and outcome of Zika disease and fetal damage.

Materials and methods: Gene expression of 15 immunological mediators including innate immune response, regulatory, pro-inflammatory, cytotoxic and immune pathological mediators were kinetically quantified in fluids (serum and urine) and PBMC of Cuban Zika patients with and without previous dengue infection as well in different Zika infected fetal tissues from mothers with and without previous dengue infection.

Results: The immunological pattern showed significant differences according to previous dengue immunity, and significant association with different Zika infection sign and symptoms and different clinical outcome. A mother dengue immunity seems to be protected from Zika fetal infection.

Discussion/conclusion: We demonstrated that previous dengue infection influence the course of Zika disease, and more important, that maternal dengue immunity seems to have a fetal protector effect.

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Phytochemicals against Zika virus, what have we done?

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Introduction: The emergence of Zika virus in the Americas has challenged the ability to quickly respond to emerging infectious diseases and highlighted the need to quickly develop tools for prevention, diagnostics, and treatment for these diseases. There are not antiviral drugs approved against flaviviruses, and usually the treatment arsenal for viral infections is limited. However, phytochemical research is a promissory field in the search of new antivirals and natural products are a valuable source of therapeutic molecules. **Objective:** To summarize the evidence about available phytochemicals with potential Anti-Zika activity. **Materials and Methods:** A systematic review was conducted in four databases (PubMed, Web of Science, EMBASE and Scopus) using ("Natural Product" OR "Plant Extract" OR "Phytochemical") AND (Zika) as search strategy. Studies that assessed the in-vitro or in-vivo antiviral activity against Zika were screened by title. In-silico studies, works assessing molecules for vector control, duplicates, reviews and opinion articles were excluded after abstract and full text assessment. **Results:** A total of 240 articles were screened by title (PubMed=142; Scopus=46; Web of Science=31 EMBASE n=21). Thirty-one were selected and ten duplicates were eliminated. Next, fifteen articles were excluded (thirteen because assessed vector control activity, one because did not assess phytochemicals or natural products, and one review article). Finally, six articles were included in which eleven promissory molecules were evaluated (nine polyphenols, one lipopeptide and one stilbene) derived from *Camellia sinensis, Colispora cavincola* and other not specified edible plants. Three proposed mechanisms of action were identified for eight molecules (Entry inhibition for Epigallocatechin gallate, inhibition of viral polyprotein processing for Cavinafungin, and Inhibition of Zika NS2B-NS3 protein by Myricetin, Quercetin, Luteolin, Isorhamnetin, Apigenin and Curcumin). None of the studies were conducted using and in-vivo model. **Conclusion**: Although some promissory phytochemicals with anti-Zika activity have been identified there is a need for promote research in this field in order to find novel promissory molecules and elucidate their mechanisms of action. Additionally, it is important to conduct research for validating their activity using in-vivo models. The knowledge gap for the identification of antivirals is still a field that requires to be covered.

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Development of African lineage Zika virus infectious cDNA clone for pathogenesis studies

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Introduction: Zika virus (ZIKV) Asian lineage is responsible for recent epidemics in the Americas and for severe disease. However, ZIKV African lineage hasn't been involved in many epidemic activity and is not usually associated with severe disease manifestations. It is possible that these differences in epidemic potential and pathogenesis may be determined by genetic variance. Thus, this study aims to develop an African ZIKV infectious clone to allow for future studies identify those markers. **Materials and Methods:** ZIKV African strain DAKAR41525 was used to construct the cDNA infectious clone, following a standard cloning procedure as previously described by Shan et al (2016). Vero, BHK, U4.4 and C6/36 cells were infected with parental and recombinant virus in a growth kinetics assay (M.O.I. 0,01). In addition, A129 mice were infected with intraperitoneally and newborn CD1 mice were infected intracranially for virulence studies. **Results:** Vero cells transfected with RNA transcript expressed viral E protein (24- 48h.p.t). Culture fluids of the transfected cells contained infectious virus with a titer of 6.25 Logs PFU/ml at 3d.p.t. The recombinant virus replicated robustly on Vero and U4.4 cells (peak titers at 3d.p.i.). A129 mice infected with the recombinant virus showed peak viremia of $1.2x10^7$ PFU/ml on 3d.p.i. On 6d.p.i., all mice had high viral loads in every tested organ, with the highest titers in the lung, spleen, eye, and testis. Newborn CD1 mice succumbed in a dose-responsive manner. **Discussion/conclusion:** Culture fluids of cells transfected with DKR in vitro RNA contained infectious virus, demonstrating that the DKR cDNA clone can produce infectious virus. Head-to-head comparison between Recombinant and Parental virus using both in vitro and in vivo models showed no significant difference. DKR ZIKV rapidly replicates in Vero cells with titers reaching 7 logs as early as 3d.p.i. Furthermore, DKR ZIKV showed notable virulence in A129 and newborn CD1. These data corroborate other studies with wild-type African ZIKV strains. When used in comparison with Asian ZIKV, this African ZIKV clone could be a valuable tool in identifying genetic determinants for ZIKV pathogenesis and dispersion.

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Placental histopathology of congenital Zika syndrome in an HIV-exposed infant

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Introduction: Zika virus (ZIKV) is a mosquito-borne pathogen that belongs to the *Flaviviridae* family. In the large ZIKV epidemic that occurred in Brazil in 2015, intrauterine fetal exposure to ZIKV was associated with a significant risk of developing microcephaly and neurological disorders in the infected infants. Moreover, the mechanism by which ZIKV crosses the placenta to establish fetus infection has not been fully elucidated, despite the efforts of numerous groups attempting to clarify placental transposition. ZIKV-associated disease has since been reported in 24 countries in the Americas. At present, definitive evidence is lacking regarding intrauterine co-exposure to ZIKV and other viral infections and whether such co-infection impacts the risk of acquiring either infection or disease severity. Objective: Here, we provide evidence of placental alterations in an intrauterine exposure to both ZIKV and HIV infection, causing congenital Zika syndrome in an HIV-exposed uninfected infant. Material and Methods: Clinical, imaging and laboratory examinations of the pregnant woman and the newborn were performed. Histopathology, ZIKV/HIV-specific immunoassays, and ultrastructural evaluation of the placenta were performed. Results: The HIV-positive pregnant underwent ultrasounds revealing fetal cerebral ventriculomegaly, microcephaly, and brain atrophy. Her baby girl was born small for gestational age and with the neurological sequelae of congenital Zika syndrome. The evaluation of the abnormally large term placenta revealed severe damage to the maternal decidua and chorionic villi. Maternal portions presented diffuse edema, fibrinoid necrosis, fibrosis, vascular endothelial thickening, degeneration, calcification, vascular congestion and focal areas of inflammatory infiltrates. An investigation of the chorionic villi presented an area of extensive hemorrhage and prominent syncytotrophoblasts. These features were not observed in the control placenta. ZIKV-E and NS1 protein were detected only in samples from the ZIKV infected patient by immunohistochemistry. Ultrastructural aspects of this sample showed endothelium thickening, damaged syncytotrophoblasts nuclei, abnormal organelles and intracellular clusters of virus-like particles approximately 25 nm in diameter. Discussion/Conclusion: Our results confirm that maternal ZIKV infection during pregnancy can result in placental and fetal injury. Furthermore, in countries where the prevalence of HIV-positive pregnant women is high, more cases of severe Zika disease in HIV-exposed fetuses are expected.
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CHARACTERIZATION OF A NEWLY EMERGED DENV SEROTYPE IN NON-HUMAN PRIMATES

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Dengue virus (DENV) is a mosquito-transmitted flavivirus that presents a substantial threat to public health worldwide. One third of the global population is at risk of infection and over 400 million cases of dengue are reported per year. DENV is maintained in two transmission cycles: a sylvatic cycle between mosquitos and non-human primates (NHP), and an urban cycle between mosquitos and human hosts. The human-endemic lineages of DENV-1-4 each emerged from sylvatic ancestors maintained in a cycle between NHP and arboreal *Aedes* mosquitoes. Ancestral strains are still persisting in both Southeast Asia and West Africa, posing a risk for the contemporary emergence of sylvatic strains into the human population. In this study, we described the discovery and characterization of a novel DENV, isolated from a febrile patient in the Malaysian state of Sarawak, which presumably represents the prototype virus of a new dengue serotype. A complete genetic and serologic analysis was performed to characterize this isolate and to demonstrate that it represents a distinct virus among the other members of the DENV serogroup. The infection was assessed in a NHP model using rhesus macaques to study pathogenesis and homotypic and heterotypic responses to this novel DENV. A productive infection in NHP was demonstrated, as well the ability of this host to transmit the virus to mosquitoes, which implies the virus can be sustained a transmission cycle in nature. Collectively, my study describes the emergence of a novel DENV serotype and its biological characteristics. It also brings new insights to the future development of diagnostics, therapeutics and vaccines.

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Highlighted Poster

AMINOPEPTIDASE SECRETED BY CHROMOBACTERIUM SP. PANAMA INHIBITS DENGUE VIRUS INFECTION

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Dengue virus (DENV) is an important arboviral pathogen transmitted by *Aedes sp.* mosquitoes. Currently, there are neither FDA-approved antivirals for any human DENV infection nor credential measurement for vector control. A *Chromobacterium sp*. Panama (*Csp_P*), isolated from the midgut microbiota of *Ae. aegypti*, is previously shown to inhibit DENV infection within the mosquito and *in vitro*. Here, we conclude that proteins secreted by *Csp_P* mediate the antiviral effect against DENV infection *in vitro*. Biochemical characterizations led to the isolation of *Csp_P* protein extract and the downstream identification of *Csp_P* secreted Aminopeptidase, which gives rise to the anti-DENV activity carried by *Csp_P*. To elucidate its mechanism, we demonstrate that the antiviral effect against DENV occurs prior to viral attachment using assays allowing separation between viral attachment and receptor-mediated endocytosis. This result is explained by the observation of degraded DENV envelope (E) protein upon treatment with *Csp_P* protein extract, since E protein is the viral ligand for receptor binding. As an outcome of degraded E protein, purified DENV virions exposed to *Csp_P* proteins displayed aberrant structure visualized by EM. Together, we uncover that an antiviral protease secreted by *Chromobacterium sp.* Panama is able to destabilize DENV virions by promoting the degradation of viral envelope and consequently, abolish DENV infection. This is the first study characterizing the anti-DENV activity of a mosquito-associated bacterium, thereby contributing toward understanding how the mosquito microbiota may limit disease transmission, and providing new tools for dengue prevention and therapeutics.
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Morphological studies of cell cultures infected with Brazilian Zika virus

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Zika virus (ZIKV) is a member of the flavivirus genus, and its genome is approximately 10.8 kilobases of positive-strand RNA enclosed in a capsid and surrounded by a membrane. Studies on the replication dynamics of ZIKV are scarce, which limits the development of antiviral agents and vaccines directed against ZIKV. Identifying a ZIKV susceptible cell line that may enable viral isolation and identification, viral stock production, and testing of drug and vaccine candidates is of utmost importance. In this study, aiming at verifying presence and replication cycle of ZIKV and ultrastructural cell alterations, *Aedes albopictus* mosquito lineage cells (C6/36 cells) and African green monkey kidney epithelial cells (Vero cells) were experimentally infected with a ZIKV sample isolated from a Brazilian patient. The cells infection was characterized by immunofluorescence staining, phase contrast light microscopy, transmission electron microscopy and real-time RT-PCR. The infection was observed in both cell lineages, and ZIKV particles were observed inside lysosomes, the rough endoplasmic reticulum and in viroplasm-like structures. The susceptibility of C6/36 and Vero cells to ZIKV infection was demonstrated. Moreover, this study showed that part of the replicative cycle may occur within viroplasm-like structures, which has not been previously demonstrated in other flaviviruses. The results presented in our study highlight the importance for additional investigation of how ZIKV replicates, disseminates into tissues and transmits to other organisms to develop therapeutic approaches against this virus.

Category:
1-Virology-Pathogenesis-Antivirals

Highlighted Poster

A single nucleotide change in dengue NS2B alters virus replication

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The two successful flavivirus live attenuated vaccines (LAVs) against yellow fever (YFV) and dengue viruses (DENV), namely YF17D and DENV2 PDK53 strains were developed through serial passaging of the respective clinical isolates to obtain an attenuated phenotype characterised by small plaque size. We have recently shown that the small plaque phenotype was due, at least in part, to a robust interferon (IFN) response from infection with either virus that prevented the spread of infection to neighbouring cells. In this study, we aim to determine if mutations elsewhere in the genome would derive a virus with the same phenotype, defined by small plaque size, increase viral replication and elevated interferon response, as PDK53. We generated random mutations within the genome of a clinical DENV2 isolate by using 5-fluorouracil (5-FU) and sorted for mutants that induce robust type-I IFN response using Huh7 cells expressing IFNβ-EGFP promoter. Using full genome sequencing and reverse genetics, we generated these mutant viruses by Gibson assembly for further characterisation. We identified a single mutation within the NS2B of DENV2 virus (T4472C; amino acid I114T) that gave rise to small plaque size, increased viral replication and elevated IFN response *in vitro*, similar to PDK53 and significantly different to the parental clinical isolate as well as DENV2 16681. Similar observations were also made on monocyte derived dendritic cells (MDDCs), suggesting that NS2B could be targeted for DENV LAV development. The identification of this single mutation provide insights into the function of NS2B in suppresing type-I IFN induction, possibly in compromising its recently discovered ability to inhibit the cGAS/STING pathway. Our findings suggest that alternative LAV candidates could be generated through chemical-induced mutagenesis, fluorescence sorting and reverse genetics.

CHIKV EXPOSURE DOES NOT ALTER ZIKV PATHOGENESIS IN CYNOMOLGUS MACAQUES

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Zika virus (ZIKV) is arthropod-borne member of the Spondweni serocomplex of the genus *Flavivirus* in the family *Flaviviridae,* and is transmitted in urban settings by *Aedes (Stegomyia) spp*. mosquitoes. Until recently, ZIKV was believed to cause a mild, self-limited febrile illness called Zika fever (ZIKF), characterized by myalgia, maculopapular rash and conjunctivitis. However, the near-pandemic of 2015-2016, has demonstrated an association with serious complications, such as congenital Zika syndrome (CZS) and Guillain-Barré Syndrome (GBS). To evaluate whether prior exposure to chikungunya virus (CHIKV) (an arthropod-borne virus of the *Alphavirus* genus within the family *Togaviridae* that recently became endemic in the Americas) alters ZIKF severity, cynomolgus macaques (*Macaca fasicularis*) that had previously been infected with, and recovered from, CHIKV were utilized. NHPs were subcutaneously inoculated with $1*10^5$ FFU of ZIKV strain FSS13025 (Cambodia 2010), and monitored for 28 days. Samples (whole blood, serum, saliva, tears, and urine) were collected over the course of the infection and general health was monitored daily. In addition, on days 1-5, 10, 18, and 28 post-infection, NHPs were anesthetized and exposed to *Ae. aegypti* mosquitos to determine efficiency of infection. Overall, the course of NHP infection closely matched previous reports in the literature, with minimal signs of illness. Viral titer peaked in whole blood 4-5 DPI, exhibiting a maximum of 4.5 FFU equivalents/mL by RT-qPCR. Mosquito infection was limited, with maximal infection of only ≈26% observed at 4DPI after oral exposure to a blood titer of 3.94 $\log_{10}$ FFU equivalents/mL. In total, our analysis demonstrated no exacerbation of ZIKV disease due to prior CHIKV exposure. In addition, this study demonstrates the relative inefficiency of mosquito infection at titers closely matched to reported human viremias, posing the question of how large outbreaks of ZIKV are facilitated and maintained despite the limited vector competence of *Ae. aegypti*.

Highlighted Poster

Functional analysis of glycosylation of Zika virus envelope protein

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ISOLATION AND BIOLOGICAL CHARACTERIZATION OF ZIKA VIRUS STRAINS, CUBA 2016

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Introduction: Virus Zika (ZIKV) belongs to the genus Flavivirus of the family Flaviviridae, which includes a large number of viruses that cause human infections. **Objectives**: In order to obtain isolates of ZIKV and realize your biological characterization, during the transmission in Cuba in 2016, were studied a total of 112 samples that were positive to ZIKV by molecular diagnosis. **Materials and Methods**: Viral isolation in Vero and C6 / 36 HT cells, determination of viral growth kinetics in Vero, BHK-21, LL-CMK2 and C6 / 36 HT cells and susceptibility of strains isolated at different temperature was attempted. **Results and Discussion**: A total of 8 strains were isolated in Vero cells. In the viral growth kinetics, all strains reached the highest infective titers in Vero cells and the lowest in LL-CMK2 cells and significant differences were observed between strain 15513/16 and the rest of the evaluated strains. All strains decreased viral titers with the temperature. The strains studied can be grouped into two groups (A and B), who's differences lay mainly in viral titer, ECP, plaque size, viral growth kinetics and temperature sensitivity. **Conclusions**: There is variability in the behavior of isolates of ZIKV suggesting the possibility that the virus has undergone changes during the evolution of the transmission either by the introduction of new variants or by the development of mechanisms of genetic variability.

Highlighted Poster

Stress-induced unfolded protein response contributes to Zika virus-associated microcephaly

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Accumulating evidence support a causal link between Zika virus (ZIKV) infection during gestation and congenital microcephaly. However, the mechanism of ZIKV-associated microcephaly remains unclear. We combined analyses of ZIKV-infected human fetuses, cultured human neural stem cells and mouse embryos to understand how ZIKV induces microcephaly. We show that ZIKV triggers endoplasmic reticulum stress and unfolded protein response in the cerebral cortex of infected postmortem human fetuses as well as in cultured human neural stem cells. After intracerebral and intraplacental inoculation of ZIKV in mouse embryos, we show that it triggers endoplasmic reticulum stress in embryonic brains in vivo. This perturbs a physiological unfolded protein response within cortical progenitors that controls neurogenesis. Thus, ZIKV-infected progenitors generate fewer projection neurons that eventually settle in the cerebral cortex, whereupon sustained endoplasmic reticulum stress leads to apoptosis. Furthermore, we demonstrate that administration of pharmacological inhibitors of unfolded protein response counteracts these pathophysiological mechanisms and prevents microcephaly in ZIKV-infected mouse embryos. Such defects are specific to ZIKV, as they were not observed upon intraplacental injection of other related flaviviruses in mice.

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Highlighted Poster

Structural Investigations of an Encephalopathic Alphavirus

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Introduction:

Alphaviruses are icosahedral, insect-borne enveloped viruses that cause arthritis and encephalitis. Eastern Equine Encephalitis Virus (EEEV) causes encephalitis mainly in horses. Although EEEV rarely affects humans, up to 70% of clinical cases result in death. At present, no approved therapeutics exist to prevent or control diseases caused by alphaviruses.

Objective of Study:

To gain insights into EEEV structure and interactions with Fabs from neutralizing monoclonal antibodies (MAbs).

Materials and Methods:

Chimeric viruses containing EEEV structural proteins were purified from BHK-15 cells in a BSL2 environment. Cryo-electron microscopy (cryoEM) data were recorded on a Titan-Krios microscope equipped with a Gatan K2 direct electron detector. Maps of EEEV and EEEV-Fab complexes were interpreted using model building and homology modeling.

Results:

(i) A cryoEM map of EEEV was determined to a resolution of 4.4 Å.

(ii). N-linked glycosylation was identified on E1 and E2 protein ecto-domains at Asn134 and Asn315 respectively. The E1 glycosylation is exposed on the virus surface unlike the E2 glycosylation, which is located below the E2 ecto-domain. S-linked palmitoylation of E2 protein was identified at Cys387 and Cys414, close to the inner membrane leaflet.

(iii). The 161 residue C-terminal chymotrypsin-like domain of the capsid protein was traced in the map. However, the N-terminal, RNA-binding, 100 residues were disordered.

(iv). Weak, approximately helical density was observed directly underneath the capsid ribosome binding site (residues 100 to 110), and may represent the RNA genome.

(v) CryoEM maps of five murine Fab fragments complexed with EEEV were determined to a resolution of 7.3-8.2 Å. Three of these Fabs were found to bind to E2 domain-A residues Lys71 and Lys74 of E2, which have been implicated in receptor-binding. The two other Fabs were observed to interact with E2 domain-B, which protects the E1 fusion loop.

Conclusions:

(i) E1 glycosyation is accessible for interactions with host proteins.

(ii) Palmitoylation contributes to the folded structure of the E2 C-terminus, which interacts with the capsid protein.

(iii) The icosahedral arrangement of the capsid protein is mainly the consequence of the capsid-E2 interactions.

(iv) EEEV neutralization is achieved by inhibition of virus entry and genome release.

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ASSESSMENT OF CURCUMIN ON POLYMERASE OF DENGUE VIRUS TYPE 2

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Dengue virus (DENV) affects a vast majority of the global population of tropical and subtropical regiones, and the disease it produces, that affects more than 400 million people, has yet to receive a specific treatment. The RNA-dependent RNA polymerase (RdRp) domain of the non-structural protein 5 (NS5), essential for viral replication, is considered a therapeutic target for drug development, given that it has no homologue in humans. Recent research has focused on the search for compounds that have the ability to inhibit the enzymatic function of proteins essential for the replication of virus. This has led to assessing pharmacologically promising compounds, like curcumin (polyphenol of Curcuma longa L.), which in previous studies has been found to alter infection by different viruses, including dengue. The objetive of this research is to evaluate the effect of curcumin, *in silico* and *in vitro*, on the polymerase activity of Dengue virus type 2. For the *in silico* assays, we constructed the polymerase structure of the NS5 protein, based on homology with the same domain in DENV3 and molecular docking analyses were performed with the optimized structure for curcumin, based on density functional theories, using Autodock. For the *in vitro* assay, the polymerase domain was expressed in *E. coli* BL21 CodonPlus (DE3)-RIL and purity assessments were conducted through Western-Blot. Polymerase activity was confirmed through a modified colorimetric assay and, finally, we assessed the effect of 5 µM of curcumin on the domain enzymatic function. The molecular docking analyses on the RdRp model showed that the curcumin is involved in non-covalent interactions with several amino acids in the RNA tunnel (Ile416, Ala415, Gln746, Arg741, Trp799, Glu463, Ser320) and cavity B (Ile416, Ala415, Gln746, Arg741, Trp799, Glu463, Ser320) of which some are considered important for domain function. We obtained 1 mg/mL of recombinant protein with RdRp enzymatic activity, which was not affected by 5 µM of curcumin.

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Low prevalence of dengue in pregnant women during a zika outbreak

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From late 2014 to early 2017, a major outbreak of arbovirus infections occurred in Brazil. Three viruses were responsible for this epidemic: the newly introduced zika (ZIKV) and chikungunya viruses, and an impressive number of cases of dengue virus (DENV) infections, endemic in the country for decades. In a prospective study involving pregnant women (symptomatic or not) carried out in the city of Ribeirão Preto, a highly endemic dengue area located on the northeast region of the São Paulo state, we detected a low incidence of dengue cases. A total of 1021 pregnant women were evaluated in the year 2016 (February to May), with sequential sample collections in prenatal appointments in the municipal health centers, and 65% of the patients had no signs or symptoms of arbovirus infections. Out of 358 symptomatic patients (fever, myalgia, skin rash, arthralgia, among others), 209 had a positive ZIKV-RT-qPCR, 143 were negative for both viruses either by molecular or serological tests and 6 were positive for dengue virus by NS1 antigen detection. NS1-positive samples were tested by RT-qPCR for dengue serotype characterization and detected four DENV-1, one DENV-2 and one sample was negative to all serotypes. There were no asymptomatic infections among these patients. Pregnant women were also tested for DENV-infections (MAC-ELISA) and 251 patients were positive. Of those, 60% were asymptomatic. Dengue IgM-positive samples were tested in a $PRNT_{50}$ for ZIKV and about 54% neutralized the infection, demonstrating that IgM positivity to dengue could be due to cross-reactivity, since the sensitivity and specificity of commercially available kits for zika diagnosis are very low in countries with a high dengue prevalence. Considering the data presented here, despite a large number of DENV infections reported to the Brazilian Ministry of Health, a low prevalence of DENV infections was detected in the pregnant women cohort followed at the city of Ribeirão Preto, with no presence of asymptomatic infections in dengue cases. The low sensitivity and specificity of ZIKV-detection kits and the cross-reactivity between ZIKV and DENV may have influenced the overall number of reported cases for both diseases in Brazil during the last years.

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DETECTION OF DENGUE AND ZIKA VIRUS CO-INFECTIONS IN RIBEIRÃO PRETO, BRAZIL

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The epidemic of arthropod-borne viruses that occurred recently in Brazil involved both dengue (DENV) and zika (ZIKV) viruses. DENVs have been responsible for outbreaks in Brazil for more than three decades, causing severe disease and, sometimes, death. ZIKV has been recently detected in the Americas and is associated with neurological complications and fetal malformations. In 2016, both diseases were responsible for a huge number of cases in Brazil and the co-circulation of these viruses occurred in most areas of the country. Although not studied in depth, much because these viruses have similarities in their antigen content, making the serologic diagnosis difficult, ZIKV and DENV co-infections may have caused severe clinical manifestations and deaths. In order to define the importance of the co-infection and using NS1 antigen detection and DENV- and ZIKV-specific RT-qPCRs (real-time reverse-transcription polymerase chain reaction), we investigated the presence of DENV and ZIKV co-infection. Acute-phase serum from a convenience sample of 400 patients with suspected of DENV or ZIKV infections, i. e., presenting with myalgia, rash and fever were initially tested for NS1 antigen detection. Forty patients were NS1 positive and RT-qPCRs for DENV (serotypes 1, 2, 3 and 4) and ZIKV were performed on these samples. Twenty-nine samples were positive to DENV-1 and 9 were positive to DENV-2. Out of these samples, 4 co-infections with ZIKV were detected, three with DENV-1 and one with DENV-2. The patient with DENV-2/ZIKV co-infection died. Two samples were negative to all viruses tested and this may be explained by the time these samples were collected (convalescence), when NS1 protein is still detected but RT-qPCR is no longer positive. In conclusion, our results suggest that co-infections between DENV and ZIKV may be more common than expected (10% in our study). Thus, patients presenting with severe disease and NS1-positive should also be tested for ZIKV infections by RT-qPCR since antibodies elicited by both infections are very cross-reactive. Also a co-infection with DENV-2 resulted in a severe disease, demonstrating the need for sensitive, accurate diagnostic tests to offer the best possible patient care, and ascertain the real epidemiologic impact of both diseases.

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Cissampelos pareira Linn: Natural Source of Potent Anti-Dengue Activity

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Introduction: Dengue, a mosquito-borne viral disease, poses a significant global public health risk. In tropical countries such as India where periodic dengue outbreaks can be correlated to the high prevalence of the mosquito vector, circulation of all four dengue viruses (DENVs) and the high population density, a drug for dengue is being increasingly recognized as an unmet public health need.
Objectives: To identify Indian medicinal plants with potent antiviral activity against all four DENV serotypes.
Materials & Methods: Using the knowledge of traditional Indian medicine, Ayurveda, we developed a systematic bioassay-guided screening approach to explore the indigenous herbal bio-resource to identify plants with pan-DENV inhibitory activity.
Results[1]**:** Alcoholic extract of *Cissampelos pariera* Linn (*Cipa* extract) was a potent inhibitor of all four DENVs in cell-based assays, assessed in terms of viral NS1 antigen secretion using ELISA, as well as viral replication, based on plaque assays. Virus yield reduction assays showed that *Cipa* extract could decrease viral titers by an order of magnitude. The extract conferred statistically significant protection against DENV infection using the AG129 mouse model. *Cipa* extract had no adverse effects on platelet counts and erythrocyte viability. In addition to inherent antipyretic activity in Wistar rats, it possessed the ability to down-regulate the production of TNF-α, a cytokine implicated in severe dengue disease. Importantly, it showed no evidence of toxicity in Wistar rats, when administered at doses as high as 2g/Kg body weight for up to 1 week.
Conclusion: Our findings, taken in the context of the human safety of *Cipa,* based on its use in Indian traditional medicine, warrant further work to explore *Cipa* as a source for the development of an inexpensive herbal formulation for dengue therapy. This may be of practical relevance to a dengue-endemic resource-poor country such as India.

[1]R. Sood, R. Raut, P. Tyagi, P. K. Pareek, T. K. Barman, S. Singhal, R. K. Shirumalla, V. Kanoje, R. Subbarayan, R. Rajarethinam, N. Sharma, A. Kanaujia, G. Shukla, Y. K. Gupta, C. K. Katiyar, P. K. Bhatnagar, D. J. Upadhyay, S. Swaminathan, N. Khanna. *Cissampleos pareira* Linn: Natural source of potent antiviral activity against all four dengue virus serotypes. *PLoS Neglected Tropical Diseases* 9: e0004255, 2015.

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Vertical transmission of dengue viruses in Cuban populations of Aedes aegypti

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Dengue is an acute viral disease of major concern in public health. The disease is caused by a complex of four related dengue serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) transmitted between humans mainly by mosquitoes, particularly *Aedes aegypti*. In natural conditions, infected females *Ae. aegypti* can transmit the virus to their offspring (vertical transmission) with a significant associated epidemiological risk. However, this phenomenon had not been studied in Cuba until now. Therefore, the purpose of this study was to determine the occurrence of dengue virus vertical transmission in natural populations of *Ae. aegypti* and its spatio-temporal variation in Arroyo Naranjo municipality. *Ae. aegypti* larvae and pupae were monthly collected during September 2013 to July 2014 in the seven Municipal Health Areas, which were analyzed by molecular techniques to detect DENVs. A total of 111 pools of immature stages were processed (4102 specimens) and 37 were positive for at least one DENV. This finding reveals, for the first time in Cuba, that natural vertical transmission of DENVs occurs in *Ae. aegypti* populations. Infected pools were detected every month (except for January), suggesting a continued circulation of DENVs in the vector populations. Although DENV-1 and 3 were the most frequent and distributed in immature stages, the four DENVs were found in the studied area. This fact supports the idea of including the surveillance of vertical transmission in the general virological surveillance program. The five new nucleotide sequences obtained in this study corresponded to genotypes V, III and II of DENV-1, DENV-3 and DENV-4 respectively, which matched those circulating in the human population of Cuba and Latin America during the studied period. The occurrence, and verification, of natural vertical transmission of DENVs in Cuban populations of *Ae. aegypti* has a major impact on the vector control programs to prevent dengue outbreaks and proposes its surveillance as a tool for virological surveillance in Cuba.

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Highlighted Poster

HUMAN FETAL ASTROCYTES ARE HIGHLY PERMISSIVE FOR ZIKA VIRUS REPLICATION AND PERSISTENT INFECTION

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Introduction: Zika virus (ZIKV) is an emerging arthropod-borne virus that can cause microcephaly and other serious neurological defects in developing fetuses. The cellular response to infection and the mechanism by which ZIKV persists in the fetal brain are not well understood. **Objective:** To show mechanisms of infection and persistence of ZIKV in human fetal brain. **Materials and Methods:** Primary human fetal astrocytes (HFAs) were infected with pandemic and non-pandemic ZIKV strains. Fibroblast growth factor 2 (FGF2) inhibition and stimulation assays were performed in HFA cultures. Cell lysates and supernatants were collected for gene expression analysis (RT-qPCR and RNAseq) and viral titration and ELISA respectively. Infected HFAs were stained and quantified by indirect immunofluorescence. **Results and discussion:** Here, we show that expression and secretion of FGF2 was dramatically increased during ZIKV acute infection of HFAs and subsequent analyses revealed that it enhances viral replication. After infection, the virus can persist for at least one month in HFAs *ex vivo*; facilitated in part by their resistance to ZIKV-induced apoptosis. In contrast, human fetal neurons are refractory to infection. TIM/TAM receptors were shown to be important for infection and persistence of ZIKV in HFAs. Sustained activation of the innate immune system was observed in persistently infected HFAs indicating that the virus can thrive in the presence of a robust antiviral response. RNAseq analyses revealed that ZIKV significantly alters gene expression in HFAs in a manner that could affect antiviral defense and developmental processes. Based on these data, we posit that HFAs are reservoirs for ZIKV in the fetal brain and that continuous viral shedding and antiviral signaling from these cells may contribute to the neurodevelopmental disorders associated with *in utero* ZIKV infections.

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Immune response to ZIKV in Dengue pre-exposed Non-human Primates

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Abstract:

In 2015 Zika virus (ZIKV) re-emerged in the Americas, particularly in Dengue endemic areas. It has been shown, *in vitro*, that antibody dependent enhancement (ADE) of ZIKV can occur in presence of Dengue virus (DENV) immune sera. The effects, *in vivo,* of Dengue cross reactive/non-neutralizing antibodies against ZIKV and its potential to enhance Zika pathogenesis are unknown. Here we analyze how declining DENV immunity affects ZIKV disease, using a NHP model previously exposed to DENV. Eight rhesus macaques (*Macacca mulatta*) pre-exposed to either DENV1 WP74 (n=4) or DENV2 NG44 (n=4) were infected with ZIKV 2.8 years after DENV infection. As a control group we included a second cohort of ZIKV and DENV-naïve macaques. Animals in both cohorts matched in age and sex. Both cohorts were infected with ZIKV H/PF/2013 strain, (10^6 pfu, s.c). We show that the long-term pre-exposure to DENV serotypes 1 or 2 induces ADE of ZIKV *in vitro*, but these *in vitro* enhancing antibodies did not increase viremia and disease when the macaques were challenged with ZIKV. Actually, previous exposure to DENV may result in modulation of the immune response induced by ZIKV without resulting in enhancement of ZIKV pathogenesis; they also presented a shorter viremia when compared to the control group. This confirms that in DENV convalescent subjects the presence of cross-reacting IgG antibodies that are able to enhance ZIKV and neutralize DENV *in vitro* are present. However these antibodies neither have the quality to increase and extend the duration of viremia nor to abrogate ZIKV replication *in vivo.* Our results reinforce the value of NHP to understand the complex serological interaction among flavivirus and to support the design of effective flavivirus vaccines.

Funding of research

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Budding induces imperfect icosahedral symmetry in flavivirus virions

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Abstract

Flaviviruses assemble initially in an immature, non-infectious state (1). When icosahedral symmetry constraints were excluded in calculating the cryo-EM reconstruction of an immature flavivirus, it was found that the nucleocapsid core touched the inside of the viral lipid membrane at the “proximal” pole. The outer glycoprotein spikes on the “distal” pole were either distorted or missing. In the asymmetric reconstruction of a mature flavivirus, the core is re-positioned, as expected, concentric with the glycoprotein shell. This suggests that the interactions between the core and glycoproteins are altered during viral assembly and budding from the endoplasmic reticulum. Similarly, in the study of many other viruses, icosahedral averaging may have hidden biologically significant events.

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Epi/Immunology

Tetracyclines in IL-6 and TNF dengue immunomodulation: A trials meta-analysis

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Introduction: Recent studies have provided information regard the effect of tetracycline and doxycycline in dengue; however global immunomodulation of cytokines effect in a meta-analysis has not been reported before. Methods: A systematic review was conducted through PubMed, Scopus, Cochrane library, EMBASE and Google Scholar up to August 1, 2016. The search strategy was “tetracycline” or “doxycycline” plus “AND” followed by the keyword “arbovirus”, “dengue”, “chikungunya”, “Zika”. Languages: English and Spanish. Randomized clinical trials (RCT) that assessed tetracyclines effect in the treatment of laboratory confirmed arbovirus infection (PCR) were included in a meta-analysis. OpenMeta[Analyst] software, standardized mean difference (SMD), and its corresponding 95% confidence intervals (CIs) were used for this meta-analysis. Results: Two studies summarizing three study arms, including 345 patients were included. Two arms used doxycycline at 200 mg/day and one tetracycline at 1500 mg/day. The results of the meta-analysis indicate a significant reduction of IL-6 and TNF at days 3 and 7 in those treated with tetracyclines: (i) IL-6 (at day 3), SMD=-0.656, 95 %CI (-1.147, -0.165), P=0.003 (I2=83%); (ii) IL-6 (at day 7), SMD=-0.915, 95 %CI (-1.667, -0.163), P<0.001 (I2=92%); (iii) TNF (at day 3), SMD=-0.531, 95 %CI (-0.977, -0.085), P=0.008 (I2=80%); (iv) TNF (at day 7), SMD=-0.851, 95 %CI (-1.584, -0.118), P<0.001 (I2=92%). Discussion: Tetracyclines showed in RCTs the potential usefulness in the treatment of dengue via immunoregulation of cytokines such as IL-6 and TNF, involved in pathogenesis, with higher effect at day 7. Mores studies assessing other arboviroses (eg. Chikungunya, Zika) as well other cytokines are required.

Highlighted Poster

Structural basis of potent ZIKV neutralization by monoclonal antibody MHC1839

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Zika virus (ZIKV) infection is a major global health concern because of its potential to cause large epidemics and severe clinical manifestations such as congenital birth defects in utero and Guillain-Barré syndrome in adults. ZIKV is an enveloped, positive-sense, single-stranded RNA virus in the flavivirus genus, which includes other medically important viruses such as dengue virus (DENV), West Nile virus, and yellow fever virus. The surface of the ZIKV virion is decorated by 180 copies of envelope (E) protein that are tightly packed to form a protein coat with icosahedral symmetry. The E protein mediates viral particle attachment and entry into host target cells. People infected by ZIKV develop strong neutralizing and protective antibodies that target type-specific epitopes displayed on the envelope of ZIKV but not other closely related flaviviruses like DENV. While investigators have recently mapped the location of several epitopes on ZIKV targeted by human MAbs, much remains unknown about the full repertoire of antibody epitopes and their overall functional significance. Here, we describe structural basis of a potent neutralizing monoclonal antibody MHC1839 isolated from a person infected with ZIKV as a primary flavivirus infection. To understand the structural basis for its specificity and neutralization potency, we determined the crystal structure of Fab fragments of MHC1839 in complex with soluble E protein at 3.3 Å resolution. The structure reveals that MHC1839 targets a quaternary epitope that spreads across the E protein dimer, with its core binding site located within the domain-II of the E protein. While the mechanistic basis of MHC1839 neutralization is currently being investigated, the complex structure suggests that MHC1839 might prevent low-pH mediated viral membrane fusion and subsequent virus entry by restraining the necessary E protein transition from dimeric to trimeric state. Our findings delineate the necessary structural details for designing and testing novel diagnostic and vaccine antigens that display this epitope in the same conformation observed in our crystal structure.

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Dengvaxia efficacy dependency on serostatus: a closer look at more recent data

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Introduction: Mathematical epidemiological models are formal frameworks that convey ideas about the components of vector-host-pathogen interactions. They have become important tools for understanding and predicting the epidemiology of dengue fever disease that affects about half of the world population [1]. A dengue vaccine, Dengvaxia, recommended by the World Health Organization is now licensed for use in 19 countries. The WHO-SAGE recommendation was in part derived from mathematical models that estimated an overall reduction of 10-30% of dengue hospitalizations over a period of 30 years, if vaccine was administrated yearly to 80% of 9 years old children [2]. Aguiar et al. have discussed the risks behind this vaccine recommendation [3,4], after analyzing an age structured model [5] which was parametrized using the year 3 CYD14 vaccine trial. Objective of the study: In order to provide a better understanding on the real dengue vaccine efficacy, he objective of this is study is to estimate global dengue vaccine efficacy by serostatus, providing a better understanding on the real vaccine efficacy.
Methods: Using a more recent data set by age and serostatus from the combined CYD14, CYD15, CYD57 trials [6], the overall vaccine efficacy for hospitalized virologically confirmed dengue cases was estimated via the Bayesian approach [7] to obtain a probability for the vaccine efficacy with infected individuals in the vaccine group and in the placebo group.

Results and conclusion: The comparison between VE distributions by serostatus, for seronegatives and seropositives sharing the same age, shows a significant difference among those individuals. However, regardless of age differences, a large overlap between the VE distributions of seronegatives of 2-8 and 9-16 years of age is observed, suggesting that serostatus is determining the efficacy of this vaccine and not age [8].

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Dengue vaccine-induced T cell protection despite enhancing, interfering maternal antibodies

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Declining levels of maternal antibodies were shown to sensitize infants born to dengue immune mothers to severe disease during primary infection, through the process of antibody-dependent enhancement of infection (ADE). With the recent approval for human use of Sanofi-Pasteur's chimeric dengue vaccine CYD-TDV and several vaccine candidates in clinical development, the scenario of infants born to vaccinated mothers has become a reality. This raises two questions: will declining levels of maternal vaccine-induced antibodies will cause ADE? and, will maternal antibodies interfere with vaccination efficacy in the infant? To address these questions, the above scenario was modelled in mice. Type I interferon-deficient female mice were immunized with live attenuated DENV2 PDK53, the core component of tetravalent DENVax candidate currently under clinical development. Pups born to PDK53- immunized dams acquired maternal antibodies that strongly neutralized parental strain 16681, but not the heterologous DENV2 strain D2Y98P-PP1, and instead caused ADE during primary infection with this strain. Furthermore, pups failed to seroconvert after PDK53 vaccination owing to maternal antibody interference. However, a crossprotective multifunctional CD8+ T cell response did develop. Thus, our work advocatesfor the development of dengue vaccine candidates that induce protective CD8+ T cells despite the presence of enhancing, interfering maternal antibodies.
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Chimeric Reporter Zika and Dengue Viruses for Fast Neutralization Assays

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Neutralization tests (NT) such as plaque and micro-focus reduction neutralization tests (PRNT and mFRNT) are critical tests for flavivirus diagnostic, vaccine study, and serosurveillance. Since Zika virus (ZIKV) and dengue viruses (DENVs) co-circulate in areas with Aedes mosquitoes, simultaneous NT for ZIKV and DENV is usually required. Wild-type (wt) DENV and ZIKV replicate slowly, making PRNT time-consuming. Previously, we developed West Nile virus (WNV)-based chimeric DENVs representing the four types of DENVs, which reduced the duration of PRNT assay from 6-9 to 3 days and mFRNT from 2-3 to 1-2 days. Using a similar approach, we generated a chimeric ZIKV, producing well-defined plaques within 72 hours or micro-foci at 24 hours post-infection. Although mFRNT is faster and capable of higher throughput than PRNT, it still requires a labor-intensive fixation and immunostaining process for foci detection. To further improve and streamline the assay, we developed live WNV-based chimeric reporter DENVs and ZIKV (R-WN/DENVs and R-WN/ZIKV) expressing intensive green fluorescent protein signals in infected cells that can be directly analyzable by cytometer and fluoro-spot reader. Reporter virus seed lots used for NT assay were evaluated by RT-PCR and flow cytometry to confirm 100% reporter gene integrity of the reporter viruses. Using these chimeric reporter viruses and an image-based cytometer, we developed a fluorescent mFRNT (F-mFRNT) that can be live-imaged and analyzed automatically within 24-30 hours after cell infection. Using panels of human clinical sera, we verified the neutralization Ab titers obtained from the F-mFRNT were identical or similar to titers obtained by PRNT or mFRNT. All of the WNV-based chimeric reporter viruses have similar replication efficiency and provide a fast and practical means to obtain NT results to ZIKV and DENVs simultaneously. In addition, the live-image based assay allows for a true high-throughput detection directly from cell culture without any cell fixation or other additional procedures.

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INVOLVEMENT OF HLA ALLELES ON DENGUE OUTCOME AND IMMUNE RESPONSE

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Introduction: Viral, human genetic and immunological mechanisms are essential for understanding dengue outcomes. Regarding host genetics, HLA is the most polymorphic human gene, encoding HLA class I (A, B, and C alleles) and II (DP, DQ, and DR alleles). Likewise, T cells have an essential role against a variety of infections; however, their role in dengue is still controversial. In fact, pioneer studies have been proposed that T cells have a detrimental role in secondary dengue infections in a process termed “original antigenic sin”. In this study, we mapped HLA class I and II alleles in acute dengue patients in a population of endemic regions of Brazil. Then, we correlated the HLA alleles with clinical outcome and the magnitude of IFNγ-T cell response to the DENV-2 whole antigen. Materials and Methods: We evaluated 60 dengue patients classified as dengue without (DF) or with warning signs (DFWS) during DENV-4 outbreak. Luminex Multi-analyte profiling system was used to typed HLA alleles. Additionally, PBMCs’ IFNγ response to DENV-2 antigen was evaluated by FluoroSpot. Non-parametric Wilcoxon and RATE analysis with Bonferroni correction were used. Results: The most frequent alleles (>15%) by direct counting were A*02, A*07, C*07 for class I and, DQA1*01, DQA1*05, DQB1*03, DQB1*02, DQB1*06 and, DQB1*05 for class II. Most of DF patients carry HLA-B*15, while DFWS carry HLA-A*03. Most of thrombocytopenic patients (platelets<150,000/mm3) carry HLA-B*35, C*04 allele and the supertype A03, while non-thrombocytopenic (platelet>150,000/mm3) carry DQA1*02, DRB1*07 and supertype A24. An increase in DENV-2 antigen-specific IFNγ-producing cells was observed in DF patients, but not in DFWS patients. In addition, the frequency of IFN-producing cells increased with days of illness, just when viremia could no longer be detected by molecular methods. No correlation was observed between HLA alleles and IFN-producing T cells after DENV-2 antigen stimulation. Discussion/ Conclusion: We suggested that certain HLA alleles act as
predictors of the dengue clinical outcome and susceptibility to thrombocytopenia. As expected, IFN-producing effector T cells are more evident in the late acute phase of dengue, probably after the activation of the innate immune response.
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Pre-clinical efficacy of an inactivated Zika virus vaccine candidate

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Since 2015, Zika virus (ZIKV) infection has been associated with Guillain-Barre Syndrome (GBS) in adults and microcephaly in newborns, prompting the World Health Organization to declare ZIKV a public health emergency of international concern. Although cases of ZIKV infection have significantly decreased, it remains a high public health concern for pregnant women. This report describes the preclinical development of a purified, inactivated ZIKV vaccine (PIZV) made from a 2015 ZIKV isolate, strain PRVABC59. The isolate was amplified and plaque purified in Vero cells to generate six ZIKV seed lots for development of PIZV candidates. Based on genetic and virus growth characterization of the six seed lots, two were chosen for lab-scale production of PIZV for pre-clinical mouse studies. Both PIZV candidates formulated with alum were highly immunogenic in CD1 and AG129 mice, generating strong neutralizing antibodies against ZIKV. Vaccinated AG129 mice (group of 5) were fully protected from lethal ZIKV challenge. No weight loss or clinical signs of illness were observed in vaccinated mice, and none had detectable infectious viremia two days post challenge (PC). In contrast, challenge of the 5 placebo control mice resulted in high viremia on day 2 PC and morbidity/mortality between day 10 and 18 PC. Results of passive transfer of sera from vaccinated AG129 mice showed a highly positive correlation between neutralizing antibody titers and protection efficacy in the AG129 mouse model. Results from this study supported further clinical development of the PIZV candidates, and a phase I clinical trial was recently initiated.
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Highlighted Poster

Expression of ligands of NKG2D/DNAM-1 receptors induced by Dengue in infected cells in vitro.

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Dengue virus (DENV) infection is regulated by the innate immune response. Natural Killer (NK) cells of the innate immunity could be implicated in the early stages of DENV infection, as they have been associated with mild manifestation of the disease. In vitro experiments suggest that NK cells are capable of direct recognition of DENV-infected cells in the absence of antibodies. Cytotoxicity is induced in part by NK cells performing degranulation of granzyme and perforine granules (CD107/Lamp1 marker). Another important mechanism for viral response is the production of Interferon-gamma, which plays a key role in inhibition of viral replication. Ligands of NK cells activating receptors such as NKG2D and DNAM-1 could be induced during early establishment of DENV infection allowing recognition of infected cells by NK cells. To verify if NKG2D and DNAM-1 ligands were altered by dengue infection, we performed in vitro experiments in immature dendritic cells (imDCs) derived from primary monocytes from peripheral blood from healthy donors and infected them with DENV-2. The ligands of NKG2D receptor in our analysis included ULBP1-6, MICA and MICB, meanwhile ligands of the DNAM-1 receptor included PVR and Nectin2. Expression of mRNA for these ligands was assessed by qPCR, and we observed induction of different ligands of NKG2D receptor, especially ULBP-2, and of DNAM-1 ligands (Nectin2, PVR) depending on the time of exposure to the virus. The expression of these ligands as cell surface proteins was evaluated by flow cytometry comparing non-infected cells versus infected cells, and ULBP-4, Nectin-2 and PVR were induced at different time points. Moreover, DENV infection could induce the expression of soluble ligands and we are currently detecting soluble sMICA, sMICB, sULBPs-1,-2,-3 using ELISA assays comparing supernatants of infected and non infected cells. We are also doing Lamp-1 degranulation and intracellular IFNɣ production assays to evaluate the activation of NK cells co-cultured with DENV-infected imDCs. Our results suggest that NKG2D and DINAM-1 ligands are induced during DENV infection and this could allow the recognition of DENV-infected cells by NK cells. Further studies will need to be realized to determine the mechanism of NK cell ligands induction by DENV infection.

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Characterizing the Immune Responses to Dengue and Zika Viruses

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Objective: To understand the human immune response to DENV and ZIKV infection and vaccines.

Methods: Monoclonal antibodies (MAbs) were made from humans and animals infected or vaccinated with DENV or ZIKV viruses. Over 200 such MAbs have been characterized, including by amino acid-resolution epitope mapping, to understand the immune response against these viruses. In addition, by scanning the entire human membrane proteome for the ability to promote virus infectivity, we were able to identify novel receptors/attachment factors that enable DENV and ZIKV to enter cells.

Results and Conclusions:
MAb Isolation. B cell cloning and phage panning were used to isolate over 200 MAbs against ZIKV or DENV. These include dozens of MAbs that bound specifically to prM/E from ZIKV but not DENV, with some MAbs that also potently neutralizing ZIKV. These MAbs may be used for diagnostic and therapeutic applications.
MAb Epitope Mapping. Over 200 DENV and ZIKV MAbs have been epitope mapped at amino acid-resolution using Shotgun Mutagenesis alanine scanning across the entire prM/E protein. These epitope maps reveal which epitopes are specific for either ZIKV or DENV, information that is now being used to create better vaccines and therapeutics.
Cellular receptors. In order to differentiate DENV and ZIKV tropism, reporter viruses for each of the DENV serotypes, as well as ZIKV, have been used to identify new receptors and attachment factors that enable virus entry. Reporter viruses were tested on our Membrane Proteome Array (MPA), comprising 5,300 unique human membrane proteins individually expressed in live human cells. Known receptors and attachment factors were identified (validating the approach), as well as a number of membrane proteins not previously known to enable virus entry. These newly-identified proteins help explain viral tropism and pathogenesis, and may be useful as therapeutic targets.

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Category 2 Immunology-Vaccines

Highlighted Poster

Dengue Envelope Domain III-Based Single Entity Particulate Vaccine Candidate

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Introduction: Dengue is one of the fastest spreading vector-borne diseases, caused by four antigenically distinct dengue viruses (DENVs). Though a live attenuated dengue vaccine has become available, there is a need for new vaccine candidates which in addition to eliciting tetravalent neutralizing antibodies must also be free of enhancement risk.
Objectives: To produce a tetravalent dengue immunogen, DSV4, displaying DENV envelope protein domain III (EDIII) of the four DENV serotypes on a Hepatitis B surface (S) antigen virus-like particle (VLP) platform using Pichia pastoris and evaluate the utility of DSV4 as a dengue vaccine candidate.
Materials & Methods: DSV4 was designed by in-frame fusion of EDIII of all four DENV serotypes and hepatitis B surface (S) antigen and co-expressed with unfused S antigen, in P. pastoris, to obtain VLPs. These VLPs were characterized extensively, both physically and biochemically. DSV4 was evaluated in mice and macaques for its utility as a dengue vaccine candidate.
Results: DSV4 VLPs displayed EDIIIs of all four DENV serotypes based on probing with a battery of serotype-specific anti-EDIII monoclonal antibodies. These were highly immunogenic, inducing potent and durable neutralizing antibodies against all four DENV serotypes encompassing multiple genotypes, in mice and macaques. DSV4-induced murine antibodies suppressed viremia in AG129 mice and conferred protection against DENV-2 and DENV-4 virus challenge. Further, anti-DSV4 antibodies, even at sub-neutralizing concentrations, did not enhance sub-lethal DENV-2 infection of dengue-sensitive AG129 mice, based on their ability to suppress pro-inflammatory cytokine production and prevent mortality.
Conclusion: Directing the immune response to a minor but functionally relevant serotype-specific dengue epitope of the four DENV serotypes, displayed on a VLP platform, can help circumvent the risk of inducing disease-enhancing antibodies while eliciting effective tetravalent seroconversion. DSV4 has a significant potential to emerge as a safe, efficacious and inexpensive subunit dengue vaccine candidate.

Funding: This project was funded by grants from the Wellcome Trust, UK (WT-40395) and Sun Pharmaceuticals India Limited, India (ND/SPI/16/027).

The role of keratinocytes in DENV spread in human skin

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Dengue virus (DENV) is introduced into human skin by the bite of infected mosquitoes. While DENV has been reported to infect a wide variety of cells including Langerhans cells and dendritic cells the dynamics of infection in human skin remain unclear.
Here we exposed skin explants from healthy human donors to DENV serotype 2 and studied the dynamics of infection by quantitative in situ immunofluorescence imaging. DENV induced a transient interferon-α response that peaked from 2-8 h and returned to near baseline levels by 12 h. Time course experiments showed that virus replication was first detected in keratinocytes within 6 h. From 8-48 h of infection abundant DENV replication was detected in a wide range of skin cells, including basal keratinocytes and Langerhans cells in the epidermis and dermal macrophages, dendritic cells, mast cells, fibroblasts and lymphatic endothelium in the dermis. However, the major target of virus replication from a quantitative standpoint was the keratinocyte, which represent 60% of all infected cells over time. Exposure to DENV resulted in significant influx of Langerhans cells, dermal dendritic cells and dermal macrophages into the site of infection, and these cells also migrated out of skin in increased numbers. A real-time quantitative PCR method showed significant up-regulation of IL-1α, IL-1β, MIP-3α, IL-8, and IL-10 at 48 h after infection relative to control-inoculated skin. Immunofluorescence revealed that infected keratinocytes were the principal cell type expressing IL-1β and MIP-3α protein after infection. Microneedle array delivery of IL-1β blocking antibody reduced infection of Langerhans cells, dermal dendritic cells and dermal macrophages by 75 to 90% and reduced the overall number of infected cells in epidermis and dermis by 35% and 65%, respectively.
These data show that the innate response of infected keratinocytes favors infection by recruiting more target cells to the site of infection, inadvertently spreading DENV into other cell types that then migrate out of skin. Our findings highlight a novel role for keratinocytes and their interplay with myeloid cells in dengue biology.

Funding of research: The Government Pharmaceutical Organization (Thailand)

Highlighted Poster

DENV4 Genotypic Diversity Impacts Protective Immunity and Vaccine Responses

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Abstract:
It is estimated that one third of the world is at risk for dengue virus (DENV) infection, which can result in fever and hemorrhagic disease. There are four antigenically distinct DENV serotypes (DENV1-4). Infection with one serotype generates a neutralizing antibody response, which does not protect against the other three serotypes. Within the DENV4 serotype, there are at least five distinct genotypes. The impact of genotypic diversity is unknown, and it is unclear if infection with one DENV4 genotype results in protection against all other DENV4 genotypes, which is crucial for vaccine design. A recent study revealed that the DENV4 vaccine component in Dengvaxia (Sanofi Pasteur's live-tetravalent DENV vaccine) is more efficacious against the vaccine matched circulating DENV4 genotype compared to other circulating DENV4 genotypes (Abstract 622, ASTMH Annual Meeting 2017). In order to measure the importance of diversity within the DENV4 genotype, we generated an isogenic panel of viruses containing envelope protein sequences from multiple genetically diverse DENV4 viruses. We evaluated the ability of these viruses to grow in multiple cell types, be bound and neutralized by a panel of monoclonal antibodies and polyclonal immune sera. We found that a small number of amino acid within the envelope glycoprotein can have a large impact on virus maturation, growth and ability to infect cells. Additionally, we observed large differences in the ability of DENV4 serotype-specific antibodies and polyclonal immune sera to neutralize the panel of viruses, suggesting that DENV4 immunity might not be protective against all circulating DENV4 viruses. We will discuss the implications of our results for understanding DENV4 genotype-specific efficacy results reported for Dengvaxia, and, more broadly, the impact of DENV intra-serotype genotypic variation on vaccine performance.

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Highlighted Poster

Detection of iNOS and IDO in DENV-2 infected CD14+CD16-/+ monocytes

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Introduction: Under physiopathological conditions, human monocytes can be activated, producing inflammatory mediators and inducing enzymes such as iNOS (induced nitric oxide synthase) and IDO (Indoleamine 2,3-dioxygenase). Human blood monocytes are heterogeneous and divided into classical CD14+CD16- and non-classical CD14+CD16+ subsets. Monocytes are the main dengue target cells but specific monocyte subset functions remain unclear. Objective: Considering that iNOS and IDO enzymes may be involved in DENV infection and that the virus is capable of activating monocytes, we determined monocyte subsets expressing iNOS and IDO as well as their association with some cytokines/chemokines produced during DENV-2 infection. Materials and methods: Monocytes population was purified from the peripheral blood mononuclear cells (PBMC) from healthy donors and infected in vitro with DENV-2. Extra and intracellular labeling were performed 24 hours after infection, by flow cytometry. MCP-1/CCL2, IL-10 and TNF-α levels were detected by ELISA in culture supernatants. Results and discussion: We observed that both subsets CD14+CD16- and CD14+CD16+ were equally infected with DENV-2 (about 64,3±11% of infection; n=7). Additionally, iNOS Mean Fluorescence Intensity (MFI) were significantly higher on CD14+CD16- monocytes compared to the CD14+CD16+ subset (p=0.0004; n=7), suggesting the iNOS association with the CD14+CD16- subpopulation. However, no significant difference was found in IDO detection on monocyte subsets (p=0.2151; n=7). LPS and IFN-γ stimulated monocytes were associated with IDO detection, but not with iNOS, and reduced DENV-2 replication (p=0.0391; n=9) and infection rate (p=0.0005; n=7) in both monocyte subsets; Moreover, increased production of IL-10, MCP-1/CCL2 and TNF-α (n=9) were detected in culture supernatants. The use of an NO inhibitor (L-NMMA) increased IDO detection (p=0.0149; n=7) and inflammatory mediators production (n=9) besides raising the non-structural protein NS1 levels (p=0.0273; n=9) compared to non-treated cells. On the other hand, the use of an NO donor (SNAP) could reduce infection (p=0.0034; n=7), confirming the NO antiviral action on dengue and its possible effect on IDO enzyme, in the disease. Conclusion: Furthermore, our data suggest an antiviral role of the IDO enzyme during DENV infection that deserves to be better investigated.

Funding of research: CAPES, FAPERJ, CNPq, FIOCRUZ

The Global CD4 specific T cell response against dengue virus

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Abstract
Introduction: Dengue disease is a large public health problem that mainly afflicts tropical and subtropical regions. Understanding of the correlates of protection against dengue virus (DENV) is poor and hinders the development of a successful human vaccine.
Objective of the study: To enable global assessment of CD4+T cell responses, we mapped HLA-DRB1 restricted DENV-specific CD4+ T cell epitopes in individuals previously exposed to DENV in the general population of the dengue-endemic region of Managua, Nicaragua.
Material and Methods: HLA class II epitopes in the population of Managua were identified by an in vitro IFNγ ELISPOT assay. CD4+ T cells purified by magnetic bead negative selection were stimulated with HLA-matched epitope pools in the presence of autologous antigen presenting cells (APCs), followed by pool deconvolution to identify specific epitopes. The epitopes identified were combined with those previously identified to generate a "megapool" (MP) consisting of 180 peptides specifically designed to achieve balanced HLA and DENV serotype coverage. The DENV CD4MP180 was validated by Intracellular Cytokine Staining (ICS) assays.
Results: We detected responses directed against a total of 431 epitopes, representing all four DENV serotypes, restricted by 15 different HLA-DRB1 alleles. The responses were associated with a similar pattern of protein immunodominance, overall higher magnitude of responses, as compared to what was observed previously in the Sri Lanka region. Based on these epitope mapping studies, we designed a DENV CD4 MP180 with higher and more consistent coverage, which allowed the detection of CD4+ T cell DENV responses ex vivo in various cohorts of DENV exposed donors worldwide, including donors from Nicaragua, Brazil, Singapore, Sri Lanka and and U.S. domestic flavivirus-naïve subjects immunized with Tetravalent Dengue Live Attenuated Vaccine (TV005). This broad reactivity reflects that the 21 HLA-DRB1 alleles analyzed in this and previous studies account for more than 80% of alleles present with a phenotypic frequency ≥ 5% worldwide, corresponding to 92% phenotypic coverage of the general population (i.e., 92% of individuals express at least one of these alleles).
Conclusion: The DENV CD4 MP180 can be utilized to measure ex vivo responses to DENV irrespective of geographical location.

MAPPING THE CD4+ T-CELL RESPONSE IN A MOUSE MODEL OF ZIKA VIRUS INFECTION

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The explosive spread of Zika virus (ZIKV) throughout the Americas since 2015 and expanding list of associated neurological sequelae has driven urgent efforts to develop ZIKV-specific methods of diagnosis and novel vaccines. However, a full evaluation of the natural history of infection, potential interactions with other arboviruses, as well as the protection mediated by therapeutics or vaccination is hindered by our limited means of assessing ZIKV-specific immune responses. Advances have been made toward the development of a mouse model for ZIKV infection (Ifnar1-/-). The CD8+ T cell response in this model (haplotype H2b) has been mapped and shown to be important for protection against ZIKV, yet no one has described an importance for ZIKV-specific CD4+ T cells. Several papers have shown an importance for antigen-specific CD4+ T cells in protection from neurovirulent flaviviruses (West Nile virus, Japanese encephalitis virus, and Yellow fever virus). Therefore we began investigating the contribution of ZIKV-specific CD4+ T cells to protection from severe ZIKV disease. Through screening of a complete ZIKV peptide library, our lab has described for the first time, the antigen-specific CD4+ T cell response to ZIKV in mice on an H2b background. The majority of the ZIKV-specific CD4+ T cell responses are directed against 4 epitopes, which are peptides of the PrM, Env, NS1, and NS5 proteins. We have shown that memory cells persist in the absence of viremia. Through depletion of CD4+ T cells from Ifnar1-/- mice, we have shown that CD4+ T cells are necessary for protection from severe ZIKV disease and accelerated death. Importantly, CD4+ T cell deplete mice have significantly higher viral titers in the brain and spinal cord, suggesting that CD4+ T cells play an important role in restricting viral replication in the CNS. Due to the neurological sequelae that are associated with infection, these findings may be of importance for the development of novel vaccines against ZIKV.

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Virus-like particles and novel adjuvant combinations as candidate Zika virus vaccine

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Abstract: Currently, there is not an FDA approved vaccine directed against ZIKV, but vaccination is an efficient and tractable strategy for combating viral pathogens. The ultimate aim of vaccination is to elicit durable and protective pathogen-specific immunity. There are numerous types of vaccine platforms that have been utilized against viruses including live-attenuated viruses (LAV), subunit vaccines and virus-like particles (VLP). LAVs have important clinical restrictions for use during pregnancy due to the potential severe outcomes. The safest vaccine platforms utilize antigens (Ag), which include VLP formulated with a suitable adjuvant. Expression of the flavivirus genomic region encoding the prM-E polyprotein has been shown to result in the secretion of virus like particles (VLP) in which the envelope confirmation is antigenically similar to native virions. We have generated a VLP-based vaccine against ZIKV by purifying VLP secreted by cells expressing the ZIKV prM/E polyprotein. We have formulated VLP with known activators of innate immune responses, alone or in combinations, in order to identify optimal formulations to elicit production of neutralizing antibodies. Following intramuscular injection of C57Bl/6 mice, VLP formulated with a combination of aluminum hydroxide (alum) with the cyclic dinucleotide STING agonist 2'3'-cGAMP resulted in higher neutralizing titers than other tested adjuvants. Antibodies produced in these vaccinated animals were largely of subtypes consistent with a TH1-type response (IgG2c). Further, this response was dependent on type I interferon signaling, as mice deficient in the type I interferon receptor (Ifnar -/-) produced Th2-type antibodies (IgG1) which had less potent neutralizing activity. We have also shown that passive transfer of antibodies from vaccinated mice is sufficient to protect AG129 (type I/II interferon deficient) mice from lethal ZIKV challenge. Additionally, we are currently extending these results into a rhesus macaque model of ZIKV infection, and further testing combination adjuvants in formulation with VLP derived from various dengue virus serotypes.

Funding of research: NIAID HHSN27201400055C

Highlighted Poster

Development of a live-attenuated DENV-2-based chimeric vaccine against Zika virus

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The development of a safe and effective Zika virus (ZIKV) vaccine has become a high priority since the epidemic ensued in 2015-16. Here we report the development of a live-attenuated vaccine candidate invoking a robust immune response and providing 100% protection against lethal ZIKV challenge in mice following a single immunization. Utilizing Dengue virus 2 (DENV-2), PDK-53 (vaccine strain), as our vaccine backbone, we introduced the PrM-E genes of ZIKV to derive chimeric D2/ZK viruses. Due to difficulty in recovering viruses from initial chimeric constructs in cells, we identified and introduced multiple Vero cell adaption mutations in the construct to improve the fitness and stability of the chimeric viruses. We systematically incorporated different combinations of the mutations and have recovered virus from 7 versions of the constructs. Each version was also made in the wild type DENV-2 16681 (parental virus for the PDK-53) backbone for phenotypic comparison. Based on previously established attenuation phenotypes of the DENV-2 PDK-53 vaccine, these chimeric D2/ZK vaccine candidates are being evaluated for plaque size, temperature sensitivity, replication in C6/36 cells, neurovirulence in neonatal mice, and infection/dissemination within mosquitoes. The candidate which has the best genetic stability in Vero cells and retains all attenuation phenotypes will be chosen for further development. Currently, we have characterized several candidates for attenuation in vitro and have conducted mouse studies for 2 of the chimeric constructs. While both wild type (wt) ZIKV and DENV-2 16681 were highly neurovirulent, the chimeric D2/ZK viruses were fully attenuated in neonatal mice. AG129 mice (group of 5) immunized with a single- or double-dose of the candidates produced strong neutralizing antibodies to ZIKV, and were fully protected from lethal ZIKV challenge. Unlike the PBS group showing high ZIKV viremia on day 3 post challenge, no viremia was detected from the vaccinated groups after virus challenge. In addition, we observed the 2nd dose of vaccine candidates, as well as the wt ZIKV challenge did not provide boost effect of the neutralizing antibody titers acquired after primary immunization, suggesting a single-dose was sufficient to induce sterilizing immunity against a high dose of ZIKV infection.

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Impact of inflammatory mediators on endothelial activation in dengue

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Introduction: Bleeding and vascular leakage are determinants of dengue severity, although plasma leakage can also be observed at critical or defervescence stages, regardless of severity. The well-regulated endothelial barrier maintains vascular permeability, controls the exchange of small solutes, gases and fluids between interstitial and intravascular spaces. In addition, endothelial cells play a role in leukocyte extravasation, angiogenesis, cytokine production, protease and extracellular matrix synthesis, and antigenic presentation. There is consensus that cytokines, chemokines and others inflammatory mediators secreted by cells play a role on the alteration of endothelial permeability in dengue. We intend to quantify inflammatory mediators in the serum of dengue patients. Next, we aim to evaluate surface molecules associated with endothelial activation through a culture of human dermal microvascular endothelial cells (HMVEC-d) in the presence of serum from these patients. Materials and Methods: Twenty-one dengue patients classified as dengue without (DF) or with warning signs/severe (DFWS/S) were evaluated during the outbreak of 2010 and 2013 in Brazil. MMP9, VEGF, ICAM-1 and TIMP-1 levels were quantified by ELISA. Monolayers of HMVEC-d were cultured in the presence of patient's serum for 24 hours, followed by collection and staining of activation markers by flow cytometry. For statistical analysis, Kruskall-Wallis followed by Dunn test, Mann Whitney test and Spearman correlation. $P<0.05$ were considered significant. Results: Our data showed lower levels of MMP9 and higher levels of sICAM-1 in dengue patients, regardless of severity, compared to healthy controls. No differences were observed between patients and healthy controls regarding VEGF and TIMP-1 levels. Addition of DF or DFWS/Sev patient's serum on HMVEC-d monolayers did not trigger significant alteration on CD31, CD106 and CD147 expression. In contrast, addition of DFWS/Sev patient's serum, but not DF, on HMVEC-d resulted in a significant loss in CD54 expression. TIMP molecules are regulators of MMP activity. In this sense, we calculated a ratio between MMP-9/TIMP-1 levels. We found that the MMP9/TIMP ratio was directly correlated with CD54 expression. No other correlation was observed. Discussion/Conclusion: We are highlighting an imbalance of MMP-9, sICAM-1 and MMP-9/TIMP-1 in dengue, in which all these molecules could be associated with loss of CD54 expression.

Funding of research: CNPq, POSGPB

Structural analyses of dengue virus-like particles using cryo-electron tomography

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Introduction: Dengue is endemic in over 100 countries, with 40% of the world population residing in at-risk areas. The dengue virus (DENV) causes mild febrile illness to severe, haemorrhagic disease. DENV belongs to the Flaviviridae family and has four serotypes (DENV1-4). Sequential infections involving multiple serotypes result in severe disease due to antibody-dependent enhancement. Despite the rapidly growing global burden of the disease, there is no effective treatment for dengue.
DENV is an icosahedral virus with a diameter of 600 and 500 Å for the immature and mature particles, respectively. The DENV structural proteins comprise the capsid protein, the pre-membrane (prM) and the envelope (E) protein. The surface of the immature virus contains 60 spikes, each containing three prM-E heterodimers. Maturation in the low pH environment of the trans-Golgi network results in a conformational change in the E protein, and the furin cleavage of the prM into pr and M. This rearrangement results in smooth, mature particles with 90 E dimers on the viral surface.
A recently reported novel, tetravalent virus-like particle (VLP) based vaccine provides simultaneous highly neutralizing antibodies against all four DENV serotypes in mice. The constituent VLPs contain prM and E; however, knowledge of the structure of the VLPs is lacking.
Objective of the study: We investigate the structure of the DENV-3 and DENV-4 VLPs and probe the disposition of the envelope glycoproteins by fitting the E protein into the resulting density averaged maps. We study the VLPs both with and without mutations in the furin cleavage site of the prM, and assess the effect on the sample heterogeneity.
Materials and methods: We use cryo-tomography and sub-tomogram averaging for the structural analyses. The software packages used in this process are SerialEM, IMOD, PEET, EMfit and Chimera.
Results and Discussion: Preliminary findings show the presence of T=1 particles with a diameter of 300-400 Å and a spiky appearance.

Uncovering the memory derived antibody repertoire against DENV and YFV

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Mosquito transmitted flaviviruses, including dengue (DENV) and yellow fever (YFV), are among the most important vector-borne pathogens of humans worldwide, with nearly one-half the world's population living in areas at risk of DENV transmission. Following initial flavivirus infection or vaccination, some host B-cells differentiate into long-lived plasma cells (LLPCs) that reside in bone marrow and secrete virus specific antibodies that circulate in the sera. Other B-cells differentiate into primary (1°) memory B cells (MBCs) that are programmed to secrete virus-specific antibodies in the event of repeat infection or vaccination booster. Therefore these 1° MBCs form a unique founder population that plays a critical role in establishing and maintaining long-term viral immunity. Despite this critical role, much remains incompletely described about lifespan, specificity, potency, and breadth of these MBCs and the antibodies they secrete. This knowledge is vital for understanding long term human immunity to flaviviruses as well as for vaccine design. Here we describe the results of experiments designed to identify and quantify MBCs specific to two prominent flavivirus antigens, DENV1 and YFV, using two complimentary approaches and a unique non-endemic human cohort. The first approach is an ex vivo approach using single cell fluorescence activated cell sorting (FACS) and fluorescently labeled virus. This allows direct characterization of antigen specific memory B cells that recognize epitopes on whole mature virions. From this we can assess frequency over time of virus specific MBCs from donor PBMCs with times since exposure ranging from 1 month to 72 years. The second approach, limited dilution assay (LDA), a functional assay in which cohort MBCs are non-specifically stimulated in culture to secrete antibodies. These antibodies can be assayed for binding (ELISA) and neutralization, and frequency of neutralizing Ab secreting donor MBCs can be quantified as well as the potency and breadth of Abs they secrete. The results of this project will provide insight into the MBC founder population and the role it plays in broader flavivirus immunity.
Research funded by OCTRI Catalyst Award and Medical Research Foundation of Oregon

UNDERSTANDING THE NEUTRALIZING ANTIBODY RESPONSE TO TAKEDA'S TETRAVALENT DENGUE VACCINE

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Funding of the research: Takeda Vaccines

Dengue viruses cause a global disease burden of approximately 100 million apparent infections annually. Takeda Vaccines is developing a live attenuated tetravalent dengue vaccine (TDV) based on a recombinant attenuated dengue serotype 2 virus (TDV-2). For dengue serotypes 1, 3, and 4, the pre-membrane (prM) and envelope (E) genes replace the corresponding TDV-2 genes in the attenuated backbone to form chimeric viruses (TDV-1, TDV-3, and TDV-4). The safety and immunogenicity of TDV was evaluated in a double-blind phase II controlled study. With the study described here, we sought to understand the magnitude and specificity of the antibody response to TDV in a phase II study (DEN-205).

For this purpose, we developed a high-throughput neutralization assay using dengue reporter virus particles (RVPs) and Raji B cells expressing the flavivirus attachment factor DC-SIGN (CD-209). This assay demonstrates increased sensitivity and precision compared with the Vero-cell-based microneutralization assay (MNT), commonly used to evaluate flavivirus neutralizing antibodies.

Using the RVP neutralization assay, we first evaluated the magnitude of the neutralizing antibody response to TDV in a randomly selected subset of 88 subjects from the DEN-205 Phase II study at 0, 30, 60, 90, 180, and 360 days post-vaccination. The neutralization antibody potency (titer) values generated by the dengue RVP neutralization assay correlate well with titers obtained using the MNT assay.

We then developed tools to investigate the specificity of the neutralizing antibody response to TDV. Using recombinant RVPs with point mutations in previously characterized epitopes of the dengue E protein, we were able to abrogate interaction with human type-specific antibodies 1F4 and 5J7 (corresponding to dengue serotypes 1 and 3, respectively), while preserving the overall antigenic landscape. Using the 1F4 mutant RVPs we demonstrate that TDV-vaccinees elicit 1F4-like dengue 1 type-specific antibodies. Individuals previously evaluated in the immunogenicity subset will be further screened using the 1F4 and 5J7 mutant RVPs to understand the type-specificity of the response elicited by TDV. The evolution of the type-specific response over time will also be studied in a subset of subjects.

Characterization of a live-attenuated Zika virus vaccine

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Abstract
Introduction
Zika virus (ZIKV) is a member of the Flaviviridae family, which includes other medically relevant viruses such as Dengue, West Nile, and Yellow Fever. ZIKV has drawn worldwide attention due to novel clinical implications such as Congenital Zika Syndrome (CZS). Since there are currently no approved vaccines, diagnostics, or therapeutics for ZIKV infection their development has become a worldwide priority. Our group has developed a live-attenuated vaccine (LAV) that has shown protection to adverse outcomes with single dose administration in current murine and NHP models.
Objective
The objective for this experiment was to further characterize the safety and immunogenicity profile of a previously developed LAV for ZIKV. Specifically, the serial passaging of this LAV to ensure attenuation and continued immunogenicity for continued validation as a vaccine candidate.
Materials and Methods
The LAV attenuation strategy was developed by deleting nucleotides from the 3' UTR. This virus was passaged ten times on Vero cells and sequenced for mutations. The resulting passaged vaccine (LAV-P10) was compared to the original vaccine (LAV-P0). The comparison profile was done in vitro and in vivo. The in vitro comparison focused on the kinetics of the LAV-P10 compared to the LAV-P0. The neurovirulence safety profile LAV-P10 and LAV-P0 was compared by injecting them intracranially into neonatal mice. To test the immunogenicity of this virus it was inoculated into 3-week old A129 mice which were then challenged twenty-one days post infection with a lethal dose of parental virus.
Results
After passaging the LAV-P10 did not revert to WT virus. The LAV-P10 also showed similar characteristics to the LAV-P0 in vitro. The immunogenicity and safety profile of the LAV-P0 and LAV-P10 was unchanged as both showed protection against a lethal challenge with no adverse outcomes to either male or female mice.
Discussion/Conclusion
With the apparent need to have a ZIKV vaccine our group has developed a vaccine that has been previously shown to have single dose protection against adverse outcomes at low doses in murine and NHP Models. This study comparing LAV-P10 and LAV-P0 continues to show the superiority of our vaccine showing that high passaged vaccine shows no reversion to WT and retains its safety and immunogenic profile.
Funding of research:
P.-Y.S. lab was supported by University of Texas Medical Branch (UTMB) startup award, University of Texas STARs Award, CDC grant for the Western Gulf Center of Excellence for Vector-Borne Diseases, Pan American Health Organization grant SCON2016-01353, and UTMB CTSA UL1TR-001439. K.M.M., W.P.K, B.S.G and T.C.P. are funded through intramural funding from the National Institute of Allergy and Infectious Diseases. This research was also partially supported by NIH grant AI120942 to S.C.W. and grants AI073755, AI104972, and AI106695 from the NIH to M.S.D.

RECOMBINANT NS1 PROTEIN DENGUE 2 VIRUS CONFERS PROTECTIVE IMMUNITY SUITABLE FOR VACCINE.

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Introduction: Dengue virus (DENV) non-structural 1 (NS1) is a glycoprotein produced in both membrane-associated and secretion forms. The DENV NS1 protein contributes to evasion of host immune defenses and induction of anti-NS1 immunity correlates with the generation of cross-reactive antibodies that recognize platelets and proteins involved in the coagulation cascade. However, within the context of the DENV life cycle, NS1 protein represents a target for vaccine developments because infected cells present the full-length NS1 protein associated with the plasma membrane representing a target for antibodies, which may recruit complement proteins or effector cells to kill the infected cells and NS1 peptides presented by MHC I molecules are target for cytotoxic T cell.
Material and methods: In this work, the full-length DENV2 NS1 gene encoding NS1 was amplified and cloned in pQE30 vector. The NS1 protein was expressed in Escherichia coli cells by employing suitable culture conditions such as: culture media, temperature, different phases of growth for the induction and concentration of IPTG were tested to maximize protein production. Recombinant protein was purified by affinity column under denaturing conditions. Also, we evaluated the immune responses and protective capacity induced by the recombinant NS1 protein in BALB/c mice.
Results and discussion/conclusion: The NS1 was successfully expressed and purified. The results showed that immunized mice showed significantly higher NS1-specific antibodies titers, corresponding to a balance of IgG subclasses response. In relation to the cellular immune responses, the production of IFN-γ was highest after the splenocytes stimulation of immunized animals. In addition, mice immunized with the NS1 recombinant protein were significantly protected from a lethal intracranial challenge. Therefore, the high level of NS1 protein expression in E. coli as well as its immunogenic capacities converts this protein as an attractive vaccine candidate against dengue virus.
Financial support: Cuban Dengue Vaccine Program.

Impact of inflammatory mediators on endothelial activation in dengue

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Introduction: Bleeding and vascular leakage are determinants of dengue severity, although plasma leakage can also be observed at critical or defervescence stages, regardless of severity. The well-regulated endothelial barrier maintains vascular permeability, controls the exchange of small solutes, gases and fluids between interstitial and intravascular spaces. In addition, endothelial cells play a role in leukocyte extravasation, angiogenesis, cytokine production, protease and extracellular matrix synthesis, and antigenic presentation. There is consensus that cytokines, chemokines and others inflammatory mediators secreted by cells play a role on the alteration of endothelial permeability in dengue. We intend to quantify inflammatory mediators in the serum of dengue patients. Next, we aim to evaluate surface molecules associated with endothelial activation through a culture of human dermal microvascular endothelial cells (HMVEC-d) in the presence of serum from these patients. Materials and Methods: Twenty-one dengue patients classified as dengue without (DF) or with warning signs/severe (DFWS/S) were evaluated during the outbreak of 2010 and 2013 in Brazil. MMP9, VEGF, ICAM-1 and TIMP-1 levels were quantified by ELISA. Monolayers of HMVEC-d were cultured in the presence of patient's serum for 24 hours, followed by collection and staining of activation markers by flow cytometry. For statistical analysis, Kruskall-Wallis followed by Dunn test, Mann Whitney test and Spearman correlation. $P<0.05$ were considered significant. Results: Our data showed lower levels of MMP9 and higher levels of sICAM-1 in dengue patients, regardless of severity, compared to healthy controls. No differences were observed between patients and healthy controls regarding VEGF and TIMP-1 levels. Addition of DF or DFWS/Sev patient's serum on HMVEC-d monolayers did not trigger significant alteration on CD31, CD106 and CD147 expression. In contrast, addition of DFWS/Sev patient's serum, but not DF, on HMVEC-d resulted in a significant loss in CD54 expression. TIMP molecules are regulators of MMP activity. In this sense, we calculated a ratio between MMP-9/TIMP-1 levels. We found that the MMP9/TIMP ratio was directly correlated with CD54 expression. No other correlation was observed. Discussion/Conclusion: We are highlighting an imbalance of MMP-9, sICAM-1 and MMP-9/TIMP-1 in dengue, in which all these molecules could be associated with loss of CD54 expression.

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Patterns of cellular immunity associated with experimental infection with rDEN2Δ30

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Abstract
A deletion variant of the DENV2 Tonga/74 strain lacking 30 nucleotides from its 3' untranslated region (rDEN2Δ30) has previously been established for use in a controlled DENV human challenge model. To evaluate if this model is appropriate to derive correlates of protection for DENV vaccines based on cellular immunity, we wanted to compare how the cellular immune response to this challenge strain compares to the response induced by natural infection. To achieve this, we predicted HLA class I and class II restricted peptides from rDEN2Δ30 and used them, in an IFN-gamma ELISPOT assay, to interrogate CD8+ and CD4+ T cell responses in healthy volunteers infected with rDEN2Δ30. At the level of CD8 responses, vigorous ex vivo responses were detected in approximately 80% of donors. These responses were similar in terms of magnitude and numbers of epitopes recognized to previously reported responses observed in PBMC from donors from DENV hyper-endemic regions. The similarity extended to the immunodominance hierarchy of the DENV nonstructural proteins NS3, NS5, and NS1 being dominant in both donor cohorts. At the CD4 level, responses were less vigorous compared to natural DENV infection, and were more focused on nonstructural proteins. The epitopes recognized following rDEN2Δ30 infection and natural infection were largely overlapping for both CD8 (100%) and CD4 (85%) responses. Finally, rDEN2Δ30 induced stronger CD8 responses compared to other more attenuated DENV isolates.

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DIFFERENCES IN IMMUNE RESPONSE CELL SUBSETS IN DENGUE AND ZIKA PATIENTS

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Introduction: Zika virus (ZIKV) has been spreading during the last three years in the same American countries where dengue virus (DENV) was endemic or hyperendemic. The high degree of amino acid sequence homology between ZIKV and DENV presuppose a wide cross reactivity in the adaptive both humoral and cellular immune response between them, however, the possible differences in immune response cell subsets between these flaviviruses remain unkown.
Objective of the study: In our study antibodies levels and immune response cell subsets against DENV or ZIKV were compared in DEN and ZIK patients using serial samples collected during infections.
Materials and methods: PBMC cells (T cells subsets, B, monocyte and NK cells) frequencies and their activation were monitored by flow cytometry.
Results: An increased CD4+ T cells activation was detected in DEN comparing with ZIKV infection. Wide CD8+ T cells activation was shown during ZIKV infection. Specific and cross-reactive antibodies were detected after sixth day after DENV and ZIKV infection. DENV and ZIKV loads and antibodies levels were correlated with frequencies of activated PBMC. Differences were detected between primary and secondary flavivirus infections.
Discussion/conclusion: These results aid to clarify the role of PBMC in the control of DENV and ZIKV infections, with an impact on vaccine design and efficacy.

Funding of research: Public Health Ministry of Cuba

The impact of obesity on arbovirus disease outcomes

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The recent outbreaks of Zika, Chikungunya, and Mayaro as well as the seasonal reoccurrence of Dengue have caused waves of social and economic turmoil throughout much of Central and South America. With all arbovirus infections, the spectrum of disease severity, and outcomes vary widely among the susceptible populations; following homologous viruses infections some individuals experience mild symptoms while others are more severely impacted. Many factors contribute to the spectrum of disease, including immune sufficiency, age, access to health care, and prior arbovirus exposure. Another factor which we believe contributes to the spectrum of disease associated with infection is obesity. Obesity is a major world health problem, notably in Latin America approximately 300 million (~60%) of the adult population is overweight, and ~20% are classified as obese. It is well established that overweight and obese individuals have a higher risk of developing many chronic diseases including cancer, diabetes and heart disease, however the association between weight and acute arbovirus infection remains poorly understood. Recently, some of the underlying causes associated with increased risks to chronic diseases have been identified, including higher levels of inflammatory cells present in fat. It is believed that this highly inflammatory environment results in the development of dysfunctional and defective adaptive immune responses leading to chronic diseases. Building on these recent observations, we hypothesized that the inflammatory environment associated with obesity alters the susceptibility and protective immune responses generated to acute arbovirus infections. To challenge this hypothesis we are examining the association of obesity with the spectrum of disease outcomes from arbovirus infections in our small animal models. Using arboviruses including West Nile, Zika, and Dengue we have identified significant differences in blood chemistry, hematology, immune responses and viral spread between infected obese and non-obese mice. Demonstrating that obesity is a previously unappreciated factor associated with altered susceptibility to arbovirus infection. We are currently investigating the underlying immunological molecular mechanisms associated with obesity and diseases outcomes.

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A single-dose live-attenuated vaccine prevents Zika virus pregnancy transmission and testis Damage

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Introduction: Zika virus infection during pregnancy can cause congenital abnormities or fetal demise. The persistence of Zika virus in the male reproductive system poses a risk of sexual transmission. Materials and Methods: Here we demonstrate that live-attenuated Zika virus vaccine candidates containing deletions in the 3′ untranslated region of the Zika virus genome (ZIKV-3′UTR-LAV) prevent viral transmission during pregnancy and testis damage in mice, as well as infection of nonhuman primates. Results: After a single-dose vaccination, pregnant mice challenged with Zika virus at embryonic day 6 and evaluated at embryonic day 13 show markedly diminished levels of viral RNA in maternal, placental, and fetal tissues. Vaccinated male mice challenged with Zika virus were protected against testis infection, injury, and oligospermia. A single immunization of rhesus macaques elicited a rapid and robust antibody response, conferring complete protection upon challenge. Furthermore, the ZIKV-3′UTR-LAV vaccine candidates have a desirable safety profile. Discussion/conclusion: Live-attenuated vaccines generally have the advantage of single dose, rapid induction of durable immunity. These results suggest that further development of ZIKV-3′ UTR-LAV is warranted for humans.

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Chikungunya: study of virus-host interaction through the Systems Biology

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Chikungunya virus (CHIKV: Togaviridae: Alphavirus) is a re-emerging arbovirus that is causing outbreaks in several countries of the Americas. The first autochthonous transmission of CHIKV in Brazil was described in 2014 in the North region (Asian genotype). Few days later, autochthonous cases of East-Central-South-Africa genotype was confirmed in the Northeast of Brazil. Brazilian CHIKV infection cases have not ceased but it has still been neglected, reaching 185,369 suspected cases in 2017 (Jan-Sep). CHIKV infection usually results in a 5-7 day viraemia, inducing a rapid type I IFN response and subsequently neutralizing antibodies, both able to control the infection. On the other hand infection also drives a pro-inflammatory response that could be considered harmful since it is associated with the arthropathy caused by the infection that can be chronic in some cases, with symptoms similar to chronic rheumatic disease. This persistent arthritis could be also associated with viral material in joint tissues. A goal of CHIKV arthritis research is to identify potential new targets for anti-inflamatory drug interventions, without compromising the viral host immunity.
Our main goal is to explore in depth the molecular mechanisms that govern the immune response to CHIKV infection in the acute phase in human samples in order to identify molecular markers and mechanisms that could differentiate the chronic arthralgia patients from those ones that solve it.
We performed whole blood RNA-Seq analysis in 60 acute phase CHIKV infection volunteers' samples and 20 non infected controls. For the blood RNA-Seq analyses, we used TopHat alignment, Cufflinks transcript assembly, the HTseq for number of reads per annotation unit. Normalization was performed using DEseq algorithm and differentially expressed genes (DEGs) analyses with DEseq R package.
Our preliminary data with the whole blood transcriptomic analysis reveled more than 3K DEGs comparing the infected and control volunteers. The top 50 genes analyzed so far, reveled that more than 95% of the DEGs were upregulated and 50% enconding proinflammatory mediators and immune cells' chemoattractants. We are currently searching for DEGs between chronic arthritis and non-chronic patients.
To our knowledge, this is the first study describing the transcriptomic changes in circulating cells of CHIKV infection patients. Most of the DEGs encountered in this study had not been described yet and could be helpful to find new targets to CHIKV infection arthritis treatment.
Funding of Research: Butantan Foundation, Fapesp, Ministry of Healthy (Brazil) and CNPq.

Highlighted Poster

Outcomes of congenital Zika disease depend on fetomaternal IFN action

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Abstract

Zika virus (ZIKV) infection during pregnancy in humans results in intrauterine growth restriction, spontaneous abortion and microcephaly. Here, we found that fetus-derived type I interferon (IFN-I) signaling can enhance anti-ZIKV responses and provide clinical benefits to the fetus. Since IFN-lambda shares signaling cascades and antiviral functions with IFN-I, we investigated the in vivo effects of IFN-lambda in ZIKV-infected pregnant mice. IFN-lambda administration during mid-pregnancy reduced ZIKV burden in maternal and fetal organs and alleviated placental injuries and fetal demise. In addition, prophylactic and therapeutic treatment of IFN-lambda in a human trophoblast line, as well as in primary human amniotic epithelial cells, greatly reduced the ZIKV burden. Our data highlight IFN-lambda as a potential therapeutic useful for women at risk for congenital Zika disease.

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CELL-MEDIATED IMMUNITY GENERATED BY TAKEDA'S LIVE-ATTENUATED DENGUE VACCINE

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Introduction: There is an unmet medical need for a vaccine that is effective against all four dengue virus serotypes (DENV-1–4) in both dengue-exposed and dengue-naïve subjects in all age groups. Takeda is developing a tetravalent dengue vaccine candidate (TDV) based on a live, attenuated dengue serotype 2 virus, which provides the genetic 'backbone' for all four vaccine viruses (TDV-1–4).
Objective of the study: Here we report the cell-mediated immunity (CMI) generated by TDV administered in different dose schedules in children and adults living in dengue-endemic countries.
Materials and Methods: Peripheral blood mononuclear cells collected pre-vaccination and post-vaccination at different timepoints from children and adults participating into two phase 2 randomised double-blind trials conducted in dengue-endemic countries were assessed for CMI responses using intracellular cytokine staining (ICS) and IFNγ ELISpot assays. These clinical trials are registered with ClinicalTrials.gov, numbers NCT02302066 and NCT02425098.
Results and Discussion/Conclusion: Peptide pools spanning the DENV-1, -2, -3 and -4 proteomes were used as antigens to assess dengue-specific T cell responses in vaccinees. The magnitude, cross-reactivity, poly-functionality and persistence of the dengue-specific CMI response elicited by TDV in children and adults in endemic areas will be presented. These data are important to fully characterize the immune responses elicited by TDV.
Funding of Research: Takeda Vaccines, Inc.

"Four-in-One" Dengue Envelope-based Particulate Vaccine Candidate

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Introduction: Dengue is one of the fastest spreading vector-borne diseases, caused by four antigenically distinct dengue viruses (DENVs). Though a live attenuated dengue vaccine has become available, there is a need for new vaccine candidates which in addition to eliciting tetravalent neutralizing antibodies must also be free of enhancement risk. Recently, we found that Pichia pastoris-expressed DENV envelope (E) protein self-assembled into highly immunogenic virus-like particles (VLPs) in the absence of the prM, a DENV protein known to induce enhancing antibodies.
Objectives: To co-express the E proteins of all four DENVs in P. pastoris to obtain mosaic VLPs and analyze their DENV-neutralizing and DENV-enhancing potential in mice.
Materials & Methods: A DNA fragment engineered to carry four DENV E expression cassettes, corresponding to the four serotypes, was integrated into the P. pastoris genome. This was induced to co-express all four E proteins to obtain tetravalent mosaic VLPs (T-mVLPs). The potential of these VLPs to elicit DENV-neutralizing and DENV-enhancing antibodies in mice was evaluated.
Results: The co-expressed E proteins co-assembled into T-mVLPs, which retained the serotype-specific antigenic integrity and immunogenicity of all four types of their monomeric precursors, based on comparison to physical mixtures of monovalent E VLPs and bivalent E mVLPs. The T-mVLPs elicited EDIII-specific antibodies which could neutralize all four DENV serotypes. Significantly, anti-T-mVLP antibodies did not augment sub-lethal DENV-2 infection of dengue-sensitive AG129 mice based on their ability to suppress pro-inflammatory cytokine production and prevent mortality.
Conclusion: The "Four-in-One" tetravalent T-mVLPs offer the essential attributes of safety (non-replicating, prM-lacking and in vivo enhancement potential-lacking), efficacy (EDIII-directed virus-neutralizing antibodies), and low cost (single tetravalent immunogen produced using P. pastoris, an expression system known for its high productivity using simple inexpensive media). The results of this work strongly warrant further exploration of this vaccine candidate.

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Unique phenotypes of human CD4 memory T cells re-expressing CD45RA

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The expression of CD45RA is generally associated with naive T cells. However, a subset of effector memory T cells re-expresses CD45RA (termed TEMRA) after antigenic stimulation with unknown molecular characteristics and functions. CD4 TEMRA cells have been implicated in protective immunity against pathogens such as dengue virus (DENV). Here we show that not only the frequency but also the phenotype of CD4 TEMRA cells are heterogeneous between individuals. These cells can be subdivided into two major subsets based on the expression of the adhesion G protein-coupled receptor GPR56, and GPR56+ TEMRA cells display a transcriptional and proteomic program with cytotoxic features that is distinct from effector memory T cells. Moreover, GPR56+ TEMRA cells have higher levels of clonal expansion and contain the majority of virus-specific TEMRA cells. Overall, this study reveals the heterogeneity of CD4 TEMRA cells and provides insights into T-cell responses against DENV and other viral pathogens.

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Pichia pastoris-expressed Zika Envelope Domain III-Based Particulate Vaccine Candidate

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Introduction: Recent worldwide outbreaks of zika virus (ZIKV) and its association with severe neurological complications underscore the need for a safe and effective ZIKV vaccine. Though ZIKV incidence has reduced significantly at the present time, it is worthwhile to continue efforts to develop a vaccine to address potential future outbreaks. The existence of the phenomenon of antibody-dependent enhancement (ADE) due to the close antigenic relationship of ZIKV with dengue viruses (DENVs) makes it necessary that a ZIKV vaccine should not mediate ADE of DENV infection and vice versa.
Objectives: To produce a Zika Subunit Vaccine (ZSV) by displaying multiple copies of ZIKV envelope domain III (EDIII) on the surface of Hepatitis B surface (S) antigen virus-like particles (VLP) using Pichia pastoris and evaluate the utility of ZSV as a potential ZIKV vaccine candidate.
Materials & Methods: ZSV was obtained by co-expression of two recombinant proteins, of which one was an in-frame fusion (ZS) of four copies of ZIKV EDIII and one copy of hepatitis B surface (S) antigen and the other was unfused S antigen, in P. pastoris. ZS and S proteins were co-purified, to near homogeneity, using conventional chromatography from induced P. pastoris cells. Their association into VLPs was analyzed by dynamic light scattering and transmission electron microscopy. ZSV was evaluated in mice for its capacity to induce ZIKV-specific and DENV-enhancing antibodies.
Results: The purified ZSV was found to contain VLPs based on DLS and TEM analyses. These were highly immunogenic in BALB/c mice inducing antibodies that were specific to ZIKV EDIII but not to any of the DENV EDIIIs, based on indirect ELISA. Efforts are underway to determine ZIKV-neutralizing antibody titers in the murine immune sera. ZSV-induced antibodies did not enhance a sub-lethal DENV-2 infection of dengue-sensitive interferon α/β and γ receptor knockout AG129 mice.
Conclusion: The VLP format of ZSV and the lack of DENV enhancement in vivo, taken together with the high productivity of the P. pastoris expression system, warrant further exploration of this novel vaccine candidate.

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Diagnostic-Prognostics-Clinical

Subacute disease predicts post-Chikungunya chronic inflammatory rheumatism: Findings in Risaralda-Colombia

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Introduction: Much of the clinical research on chikungunya (CHIK) has been focused on the acute disease (<3 weeks) (>500 publications on PubMed) and the post-CHIK chronic inflammatory rheumatism (pCHIK-CIR) (≥12 weeks) (>200 publications on PubMed), but subacute stage (3-11 weeks) have been neglected (6 publications on PubMed). Relevance, relationship and predictability of pCHIK-CIR based on the occurrence of subacute disease has not been properly addressed. **Methods:** In a cohort study, with data analyzed at occurrence (acute phase, <3 weeks), subacute (3-11 weeks) and chronic phases (≥12 weeks, 12 months and 2 years), of cases serologically diagnosed in La Virginia, Risaralda, Colombia, the risk of pCHIK-CIR according the progression to the subacute phase (from the acute phase), was analyzed. Phases were classified according WHO/PAHO (2015) chikungunya criteria. Incidence according exposition (risk difference, RD and relative risk, RR) and proportions (χ^2) with their respective 95% confidence intervals (95%CI), were calculated using Stata-IC 14.0® licensed, p significant <0.05. Those with other arboviroses during follow-up were excluded. **Results:** At the end of this follow-up, 62 patients were valid for analyses, 43 (69.4%) corresponding to those that progressed to the development of pCHIK-CIR+ and 19 (30.6%) that were free of disease after 2 years (pCHIK-CIR-). From the total, 83.9% had subacute disease and 16.1% not. From those patients that developed pCHIK-CIR, all of them had subacute disease first. Among those free of disease (pCHIK-CIR-), 9 presented subacute disease. Among patients that presented subacute disease 82.7% developed pCHIK-CIR+, compared to 0% of those that did not progressed to subacute disease, RD=83/100 (95%CI 58-100) (p=0.0076). Among those with subacute phase polyarthralgia 84% developed pCHIK-CIR+ (vs 16.7% without it) (RR=5.208; 95%CI 2.51-10.797). The median number of joints affected during subacute disease was 8 (reaching up to 16 simultaneously). **Discussion:** Based on the current findings, once a patient reaches the subacute phase (presenting rheumatological persistent symptoms, such as polyarthralgia, rigidity and/or edema), interventions to mitigate the impact of pCHIK-CIR should be performed. These results would be also in the current consideration of immunomodulatory therapeutics, since cytokines, among other factors, appear to be related to the progression to chronic disease.

Zika and Dengue antibodies in a general population prospective cohort

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Introduction
The emergence of Zika virus (ZIKV) in Brazil presented new challenges to both clinicians and public health authorities. Brazilian authorities largely rely on clinical and epidemiological data for the diagnosis of most ZIKV cases, but the clinical presentation of this arboviral infection is diverse, from severe cases with neurological complications to asymptomatic or mild infections.
Objective
We performed a serological survey in an ongoing general population cohort in São José do Rio Preto, São Paulo, Brazil.
Material and Methods
Blood samples of the same individuals were collected in two moments: in 2016, before large ZIKV circulation in Brazilian southeast, and in 2017. They were submitted to detection of specific anti-Zika and anti-dengue Immunoglobulin G (IgG) by ELISA (enzyme linked immuno sorbent assay).
Results
808 paired samples were tested. In the first year, the IgG anti-dengue prevalence was 74.0%, and in the second year 85.5%. About ZIKV, the IgG anti-Zika prevalence was 10.1% in the first year, and 29.7% in the second year (incidence of 217 cases/1000 habitants). The incidence of Zika infection was equal in age and gender. In the logistical regression analysis, the education level was related to Zika incidence. Among the Zika-seroconverted patients only 7 (7/158; 4.4%) were assisted by health service and no one had acute ZIKV diagnosis by PCR (polymerase chain reaction). In contrast, among 93 (13.5%) dengue-seroconverted patients, 16 (16/93; 17.2%) were assisted by health service and 3 (3/16; 18.7%) were diagnosed as acute dengue by PCR/NS1 (non-structural 1 protein) detection.
Discussion and conclusion
Our data suggests that despite of a ZIKV outbreak, there is still a large number of susceptible individuals in our region. Furthermore, in our region (with large number of dengue IgG patients and widespread YFV vaccination) the vast majority of Zika cases were asymptomatic or mild disease. The epidemiological frame is imperative to understand of the ZIKV outbreaks and future of vaccine against arboviral infections.
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CATEGORY 4 - DIAGNOSTIC-PROGNOSTICS-CLINICAL

Multiplex rRT-PCR for Dengue Virus and Yellow Fever Virus Detection

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Introduction. The differential diagnosis of dengue virus (DENV) and yellow fever virus (YFV) infections in endemic areas is complicated by non-specific early clinical manifestations. Laboratory diagnosis typically involves serological methods, which have well-documented limitations for these flaviviruses, and/or individual molecular tests for each virus.
Objectives. To develop an internally-controlled multiplex rRT-PCR for DENV and YFV detection based on a re-designed pan-DENV assay and a new assay for YFV.
Methods. DENV and YFV assays were designed from alignments of all publicly-available whole genome sequences. Assays were optimized and analytical evaluation was performed in a multiplex format, which included an internal control assay for RNase P. Clinical performance of the DENV-YFV assay was then compared to published reference assays.
Results. The dynamic range of the DENV-YFV assay extended from 2.0 to 8.0 $\log_{10}$ copies/µL of eluate for each DENV serotype and YFV. The 95% LLOD for each target, expressed in copies/µL of eluate, was the following: DENV-1, 17.6; DENV-2, 9.4; DENV-3, 3.8; DENV-4, 150.1; YFV, 11.3. The assay proved specific for DENV and YFV detection when tested using genomic RNA from reference arbovirus strains, including Zika virus.
To evaluate clinical performance, samples from 48 dengue cases [DENV-1 (n=12); DENV-2 (12); and DENV-3 (24)] and two isolates of DENV-4 (strain H241) were tested side-by-side using the DENV-YFV assay and a published pan-DENV assay. All samples yielded similar results in both tests. For YFV detection, mock samples were prepared using serial dilutions of RNA from nine YFV strains: one 17D isolate and four strains each from Africa and the Americas. Mock samples were tested side-by-side in the DENV-YFV assay and a published YFV rRT-PCR. The DENV-YFV assay detected 42/54 samples versus 41/54 detected in the comparator.
Discussion. Despite control efforts, the number of DENV cases worldwide has increased over the past two decades, and YFV continues to cause outbreaks in Africa and the Americas. The DENV-YFV assay provides accurate detection of these pathogens in an internally-controlled multiplex format, and it is expected to be a useful tool to narrow the differential diagnosis and provide early case detection.
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Comparison between dengue classifications (1997 vs 2009): 30670 cases analyzed.

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Changes in the epidemiology of dengue has resulted in better availability of research and improved understanding of its clinical manifestations. Additionally, some limitations of the 1997 WHO's guidelines for dengue classification also became apparent, leading to the revision of the dengue classification in 2009. With the adoption of the new guidelines, comparability between studies—over time and across countries—became affected, which is important for the accurate diagnosis, reporting, and tracking of dengue patients' recovery. Here, we attempt to bridge this gap by comparing the traditional (1997) and revised (2009) classifications using a large number of patients of all ages, with different severities of dengue. We evaluated 30670 laboratory-confirmed dengue cases from a Brazilian endemic area. The degree of agreement between the variables and the two dengue classifications were determined using the Cramer's V test and the Kendall's tau-b correlation coefficient, respectively. The degree of agreement between the independent variables of each final model and their respective dependent variables was found to be very poor (Cramer's V < 0.2) in both classifications. Exceptions were ascites (Cramer's V = 0.81) and pleural effusion (Cramer's V = 0.45) for the 1997 classification, and hypotensive shock (Cramer's V = 0.22) for the 2009 classification. When we compared the two dependent variables (1997 classification *vs* 2009 classification) of both models, we found that there was only a slight agreement between the two classifications (Kendall tau-b = 0.2, P = 0.01). Limited comparability was noted between the WHO 1997 and 2009 guidelines for dengue case classification. It is important to exercise caution while considering the clinical applicability of both classifications for evaluate dengue severity.

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Zika virus-associated urinary bladder agenesis, Colombia

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Introduction: Recent studies have demonstrated an association between congenital Zika virus (ZIKV) infection and multiple birth defects, particularly microcephaly; however, to date, there have been no reports on the consequences of ZIKV infection on the development of the urinary tract. **Methods:** Herein, we reported on the first case of a pregnancy having ZIKV-related urinary bladder agenesis. **Results:** A 22-year-old patient who conceived her third pregnancy and became infected with Zika virus at 16 weeks' gestation in Sucre, Colombia. Zika infection was confirmed by real-time PCR; ultrasound revealed biometry with decreased fetal movements, and amniotic fluid decreased (index <3) (severe oligohydramnios) and anterior placenta (I/III). In a new ultrasound at 17 weeks, an estimated weight of 168 gr was calculated, with anhydramnios and intrauterine growth restriction (IURG). At this point a complete absence of the urinary bladder was evidenced. Because of the poor prognosis, pregnancy was interrupted. The fetal and placental tissue samples for RT-PCR were positive for ZIKV. **Discussion:** Given the originally not adverted wide teratogenic potential of ZIKV, the severe damage caused at urinary tract would be related to this flavivirus infection. Complete agenesis of urinary bladder is an extremely rare anomaly with only a few live cases reported so far. To understand the impact and mechanisms of ZIKV infection on human tissues development and the link of ZIKV to birth defects, a key step is to identify cell types that are particularly vulnerable to viral infection in the developing tissues after ZIKV breaches the placental barrier. This, particularly outside the central nervous system is not properly understood yet. Arthrogryposis, IURG, uveitis and retinal degeneration have been reported in association with gestational ZIKV infection. ZIKV epidemics has reminded us the fragility of the human beings to emerging infectious diseases, as previously experienced with many other agents. Moreover, ZIKV also changed the way researchers and physicians deal with flavivirus infections. This is due mainly to the severe impact of ZIKV infection during pregnancy and the resulting congenital ZIKV syndrome (CZS) and other birth defects that would include potentially the urinary tract abnormalities, as seen in our case.

Asymptomatic dengue in Sucre, Colombia: a serological and molecular survey

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Introduction: Asymptomatic infections would be considered relevant for public health in terms of transmission risk. In the case of dengue, this has not been comprehensively assessed in certain endemics countries, such as Colombia, as this is not part of the routine epidemiological surveillance. **Methods:** This descriptive, prospective epidemiologic study assessed the presence of antibodies but also of viral RNA in a random sample (n=253) of people who live in a coastal region of a tropical country where dengue is endemic (non-symptomatic). An enzyme-linked immunosorbent assay was used to test the serum for dengue virus IgM and IgG antibodies. Seropositive were assessed also with NS1 antigen. RT-PCR was also used. Samples were collected between January-February 2014 (for reference, chikungunya [CHIKV] arrived to Colombia apparently in September 2014 and Zika [ZIKV] on September 2015). **Results:** Seroprevalence by IgM was 20.42% (95%CI 15.5-26.1), whilst by IgG was 13.8% (95%CI 9.4-18.3). RT-PCR for DENV-RNA was positive in 12.5% of those serologically positive and 2.8% of the total. Serotypes: DENV-1 (n=4) DENV-2 (n=12), DENV-3 (n=0), DENV-4 (n=1) and one coinfection DENV-1/DENV-2. Estimated general prevalence was 428 cases/1,000 pop. **Discussion:** Because dengue viremic people without clinical symptoms may be exposed to more mosquitoes through their undisrupted daily routines than sick people and represent the bulk of DENV infections, this and previous studies indicate that they have the potential to contribute significantly more to virus transmission to mosquitoes than previously recognized. Sucre department is endemic for this and other arboviruses (that arrived later, e.g. CHIKV and ZIKV). DENV serotype coinfections are possible as we observed in this study, but also possible with other flaviviruses and alphaviruses, as we documented in other studies. All of this, has significant clinical and public health implications and impact.

Autoimmunity triggered by Chikungunya and other pathogens: a diagnostic challenge

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Introduction: Chikungunya (CHIK) emerged in the Americas as an epidemic-associated pathogen leading to significant morbidity and disability, particularly from chronic sequela (post-CHIK chronic inflammatory rheumatism). Autoimmune polymiositis, adult onset Still's disease and antiphospholipid syndrome, have been reported in association with CHIK infection. To date, there have been no reports on the potential association and triggering of other autoimmune disorders, such as angioedema, RA or SLE, due to CHIK infection. **Methods:** Herein, we reported a diagnostic dilemma case, where after CHIK infection patient presented clinical findings compatible with angioedema, RA and SLE, with detected autoantibodies. **Results:** A 38-year-old patient from Mompox, Bolivar, Colombia (CHIK endemic area), consulted with a history of 3 years (2014-2017) after CHIK infection, presenting generalized facial edema, polyarthralgia and periarticular edema. These findings according her recurred every 2 months since the CHIK infection was initially diagnosed. Use of naproxen and prednisolone during these recurrences, significantly decreased symptoms. Laboratory findings (at beginning of last episode) were significant for the following results: ANAs-1/160+, IgM-FR+ (177.38), anti-dsDNA+++ (1042.74), ACA-III-IgG+ (43.68), Sm++ (73.94), RNP++ (48.14), SS-A+++ (141.06), SS-B+++ (132.59), CHIKV IgG+ (68.156), DENV IgG+ (28.949), ZIKV-IgG+ (4.033), EBV IgG+ (28.295), *Mycoplasma* IgM+ (18.03), *Campylobacter jejuni* IgG+ (39.164), C3 normal (84mg/dL), C4 low (6mg/dL). lymphocytes T CD4+ 1.3%, TCR γ:δ 14.3%, TCR α:δ 74.9%, effector memory 10.5%, central memory 22.4%, effector 22.6%, naïve 44.5%, lymphocytes T CD8+ 2%, effector 100%, lymphocytes B CD20+ 13.2%, transitional 96.6%. Anti-CCP3, TPO, Tg, B2GPI-IgM, -IgG, ACA-III-IgM, CHIKV-IgM, DENV-IgM, ZIKV-IgM, EBV-IgM, CMV-IgG, -IgM, and *Mycoplasma*-IgG, antibodies were all negative. Patient was treated with azithromycin and still under study to define treatment. **Discussion:** The innate and adaptive immune responses to acute CHIK infection have received much attention, yet the pathogenesis of chronic arthralgia and the basis for the variation in long-term outcomes among patients remain elusive. In this case, the autoimmune ecology is illustrated by the development of SLE triggered after CHIK infection and probably other environmental factors. Further longitudinal studies of patients infected with CHIK virus are warranted to confirm this observation.

Highlighted Poster

Zika viral RNA persistence among pregnant women – Puerto Rico, 2016-2017

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Introduction: Although Zika virus (ZIKV) screening has been recommended for pregnant women living in areas with ZIKV transmission, the length of ZIKV persistence among pregnant women after infection is unknown. A cohort study of primarily non-pregnant persons found the median time to loss of ZIKV RNA in serum was 14 days after symptom onset, and a case series suggested that ZIKV RNA persistence among pregnant women might be longer than among non-pregnant persons.
Methods: During the 2016 ZIKV outbreak in Puerto Rico, ZIKV screening was recommended for pregnant women during each trimester. All specimens from pregnant women were submitted to the Puerto Rico Department of Health for testing using the TrioPlex RT-PCR or the ZIKV IgM ELISA assay, depending on the time after symptom onset and phase of the outbreak. More than 50,000 testing events were recorded, and nearly 4,000 ZIKV infections were identified. Because of repeated testing during pregnancy, many women were tested multiple times, including before and after positive ZIKV results. To determine the duration of ZIKV RNA detection among pregnant women and inform screening recommendations, we analyzed ZIKV RNA detection by time after symptom onset among symptomatic pregnant women with confirmed ZIKV infection by RT-PCR.
Results: In total, 602 symptomatic women had 686 additional specimens submitted after an initial RT-PCR-positive result for ZIKV, for a total of 1,288 specimens. ZIKV RNA was detected in 54% of specimens submitted in the first month after symptom onset. Frequency of positivity decreased each week after illness onset from 77% in week 1, 59% in week 2, 39% in week 3, and 35% in week 4. The proportion of women with an additional positive specimen by RT-PCR further decreased to 17% in the second month and 7% in the third month after symptom onset. The latest detection of ZIKV RNA by RT-PCR occurred 140 days after symptom onset.
Conclusion: Approximately half of the pregnant women cleared ZIKV RNA in the first month after symptom onset, although prolonged ZIKV RNA detection was identified up to 140 days after symptom onset.

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Category 4: Diagnostic-Prognostics-Clinical
References:
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CLINICAL MANIFESTATIONS OF ARBOVIRUSES IN LATIN AMERICA

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Introduction: Several arboviruses belonging to the *Alphavirus*, *Flavivirus* and *Bunyavirus* genus circulate in Latin America. Among the challenges health practitioners face, probable diagnosis is the most difficult because of the similar clinical presentation among these viruses. NAMRU-6 established passive febrile surveillance for more than 15 years in several Latin American countries (Honduras, Venezuela, Colombia, Peru and Paraguay) in order to identify the etiology of acute febrile illness. **Objective:** To identify symptoms and signs associated with these viruses. **Methods:** Male and female subjects (≥ 5yrs) who sought care in health centers or hospitals with fever (≤ 5d of disease) were enrolled. Epidemiological and clinical data were recorded and blood samples were analyzed for dengue (DENV) by RT-PCR and/or viral isolation to detect Mayaro virus (MAYV), chikungunya virus (CHIKV), Venezuelan Equine Encephalitis virus (VEEV), Group C virus (GRCV), Guaroa virus (GROV) and Oropouche virus (OROV). **Results:** Between Dec 2010 and July 2017 a total of 12,349 participants were enrolled, 49% were women, and the median age was 29.3 (Q_{25}: 18, Q_{75}:39). The study detected 3,358 DENV, 88 CHIKV and 129 other arboviruses. The median age for CHIKV cases was 31 (Q_{25}: 18.25, Q_{75}:43.75) and for DENV was 24 (Q_{25}: 18, Q_{75}:36) ($p<0.05$). Headache was associated more with DENV cases (95.4%) and other arboviruses (98.4%) as compared to CHIKV (84.1%). Anorexia, retroocular pain, nausea, dysgeusia, abdominal pain, vomiting and dizziness were more frequent in DENV and other arboviruses than in CHIKV ($p<0.05$). Gastrointestinal symptoms were similar between DENV and other arboviruses cases. Arthralgia was more frequently associated with CHIKV (96.6%) in comparison with DENV (78.8%), and rash was also more frequent in CHIKV (52.3%) than DENV (36.3%). Among those with fever at the time of enrollment, CHIKV cases presented higher median temperature (38.8°C, Q_{25}-Q_{75}:38.5-39.0) compared to DENV (38.2°C, Q_{25}-Q_{75}:38.0-38.8, $p<0.05$). **Conclusion:** Gastrointestinal symptoms and headache were more associated with DENV and other arboviruses cases. CHIKV cases presented higher temperatures associated with the presence of arthralgia and rash. However, the low diagnostic value of these clinical findings supports the continued application of pathogen detection platforms for patients care.

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Long term vaginal shedding of Zika virus in Nicaraguan women.

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Introduction. Case studies have described detection of Zika virus(ZIKV) RNA in vaginal fluids up to 37 days post infection (Sanchez, et al., 2017); however, ZIKV RNA has been detected for 6 months or more in other body fluids like semen.
Objective of the study.To define the duration of ZIKV shedding in the female genital tract, we monitored the presence of ZIKV RNA in vaginal fluid of Nicaraguan women with confirmed ZIKV infection.
Material and Methods. Between October 2016 and November 2017,women ≥18 years of age presenting to public health facilities in León, Nicaragua with symptomaticZIKV infection(confirmed by RT-qPCR in blood, urine, or saliva)were enrolled in this study. Following confirmation of ZIKV infection, these women were asked to provide self-collected specimens of vaginal fluid at 7, 14, 21, 28, 60, 90 and 180 days post onset of symptoms (DPOS). Viral particleswere eluted with PBS from vaginal swab and viral RNA was purified by using the QIAamp Kit. Two separate reactions of one-step real-time RT-PCR (AgPath-ID™) were used for ZIKA RNA detection[Lanciotti et al., 2008]. Any sample with a cycle threshold of (Ct) <38 was defined as Zika-positive.
Results. The mean age of the 14 participantsenrolled was 25 years (range 18 – 60 years); 5 were pregnant and 2 had a concomitant chronic disease. In total, 40 vaginal fluid specimens were analyzed from the 14 participants, of which, 57% (8/14) had at least one positive sample; no woman provided samples at all time points due to menstruation, etc. Of the 40 specimensexamined, 14 (35%) were ZIKV-positive by RT-PCR at any sampling time. None of the 4 samples collected at 7 DPOSwerepositive, but 3/8 (38%), 4/9 (44%), 1/7 (14%), 2/4 (50%),2/5 (40%) and 2/3 (67%) were Zika-positive at 14, 21, 28, 60, 90, 180 DPOS, respectively.
Conclusion. Detection of ZIKV RNA in vaginal fluid between 30 - 180 days after the onset of symptoms is a novel observation, but the presence of ZIKV in vaginal fluid for as long as 6 months has important public health implications for couples aiming for pregnancy and for the potential for sexual or mother-to-child transmission.

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Primers for full genome amplification and sequencing DENV serotypes 1-4.

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Introduction Dengue virus (DENV) is the most prevalent arbovirosis in the world. Infection with DENV results in either unapparent disease (up to 75% of infections) or a spectrum of clinical outcomes, ranging from self-limiting uncomplicated dengue fever, to the more severe life-threatening syndromes. The DENV consist of four antigenically related but genetically distinct serotypes DENV1 to 4; all of them have the ability to cause severe disease. The viral genome is a positive-sense, single-stranded RNA of approximately 11 kb in length, encoding a single open reading frame for three structural and seven nonstructural proteins flanked by 5´ and 3´ non-translated regions. DENV serotypes share 65–70 % amino acid sequence similarity and each one comprises several genotypes, which can vary by 3% at the amino acid sequence and 6–8% at the nucleotide sequence. Genetic differences among DENV genotypes have been associated with virulence and with the severity of the disease; however, many of sequence analyses have focused on envelope gene mainly due to the high cost involved in complete genome sequencing. **Objective**: To develop a simple and affordable method for sequencing complete genome for all four serotypes of DENV. **Materials and methods**: A multiple sequence alignment with genomes from all serotypes was done using Clustal W program and maximum similarity regions were selected to design oligonucleotides. The utility of this set of oligos was tested in PCR reactions in which cDNA from each serotype was used as template. Four primer pairs were selected to amplify contiguous overlapping fragments of approximately 3000 bp spanning the entire sequence of the viral genome. The amplified PCR products were sequenced and edited with the Snapgene software to assemble the full-length genome. **Results:** Designed oligos allowed to amplify the genome of the four serotypes and to obtain sequence fragments near 1000 pb, from which the complete genome for one virus by serotype was assembled. **Conclusion:** The implemented method is simple and useful to get the genomic sequence of any DENV isolate.

Persistence of Zika virus in urine and breast milk samples.

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Zika virus (ZIKV) is a flavivirus mainly transmitted by infected *Aedes* mosquitoes, although blood transfusion, mother-to-child (intrauterine and intrapartum transmission) and sexual transmission have also been described. ZIKV RNA has been detected in serum, saliva, urine, cerebrospinal fluid, semen, between others body fluids in patients with acute illness but also in samples collected up to several months after acute infection. In the tears of infected patients for up to 30 days post-illness, in urine for 6 weeks, in serum and in whole blood up to 2 months and in semen up to six months; however persistence of ZIKV in breast milk has not been well determined. **Objective:** To analyze the persistence of ZIKV in breast milk. **Methods:** Three samples of serum, urine and breast milk obtained from the same patient at different times, were analyzed to investigate the persistence of the virus in those body fluids. Viral RNA was extracted using the QIAamp viral RNA Mini Kit according to the manufacturer's instructions and tested for the detection of ZIKV, CHIKV and DENV using a RT-nested PCR reported earlier. Viral isolation in Vero cell cultures was attempted for all the specimens. Seven days after inoculation, cells were tested by immunofluorescence and supernatants by RT-nested PCR. **Results:** ZIKV was the unique arbovirus detected in the samples. ZIKV was detected in urine and breast milk specimens up to 90 days after the first samples were taken. Attempts for virus isolation were unsuccessful. **Conclusions**: Our results confirm the persistence of ZIKV genomic RNA in breast milk and urine samples up to three months after infection.

Seroepidiology of pregnant women and adverse outcome during Zika emergence

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The Zika epidemic has underscored the importance of tracking emerging infectious diseases, particularly in at risk populations such as pregnant women; however, expeditious implementation of surveillance systems is a major challenge. In León, Nicaragua, we established a cohort of pregnant women in 2016 (n=253) with the following objectives: 1) Monitor for adverse fetal outcomes associated with Zika infection 2) Determine the rates and risks for vertical Zika transmission in maternal infection 3) Establish cost-effective methods for surveillance of emerging pathogens in pregnant women that could be readily implemented in resource-limited settings. Our interim analysis is presented here (n=123). We leveraged the existing public health infrastructure for prenatal care to follow women from presentation through birth. We then utilize a reverse chronology testing algorithm to serologically screen samples for evidence of recent and remote Zika infection, finding the vast majority of women in this region (98%) have IgG reactivity in a Zika antigen capture ELISA. A very high degree of correlation between IgG results using cord blood or maternal peripheral blood (98% concordant, R^2=0.814, p<0.0001) was observed, indicating that cord blood can effectively be used for serologic studies on pregnant women for clinical, research, or epidemiologic purposes without having to perform venipuncture on the mother. We next used a 96 well, high throughput screen for Zika-neutralizing antibodies, defining positive as an estimated FRNT50 values of 800 or greater (64% positive). On a subset of samples, full neutralization testing and NS1 BOB assay were used as confirmatory tests, with 78% of screen-positive results validated by one or both tests. Taken together, these data indicate high rates of Zika infection (approximately 50% of this cohort). Adverse outcomes occurred in 7% of pregnancies; however, rates did not differ by Zika FRNT50 screen positive (5/73=7%) vs negative (3/41=7%). Our study is limited by incomplete testing for alternative cause of congenital anomalies. Analysis of the remaining subjects is ongoing and will determine if risk for adverse pregnancy outcomes segregates by our screening method or other serologic variables. This attractive approach to efficiently gather seroepidemiologic information could be applied to other outbreak settings.

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Guillain Barré Syndrome during the Zika outbreak in Mexican southeast

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Abstract.
Introduction. There is an increase in the incidence of Guillain Barré cases during the Zika outbreak of 2015 to 2017 in Latin America, however the Zika virus is not always identified as the precise causative agent, resulting in disparate percentages in the different series of cases studied.

Objective. To determine the incidence of cases and the direct causal relationship between Guillain Barré and Zika virus during 2016 and 2017, in a general hospital in the city of Veracruz, Mexico.

Material and methods. We documented the suspected cases of Zika that had acute flaccid paralysis, and verified Guillain Barré according to Brighton criteria (clinical, cranial tomography, cerebrospinal fluid analysis, neuroconduction studies) during the 2016 and 2017 seasons. presence of Zika, Dengue and Chikungunya (PCR-RT-serum/urine-, IgM/IgG); extending to HIV, Hepatitis B/C, TORCH, Enterovirus, Campylobacter, Brucella and Salmonella in 2017.

Results. A total of 14 patients were documented (8 in 2016 and 6 in 2017), the male gender was the most affected and the age group was 50-59 years old; September had a higher incidence in 2016 with 7 cases, January with 3 cases in 2017; all were negative to Zika; He highlighted that the cases of 2017 were positive for Campylobacter (only two with acute diarrhea), one case with IgM+ to Dengue and another case with IgM+ Chikungunya; there was a report of IgG+ to Zika and Dengue, however it was considered a cross reaction with other arboviruses. All were treated with immunoglobulin, 13 had good functional prognosis (Hugues initial 4, Hugues final 2), a death was reported. Incidence rate 0.039 individuals/year, cumulative incidence 3.9% during 2016; The 2-year incidence rate was 0.020 individuals/year.

Conclusion. There was an increase in the incidence of Guillain Barré; however, there was no direct causal association with Zika; there was evidence of involvement by Dengue and Chikungunya in two individual cases; in the cases of 2017, the presence of Campylobacter predominates, the asymptomatic carrier state prevailing, this characteristic being able to favor or predispose the appearance of the cases of Guillain Barré together with the presence of viral co-infections.

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Pregnancy and Zika Disease, Surveillance Method for Vaccine Efficacy Trial

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Introduction: Surveillance for suspected or confirmed cases of specific diseases allows the capturing and classification of cases for the assessment of efficacy in vaccine clinical trials. In the specific case of Zika Virus Disease surveillance, there are major limitations including the lack of a fully characterized disease epidemiology, and the partially understood burden of disease.

Objective: To develop a surveillance methodology for detection and follow-up of Zika Virus infection cases in pregnant women and their infants up to 1 year of age, in the context of the preparation for a phase III vaccine efficacy trial.

Methods: A systematic review of current validated national guidelines for Zika surveillance in pregnant women and newborns in 4 countries of Latin America and the US was performed. The analysis included a comparison between the documents and with those from other international and regional organizations. A Consensus Surveillance Method document was drafted and shared with clinical experts on the topic in the selected countries; the background of these experts included neuropediatrics, pediatric infectious diseases, obstetrics/gynecology and perinatology.

Results: After a systematic review of available country guidelines and the supporting capabilities in the clinical trial target countries, the Experts group produced a proposal for a regional Zika disease surveillance methodology for pregnant women and newborns that could be integrated in the assessment of cases for vaccine efficacy phase III trials. This document proposes parameters for the identification of probable Zika infection in pregnant women, ascertaining a probable Zika infection in the fetus and defines early findings of Congenital Zika Syndrome (CZS) for the follow-up of infants during the first year of life.

Conclusions: Vaccine efficacy trials should have strong disease surveillance methodologies that enable the demonstration of the efficacy or futility of the intervention. In the case of a Zika vaccine candidate efficacy phase III trial, it is important to have a clear suspected clinical case definition, laboratory tests for their confirmation, and other specific surveillance methods for subgroups of interest (i.e. pregnant women and their children). All these actions will increase the likelihood for a Zika vaccine candidate efficacy clinical trial to prove its hypotheses.

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ADVERSE BIRTH OUTCOMES ASSOCIATED TO ZIKA VIRUS EXPOSURE DURING PREGNANCY

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Introduction
Zika virus (ZIKV) infection has been associated with severe birth defects, including newborn microcephaly and neurological complications. Intrauterine abnormalities have also been associated with ZIKV infection during pregnancy. These abnormalities reflect the teratogenic effect of this arbovirus and are now collectively referred to as congenital Zika syndrome (CZS).
Objective
This descriptive study reports clinic outcomes in newborns exposed to ZIKV during pregnancy, from February to October 2016, in São Jose do Rio Preto, São Paulo, Brazil.
Material and methods
After a ZIKV qPCR positive in urine or blood samples of Zika-suspected pregnant women, 54 ZIKV-positive women, from 216 Zika-suspected cases, were included in the study.
Results
None of the women miscarried, and 15 (27.8%) exhibited adverse outcomes at birth. The highest number of ZIKV infections occurred during the second and third trimesters. No cases of microcephaly were reported, though the broad clinical spectrum of outcomes included lenticulostriate vasculopathy, subependymal cysts, unilateral abnormal otoacoustic emission test (OAE) results, and chorioretinitis. The phylogenetic analyzes showed that the ZIKV identified in our mothers during outbreak in 2016 was the same virus circulating in other areas of the country.
Discussion and Conclusion
Among the 15 newborns with adverse outcomes, eight (53.3%) had ZIKV-positive samples. Although other studies have associated many newborn outcomes with ZIKV infection during pregnancy, adverse outcomes were mild or non-existent in our newborns, but their occurrence may affect neurological development, thus having an important negative impact on the patient specifically and on the population more generally. The clinical presentation of CZS in newborns in our study was mild compared to other reports, suggesting that there is significant heterogeneity in CZS rates and severity.

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Co-infection between Zika and Dengue during Zika outbreak in Brazil

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São José do Rio Preto Regional School of Medicine Foundation (FUNFARME). São José do Rio Preto, São Paulo, Brazil.

Introduction
Prior to the arrival and subsequent spread of Zika virus (ZIKV) in Latin America and the Caribbean, Dengue virus (DENV) was the predominant arbovírus. With the recent increase of ZIKV and Chikungunya (CHIKV) circulation worldwide, several instances of human co-infection have been reported and characterized, being co-infection with more than one DENV serotype associated to more severe forms.
Objective
We reported twelve cases of co-infection of ZIKV and different DENV serotypes in a city located in the northwest region of São Paulo State, Brazil, which is hyper-endemic to Dengue.
Material and Methods
Between January and November 2016, 1,254 suspected cases of arboviral infection were available by our surveillance program in São José do Rio Preto. All suspected patients were examined and, when they were arboviral disease-suspectd, had sera separated and viral RNA analyzed by PCR/qPCR assays to determine the diagnosis of DENV 1-4, ZIKV, or CHIKV in the same samples. After the molecular results, twelve patients with ZIKV-DENV coinfection were identified and their clinical and laboratory characteristics were described.
Results
The mean between symptoms onset and collected sample of 3 days. DENV-1 was identified in seven co-infected patients and DEN2 in other five. Two patients presented alarm signs of Dengue and no one was hospitalized.
Discussion and Conclusions
The knowledge about arboviral coinfection is limited, and few cases are already described in the world. The impact of coinfection is also unknown, and co-circulation of theses arboviruses should be faced as a serious public health issue and challenge, in terms of transmission dynamics, vector competence, clinical spectrum, and health outcomes and complications. The constant presence of co-circulating arboviruses increases the chance of co-infection and demonstrates the importance of the differential diagnosis, especially during periods of arboviral outbreaks. The impact of this co-infection is known individual and collectively.
Word count: 297 words.

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Dengue and Zika IgM-antibodies in pregnant women in Tegucigalpa-Honduras 2016-2017

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Introduction

Zika infection is often described as mild or asymptomatic, nonetheless the infection in pregnant women is associated with congenital abnormalities. It has been shown the difficulty to differentiate Zika infection from Dengue serologically in endemic areas, due to cross-reaction. Dengue is considered endemic and one of the most important public health problems in the country.

Objective of the study

To determine the prevalence of IgM Dengue and Zika antibodies in pregnant women from Tegucigalpa, Honduras during 2016-2017.

Brief material and methods

A total of 1,429 plasma samples from pregnant women, 1,329 enrolled in 2016 and 100 during 2017 were included in the study. All were asymptomatic. The 1,429 samples were tested by *Panbio® Dengue IgM* Capture *ELISA (Alere* TM US). A subgroup of 50 samples, were randomly tested by Zika IgM InBios ZIKV *Detect*™ (InBios US).

Results

Among the 1,429 samples tested for Dengue IgM antibodies, 19% (267/1,429) were positive: 19% for 2016 (257/1,329) and 10% for 2017 (10/100); p=0.02.

Out of 50 samples tested for Zika IgM antibodies, 50% (25/50) were positive; 57.5% for 2016 (23/40) and 20% for 2017 (2/10); p=0.03. From these 50 samples: cross reactions were observed in 14% (7/50); 24% Dengue seropositivity (12/50) and 36% only for Zika (18/50). There was 12.5% (5/40) cross reactions in 2016 and 20% (2/10) in 2017, p=0.5; positivity only for Dengue 12% (5/40) in 2016 and 70% (7/10) in 2017, p=0.00014; positivity only for Zika: 45% (18/40) in 2016 and none in 2017.

Discussion/conclusions

Since the introduction of Zika in Honduras, main attention is to pregnant women. These results suggest that Zika had a statistically significant higher circulation in 2016 when the virus was introduced and the later outbreak of Zika p=0.03, one must keep in mind that Dengue is endemic in Honduras, with statistical significance between the prevalence's by year 2016 & 2017 p=0.02; p=0.00014. Zika serology could be a useful tool to report evidence of possible Zika virus infection during pregnancy.

Funding of research

Dirección de Investigación Científica y de Posgrados (DiCyP) - UNAH, Tegucigalpa-Honduras.

Prevalence of Zika IgM-antibodies in asymptomatic-pregnant women in Honduras 2016-2017

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Evaluation of a Commercial SD Dengue Virus NS1 Antigen Capture Enzyme-Linked Immunosorbent Assay Kit for Early Diagnosis of Dengue Virus Infection

Introduction

The emergency of Zika infection was declared globally in February 2016. The infection is asymptomatic in approximately 80% of cases. There is an association between Zika infection during pregnancy and serious congenital syndrome, therefore when there is virus circulation, pregnant women should be studied for the presence of this infection.

Objective of the study

To determine the prevalence of Zika IgM in asymptomatic pregnant women collected in Tegucigalpa Honduras, during the period of 2016-2017.

Brief materials and methods

Fifty plasma samples were tested from asymptomatic pregnant women, possibly exposed to Zika virus during the outbreak in 2016 and the following year. Samples were collected between March 2016 to September 2017 during prenatal visit in different health centers from Tegucigalpa, Honduras, then they were analyzed by the FDA approved serological method Zika IgM InBios ZIKV *Detect*™ (InBios US).

Results

The global seropositivity was 50% (25/50). Twenty three samples out of the 40 collected in 2016, were positive (57.5%). Only 20% (2/10) were positive from 2017. Clearly there is a significant decrease in infection rates between year 2016 to 2017; probably due to the pre-existing immunity in the population or due to the low circulation of the virus in this geographical area.

Discussion/conclusions

With the results obtained in this study we can report high rates of presumptive infections by the Zika virus in 2016, in a vulnerable population such as pregnant women, and a decrease seroprevalence in 2017 in Tegucigalpa, Honduras. It is important to emphasize that it is a serological method and Honduras is an endemic dengue area, the results should be considered with caution.

Funding of research

Dirección de Investigación Científica y de Posgrados (DiCyP) - UNAH, Tegucigalpa-Honduras.

ZCD rRT-PCR in patients with suspected Zika infection Honduras-outbreak 2016

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Introduction

There are several efforts to develop molecular techniques with high sensitivity to accurately detect Zika infection in suspected cases. In 2016 it was published a molecular test a simple-reaction multiplex reverse transcription PCR for detection of Zika, Chikungunya and Dengue viruses (ZCD). We evaluated the usefulness of this test in a set of samples with suspicious Zika infection.

Objective of the study

To evaluate a set of samples using ZCD rRT-PCR with one of two PCR runs positive in the Lanciotti (et al 2008) method.

Brief materials and methods

A total of 50 samples were analyzed (45 plasma and 5 urine samples) of symptomatic patients with Zika suspected infection, collected during Zika outbreak in Tegucigalpa, Honduras in 2016. The samples were taken in the first seven days of the onset of symptoms. All samples were positive with the first pair of primer set in the Lanciotti et al protocol 2008 (PCR1: CT values range of 28.2-38.2), and negative with the second set of primers (PCR2).

Results

With ZCD rRT-PCR, an increase of 12% in positivity (6/50) for Zika was observed. It was also found 2% (1/50) of Dengue-Zika co-infection and 2% (1/50) of Chikungunya infection. All participants had symptoms compatible to Zika infection and were collected during the months of the highest incidence in the country.

Discussion/conclusions

The increase in positivity observed using ZCD method in a 12%, shows its usefulness in the detection of Zika infection, otherwise these cases would have been considered as negative by using the Lanciotti method. Although in general, the multiplex assays are usually considered less sensitive, in this study we reported an increase positivity rate and the possibility to detect simultaneously other pathogens and were able to report and differentiate the infecting agent.

Funding of research

Dirección de Investigación Científica y de Posgrados (DiCyP) - UNAH, Tegucigalpa-Honduras.

LIMITATIONS TO AN EFFICIENT ARBOVIROSIS SURVEILLANCE IN RIO DE JANEIRO-BRAZIL

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The epidemic history of Zika virus (ZIKV) revealed the risk associated with emerging pathogens and exemplified that preparation for the worst scenario is necessary. As ZIKV infection shares signs and symptoms with other arboviruses infection, differential clinical diagnosis is not unequivocal. Due to this, laboratory diagnosis is crucial in areas of arboviruses co-circulation, as in Brazil. Depending on the window of infection, the combination of different methodologies can be essential for the disclosure of the etiological agent. So, the aim of this study was to evaluate clinical samples of patients with potential arbovirus infection through molecular and serological tools. For this, 2,054 patients presenting fever and/or skin rash, followed from March 2016 to November 2017 at Emergency Care Units in the city of Rio de Janeiro, were mainly screened for ZIKV, dengue (DENV) and chikungunya (CHIKV) viruses by qPCR. Serology was performed for patients with negative molecular diagnosis, whose blood collection date varied between 5-15 after the onset of symptoms. Cross reactivity between ZIKV and DENV was evaluated by ELISA. ZIKV was quantified in blood and urine specimens by qPCR. We observed 21% of positivity by qPCR (Zika- 6%, dengue- 3% and chikungunya- 12%). When molecular analysis is performed up to 4 days after the onset of symptoms, 50-100% diagnostic positivity can be achieved for ZIKV, whereas for DENV and CHIKV this number reaches 30%. Serological tests performed on 578 samples allowed the attribution of diagnosis in 25% (147/578) of suspected cases previously negative by qPCR. Among 7 (100%) samples positive for ZIKV through qPCR, 5 (71%) demonstrated cross reactivity for DENV IgM and 2 (29%) were classified as borderline. Selected negative samples were investigated for Mayaro and Yellow Fever viruses and tested negative. Comparing the viral load of ZIKV in blood and urine from 35 patients, the mean value of Ct was 29 in both specimens, corresponding to 833.8-1271.8 copies/uL. Inclusion of serology in epidemiological surveillance may increase the number of positive cases, especially when the patient is out of the convenient window of molecular diagnosis. However, cross reactivity between DENV and ZIKV deserves attention to avoid false misdiagnosis.

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IN-HOUSE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR ZIKV ANTIBODY DETECTION

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Zika virus (ZIKV) is an emerging flavivirus transmitted by *Aedes spp.* mosquitoes and is currently circulating in South America, where dengue (DENV) and chikungunya (CHIKV) viruses have been co-circulating. Diagnostic testing for ZIKA virus infection includes virus isolation, as well as molecular and serological methods. However; due to the high rates of antibody cross reactivity between DENV and ZIKV even if an immunoglobulin (Ig) M result is positive or negative, performing a plaque reduction neutralization test (PRNT) or microneutralization (MNT) is needed to confirm the diagnosis. With a newly developed ELISA using antigen from the circulating ZIKV strain in the Americas, we analyzed 52 paired human serum samples obtained from a multicenter, IRB-approved protocol in Colombia, Honduras, Venezuela and Peru during acute (enrollment within 5 days of symptom onset) and convalescent phases (2 weeks) of illness. Acute samples were tested by RT-PCR for ZIKV (25 were positive and 27 negative). RT-PCR for DENV and CHIKV were all negative. Paired samples were tested with ELISA-IgM for DENV, CHIKV, and ZIKV; and MNT for ZIKV were performed as confirmatory testing. From the RT-PCR positive group; 24 were negative and 1 positive by IgM (acute samples) while 24 were positive and 1 was negative by IgM in convalescent sampling. From the RT-PCR negative group; 25 were negative and 2 were positive by IgM (acute sample), while 22 were negative by IgM and 5 samples were positive in convalescent cases. Testing for co-circulating arboviruses with IgM showed cross reactivity of 11 convalescent samples to DENV and none to CHIKV. The correlation between ZIKV ELISA-IgM and RT-PCR showed 96% sensitivity (95%CI: 82.78-99.92%) and 81.4% specificity (95%CI: 88-94%); whereas, correlation between ZIKV ELISA IgM and MNT assay revealed 96.67% (95% CI 82.78 to 99.92%) and 100% (95% CI 84.56 to 100 %) sensitivity and specificity, respectively. The development of an in house ZIKV ELISA-IgM from a locally circulating strain, could improve serologic diagnostics and discriminatory capabilities for ZIKV infections in flavivirus endemic areas.

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NEUTRALIZING ANTIBODIES TO YFV mIGHT protect against zIKA cONGENITAL sYNDROME

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The increase in the number of cases of microcephaly demonstrated related to Zika virus (ZIKV) infection during the pregnancy has led the scientific community to seek answers about the reason, since in other populations this fact has not been observed or detected before. The co-circulation of several flavivirus in this population has raised hypotheses about the existence of antibodies derived from prior infection or by vaccination for different virus may be influencing the severity of the disease. This study aims to analyze the levels of antibodies against yellow fever in ZIKV-infected pregnant women to ascertain the existence of correlation between outcomes in newborns and mothers' antibodies levels. We analyzed the serum of 34 pregnant women with ZIKV confirmed by qPCR by seroneutralization assay angainst Yellow Fever virus. These samples came from 2 different cohorts from two different cities in the country with different politics regarding YFV vaccination. The result obtained was compared through geometric means and the clinical and/or virological outcomes in newborns using a non-parametric 2 Sample T-test. The antibodies titles were compared to two categories based on outcomes: i) with outcome (clinical, radiological and/or virological manifestation in the newborn); ii) no outcome (no clinical, virological or radiological manifestation in the newborn). We found in the with outcome group 49.1 of geometric media and 135.9 to no outcome group. In this analysis, a significant difference was observed between neutralizing antibodies titles against Yellow Fever and clinical outcomes in newborns exposed to ZIKV in pregnancy, when we observed the means (p=0.0326). This data suggests that the YFV vaccine may provide a degree of protection to Zika virus manifestation in newborns exposed to ZIKV in pregnancy.

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RT-QPCR APPROACH FOR DETECTION OF ZIKA AND CHIKUNGUNYA IN *Aedes spp.*

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The global public health threat caused by the spread of arboviruses as Chikungunya and Zika increase the need for better surveillance plans that include early detection of mosquito-borne viruses co-circulating in the same vector in Panama. Consequently, it is important to implement sensitive and specific molecular tools able to detect very low viral load in field-caught mosquitoes. We engined a RT-qPCR assay based upon the specific amplification of the RNA polimerase gene of Chikungunya (NSP4) and Zika viruses (NSP5) by retrieving and analyzing all complete genomes of Zika and Chikungunya virus available in the GenBank obtained from America. All conditions for both assays were set independently in a SYBR green based RT-qPCR following the MIQE guidelines and the in silico exclusivity and inclusively assays performed indicated specificity for both targets as confirmed by conventional RT-PCR amplification and Sanger sequencing of the obtained amplicons of 174bp and 163bp that showed an average melting temperature of 81°C and 80°C for Zika and Chikungunya, respectively. No primer dimmers, unspecific products and Ct changes were detected in the reaction and melting curve when the two sets of primers were used in a single or duplex RT-qPCR amplification reaction. Also, we designed specific Taq man probes by the sofware primer3 included in the bioinformatic package Ugene 1.27 and set a Taq man assay using the primers of the SYBR green approach which specificities were evaluated in silico using the software Primer-BLAST. The preliminary results point out that both Taq man approaches specifically detect Zika and Chikungunya viruses and the probes do not incorporate in any off-target gene or RNA polimerase genes from other related viruses as Dengue. Additionally, further assays will allow us to assess the sensitivity of the SYBR green and Taq man approaches individually or in combination in a multiplex RT-qPCR to detect and quantify viral load of both viruses in *Aedes spp.* mosquitoes.

Keywords: Chikungunya, Zika, multiplex RT-qPCR, field-caught mosquito.

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SEVERE YELLOW FEVER OUTBREAK IN THE SOUTHEAST REGION OF BRAZIL, 2017: LABORATORY SURVEILLANCE IN CASES CONFIRMATION

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Introduction. In 2017, Brazil experienced the largest outbreak of Yellow Fever (YF) observed in the last decades. The epidemics has started in Minas Gerais State and has rapidly spread eastward, reaching the states of Bahia, Espirito Santo, Rio de Janeiro and São Paulo; including an extensive area in close proximity to major urban centers where yellow fever vaccine coverage is low. **Objective.** Here, we report the results of the molecular and serologic detection tests for diagnosis of yellow fever infection during the Brazilian epidemics in 2017. **Materials and Methods**. Between January 1st and April 30rd 2017, 1288 samples of human serum, blood, plasma, CSF, urine, fragments of liver, spleen, heart, lung and kidney collected from 970 patients included as an YF suspect case were performed by real-time Reverse Transcription Polymerase Chain Reaction (real time RT-PCR) and IgM-capture enzyme-linked immunosorbent assay (ELISA) to evaluate the effectiveness of the diagnostic tool during the yellow fever outbreak. **Results.** Using the RT-PCR protocol, YF infection was confirmed in 31.4% of serum samples. IgM serology presented 35.4% of confirmation in serum. CSF samples presented 9% positivity with RT-PCR protocol and 50% positivity with IgM protocol. Urine samples sent to RT-PCR detection presented 28% of positivity. The performance of the RT-PCR and IgM serology in serum samples stratified by the day of onset of symptoms shows that the molecular method was more efficient until the sixth day of illness, and IgM serology was more efficient after the eighth day of illness. **Conclusion**. Considering that, the molecular diagnostic methods represent essential tools for early diagnosis, as they are able to detect infections during the viremic phase, early detection of cases is crucial to provide efficient patient management, rapid outbreak response and emergency vaccination measures.

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Performance Comparison of two commercial RT-qPCR tests for detection of Zika virus RNA on clinical samples

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Introduction: Zika virus represents a challenge for diagnostic labs and urgent data is needed for testing of commercial tests on clinical samples. Objective: Verification of the usefulness of two commercial kits for detecting RNA from virus Zika on clinical samples. Materials and methods: 40 retrospectively selected (previously anonymized) clinical samples (20 positive and 20 negative to Zika virus infection) were used following recommendations from CLSI document EP09-A3. The commercial Zika Virus Polyprotein gene Genesig Advanced Kit ® (Primerdesign, UK) and RealStar® Zika
Virus RT-PCR Kit (Altona diagnostics, Germany) were used and results were compared against reference RT-qPCR methods [Lanciotti et al, 2008 (25 copies sensitivity test) and Lanciotti unpublished (confirmatory test-C)]. Each test was ran by duplicate (with exception of confirmatory test) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA). The statistical analysis was performed using Excel Tool Passing-Bablok Regression, V.3 (ACOMED statistic, Germany) and Reliability with Kappa (k) for 2 by 2 tables with CI and McNemar test (ACOMED statistic, Germany). The C reference test was ran at early stages (as part of the regular screening of samples for surveillance purposes).
Results: when only positives were analyzed on reliability tool, using confirmatory test as reference the results were: Altona kit and 25 copies reference test are equivalent; Genesig kit was not equivalent. When only negatives were analyzed the results were: Altona kit had significant deviation from consistency while Genesig kit and 25 copies reference test were both equivalent. Regarding Passing-Bablok Regression the results when comparing against C were: Altona [(slope = 0,8885) and Cq lower for about 5,3553 (intercept)] and 25 copies test [(slope = 0,7278 and Cq lower for about 11,7430 (intercept)] performed slightly lower; Genesig performed poorly (slope = 0,2218 and intercept = 30,4210). Two samples within the negative group of Confirmatory (C) test results were confirmed as positives by the three other tests (Altona, Genesig and 25 copies reference test).
Discussion/conclusion: under this premises we conclude that it is necessary to test
in parallel any sample under suspect of Zika virus infection using at least two tests targeting two different regions of the genome.
Funding of research: National Institute for Public Health Research “Dr. Leopoldo Izquieta Pérez”-INSPI, Guayaquil-Ecuador.

Envelope domain-III based serological assay differentiates Zika from dengue infection

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Zika virus (ZIKV) can cause Guillain-Barré syndrome in some adults and fetal malformations, including microcephaly. Laboratory detection of ZIKV infection by viral nucleic acid or antigen detection assays is limited to a brief period of several days after onset of symptoms. Serology is the only reliable method for detecting infection after the viremic period both in the context of individual patients and population level surveillance. However, most serological assays have limited specificity and utility because Zika and related flaviviruses like dengue virus (DENV) stimulate highly cross-reactive antibody responses. Distinguishing ZIKV from DENV infection is particularly important because these two viruses co-circulate in the same populations and cause similar symptoms including a large number of asymptomatic infections. Since flaviviruses also elicit type-specific antibodies directed to unique epitopes on the infecting virus, we hypothesized that specific domains or subdomains within the ZIKV envelope (E) protein may be better diagnostic antigens than the entire E protein or virion. Guided by comparative epitope modeling of ZIKV envelope protein, we designed two recombinant antigens displaying non-cross-reactive epitopes on domain I (Z-EDI) and domain III (Z-EDIII) of ZIKV envelope protein. Unlike whole Zika virion or soluble envelope protein, the Z-EDI and Z-EDIII antigens consistently detected ZIKV-specific IgG in ZIKV- but not DENV-immune sera in a panel of 22 late (>12 weeks after infection) convalescent serum specimens from

NS1 ELISA Accuracy for dengue diagnosis during an arboviruses epidemic

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Brazil has recently suffered a triple epidemic due to co-circulation of dengue virus (DENV), Zika virus (ZIKV) and Chikungunya virus (CHIKV). Diagnosis of those diseases can be performed, based on clinical-epidemiological and/or laboratory approaches. The concomitant presence of those viruses may result in a difficult differential diagnosis for doctors because the infections caused by those arboviruses share similar signs and symptoms. Although, due to the similarities shared by ZIKV and DENV as they belong to the same family, serological assays show a high rate of cross-reactivity. Here we aimed to evaluate a Dengue NS1 antigen capture assay for early and differential diagnosis of dengue during the zika epidemic occurred in Campo Grande, MS, Brazil in 2016. A total of 227 samples were tested and characterized as follows: RT-PCR positive ZIKV samples (n=57), dengue positive samples (n=51); CHIKV positive sample (n=1), ZIKV/DENV co-infected cases (n=24) and negative cases (n=94), based on the molecular and serological diagnostics tests available in the Viral Immunology Laboratory, IOC, FIOCRUZ. In our study, the Platelia NS1 ELISA performed for dengue diagnosis, showed a specificity of 98.2% based on the analysis of zika confirmed cases. No positive results were observed for zika, but one case presented an inconclusive result. Based on the results obtained here, it is suggested that the Platelia NS1 test is very specific for dengue diagnosis even in the co-circulation with ZIKV. This test exhibited a high accuracy in the non-detection of acute zika infections (93.5%). Our findings showed the non-ability of the dengue NS1 capture test to recognize the NS1 of ZIKV and its potential for cross-reaction. Moreover, this is particularly important for epidemiological surveillance in the choice of the best serological test for the differential diagnosis between dengue and zika.

Key Word: Dengue diagnosis, Zika diagnosis, NS1 antigen capture ELISA

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RT-Loop-Mediated Isothermal Amplification assays for detection of Dengue and Chikungunya in Mexico

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The emergence of arboviruses in America has become an important health problem. During 2015, chikungunya virus (CHIKV) infection was reported in Mexico, and the clinical diagnosis became a challenge in these endemic regions for dengue (DENV). DENV and Zika (ZIKV) are flaviviruses, while Chikungunya (CHIKV) is an alphavirus. All three viruses have a single-stranded RNA genome and can be detected by molecular assays during the first days after the onset of symptoms. However, detection requires the transport of the sample to reference laboratories for diagnosis. Loop-Mediated Isothermal amplification (LAMP) technology is simple, do not require thermocyclers and is rapid to perform. The aim of the study was to develop and standardize a detection reverse transcription-LAMP for CHIKV and DENV genomes. LAMP primers for DENV genome capsid (C), envelope (E1) in CHIKV and NS1 in ZIKV were used. The RT-LAMP assay was performed at 65°C for 1 h, and the amplification products were evaluated by electrophoresis in agarose gels. The evaluation of the assays was performed in 95 acute-phase patient plasma samples collected during the 2015 outbreak in Veracruz, Mexico. Viral RNA was extracted and analyzed both by a two-stage reverse transcription-real time PCR for all serotypes of DENV, CHIKV, ZIKV, West Nile Virus and Yellow Fever Virus detection, and by the standardized RT-LAMP assays. The presence of DENV and CHIKV IgM antibodies was determined by ELISA. The RT-LAMP assay confirmed to be specific to CHIKV, no cross reaction was found with DENV and ZIKV. Acute DENV infection was confirmed by RT-PCR, RT-LAMP and IgM reactivity in 6/95 cases (6.3%). RT-qPCR assay detected 41/95 (43.2%) positive samples for CHIKV, while 45/95 (47.4%) were positive by RT-LAMP assay. Anti-CHIKV IgM was detected in 36/95 (37.9%) samples. Acute CHIKV infection was confirmed by molecular and serological assays in 70/95 cases (73.7%). The evaluation of sensitivity for RT-LAMP CHIKV assay was 78% and specificity was 75% compared to the RT-qPCR. The RT-LAMP assay is a promising molecular tool that can be used for CHIKV genome detection and DENV differential diagnosis in the field for resource limited settings.
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Zika infection in Tegucigalpa, Honduras during year 2016

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Introduction: Zika has become one of the most serious arthropod-borne viral diseases in humans. In 2016 Honduras an estimated 32,113 suspected cases; majority of cases were in pregnant women (n=700), 133 microcephaly, 167 GBS cases. Since the introduction of Zika, efforts have been done for an accurate diagnosis

Objective of the study: To describe the Zika outbreak in Tegucigalpa, Honduras in year 2016

Materials and methods: Analysis of 751 subjects, 464 pregnant women (PW), 24 microcephaly, 69 GBS cases, general population (n=194). Plasma and/or urine were tested by rRT-PCR (Lanciotti 2008 & Wagonner 2016). Plasma from 83 cases were tested by STANDARD-E-Zika/IgM-SD-BIOSENSOR (Republic of Korea). rRT-PCR was done to plasma and urine in 92 subjects

Results: A 37% of positivity for Zika by rRT-PCR & serology is reported [33% by PCR (247/751); 42% by serology (35/83)]. Positivity per group with molecular and/or serological tests was 44% in PW (205/464), 46% in microcephaly cases (11/24), 32% (22/69) in GBS and 19.5% in acute infections (38/194). Positivity by rRT-PCR was: 44% in PW (205/464), 8.3% (2/24) in microcephaly cases, 3% (2/69) in GBS and 19.5% in acute infections (38/194). A Dengue-Zika co-infection and a Chikungunya case were detected using ZCD protocol. Serologically 69% microcephaly cases (9/13), 33% GBS (20/60) and 60% of convalescent patients (6/10).Regarding urine & plasma analysis, 15% were positive only in urine; 21% only in plasma

Discussion/Conclusion: Molecular techniques are the recommended methods for Zika diagnosis. Serology is considered difficult due to cross reactions with other Flaviviruses, an increase in positivity rate was observed when using both methods. Despite that the diagnosis of Zika in newborns and GBS cases is a real challenge because of the low probability of detecting viral RNA, it was possible to determine in 8% of newborns and 3% of GBS by rRT-PCR.This positivity could increase to 46% and 32% respectively if serology is used. We found that both, urine and plasma samples, contribute an added value to the Zika diagnosis, since if both samples were not analyzed there would been lost (15% positivity in urine and 21% in plasma).

Funding of research: Dirección de Investigación Científica y de Posgrados (DiCyP) - UNAH, Tegucigalpa-Honduras.

Dengue severity assessment in patients previously vaccinated against yellow fever.

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Background: The immune response of individuals undergoing to protection by the vaccine against yellow fever (YF) with 17DD strain is highly linked to activation of CD4 + T lymphocytes, CD19 + B lymphocytes, and CD8 + T lymphocytes, generating a modulating effect between the innate response and adaptive, and possibly associated to the evolution of more severe forms of individuals subsequently affected by other arboviruses, such as dengue. The objective of this study was to compare the severity of patients diagnosed with dengue and who were vaccinated with those not vaccinated against yellow fever. Materials and methods: We evaluated 11,448 cases of dengue reported in a Brazilian endemic city and who had information about the yellow fever vaccine status. Demographic, clinical, and laboratory variables were compared to the 2009 World Health Organization (WHO) dengue classification. Two comparison groups were created: the first formed by patients who were vaccinated against YF and the other by non-vaccinated. Results: The statistical models for the two groups were composed of the same variables (sex, age, previous dengue, retroocular pain, fever, headache, myalgia, arthralgia, petechia, rash and hypotensive shock) and with similar odds ratio in both groups. There was no statistically significant difference in the comparison of the variables between the groups ($p < 0.05$), Kendall's tau-b test showed a very poor agreement of the severity of the cases between both groups (Kendall's tau-b = 0.004 ; $p = 0.78$). Conclusion: The YF vaccine previously applied does not represent a risk of more severe cases and neither confer any protection to individuals with dengue.

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Serological markers for hepatitis in patients with clinical suspicion of dengue

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Introduction: Dengue is a disease with a broad clinical spectrum, and it presents as an asymptomatic or symptomatic infection with an undifferentiated fever. Therefore, it is difficult to distinguish dengue from other acute febrile diseases by using only clinical-epidemiological criteria. It is necessary to perform a differential diagnosis with other infectious diseases, such as viral hepatitis.

Objective: To investigate the potential presence of markers for hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV) in patients with clinical suspicion of dengue.

Materials and methods: This is a retrospective descriptive study of patients with clinical suspicion of dengue at primary and tertiary care health services in the city of Fortaleza-CE (Brazil) between February 2010 and March 2016. The participants had at least two of the following signs and/or symptoms: headache, retro-orbital pain, myalgia, arthralgia, rash, and prostration.

In this study, 259 serum samples were analyzed, and they were initially tested for dengue with IgM enzyme-linked immunosorbent assay (ELISA) and/or RT-PCR methods and subsequently with HAV-IgM ELISA (for hepatitis A), HBsAg ELISA (hepatitis B), and anti-HCV ELISA (hepatitis C).

Results: Of the 259 participants, 131 were female and 128 were male. The mean age of the participants was 26.4 years. Of the 259 samples tested for dengue, 163 and 96 yielded positive and negative results, respectively. Of the 163 dengue-positive samples, 85 were of female and 78 were of male participants. A sample from a female participant showed positive results for hepatitis A and dengue. Eight samples showed positive results for hepatitis B, of which 7 also showed positive results for dengue. One sample from a female participant showed positive results for hepatitis C.

Evaluation of reporter Zika virus neutralization test in a flavivirus epidemic area

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Introduction: PRNT is the gold standard for flavivirus serologic test, but is labor intensive, technically complex and not easily adaptable for high throughput analysis. Alternatively, Reporter Virus Neutralization Test (RVNT) has also been used in flavivirus diagnosis, showing faster result as well as high sensibility/specificity. Thus, this assay may contribute to Zika virus (ZIKV) serological diagnostics, especially during outbreaks or in epidemic area. This study aims to evaluate ZIKV m-Cherry RVNT in comparison with ZIKV R-luc RVNT and PRNT using samples from a flavivirus endemic area. **Materials and methods:** It was selected 36 ZIKV RT-qPCR positive samples from Brazilian outbreak tested in Evandro Chagas Institute. Previous antibodies against flaviviruses were confirmed by Hemmaglutination Inhibition test. They were divided into two groups based on time of disease: <5 days and >5 days of disease onset. Samples were tested by $PRNT_{90,}$ R-luc and mCherry ZIKV $RVNT_{90}$, and DENV R-luc RVNT following Shan et al (2017) protocol. **Results:** Seven samples were positive (19.4%) by ZIKV $PRNT_{90,}$ showing neutralization titers ranging from 1:20 to 1:320. However, for $RVNT_{90}$, the percentage of positivity increased to 61.1% for Rluc ZIKV and 52.7% for mCherry ZIKV, as well as increased titers of about 9 folds. The analysis based on time of disease showed that the titers of samples with less than 5 days of disease onset were low for ZIKV and DENV, but the number of DENV positive samples was higher comparing to ZIKV. After 5 days of disease, all samples were positive for DENV and ZIKV by RVNT, and just 3 samples showed antibodies titer for ZIKV more than 4 fold higher than DENV. **Discussion/conclusion:** Despite the fact that Rluc ZIKV seems more sensitive than mCherry ZIKV, both $RVNT_{90}$ assays showed better performance than conventional PRNT, corroborating the previous studies. However, use of a reporter virus panel including other flavivirus is necessary to better evaluate these new $RVNT_{90}$ assays regarding the well-known cross-reactivity inside the genus, especially in flavivirus endemic regions such as Brazil.

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Hemophagocytic lymphohistiocytosis associated to Chikungunya virus infection in Colombia

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Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening, hyperinflammatory syndrome, characterized by the uncontrolled activation of macrophages and T cells, eliciting key symptoms such as persistent fever, hepatosplenomegaly, pancytopenia, hemophagocytosis, hyperferritinemia, and coagulopathy. Viral infections are frequently implicated in the onset of active HLH episodes, both in primary, genetic HLH as in the secondary, acquired form. Despite that, and a large epidemics of Chikungunya (CHIKV) no cases have been associated with this arbovirus. **Methods:** Case report and systematic review of literature. **Results:** A 42-year-old man consult for 15 days of fever, rash and evanescent symmetrical polyarthralgias predominantly in trunk and upper limbs and hepatosplenomegaly. Laboratory tests evidence thrombocytopenia and anemia without leukopenia, hyperbilirubinemia (indirect fraction), elevated ESR, high ferritin, positive serology (IgG/IgM) for CHIKV. A bone marrow aspirate showed lymphohistiocytosis and haemophagocytosis. **Discussion:** HLH, can be familiar or acquired, it is rare cause of fever of unknown origin. Given the growing epidemic of arboviral infections, including CHIKV, it is necessary to describe the finding of hemophagocytic lymphohistiocytosis, triggered in this case by such alphavirus infection. HLH is a multisystem condition in this case associated with CHIKV infection. Given the context of CHIKV as endemic disease in Colombia and other countries in Latin America, other similar cases can occur.

Highlighted Poster

DEVELOPMENT OF SEMI-QUANTITATIVE BLOCKADE-OF-BINDING ELISA TO DETECT ZIKA VIRUS EXPOSURE

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Zika virus (ZIKV) serological diagnostics are compromised in areas where dengue viruses (DENV) co-circulate due to their high proteome homology and require confirmation of positive results with more specific tests (i.e. neutralization assays). Here we describe the development and optimization of a Blockade of Binding (BOB) Enzyme-Linked Immunosorbent Assay (ELISA) for the semi-quantitative detection of ZIKV-specific antibodies. Parameters evaluated during assay development were aimed at optimizing the detection of ZIKV-specific antibodies in subjects with prior DENV exposure. Multiple monoclonal antibodies specific to ZIKV NS1 were characterized and evaluated for specificity and ability to compete with ZIKV and other flavivirus immune sera. Analysis of serum samples from PCR positive virologically confirmed ZIKV (VCZ) subjects, before and after infection, with previous DENV immunity were utilized to evaluate specificity and sensitivity of the assay. In addition, longitudinal analysis was performed using serially collected samples from subjects with VCZ infection to examine the durability of the ZIKV immune response in the BOB ELISA. Collectively, the results of this study demonstrate that the method is specific and suitable for the semi-quantitative detection of ZIKV-specific antibodies despite prior DENV exposure.

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CHARACTERIZATION OF POTENTIAL RISK FACTORS ASSOCIATED WITH DENGUE MORTALITY IN BRAZIL

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Dengue is a major arboviral disease in tropical and subtropical regions worldwide. In Brazil, since dengue introduction in the 80's more than 15 million cases have reported. Currently, the country experiences a hyperendemic scenario, with the co-circulation of the four serotypes and occurrence of secondary infections. The disease has a spectrum ranging from clinically asymptomatic to a more severe outcome, and shock may progress to death. Here, we analyzed the clinical, epidemiological and viral parameters in dengue fatal and non-fatal cases, in order to characterize potential risk factors related to the evolution to death. A total of 4521 fatal (n=189) and nonfatal (n=4344) dengue fever cases received during epidemics occurred in 30 years (1986-2015) in Brazil, were subjected to viral detection, serological and molecular methods in order to study the influence of gender, age, serotype, viremia, NS1 antigenemia and type of infection (primary / secondary) as risk factor to a fatal outcome. DENV-2 was more associated with fatal cases, followed by DENV-3 and DENV-4. Moreover, DENV-1, -3 and -4 were more frequently on deaths occurred in elderly, while DENV-2 on both children and elderly. No association among the type of infection and the evolution to a fatal outcome was observed. Nevertheless, children 15 years old and under, presenting a secondary infection had almost a 4-fold risk of death. Overall, higher NS1 levels and viral load were observed in fatal cases when compared to non-fatal ones, in all serotypes. Except by the DENV-1, the fatal cases from the three other serotypes presented higher NS1 antigenemia. Dengue is a multifactorial disease and factors such as viral strain, serotype, occurrence of secondary infections with heterologous serotype and co-morbidities may lead to a severe outcome.

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NS1 antigenemia and viraemia load: potential markers of progression to dengue fatal outcome?

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Dengue is a worldwide problem and, in Brazil, a hyperendemic scenario has been established during the past 30 years. The disease pathogenesis is a multifactorial and factors such as viral strain/serotype, occurrence of secondary infections and co-morbidities may lead to a severe outcome. Considering the viral component, it is known that high DENV load or secreted NS1 levels have been associated to a more severe disease in endemic populations. We aimed here,to characterize the NS1 antigenemia and viraemia in dengue fatal and non-fatal cases, as potential markers of progression to a fatal outcome. NS1 antigenemia and viraemia were determined in confirmed dengue fatal cases (*n*=40) and non-fatal cases (*n*=40), representative of the four serotypes, occurred in Brazil analyzed from 1990 to 2013. Overall, the NS1 antigenemia were significantly higher on dengue fatal cases when compared to non-dengue fatal ones. No differences were observed on NS1 levels between non-fatal and fatal primary cases, however, fatal cases from secondary infections showed significantly higher NS1 levels. DENV-1 showed significantly higher NS1 antigenemia, followed by DENV-3, DENV-4 and DENV-2. On DENV-2 and DENV-4 fatal cases, the NS1 levels were significantly higher that those observed on non-fatal cases. Despite the high NS1 levels observed on DENV-1 and DENV-3, no differences were observed between fatal and non-fatal cases from those serotypes. A higher viral load was reported for dengue fatal cases in comparison to the non-fatal ones, but differences were not statistically significant. DENV-3 and DENV-4 had higher viraemia compared to DENV-1 and DENV-2. Moreover, DENV-2 and DENV-3 fatal cases had significantly higher viral load compared to the non-fatal pairs. Fatal cases presented higher NS1 levels and viremia, despite the type of infection. As dengue pathogenesis is multifactorial, viral components, such as NS1 and viraemia, may be factors influencing the disease outcome. However, the host immune status, comorbidities and access to adequate medical support cannot be ruled out as interfering in the disease outcome.

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Performance characteristics of the Trioplex RT-PCR and its Global implementation

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The diagnosis of Zika, dengue and chikungunya is challenging in areas of co-transmission. In response to this public health emergency, the Centers for Disease Control and Prevention developed the Trioplex Real Time RT-PCR Assay designed for the simultaneous detection of dengue, chikungunya and Zika virus RNA in a variety of human clinical specimen types. We determined the analytical and clinical performance characteristics of the Trioplex in detecting each target virus serum, urine and whole blood-EDTA specimens. The assay was adapted to RNA extraction, PCR equipment and procedures commonly found in US and international public health laboratories. To determine the limit of detection (LoD) of the Trioplex for each target virus on each these modalities, inactivated dengue, chikungunya and Zika viruses were suspended in human serum, urine or whole blood and diluted to undetectable levels. The overall LoD for the assay was determined to approximate 10^3 genome copy equivalents per milliliter of specimen (GCE/mL) for every target virus. The assay modality that achieved the highest sensitivity included RNA extraction from 1 mL of specimen. Independent studies confirmed that the sensitivity of Trioplex is similar to other CDC and non-CDC tests; and that the Trioplex modality with the highest sensitivity compares to the most sensitive commercial tests. In order to determine the clinical sensitivity of Trioplex during the first 6 days of illness, 373 concurrently collected serum, urine and whole blood samples from patients with positive Zika IgM after 7 days were tested. In 373 confirmed cases in Puerto Rico, the Trioplex detected 85% (317/373) in serum, 83% (311/373) in urine and 82% (285/347) in whole blood. Testing simultaneously collected serum and urine provides an additional 3% sensitivity over serum alone; whereas the value of testing serum-whole blood provides an additional 5% over serum alone. The high sensitivity of the Trioplex demonstrates the utility of the assay resolving Zika cases in endemic areas. More than 39 thousand Zika cases in Puerto Rico have been confirmed with the Trioplex. Globally, more than 300 laboratories implemented the Trioplex and tested nearly 2.5 million samples.

Word count: 340

Zika Virus Isolations beyond symptomatic period in Puerto Rico

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ZIKV is transmitted by a mosquitos, but it can also be acquired through sexual contact, blood transfusions, or congenitally. For these reasons, there is a great deal of interest in determining the length of duration of infectious ZIKV in human specimens with a special emphasis on semen. The objective of our study was to isolate ZIKV from serum, urine and semen specimen from quantitative RT-PCR confirmed Zika positive symptomatic individuals, and to establish the cycle threshold (Ct) limit in the CDC Real Time RT-PCR Trioplex assay where virus isolation is likely successful. Specimens for virus isolation were from individuals identified through passive surveillance and a prospective cohort of ZIKV RT-PCR positive participants. All of the virus isolations were in Vero cells and two subsequent passages in Vero or C6/36 mosquito cells. Initially, we attempted to quantify infectious virus among ZIKV RT-PCR positive serum, urine and semen specimens by plaque assays but were not successful. Therefore, we redirected our efforts to isolate ZIKV through cell culture from samples with RT-PCR Ct values ≤30, which suggest viremias higher than $1x10^6$ GCE/mL.

Our findings show that isolation of ZIKV is feasible in specimens with at least $1.72x10^6$ GCE/mL (Ct=27 or lower) in the CDC Real Time RT-PCR Trioplex assay. Isolation of ZIKV was successful in 6/57 (11%) serum specimens (Ct range: 21-26, days post onset [DPO]: 0-4); 1/9 (11%) urine specimens (Ct=22, DPO=5); and 8/94 (9%) semen specimens (Ct range: 19-27, DPO: 14-38). All semen isolates were from different cases. An isolate was obtained from 8/32 individuals. Our preliminary results suggest that ZIKV virus is infectious in serum and urine up to 3-5 days after onset of symptoms, and in semen up to 38 days.

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Diagnosis of Zika Virus Infections: Challenges and Opportunities

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Accurate diagnosis of Zika virus (ZIKV) infections has become a pressing need for the effective prevention and control of the epidemic. The findings that ZIKV infections are associated with birth defects and neurologic disease, and that the virus can be sexually transmitted accentuate the need for accurate diagnostic testing for different applications new to the arbovirus field. This represents a diverse and ambitious testing algorithm that has challenged public health agencies in endemic and non-endemic countries. Antibody response to related flaviviruses has long been known to be cross-reactive; and antibody detection of ZIKV is nonspecific in populations previously exposed to any of the four dengue viruses (DENV-1-4), West Nile virus, or vaccinated against yellow fever virus. Therefore, the diagnosis of ZIKV infections has increasingly depended on detection by nucleic acid tests. In some instances, ZIKV RNA has been detected more frequently or for longer times than in serum by RT-PCR. Publications show that among ZIKV RNA can be detected in serum and urine specimens from 25-55% and 60-95% individuals, respectively. The lack of distinction between Zika and dengue by current serological tests is a major hurtle for case ascertainment particularly in pregnant women; however, several test options have recently emerged that may distinguish IgM antibodies. During the recent epidemic, authorized tests for emergency use have been used by public health laboratories and the commercial sector, but a more dependable and responsive diagnostic testing is yet to be developed. Analysis of different diagnostic testing algorithms will be discussed to provide a better understanding of best diagnostic practices with the highest sensitivity and specificity in populations exposed to Zika and dengue.

Seroprevalence of Zika in individuals from Tegucigalpa-Honduras, during outbreak-2016

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Introduction: Zika has become an important vector borne disease in Latin American countries. After the introduction of Zika at the end of 2015, more than 39,000 suspected cases were registered in Honduras. The diagnosis trough serology is considered difficult due to the methods are not able to consistently differentiate Zika from other Flaviviruses, making also difficult the use of these techniques to support in the calculation of the true rates of Zika infection.

Objective of the study: To report the prevalence of Zika IgM antibodies obtained by the commercial EIA method STANDARD E-Zika IgM from SD BIOSENSOR (Republic of Korea) in individuals with and without suspected Zika infection, during the Zika outbreak in Tegucigalpa, Honduras 2016.

Materials and methods: A total of 143 individuals were tested, 60 asymptomatic individuals and 83 patients with suspected Zika infection collected one point in time during the months of high incidence of Zika cases. Ten were convalescent patients with Zika diagnosis confirmed by rRT-PCR; 60 were Guillan-Barre Syndrome (GBS) patients and 13 were newborn with microcephaly. Plasma samples were tested from all participants using the STANDARD E-Zika IgM from SD BIOSENSOR.

Results: The overall seropositivity was 40% (57/143). Positivity of IgM antibodies were detected in 50% of asymptomatic individuals (30/60), 60% of convalescent patients (6/10), 33% GBS patients (20/60) and 69% of newborn with microcephaly (9/13). The OD values were 2 fold higher in microcephaly and GBS patients than OD obtained in rest of the population studied.

Discussion/Conclusion: These results suggest that Zika IgM-antibodies detected by STANDARD E-Zika IgM from SD BIOSENSOR, could be a promising technique in the presumptive diagnosis of Zika infection in endemic areas. More studies are needed to determine cross reactions with other Flaviviruses, as well test its performance in non-endemic areas to determine the usefulness of this method.

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Category: Diagnostic-Prognostic-Clinical.

Neurobehavioral findings among children participating in the Pediatric Outcomes of Prenatal Zika Exposure (POPZE) study in Puerto Rico.

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Introduction: Zika Virus (ZIKV) infection is an arboviral disease that results in a variety of illness outcomes ranging from asymptomatic infection to severe disease. Furthermore, ZIKV infection during pregnancy may lead to Congenital Zika Syndrome (CZS), characterized by microcephaly or other central nervous system (CNS) abnormalities in neonates. Due to the effect of the virus on the brain, exposed neonates are at risk for sensory, neurologic and developmental disabilities that can become evident over time, therefore their developmental trajectory must be monitored closely. The full spectrum and timing of such outcomes as caused by ZIKV infection is still undefined, as is the impact of family and socioeconomic determinants.
Objectives: To determine the occurrence of CNS abnormalities and developmental delays among ZIKV prenatally exposed infants throughout the first year of life.
Materials and methods: Participants were infants born to women exposed to ZIKV during pregnancy recruited at a tertiary hospital located in Southern Puerto Rico from May through November 2017. Following informed parental consent, a trained general pediatrician performed physical, neurological, and neuro-behavioral examinations using the NICU Neurobehavioral Network Scale (NNNS) to assess neurological integrity and behavioral function. NNNS is a validated tool with clinical and research applications that can predict neurobehavioral and psychomotor outcomes of infants at risk. Recruitment and examination occurred at birth and at 1 month. We report percentages of physical abnormalities. Further analysis to describe NNNS results, developmental delays, ophthalmic manifestations, and auditory function is ongoing. Participant follow up will to continue through July 2018.
Results: 48 infants ≤1-month old born to ZIKV-positive mothers were recruited and 25 of these infants were evaluated at birth and at one month of age. Ten infants had skull abnormalities; 8 (32%) presented skull abnormalities at both points of evaluation, whereas 2 (8%) only presented skull abnormalities during the one-month evaluation. Two (8%) infants presented decreasing head circumference percentiles at 1 month.
Conclusions: Abnormalities and developmental delays identified in this study may widen the spectrum of disease associated with CZS to inform clinicians and expedite early intervention.
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Clinical determinants of hospitalization due to dengue in 7613 patients

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In Brazil, the incidence of hospitalization due to dengue, as an indicator of severity, has drastically increased since 1998. The objective of our study was to identify risk factors associated with subsequent hospitalization related to dengue. We analyzed 7613 dengue confirmed via serology (ELISA), non-structural protein 1, or polymerase chain reaction amplification. We used a hierarchical framework to generate a multivariate logistic regression based on a variety of risk variables. This was followed by multiple statistical analyses to assess hierarchical model accuracy, variance, goodness of fit, and whether or not this model reliably represented the population. The final model, which included age, sex, ethnicity, previous dengue infection, hemorrhagic manifestations, plasma leakage, and organ failure, showed that all measured parameters, with the exception of previous dengue, were statistically significant. The presence of organ failure was associated with the highest risk of subsequent dengue hospitalization (OR = 5·75; CI = 3·53–9·37). Therefore, plasma leakage and organ failure were the main indicators of hospitalization due to dengue, although other variables of minor importance should also be considered to refer dengue patients to hospital treatment, which may lead to a reduction in avoidable deaths as well as costs related to dengue.

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Sleep Quality and Fatigue in patients with pCHIK-CIR in Colombia

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Introduction: Long-term consequences of chikungunya (CHIK) and its chronic inflammatory rheumatism (pCHIK-CIR) are not fully understood yet and include and increasing number of non-rheumatological effects, among them, sleep disturbances and fatigue. **Methods:** In a cohort study among 62 cases serologically diagnosed in La Virginia, Risaralda, Colombia, followed-up by 2-years, demographic and clinical characteristics were collected at baseline. Pittsburgh Sleep Quality Index (PSQI) (used with written permission) and Fatigue Severity Scale (FSS), validated in Spanish and for Colombia (Cronbach α=0.78 and 0.88, respectively), were applied to patients with pCHIK-CIR (+) and without (-), all under follow-up at 2 years. Score comparisons (Wilcoxon signed-rank test) and prevalence proportion (χ^2) significances were calculated using Stata IC 14.0® licensed, p significant <0.05. **Results:** We compared 43 patients with persistent rheumatological symptoms (pCHIK-CIR+) and 19 without it (pCHIK-CIR-), all them followed-up for 2 years. Mean age was 44.1 y-old, 69% female. At the PSQI we found significant differences in the wake-up time (item-3), pCHIK-CIR+ do early (median 5:00am versus 6:00am in those pCHIK-CIR-) (z=2.699; p=0.0069), sleep less time (item-4) (mean 6.46 hours vs 7.79) (z=2.51; p=0.0121), they have more somnolence from <1 times/week to ≥3 times/week (item-8) (46.5% vs 10.5%) (χ^2=7.454; p=0.006), have somewhat to very big problem keep up enough enthusiasm to get things done (item-9) (46.5% vs 10.5%) (χ^2=7.454; p=0.006). FSS were significantly higher in those with pCHIK-CIR+, 55.2 vs 37.5 (z=2.303; p=0.0213). **Discussion:** Sleep is significantly impaired among pCHIK-CIR+ patients as well they also presented with more fatigue, compared to those that recovered during acute phase of disease. This is the first study in screen these aspects with validated questionnaires, raising the relevance of such assessments in patients with acute but also chronic chikungunya consequences. Findings have significant implications in areas affected by chikungunya epidemics, as well later endemic areas with chronic disease.

Depression as manifestation of dengue and chikungunya: A meta-analysis

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Introduction: Recent studies have provided information regard mental impairment in chronic chikungunya patients (CHIKV) when assessed quality of life (QoL) (eg. by SF-36). However, occurrence of depression during acute phase, as well on dengue (DENV) have been neglected, at systematic reviews and meta-analysis. **Methods:** A systematic review was conducted (PubMed/Scopus/WoS) up to November 1, 2017. The search strategy was "mental" or "psychiatric" or "depression" plus "AND" followed by "chikungunya" and "dengue". Languages: English and Spanish. Observational studies that assessed at least self-reported depression (or using questionnaires) in patients with acute or chronic chikungunya or dengue, were included. Comprehensive Meta-Analysis 3.3.070® licensed (UTP), was used for this meta-analysis. **Results:** Our literature search yielded 241 citations. The pooled prevalence of depression associated to CHIKV/DENV in 8 selected studies among 1,649 patients was 39.3% (95% CI 36.6-42.1; τ^2=1.471; I^2=97.8; p<0.001). When considering just CHIK studies (n=6), among 911 patients, estimate was 25.1% (95% CI 22.0-28.5; τ^2=1.338; I^2=96.2; p<0.001), while for DENV studies (n=2) with 738 patients, estimate was 52.6% (95% CI 48.7-56.4; τ^2=1.664; I^2=98.9; p<0.001). For studies at acute phase (n=4 [2 with DENV, 2 with CHIKV), with 798 patients, estimate was 51.3% (95% CI 47.5-55.1; τ^2=1.730; I^2=97.2; p<0.001), whilst at chronic phase (n=4, all for CHIKV), with 851 patients, was 25.5% (95% CI 22.3-28.9; τ^2=1.378; I^2=97.6; p<0.001). Globally, the funnel plot and the Egger's regression suggested bias publication (-7.46; p=0.06) (confirmed with Duval and Tweedie's trim and fill). **Discussion:** According to our results in the most conservative scenario, approximately one third of patients at any stage of DENV or CHIKV would report depression, being higher during acute phase and for dengue (around half of patients), in comparison to chronic chikungunya (a fifth). It is important to highlight that the findings suggest a clear need to increase the number of studies about depression and other mental disorders in these and other arboviral diseases, including also Zika, as well to use standardized and validate questionnaires, such as the Zung Self-Rating Depression Scale (SDS), Hamilton Depression Rating Scale (HDRS) and Beck Depression Inventory (BDI), among others, given consequently the implications for diagnosis and therapeutics.

Depression and anxiety among patients with post-Chikungunya chronic inflammatory rheumatism

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Introduction: Despite recent significant epidemics of chikungunya (CHIK) (>3 million cases in the Americas during 2014-2015), long-term psychiatric impact of CHIK infection and its chronic inflammatory rheumatism (pCHIK-CIR), has not fully addressed with standardized and validated questionnaires. **Methods:** In a cohort study among 62 cases serologically diagnosed in La Virginia, Risaralda, Colombia, followed-up by 2-years, demographic and clinical characteristics were collected at baseline. Zung Self-Rating Depression Scale (SDS) and Zung Self-Rating Anxiety Scale (SAS), validated in Spanish and for Colombia (Cronbach α=0.92 and 0.80, respectively), were applied to patients with pCHIK-CIR (+) and without (-), all under follow-up at 2 years. Mean score comparisons (Student's *t*) and prevalence proportion (χ^2) significances were calculated using Stata IC 14.0® licensed, p significant <0.05. **Results:** We compared 43 patients with persistent rheumatological symptoms (pCHIK-CIR+) and 19 without it (pCHIK-CIR), all them followed-up for 2 years. Mean age was 44.1 y-old, 69% female. SDS and SAS scores were significantly higher in those with pCHIK-CIR+, 49.6±15.9 versus 40.1±12.4 (p=0.025) and 49.6±15.4 versus 40.1±10.9 (p=0.019), respectively. When using cut-off scores, depression and anxiety symptoms were significantly higher among those with pCHIK-CIR+, 48.8% vs 21.1% (p=0.04) and 97.7% vs 84.2% (p=0.047). Moderate-to-severe scores at SDS and SAS showed more marked difference in those with pCHIK-CIR+, 27.9% vs 5.3% in both scales (p=0.039). At subscales for SDS and SAS, significant differences (p<0.05) were found for the cognitive (9.2 vs 6.4) and somatic factors (5.6 vs 4.4) of SDS and motor (8.8 vs 6.5) and central (CNS) (9.5 vs 7.1) factors of the SAS, between pCHIK-CIR+ and pCHIK-CIR- patients. At SDS, items 1,6,9,10 and 12, as well, at SAS, 3,6,7,8,10,14,15 and 18, were significantly different among groups (p<0.05). **Discussion:** Depression and anxiety symptoms, at multiple levels, were significantly higher among pCHIK-CIR+ patients. This is the first study in screen these psychiatric long-term consequences with validated questionnaires, raising the relevance of mental health assessment in patients with acute but also chronic chikungunya consequences. Findings have significant implications in areas affected by chikungunya epidemics, as well later endemic areas with chronic disease.

Guillain-Barré Syndrome Associated with Zika Virus Infection in Honduras, 2016

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Introduction: Guillain-Barré syndrome (GBS) has been reported to be associated with Zika virus (ZIKV) infection in still relatively few studies in Latin America. This study aimed to describe the clinical profiles and the frequency of GBS associated with ZIKV during the ZIKV outbreak in Honduras in 2016. **Methods:** Observational study, where we recorded data from GBS meeting levels 1 or 2 of diagnostic certainty for the Brighton Collaboration, with proof of recent ZIKV infection and screening for other etiologies of GBS. Cases of Zika were clinically and laboratory confirmed (RT-PCR). **Results:** We studied 107 cases of GBS. Of them, 6 (5.6%) were due to ZIKV infection, 1 (0.9%) ZIKV/DENV coinfection, 0.9% were positive for DENV IgM (negative by RT-PCR), 0.9% for CHIKV IgM (negative by RT-PCR) and 0.9% positive for RT-PCR for CHIKV. Mean age of patients was 34.9 years old, 58.9% male. Electrophysiological tests were consistent with the primary demyelinating form of the disease. Among those with ZIKV-GBS, mean age of patients was 35.4 years old, 67% male. Lag between symptoms and diagnosis was in a mean of 4.38 days (ranging 2-9 days), mean time at ICU was 14.5 days. Treatment comprised intravenous immunoglobulin (IVIg) in all the patients. Two patients required intubation and assisted ventilation. No fatal cases were reported. **Discussion:** ZIKV infection is usually benign, although from our group we have reported fatal cases (Lancet Infect Dis). But in countries at risk of ZIKV epidemics, adequate intensive care bed capacity is required for management of severe GBS cases. Arbovirus RNA detection by RT-PCR should be part of the management of GBS cases. Studies have suggested that the number of cases of GBS significantly increased in the Americas during the ZIKV epidemics of 2016. Adherence to protocols for the care of patients with acute neurological syndromes in ZIKV endemic areas is clearly vital and the training and education of healthcare workers, including travel medicine practitioners dealing with travelers visiting endemic areas, particularly in Latin America should be prioritized.

EVALUATION OF ZIKA VIRUS CLINICAL DEFINITIONS

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Introduction
Estimating Zika virus (ZIKV) burden is challenging due to a high rate of asymptomatic infections, generally low, declining viremias at the time of symptom onset, and concomitant circulation with other arboviruses that have comparable symptoms limiting detection and diagnosis. In May 2016, in the Peruvian Amazon city of Iquitos, Naval Medical Research Unit No.6 confirmed its first ZIKV case through an ongoing hospital-based febrile (i.e. ≥38°C, ≤5 days) surveillance system (later modified to include afebrile cases with exanthematous symptoms). Between May 2016 to Jan 2017, 692 febrile participants were enrolled and between Feb to May 2017, 117 cases with the modified criteria were enrolled. Clinical features of ZIKV included low-grade fever, rash, conjunctivitis, headache, myalgia, and pruritus.
Objetives
To evaluate the specificity and sensitivity of three ZIKV case definitions: 2016 Pan American Health Organization (PAHO), World Health Organization (WHO), and the Peruvian Ministry of Health (PMOH) with participants enrolled under the surveillance program.
Methods
Of the acute samples collected during febrile surveillance, 47 (6.8%) and 8 (6.8%) of the modified criteria were confirmed by RT-PCR for ZIKV, we compared the sensitivity and specificity of the case definitions among the positive cases of ZIKV and DENV to determine the capacity of the ZIKV case definitions to discriminate within ZIKV and DENV cases.
Results
Sensitivity of the PMOH and WHO ZIKV case definitions ranged from 72 to 88%, improving with the modified criteria; whereas sensitivity was lower for the PAHO definition (60% before, 50% after, p=0.08). Specificity was poor for all case definitions, ranging from 11-60%. In particular, the WHO definition had a specificity of 11% among patients enrolled with only fever improving to 27% for patients enrolled with the modified criteria.; no change in these parameters were observed, indicating that DENV cases were not the primary cause of poor specificity.
Discussion and conclusion
Overall, comparison of enrolled participants where fever was a required inclusion criterion to inclusion of afebrile patients with exanthematous symptoms improved sensitivity for PMOH and WHO definitions with a minimal impact on specificity. Further evaluation of the ZIKV case definition is critical to accurately improve detection, specifically in high-risk patients or resource limited settings

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Clinical and laboratory findings of a Zika and Dengue Outbreak in the Caribbean Region of Costa Rica during 2017

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Zika virus (ZIKV) is an emerging mosquito-borne Flavivirus in the Americas. Since its first recognized autochthonous case in Costa Rica during 2016, several outbreaks of ZIKV have been reported in dengue (DENV) hyperendemic regions. In 2017 the most affected region was the Huetar-Caribbean, with more than 1500 clinically suspected ZIKV cases. In this study, we describe the major clinical and laboratory findings of ZIKV and DENV patients in two healthcare facilities in this region. A total of 307 patients with a presumptive diagnosis of a dengue-like illness where included in this study. Serum and urine samples were analyzed by real-time PCR (RT-PCR) to detect DENV, ZIKV and Chikungunya (CHIKV) RNA. Samples from 34 (11%) patients were positive for DENV, 104 (34%) for ZIKV, 1 (0.3%) for CHIKV and 4 (1.3%) were positive for both DENV and ZIKV simultaneously. Virus isolation was attempted from positive samples. ZIKV virus was successfully isolated from serum but not from urine, while DENV was isolated from 50% of the positive samples. Phylogenetic analysis showed DENV-2 American/Asian as the circulating DENV genotype in this outbreak. Overall symptoms and laboratory reports were very similar between DENV and ZIKV cases; nevertheless, patients with confirmed ZIKV infection were more likely to report a maculopapular rash and DENV cases were more likely to present thrombocytopenia. Given the diversity and overlapping symptoms of these arbovirosis, laboratory confirmation is fundamental for epidemiological and clinical management. Moreover, further studies are crucial to address the effects of co-circulation of these arbovirus in Costa Rica.

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A NOVEL MOBILE PHONE APPLICATION FOR REMOTE RESEARCH DATA COLLECTION

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Introduction

Although much is known about the presentation of acute viral infections such as dengue infection, the long-term sequelae of such infections has not been systematically studied and thus remains poorly understood. Anecdotal evidence suggests that many viral infections, particularly flaviviral infections, may have long term sequelae with chronic health outcomes. Barriers to conducting a prospective longitudinal study evaluating such outcomes include the high financial costs associated with frequent patient follow-up and a significant participant drop out rate over time. A proposed solution is to harness technology to collect important research data remotely via a mobile phone application.

Objective

Using acute dengue and other febrile illnesses (OFI) as test cases, we explored the utility of mobile phone application to evaluate the long-term sequelae and health outcomes in a cohort of 450 patients for one year post-infection.

Materials and methods

We designed a Mobile-phone Application for Information extraction in Dengue (MAIDEN) to study the long-term effects and health outcomes of acute dengue compared to OFI. The MAIDEN platform is compatible with both iOS and Android operating systems. Datasets are stored on a secure back-end database with an object-based relational structure.

Results

Demographic and clinical information was collected from the study participants upon recruitment. Participants were then sent a link to download the MAIDEN application onto their mobile phone via Short Messaging System (SMS). Participants were followed-up daily for the first 7 days, then weekly from Weeks 2-8, then monthly from Months 3-12 up to a total duration of 1 year. Except for the Day 1 visit, participants were not required to attend the study site in-person, but instead used MAIDEN to remotely enter clinical information and complete the SF-12v2 Health Survey. Data collected on MAIDEN was automatically fed back into a centralized database for analysis.

Conclusion

Mobile application tools such as MAIDEN allow for research data to be collected remotely, removing the need for frequent in-person study visits thus potentially reducing the high costs of running prospective longitudinal studies. If successful, such remote data collection tools could prove to be useful in a variety of research and clinical settings.

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Evidences of intrauterine ZIKV exposure in pregnant women in Brazil.

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Zika virus (ZIKV) infection has been associated with severe birth defects, including microcephaly and neurological complications. Intrauterine abnormalities have also been associated with ZIKV, reflecting the teratogenic effect of this arbovirus and are referred to as Congenital Zika Syndrome (CZS). The aimed was to report the virological outcomes in newborns exposed intrauterine to ZIKV in a cohort of ZIKV-infected pregnant women. From February to October 2016, the Public Health Authority of São José do Rio Preto, SP, Brazil, detected pregnant women with acute ZIKV-like symptoms. After, 54 of them, with 5 to 38 weeks in their pregnancies, were enrolled in the study. All patients were tested to TORCHS and were monitored by a multidisciplinary medical team. After the delivery, newborns umbilical cord blood and/or urine were collected and tested to ZIKV by RT-qPCR and ELISA. Evidence of ZIKV exposure was detected in 18 of the 51 newborns (35%) evaluated at birth. The ZIKV RT-qPCR in the umbilical cord blood of 48 newborns showed positivity of 29% (14/48; mean C_t 36.5; IQR 36-37), while the urine of 46 newborns showed a positivity of 9% (4/46; mean C_t 36.5; IQR 31-36.6). Sixteen of 18 newborns (89%) had been exposed in the second or third trimester. ZIKV NS1 was reactive in the umbilical cord blood (12/15; 80%). ZIKV IgM was non-reactive. The most common symptoms reported by the mothers were rash, pruritus, and headache. Conjunctivitis/conjunctival hyperemia were not a prominent finding. Seven pregnant women had ZIKV and other infections as determined by laboratory testing. All the pregnant women from the study delivered by the time of this study, and there were no miscarriages, fetal deaths or microcephaly. Only one newborn was born pre-term. The link to ZIKV may not be clearly established, but the only infectious agent detected in this case was ZIKV in an umbilical cord blood. More studies are necessary to understand the dynamics of the ZIKV infection during pregnancy. Thus, the monitoring of ZIKV infection during prenatal and postnatal care is extremely important to

CHIKUNGUNYA VIRUS SURVEILLANCE DURING THE 2015-2016 BRAZIL TRIPLE ARBOVIRUS EPIDEMIC

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Introduction: Chikungunya virus (CHIKV) is an arbovirus that causes an acute febrile syndrome with a debilitating arthralgia. Currently, Brazil lives a triple arbovirus epidemic caused by the concomitant circulation of CHIKV, dengue virus (DENV) and zika virus (ZIKV), which makes the differential diagnosis extremely difficult for health professionals. Objectives: we aimed to investigate CHIKV suspected cases and the possible occurrence of co-infections among the three circulating arboviruses, as well as perform the partial sequencing of the E1 region of CHIKV strains from epidemics that have occurred in Amapa (AP) and Feira de Santana (BA) in 2014-2015 and in Campo Grande (MS) and Rio de Janeiro (RJ) during 2016. Material and methods: all cases were submitted to the qRT-PCR for CHIKV genome detection, to anti-CHIKV IgM ELISA and also to the differential diagnosis for DENV and/or ZIKV. Chikungunya positive cases were randomly selected and partially sequenced (CHIKV E1 gene). Results and discussion: In AP, the results show that 107/208 (51.44%) of the cases were confirmed for CHIKV. Of these, 24 co-infections by CHIKV and DENV, 2 by CHIKV and DENV-1 and 2 by CHIKV and DENV-4 were observed. In BA, 24/28 (85.71%) cases were confirmed for this virus and of these, 12 co-infections by CHIKV and DENV were found. In MS, 7/134 (5.22%) of the cases presented only serological evidence of this infection. In RJ, 59/91 (64.83%) of the cases were confirmed for CHIKV. Of these, 3 co-infections were observed by CHIKV and DENV-4, 16 by CHIKV and ZIKV and 1 by ZIKV and DENV-4. Finally, although no amino acid change was observed, it was shown that all the CHIKV strains analyzed from AP belong to the Asian genotype. In RJ, ECSA circulation was demonstrated for the first time in this region and no E1-A226V mutation was observed. Despite this, an E1-V156A alteration was revealed in two samples and for the first time the E1-K211T mutation was revealed in all analyzed samples. Conclusions: it's fundamental the monitoring of the CHIKV suspected cases and the dispersion of circulating genotypes, besides the identification of possible mutations that facilitate mosquito vectors transmission.

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Evaluation of persistent musculoskeletal involvement in Chikungunya infection, Colombia

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Abstract

Chikungunya is an emerging disease in Colombia, which produces an important compromise Musculoskeletal which can be long lasting and significantly affect patient health and quality of life. The aim of the study was to determine the incidence of chronic musculoskeletal pain and its factors clinical and sociodemographic associated and the degree of severity and disability of Chikungunya infection.

A retrospective cohort study of 111 Chikungunya patients from three states was performed; infection was confirmed by IgG antibodies. Persistence of musculoskeletal pain was considered as positive if the duration of musculoskeletal symptoms, regardless of severity, continued after three months from the date of symptom onset. Severity was measured by the presence of arthritis (pain and inflammation), the degree of pain was measured through the visual analogue scale (VAS) and the degree of disability was measured through the health assessment questionnaire (HAQ20). The data was analyzed using a binary logistic regression analysis. We found that the incidence of persistence of musculoskeletal compromise was 74.8% (83/111). People who experienced severe joint pain in the acute phase of the infection and those who had inflammatory-type involvement (arthritis) had a higher risk of the persistence of musculoskeletal compromise (85.2 vs. 34.8%, RRa = 7.6, 95% CI = 1.3-44.3, p <0.01 and 88.1% versus 34.6%, RRa = 11, 95% CI = 2.4-49.9, p <0.01 respectively). We observed a reduction of acute and chronic severity. For arthritis it was 75.7% (84/111) vs. 17.1% (11/191); moderate disability

Postnatal acquired fatal Zika Virus Infection: A Systematic Review

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Introduction: It seems likely that many cases of ZIKV infection were unrecognized or misdiagnosed in the past including fatal cases. Notwithstanding, having in to account that study and description of fatal cases varies among reporting countries, it is necessary to evaluate with a systematic review (SR) the casualty linkage between infection and outcome, and the clinical spectrum of ZIKV associated deaths. **Methods:** Eligibility criteria were: peer-reviewed articles reporting fatal cases associated with ZIKV infection. We have included English-, Spanish-, Portuguese- and French-language studies. Eligible designs: case-control, cohort studies, case reports and case series. Information published in epidemiological reports and bulletins(CDC/ECDC/WHO/PAHO) and National Governments through their surveillance systems or Health Ministries were also included. In order to assess casualty linkage between ZIKV and fatal cases a modified version of the WHO Zika Causality Working Group casualty framework was used. Protocol was registered in PROSPERO(ID:58347). **Results:** The research initially rendered a total of 311 articles: duplicates across the databases and articles about other viruses were eliminated. Finally, 24 articles were selected based on their relevance and pertinence of the title or abstract to the severe disease that was being evaluated, with all of them mentioning fatal outcomes. A total of 84 deaths, from 575,677 cases were obtained, for a case fatality rate (CFR) of 1.5/10,000 cases. Country with the highest CFR was Dominican Republic (32.4 deaths/10,000 cases), followed by Suriname 11.46 and Bolivia 9.72. All the deaths begun to be reported since 2015. No deaths were [globally] reported before the 2015-2016. In >70% diagnosed used RT-PCR, in the rest MAC-ELISA. The most of the reported cases assessed temporality, and analogy as casualty dimensions, only four fully discarded other possible causes, and only two stablished a dose/response relationship between ZIKV infection and death. **Discussion:** Until 2015, in general, ZIKV was not considered a dangerous pathogen, but after the first death, reported by our group in EID 2016, an increasing number of fatal cases were published. Although the pathogenicity of severe infection is poorly understood, based on in vitro and animal evidence, it is possible that antibody dependent enhancement has a role.

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Highlighted Poster

Aedes aegypti RESISTANCE IS ASSOCIATED WITH MUTATIONS IN SODIUM CHANNEL

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INTRODUCTION: The use of insecticides pyrethroids, is one of the most common vector control strategies to interrupt the arboviruses transmission of Aedes aegypti. However, their massive use without knowledge of the resistance status generates a selective pressure in mosquitoes populations. In this way, the identification and characterization of resistance associated mutations can be used as bioindicators for resistance monitoring. The aim of this work was to characterize the resistance status to Lambda-cyhalothrin and its possible mechanism in ten Ae. aegypti natural populations of Colombia.
MATERIALS AND METHODS: The resistance status to lambda-cyhalothrin was determined in ten Ae. aegypti populations. One of the most resistant populations were analyzed by sequencing of the coding region of sodium channel gene. Three specific allele PCRs were implemented to identify at population level each of the mutations in the voltaje-gated sodium channel gene. Allelic and genotypic frequencies were calculated and compared with the resistance ratio to lambda-cyhalothrin.
RESULTS: Seven of the Ae. aegypti populations were resistant to lambda-cyhalothrin, while two were tolerant and one presented a susceptibility profile. The gene sequencing allowed us to identify three mutations: V419L reported for first time in Ae. aegypti Colombian populations and for second time in worldwide. The second one V1016I, which has been widely reported to be associated with the pyrethroid resistance in LatinAmerica and finally the mutation F1558C. We found that the populations classified as resistant showed a higher frequency of the mutated alleles 419L and 1016I with respect to those populations that were determined as susceptible and tolerant. A polynomial correlation was found between the presence of these two mutated allele in mosquitoes (r^2= 0.9441 for 419L and r^2= 0.9541 for 1016I). For the mutation at position 1558 was found the presence of the mutated allele at a high frequency for all populations.
CONCLUSIONS: In this work, we characterized the existence of mosquitoes populations resistant to pyrethroid lambda-cyhalothrin and identified the presence of mutations in the coding region of the volt-aged-gated sodium channel, which could explain the resistance found. The mutation F1558C could be involved in the resistance to other insecticides.

Biological Control of Dengue, Zika, and Chikungunya using Wolbachia

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The World Mosquito Program (worldmosquitoprogram.org), known until recently as the Eliminate Dengue Program, has developed *Wolbachia* as an effective, safe and self-sustaining biological control method to reduce transmission of human pathogens such as dengue, chikungunya and Zika. *Wolbachia* are naturally occurring bacteria that are present in many insects, including some mosquito species that commonly bite humans. *Aedes aegypti,* the primary mosquito species that transmits dengue, is not naturally infected with *Wolbachia*. The World Mosquito Program has successfully transferred *Wolbachia* from the fruit fly, where the bacteria occur naturally, into *Aedes aegypti* mosquitoes. The presence of *Wolbachia* reduces their ability to transmit viruses, including dengue, chikungunya, and Zika. To implement the approach, the Program has developed low cost and effective methods to introduce *Wolbachia* into mosquito populations in order to reduce the transmission of these diseases. This is done through field releases of relatively small numbers of mosquitoes that contain *Wolbachia.* Released mosquitoes breed with local mosquitoes and the *Wolbachia* is passed on to the mosquitoes in the local area. The method has been successfully implemented in communities across a range of countries, including Australia, Vietnam, Indonesia, Brazil and Colombia in partnership with key government agencies, community groups and householders. In areas where *Wolbachia* has established in the mosquito populations there has been no evidence of local dengue transmission. Overall, the approach has also been shown to be acceptable to stakeholders and communities, is safe for humans and the environment, and effective at reducing transmission of dengue and other viruses that are transmitted by *Aedes aegypti* mosquitoes.

Vector competence of Mexican Aedes aegypti for Zika virus

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INTRODUCTION: *Aedes*-borne viral diseases are a significant threat to the health of human populations. Zika virus (ZIKV) gained worldwide attention after spreading to Brazil in 2015, because its ability to cause neurological complications such as newborn microcephaly and adults Guillain-Barré syndrome. Mexico notified the first autochthonous cases of ZIKV infection on late 2015, and so far, have been reported approximately 10,000 cases of ZIKV infection, including 5,925 pregnant women, in 26 states out of 32 (revised on December 2017).
ZIKV is transmitted to human primarily by the bite of an infected mosquito *Ae. Aegypti*, However, little is known about the geographically distribution in the level of vector competence (intrinsic characteristic of the vector to acquire, maintain, and transmit a pathogen) of Ae. aegypti for zika virus. Here, we study the vector competence of field populations of *Ae. aegypti* from three different States of Mexico to transmit ZIKV.
METHODOLOGY: We collected eggs inside houses from mosquitoes *Ae. aegypti* in Morelos, Guerrero and Chiapas. Following, the F1 generation were propagated in insectary conditions. Female mosquitoes were infected with artificial blood meals containing ZIKV (10^7 FFU/mL). Virus concentration was measured in midgut, legs-wings and salivary gland at 7, 14 and 21 days after infection, respectively, by qRT-PCR. Vector competence of mosquitoes was evaluated by calculating potential transmission rate (no. infected salivary glands/no. tested mosquitoes).
RESULTS AND DISCUSSION: Our results showed that Mexican *Ae. aegypty* evaluated in this study, were susceptible to zika virus infection, although, the infection, dissemination, and transmission rates were different among *Ae. aegypti* mosquito populations from 3 different states of Mexico, suggesting that regional origins of vector populations influence transmission efficiency. Additional studies are needed to make a Mexican map of entomological risk of ZIKV dissemination.

POTENTIAL MOSQUITO VECTORS OF ARBOVIRUSES IN TURBACO, BOLÍVAR, COLOMBIA

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Introduction: Located 10 km from the city of Cartagena, Colombia, in a dry tropical forest on the Northern Atlantic coast, the municipality of Turbaco has had an increase of mosquito-borne disease transmission since 2014. Despite the disease burden, there are no studies characterizing local mosquito vector fauna in this municipality. **Objective:** The objective was to characterize the mosquito species present in Turbaco municipality, Bolivar department, in Colombia. **Methodology:** Entomological surveys were carried out during September and December of 2016 in 7 rural sites and 15 urban houses. In the rural sites, mosquito collections were performed for five consecutive days using CDC light (1800-0600) and Shannon (1800-2000) traps as well as human landing collections (2-3 collectors, 1800-2000). In urban areas, Prokopack® aspirator collections were performed inside household between 0900-1200. **Results:** A total of 1,510 adult mosquitoes (11 genera and 26 species) were collected. 15 of the 26 species were identified as potential arboviral vector species, including *Psorophora cyanescens* (10.1%) using CDC and Shannon and *Aedes aegypti* (4.1%) using CDC (7.1%), Prokopack® aspirator (85.7%) and human landing (7.1%). The most abundant species overall was *Culex quinquefasciatus* (52.5%), accounting for 22.4% of rural collections and 38% of urban collections. *Aedes aegypti* accounted for 4.1% of the overall collection, but was the most abundant species collected in urban houses with densities of 5.36 mosquitoes/house. **Conclusion:** *Aedes aegypti* was identified mostly in urban centers in Turbaco, Bolívar, demonstrating the risk for dengue, chikungunya, and other arbovirus transmission. Households inspections in rural areas should also be carried out to better access risk of *Aedes*-borne viruses in rural areas. Interestingly, no *Anopheles* species were identified. Culicidae fauna was diverse with previously incriminated vector species *Culex* (5), *Limatus* (1), *Mansonia* (2), *Ochelrotatus* (2), *Psorophora* (3) and *Sabethes* (1) species. Subsequent studies must characterize

bionomics of these species, including infection rates with arboviruses.

Funding of research: 800000.82000.25GB.B0016, US Dept. of Defense Armed Forces Health Surveillance Center (AFHSC), Global Emerging Infections Surveillance and Response Systems (GEIS)

A stable isotope mark-capture study of Aedes aegypti source habitat in South Texas

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South Texas has been recognized as an area of emergence of mosquito-borne viruses due to the abundance of *Aedes aegypti* and proximity to disease endemic areas in Latin America. The control of *Ae. aegypti* targeting immature stages with larvicides and source habitat reduction is challenging due to difficulty in identifying all habitat that contributes to the adult population. We conducted a novel field experiment using stable isotope amendment of natural container habitat in South Texas to identify the success of a simulated source reduction campaign and relative contribution of different larval habitat types to the adult *Ae. aegypti* population. The study was conducted in the community of Indian Hills, Mercedes and all containers with water from the houses that agreed to participate (42/92) were enriched on a weekly basis during the months of September–November, 2017. All used tires received ^{15}N-enriched potassium nitrate and all other type of containers received ^{13}C-enriched glucose. Adult mosquitoes were captured in the study region using BG2 Sentinel Traps from 45 locations, up to 350 m from enriched habitat. To confirm isotopic enrichment was achieved, we collected pupae from enriched containers, allowed the adults to emerge, and processed them for isotope analysis. Mosquitoes had values of about 500 δ^{13}C and 1,000 δ^{15}N from glucose and potassium nitrate treated habitat, respectively. These results were well above natural abundance isotope values from specimens collected from un-enriched habitat, confirming enrichment was achieved. We collected over 4,500 *Ae. aegypti* mosquitoes from BG Traps that are in the process of being analyzed to identify marked pools and results will be presented.

Funding of research: Centers for Disease Control and Prevention - Advanced and Innovative Solutions to Improve Public Health

Knowledge Aptitude and Practices of South Texas communities towards Aedes aegypti.

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Introduction: *Aedes aegypti* mosquito is the main vectors of arboviral diseases such as Dengue, Chikungunya and Zika. In the continental United States, Florida and Texas, are the only states, with autochthonous Dengue and Zika cases. In Texas, the Lower Rio Grande Valley (LRGV) has periodically experienced local transmission of Dengue and more recently Zika virus. Risk of disease transmission in the LRGV region has been associated with socioeconomic condition, knowledge of mosquitoes, and behavioral practices. Our objective was to identify key knowledge gaps in community awareness for vector control programs.

Methods: In November of 2017, a Knowledge Aptitude and Practices (KAP) survey regarding mosquitoes, the diseases they transmit and house structure was conducted in eight LRGV communities located in Hidalgo (Balli, Christian Ct., La Vista, Chapa, Rio Rico, Mesquite, Progreso) and Cameron (La Feria) Counties. The selected houses have participated in a weekly indoor and outdoor Autocidal Gravid Ovitrap (AGO) surveillance program since August of 2016.

Results: We conducted the KAP's survey in 85% (34/36) of the households under surveillance. Of those 88% could recognize an adult mosquito and 50% larvae or pupae. More than 80% considered mosquitoes as posing a negative impact in their lives, of which 60% considered it as a health risk additionally; they knew mosquitoes could transmit Zika and/or Dengue. In contrast, only 34% considered mosquitoes as a problem in their communities, with <50% considering Zika as an issue, being their main concern the health of children or relatives with children.

Discussion: Vector control programs for *Aedes* mosquitoes are extremely needed, especially along the Texas-Mexico border given the abundance of *Ae. aegypti* and that dengue disease is endemic in Mexico. Such programs will relay in community understanding to ensure support and participation in vector control programs. This urge us to develop inclusive programs which takes into consideration the current gaps in knowledge from communities at higher risk of disease transmission through the development of community centered education campaigns.

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Spatial Diffusion of Dengue in the Pioneer Amazonian Front, 2003-2012

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In the last decades, the occupation of the Amazon, along with the expansion of economic activities on a large scale, had a great negative impact on the environment and health. These processes altered the context of manifestation of health problems in time and space and also modified the spatial diffusion characteristics of health problems in the region. This research aimed to evaluate the relationship between the various economic processes of territorial occupation of the Amazon and the spatial diffusion of dengue, through the configuration of networks of economic production and circulation of people. The study used statistical data on dengue, city network, socioeconomic production and environmental variables, georeferenced and available for the 807 municipalities of the Legal Amazon. Thematic maps were constructed using the MapInfo 9.1 program. The mapping was performed through the application of a geostatistical treatment denominated Inverse Distance Weighted Interpolation – IDW. The results suggest that the spatial diffusion of dengue closely follows the area of Consolidated Settlement closely, Central Amazonia and the major cities of the region, strongly related to ecological changes such as deforestation, opening highways, construction of hydroelectric power stations, ports and railroads, increasing the exposure of the population to the insects, and also the process of disordered urbanization as an important factor for the emergence of dengue and the persistence of the vector in the region, what can evidence stages and typologies of recent occupation of the Brazilian Amazon that promotes the occurrence of the disease. These spatial patterns reveal environmental and socio-economic macrodeterminants, which materialize in geographic space by building roads and forming networks of cities.

Keywords: Spatial Analysis. Space Diffusion. Dengue. Mobility. Risk Areas. Public Health. Frontier

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Evaluation of an Autocidal Gravid Ovitrap intervention for the control of Aedes aegypti in the Lower Rio Grande Valley, Texas

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Introduction: Mosquito-borne viruses driven by *Aedes aegypti* continue to emerge and re-emerge globally. The Lower Rio Grande Valley (LRGV) has now experienced local transmission of Dengue, Chikungunya, and Zika virus. Our objective was to evaluate an Autocidal Gravid Ovitrap (AGO) intervention to identify strengths and limitations in South Texas for this form of mosquito control.

Methods: Eight communities from the LRGV region were selected for our study. Our intervention was carried out from August–December of 2017, concurrent with the recognized arboviral transmission season. Two middle-income and two low-income communities were randomly allocated into either control or intervention. The intervention communities had systematic recruitment of all available houses within those communities. Each recruited house had three AGO's placed in their premises, that were reset every two months. An average of five houses in each community served as our reference houses, weekly data was collected from indoor and outdoor sentinel AGO traps.

Results: For the intervention communities, we were able to recruit 50-75% of the houses. The most common cause for recruitment failure was absence of homeowners (35%), followed by abandoned houses (15%). For the October reset in the intervention communities we found an average of 80 mosquitoes per house, for both middle and low income communities. In the December reset we found an average of 50 mosquitoes per house. Mosquito densities between the control and intervention communities in either indoor and outdoor AGO reference traps were not statistically different.

Conclusion: Vector control programs for *Aedes* mosquitoes are urgently needed, especially within the Texas—Mexico border to fight off the introduction and establishment arboviral diseases such as Zika. The results of the first year of the AGO intervention yielded no evidence of reduced mosquito populations, likely due to the low recruitment of homes into the study. During year two, we plan to recruit more homes to achieve 80% of them having 3 AGO traps per house in a community.

Funding of research: NIH R21 (5R21AI128953-02)

Dengue Outbreak in Barra Longa Associated to Mine Dam Burst, the worst environmental disaster in Minas Gerais State Brazil

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Abstract

INTRODUCTION: In November 2015, the iron mine dam spilled 60 million m^3 of mud in the Rio Doce Basin (RDB), contaminating its water with iron residues, as well as transporting human and animal sewage to this river. This was the worst environmental disaster in Brazil. The waters at RDB are widely used.This study examined the relationship between the low sanitation of Barra Longa after the disaster and the emergence of new cases of dengue. With this event it was possible to prove how low sanitation is a important element for *Aedes aegypti* proliferation. Currently, dengue has been the most important epidemic in Brazil since 2014. MATERIALS AND METHODS: The Ministry of Health has implemented the Contingency Plan based on protocols for the control and prevention of outbreaks and deaths and has encouraged municipalities to create their sanitation database. Municipal database and Geoprocessing dengue cases points and contamination of water supply have been used to improve the actions of the Contingency Plan in the Barra Longa. RESULTS: Until 2015 Barra Longa was one of the cities of Minas Gerais with a lower incidence of dengue fever. However in 2016, after the disaster,

the dengue cases was three times higher than in previous years. The work of the epidemiological surveillance in Barra Longa in the last two years have shown that the incidence of dengue has increased as well as the vector *Aedes aegypti* detection and this event was correlated to increased contamination of water supply observed too. Other indicators of low sanitation were also determined in Barra Longa as an increase in the volume of waste in the streets. DISCUSSION AND CONCLUSIONS: Many researchers have demonstrated low sanitation and crowded cities are correlated to proliferation of *Aedes aegypti* population.The University of Ouro Preto has used geoinformatic methods as an innovative action in Epidemiological Vigilance of dengue in Minas Gerais cities. Municipal database and Geoprocessing dengue cases points and contamination of water supply has been used as the actions of the Contingency Plan in the Barra Longa as an innovative model of action in Epidemiological Vigilance for containment and prevention of dengue. Financial Support: CAPES, UFOP, UFMG.

Dengue Outbreak Associated to the worst environmental disaster in Brazil

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COMPARISON OF THE EFFICACY OF THE BG SENTINEL AND SUNA TRAPS FOR CAPTURE OF AEDES AEGYPTI MOSQUITOES

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Aedes aegypti is the primary vector of dengue, chikungunya, and other viruses. *Aedes*-borne virus prevention and control programs have traditionally relied on household surveys, primarily of immature stages to access transmission risk. Fixed traps have numerous advantages over house to house surveys, as they collect adult stages and sampling can be standardized in space and time. The BG sentinel trap uses color contrast and a chemical lure to attract mosquitoes. The SUNA trap was developed for *Anophelines* in Africa and utilizes MB5 as a lure. Our objective was to evaluate both traps, with different combinations of lures and attractants for *Ae. aegypti*. Two traps (SUNA, BG), two lures (BG, MB5, no lure), and CO_2 (with or without) for a total of 9 treatments were compared in an abandoned lumberyard, heavily infested with natural populations of *Ae. aegypti*, in the city of Iquitos, Peru. Five SUNA and four BG traps were placed in covered locations a minimum of 100m apart. Traps were run from 0600-1800 and positions were rotated each day for 9 consecutive days; this process was repeated 2 additional times (3 replicates). A total of 10,654 mosquitoes comprised of 9 species were collected during the experiment. *Ae. aegypti* was the most abundant species (N=6,534, 61%). The average number of *Ae. aegypti* captured per trap night in BG (23) and SUNA (30) was not statistically different (p=0.60). Traps using the BG lure captured significantly more *Ae. aegypti* than no lure (LSmeans p=0.013) but this difference was not significant when compared to the MB5 lure. CO_2 had the highest impact on *Ae. aegypti* counts (p<0.0001); traps with CO_2 averaged 34 *Ae. aegypti* per trap-night compared to 14 *Ae. aegypti* per trap-night without CO_2. Our study demonstrated that the SUNA trap was as effective as the BG sentinel at capturing adult *Ae. aegypti* mosquitos. CO_2 significantly improved collection efficacy and appeared more attractive than lures. Both traps provide practical alternatives to labor intensive household aspirator collections that are subject to significant collector bias and could contribute to entomological surveillance and vector control evaluations.
Funding of research: 1PO1AI098670-01A1, Foundation for National Institutes of Health

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Funding of research: 1PO1AI098670-01A1, Foundation for National Institutes of Health

Highlighted Poster

ANALYSIS BETWEEN A NEW METHODOLOGY AND BRETEAU INDEX TO DETECT AREAS WITH HIGHER RISK TO ARBOVIRUS OCCURRENCE

Parra, Maisa Carla Pereira[1]; Fávaro, Eliane Aparecida[1]; Dibo, Margareth Regina[2]; Chiaravalloti-Neto, Francisco[3]; Nogueira, Mauricio Lacerda[1].

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In the last years, the arboviruses caused thousands cases around the world. Sao Jose do Rio Preto city (SJRP) notified a lot of cases of the Dengue virus (DENV1-4), Zika virus (ZIKV) and Chikungunya virus (CHIKV). *Aedes aegypti* is the main mosquito responsible for the urban transmission of these viruses. The control of arboviruses is performed using methodologies based on immature forms of the vector. The aim of this study were to analyze the cost-benefit to perform the new methodology (NAAaF) based on adult mosquito and Breteau Index (BI) and to associate these results with the arboviruses occurrence. The study area was Vila Toninho neighborhood SJRP city, Sao Paulo, Brazil. During two years, the BI and NAAaF were realized every month (First three weeks: BI; Last week: NAAaF). To perform the NAAaF were installed adult mosquito traps during 24 hours in pre-select residences distant a minimum 100 meters each other, totalizing 60 residences by month. To realize the BI, the blocks were drawn and all the residences the chosen block were visited, totalizing approximately, 1650 residences visited by month. The NAAaF results were calculated by 100 similar to BI. It was collected more than 3,500 culicids mosquitoes divided in 799 *Ae. aegypti*, 9 *Ae. albopictus* and 2775 *Culex sp.*. The *Ae. aegypti* were tested by RT-PCR to detect DENV, ZIKV and CHIKV. It was found 12 pools positive to DENV (8 *Ae. aegypti* female; 4 *Ae. aegypti* male), 36 positive to ZIKV (19 *Ae. aegypti* female; 16 *Ae. aegypti* male; 1 *Ae. albopictus* female) and 22 positive to CHIKV (15 *Ae. aegypti* female; 5 *Ae. aegypti* male; 2 *Ae. albopictus* female). The NAAaF was realized using one driver and one field agent during one week while BI needed one driver and four-field agent during three weeks. In conclusion the NAAaF seemed to be more efficient and cheaper than BI. The association between the NAAaF and the arboviruses occurrence can be visualized in maps showing areas with higher risk to arboviruses occurrence. Then, this methodology can be used by the health authorities to make decisions about epidemiological surveillance.

FINANCIAL SUPPORT: FAPESP, BUTANTAN INSTITUTE.

Author that will present the work: Maisa Carla Pereira Parra

Sessions to apply: Vector Biology-Ecology-Control

Modality of presentation: Oral presentation or Poster under consideration of the Scientific Committee

Highlighted Poster

CHARACTERIZATION OF Aedes Aegypti CULTIVABLE MICROBIOTA BY TANDEM MASS SPECTROMETRY PROTEOTYPING

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The characterization of A*edes aegypti* microbiota is relevant since it plays an important role because affects the vector physiology and influences the vector competence. Proteotyping is a technic that analyze microbial proteome through peptide sequences obtained by mass spectrometry to typing microorganisms from complex samples that contain multiple microorganisms or from isolated. In this study, we compare in-solution and in-gel digestion with trypsin to characterize *Aedes aegypti* microbiota by tandem mass spectrometry proteotyping.
Methods. Abdomen of two female *Aedes aegypti* mosquitoes from hatchery were sterilely dissected 48 hours after blood feeding. They were macerated in 1X PBS and then cultivated in BHI and TSB broth. Soluble bacterial proteins from BHI and TSB samples were extracted and digested with trypsin by in-solution method while only bacterial protein from BHI samples were digested by in-gel method. Peptides were analyzed by MALDI TOF/TOF (Sciex TOF/TOF™ 5800 System) and their sequences were inferred with PARAGON™ algorithm implemented in ProteinPilot™ software. Obtained sequences was annotated using BLAST to find peptides associated with a unique taxonomic group.
Results. Twenty-seven out of ninety-three peptide sequences obtained by in-gel digestion had 100% similarity with proteins related to unique taxonomic group. The major taxonomic groups were Gammaproteobacteria (33 %), Alphaproteobacteria (26 %) and Betaproteobacteria (15 %). Moreover, three different *Pseudomonas* species were found by in gel-digestion. In-solution digestion showed less number of inferred peptides, fourteen out of thirty-six peptide sequences obtained had 100% similarity with proteins related to unique taxonomic group and the major taxonomic group was Alphaproteobacteria (43 %).
Conclusion. These results give an insight about how powerful is tandem mass spectrometry to characterize complex bacterial community. Furthermore, the results showed the importance of methods to decrease the metaproteome sample complexity since it improve separation and ionization efficiencies, which enhance the detection of peptides.

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Factors associated with container infestation by Stegomyia aegypti in Mexico.

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Introduction: Dengue is an important public health problem in tropical and subtropical countries. Mexico spent approximately $88.5 million dollars in surveillance and vector control measures in 2011. However, these programs have not achieved the expected impact on dengue incidence.

Objective: To determinate factors associated with container infestation by *Stegomyia aegypti* in two dengue endemic municipalities from Mexico.

Materials and methods: We carried out a cross sectional study in two dengue endemic towns (Axochiapan and Tepalcingo, Morelos, Mexico). Overall, 391 houses were visited between June and November/2011. During the visit, a questionnaire was applied and the patio and containers were inspected. Through multilevel analysis, we evaluated the factors associated with container infestation including sociodemographic and environmental variables, and vector measures. Characteristics of the container and the household were analyzed in individual and contextual levels, respectively.

Results: From 43,046 containers inspected, 2,696 contained water. 96.3% of household had at least one container with water**.** Adjusted by contextual variables, infestation of containers was more frequent in small and medium containers, for example small utensils, livestock waterer, plastic tubs, buckets and plastic drums (ORa: 3.0 CI95% 1.59-5.64), and in tires (ORa: 30.3 CI95% 7.71-119.3) compared with big container as a water tanks and cement tanks. Regarding contextual variables, Odds of infestation increased as the number of cohabiting also increased (ORa per cohabitant: 0.82 CI95% 0.73-0.92) and decreased as the months passaged towards rainy to dry (June to November; ORa per month: 0.77 CI95% 061-0.96).
Discussion/conclusion: Increasing campaigns for removing tires and small and medium containers would be expected to decrease considerably the vector density. Passing from rainy months in summer (July, August, September) to dry months in autumn (October, November) was associated with a decreased odds of vector potential breeding. Knowing these factors is useful for assessing arbovirus risk trends as well as for planning and evaluating the impact of community control strategies.
Founding: CONACyT – Fondo Sectorial de Investigación en Salud y Seguridad Social: 138511.
Category: Vector Biology-Ecology-Control

EFFECT OF NS1 PROTEIN ON ARBOVIRUS COINFECTION IN MOSQUITOES CELLS

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Introduction: *Aedes aegypti* mosquito is the most important vector for transmission of different arboviruses, which include dengue (DENV) and chikungunya virus (CHIKV), both of them coexisting in the Americas. In mosquitoes the existing innate immune response involves different pathways, among them are: Toll, IMD, Jak-stat and iRNA pathways. In humans and mosquitoes coinfections with both arbovirus have been reported. An additional important element in natural infections with DENV, is the NS1 viral protein. Its effect on the mosquito immune response and viral replication remains undetermined.
Objective: Evaluate the effect of NS1 viral protein on molecules related to immune response in mosquitoes and its the effect on arboviral replication in Aag-2 cells coinfected with both DENV and CHIKV.
Methodology: Mosquito Aag-2 cells were infected with DENV-2 and CHIKV in combination or absence of NS1 viral protein. We used qRT-PCR to detect the viral genome and flow cytometry to determine either infection or coinfection in cells. Also expression of some molecules signalling in immune pathways was assesed.

Results: Our results showed that NS1 protein of DENV modulates the viral replication shorthening the time required to release infective DENV particles. Moreover, in coinfected cells DENV viral titer are significant higher than CHIKV, when NS1 protein is present. Finally, NS1 protein induces significant changes in the mRNA levels for REL1A, Cactus, STAT, and defensin C. Also, we can see NS1 protein in RE in the Aag-2 cells.
Conclusion: DENV NS1 protein not only modulates immune response in mosquitoes cells , also impacts the efficiency of DENV replication. This is the first report showing that NS1 virus protein affects directly the viral replication and the immune response in mosquito cells.

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Insecticide activity of extracts of Turmeric powder (*Curcuma longa L.)* against immature forms *Aedes aegypti*.

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Arbovirusescause important public health problems worldwide. One of the main vectors of several arboviruses is Aedesaegypti, which is highly adapted to urban settings and transmits the four serotypes of dengue (DENV 1-4), zika (ZIKV) and Chikungunya viruses (CHIKV). Several strategies to control infestation have been tested, but synthetic insecticides were popular for many years. However, they have become obsolete due to selection of resistance genes and the damage that they cause to the environment. An alternative to control mosquitoswould beplant extracts, which are free from the aggravating effects caused by the synthetic predecessors. The objective of this work was to evaluate the larvicide activity of the aqueous extract of *Curcuma longa* L., as well as the impact of the extract in larval development. Serial dilutions of the aqueous extract of *C. longa*were applied in fourth stage larvae. Different concentrations were tested in quintuplicates. Larvae from untreated controls were used to assess morphometric changes. Death rates of larvae were evaluated 24 hours after the exposition to different concentrations.Morphological analysis was performed with the aid of image capture programs and computer-coupled measurement systems. We assessed morphometric parameters such as size of respiratory syphon, width and length of the body. Larvicide activity was promising.Low concentrations obtained lethalityranging from 60 to 85% in comparison to untreated controls. Treated larvae presented significant morphometric changes, especially in respiratory syphon. The mean size of the respiratory syphon was increased in treated

larvae, varying from 0.84 to 0.86 mm. The mean size of respiratory syphon in untreated controls was 0.77mm. Histologic and cytogenetic analyses have not been performed yet. Larvicide activity demonstrated by low concentrations of aqueous extracts of *C. longa* reveals a potential use of this plant extract in controlling infestation. This is the first work to date showing significant morphometric alterations in the respiratory syphon. Histologic and cytogenetic analysis may shed light in biological features underlying the action of *C. longa* extracts in larval development.The next step of our work is to evaluate antiviral activity of *C. longa* extract against different serotypes of the DENV.

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Highlighted Poster

GUT MICROBES ENGINEERED TO INHIBIT ZIKA VIRUS INFECTION IN MOSQUITOES.

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Introduction Microbes residing within mosquitoes form a complex tripartite relationship with pathogens that can alter vector competence. Furthermore, microbes can be engineered to produce molecules that interfere with pathogens within the mosquito gut. As such, the use of engineered gut microbes is a promising strategy to investigate as a novel approach to control arthropod-borne disease.
Objective: Use vertically transmitted bacterial symbionts to deliver double stranded RNA (dsRNA) to reduce vector competence of *Aedes aeggypti* mosquitoes to Zika virus (ZIKV).
Methods: We assessed the ability of *Escherichia coli* to deliver dsRNA to mosquitoes *in vitro* and *in vivo*. For *in vitro* infections, we transfected total RNA extracted from bacteria expressing dsRNA. For *in vivo* delivery, we fed mosquitoes on sugar meal spiked with *E. coli* that expressed RNA hairpins.
Results: We designed RNA hairpins to up-regulate mosquito immune pathways as well as hairpins that target the ZIKV directly. In Aag2 cells, hairpins targeting the viruses significantly reduced ZIKV titer. We then assessed the ability of bacteria expressing dsRNA to target PIAS, the negative regulator of the Jak-Stat pathway. At 4 and 7 days post supplementation, PIAS expression was significantly reduced in the treatment compare to the control. Next, we supplemented bacteria in a sugar meal to *Ae. aegypti* for 7 days, and then fed mosquitoes with a ZIKV-infected blood meal. At 14 dpi, we saw significant reduction in ZIKA in treatments that targeted the Jak-Stat pathway as well as hairpins that targeted the virus.
Conclusion: Our encouraging results suggest further investigation into the development of a bacterial dsRNA delivery system is warranted.

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Highlighted Poster

Colonization of Aedes aegypti and associated changes affecting vector competence

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Aedes aegypti is the primary vector for a number of significant arboviruses, including dengue, zika, chikungunya, and yellow fever viruses. Understanding the transmission of these viruses is vital in developing vector-based tools to combat global spread. Consequently, transmission studies are a major focus of arbovirus research, most recently exemplified by dozens of zika virus competence publications. Unfortunately, many studies utilize strains of *Ae. aegypti* that have been colonized for many generations, which is known to affect competence such that these mosquitoes no longer reflect the wild-type mosquitoes. Using colonized mosquitoes may therefore lead to results that are difficult to replicate as well as to erroneous conclusions. However, it is not established what mechanisms are responsible for this change in transmission phenotype. However, it has been established that the microbiome of insectary-reared mosquitoes differs from those collected from the field. This study aims to elucidate changes that occur during colonization of *Ae. aegypti*, specifically those associated with the microbiome and immune response. Adult female mosquitoes were collected from collected from Austin, TX and subsequently reared under insectary conditions. Vector competence for Zika virus was determined for each generation via artificial bloodmeals followed by collection of bodies, legs, and saliva to test for the presence of virus. Vector microbiome was determined by next generation sequencing to detect changes with regards to specific bacterial genera as well as overall diversity. Activation of immune pathways, including JAK-STAT, Toll, and IMD, was determined at baseline (prior to infection) for each generation by qRT-PCR. Susceptibility differences associated with early colonization of *Ae. aegypti* and potential mechanisms responsible will be discussed, along with methods to improve experimental vector competence approaches.

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Aedes aegypti and other mosquitoes in two municipalities of Colombia.

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Category 5-Vector Biology-Ecology-Control

The characterization of basic aspects of mosquito's ecology is crucial to understand factors involved in their distribution and the diseases that they transmitted, in order to improve control strategies. The municipalities of Anapoima and Viotá (Cundinamarca) have different characteristics in terms of house density, vegetation cover, and urban area extension, but both have the presence of vector-borne diseases such as dengue and Zika. The aim of this study was to evaluate some aspects of mosquitoes community composition related to environmental factors and spatial distribution in urban areas of Anapoima and Viotá, Cundinamarca. Entomological surveys for adult mosquitoes using Prokopack aspirators from houses selected randomly by block sizes during wet and dry season on 2017 were applied. Mosquitoes were taxonomically determined, mosquito infestation (number of mosquitoes divided on the number of sampled houses per each block) species richness and evenness were analyzed in terms of seasonality, median temperature, normalized difference vegetation index (NDVI), distance to rivers and municipality. A total of 406 houses were tested (243 Anapoima and 163 Viotá), 7619 mosquitoes were classified, which belong to nine species. The most common species identified were *Aedes aegypti* (7030 individuals) and *Culex quinquefasciatus* (543 individuals). Species richness and evenness were positively associated with NDVI, while the highest infestations were related to areas with lowest NDVI. Regarding municipalities Anapoima showed a higher infestation by *A. aegypti* and *C. quinquefastiatus*, lower richness and low changes on mosquitoes spatial distribution between seasons than Viotá. The dynamics observed in terms of mosquitoes richness could be associated to the availability of breeding and resting places for the different species associated to presence of vegetation. Regarding the density of commont mosquitoes species it could be associated with socio-economic conditions such as problems with water supply that cause water storage producing potential breeding places for mosquitoes such as *A. aegypti and C. quinquefasciatus*. Given the importance of *A. aegypti* and *C. quinquefasciatus* as vectors, these results are important to identify the main areas to improve vector control and surveillance of arboviral disease and contribute to understand the mosquitoes`dynamics at the local scale.

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Detection of Aedes aegypti above 1.800 masl in Cundinamarca, Colombia.

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Category: 5-Vector Biology-Ecology-Control

Dengue virus (DENV) spread is determined by several factors such us environmental, ecological, biological and socio-economic conditions, that influence vector (*A. aegypti*) life cycle, their adaptation and virus transmission. Despite the reports that dengue transmission in Colombia happened at 1800 masl (meters above sea level), DENV and the mosquito have been identified at high elevations in the country. The aim of this work is to evaluate the presence of *A. aegypti* and potential dengue cases on altitudes above 1800 masl, on urban areas of eight municipalities of Cundinamarca, Colombia. A cross sectional study was conducted between June to September 2017 on urban areas of the municipalities: Ubaque (1850 masl), Pacho (1900 masl), Choachí (1960 masl), Machetá (2100 masl), Fosca (2100 masl), Pasca (2200 masl), Junín (2300 masl) and Chipaque (2400 masl). Entomological surveys (searching for adults and immature stages) were applied on houses randomly selected for each municipality. Additionally, dengue suspected cases were identified from local health entities, and patients were visited to know if they had traveled to endemic areas before dengue infection. We found the presence of *A. aegypti* only on three of the inspected municipalities, the highest elevation observed for the mosquito was 1950 masl in Choachí showing a House infestation index(HI): 6.6, Breteau index: 1.6, and adults *A. aegypti* female density(FD): 0.06, in Pacho, the HI was: 22, Breteau: 8.47 and FD: 0.32, finally in Ubaque HI: 16.6, Breteau: 33.3 and FD: 0.33. Regarding the potential dengue cases, the information obtained from suspected cases in five municipalities, showed the presence of autochthonous cases in Choachí (five cases), Junín (three cases) and Pacho (six cases). These results suggest that the elevational range for *A. aegypti* is increasing respect to the previous records for Cundinamarca, being especially important in municipalities where the local authorities are not aware of vector presence. This study identified areas over 1800 masl at risk of dengue transmission or other arboviral diseases. These results highlight the importance to increase the elevational range for the entomological surveillance of *A. aegypti* as well as potential dengue transmission.

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Epidemiological implications of the differential effect of local temperature on DENV infection in mosquitoes

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Dengue fever is endemic in numerous cities in Colombia. Vector control is addressed according to entomological indices like larval index. However, the city of Riohacha, with notably high values of Breteau index shows an incidence comparable to cities like Bello, with low values of Breteau index. On its hand, Villavicencio displays Breteau index lower than Riohacha but higher incidence.

To try to explain the difference on incidences, we collected mosquitoes five different times in those three Colombian cities and estimated infection rates of dengue virus (DENV). We found that infection rates in mosquitoes are negatively correlated with high temperatures of Riohacha while the relationship was positive for Bello and Villavicencio, cities with mean temperatures lower than Riohacha, which suggests a negative influence of high temperatures on infection in mosquitoes by DENV.

We tested if such negative correlation has an impact on public health and if it is replicated in other cities with similar characteristics of population, endemicity, temperature and incidence. For this, we took epidemiological and climatic data for 20 cities across the country and performed correlations at different lag times. We found that cities with higher temperatures displayed negative correlations with incidence while cities with lower temperatures displayed positive correlations, suggesting that relationship between DENV infection in mosquitoes has a non-linear correspondence with temperature. Finally, a *LOESS* analyses were performed among mean weekly incidence of each city and average, maximum and minimum temperatures. The resulting model proposed an increasing effect of temperature on incidence until an inflection point near to 28°C of mean temperature where incidence fall down.

The results of this work suggests that infection of vectors is influenced by local temperature, which prime the idea that dengue fever risk can be stratified in cities in order to make a more rational use of resources.

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Highlighted Poster

Mechanisms of pyrethroid resistance in Aedes aegypti from Colombia.

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Introduction: In Colombia *Aedes aegypti* is the main vector of arboviruses as dengue, chikungunya and Zika. This urban mosquito has the capacity to develop multiple mechanisms to insecticide resistance which may affect vector control.
Objective: To identify the biochemical and molecular mechanisms associated with pyrethroid resistance in laboratory-selected strains and field-collected strains of *Aedes aegypti* from Colombia.
Methods: Three strains, selected in the laboratory for resistance to DDT, Propoxur and lambdacyhalothrin, were compared with7 field collected strains. Rockefeller strain was used as a susceptible control. CDC bioassays were performed to measure the susceptibility status to pyrethroids type I (permethrin) and II (Deltamethrin and lamdacyhalothrin), and potential cross resistance with different types of insecticides, including organochlorine (DDT), carbamate (Propoxur) and organophosphate (Malathion). The enzymatic activity of esterases, Glutathione S transferases and cytochrome P^{450} was measured by biochemical tests. The detection of frequencies of kdr mutations Val1016Ile and Phe1534cys were determined through real-time PCR. A proteomic analysis of midgut and Malpighian tubules using high definition mass spectrometry was performed.
Results: Almost all strains were resistant to permethrin except 1 field strain and the laboratory-selected “DDT” strain. Three strains (selected “propoxur” and “Lambdacihalothrin” and 1 field strain) were resistant to both types of pyrethroids. All evaluated strains were resistant to DDT. Although we evidenced cross resistance between lambdacyhalothrin and propoxur, all field-collected strains were susceptible to propoxur. There was no evidence of malathion resistance.
The main enzymatic mechanism observed in the resistant strains was GST. The kdr mutations were detected in all strains. The allelic frequencies of Cys1534 (ranged 0.44 to 0.99) were greater than for Ile1016 (0.02 to 0.72). The strains that had higher frequencies of both mutations were resistant to both type I and II pyrethroids insecticides. Finally the proteomic analyses confirmed the pathway Glutathione metabolism and additionally evidence an up regulation of translation and ribosomal proteins in resistant mosquitos.
Conclusion: Pyrethroid resistance in *Ae. aegypti* from Colombia are mainly associated to increased GST enzymes and the high frequency of Kdr mutations. The up regulation of ribosomal proteins in the resistance strains may suggest increase protein turnover and/or altered gene expression.

VECTOS: an integrated system for monitoring risk factors associated with arbovirus transmission

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INTRODUCTION: In Colombia, as in many Latin America countries, decision-making and development of effective strategies for vector control of urban diseases such as dengue, Zika and chikungunya is challenging for municipal health authorities. The heterogeneity of urban areas requires a more rational and appropriate approach to prioritizing control measures, in order to direct them according to transmission risk, which can vary greatly within a single city.
OBJECTIVE: To strengthen the capacity of local surveillance systems to identify variables that favors the transmission of urban arboviruses.
METHODOLOGY. A multidisciplinary team with experience in epidemiology, entomology, anthropology, and systems engineering worked together with the local Secretary of Health officials of the Municipalities of Girón, Yopal and Buga (Colombia), in the design of an integrated information system called VECTOS. Information and communication technologies were used in the development of two mobile applications, for capturing entomological and social information, and a web-based system for the collection, geo-referencing and integrated information analysis using free geospatial software. This system facilitates the capture and analysis of information from the Colombian national surveillance system (SIVIGILA), periodic entomological surveys (mosquito larvae and pupae) and social surveys (KAP), in a spatial and temporal context at the neighborhood level.
RESULTS:
The information collected in VECTOS, allows the local-level mapping of data and the creation of graphical reports, using standard analyses that local Secretary of Health officials can easily understand. The system facilitates real-time monitoring of epidemiological indicators, as well as the evaluation of entomological and social risk variables. The analysis of the different variables through generalized linear spatial models (GLSM) has allowed risk stratification taking into account epidemiological, entomological, demographic and environmental variables identified in the neighborhoods.
Conclusion: The use of the VECTOS information tool is facilitating the analysis and understanding of surveillance results and therefore strengthening the capacity of the vector control groups in the participating municipalities.

Assessing the larvicidal activity of intestinal *Aedes aegypti* bacteria

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Vector control is still the main intervention strategy to reduce dissemination of the main urban arboviruses transmitted by the mosquito *Aedes aegypti*. However, mechanical mosquito elimination has become very challenging in the modern urban environment, whereas the increasing occurrence of insecticide resistance has made the use of such chemical compounds less and less effective. In this context, the development of new mitigation interventions is urgently needed. The mosquito gut microbiome has recently been proven to be a great resource for both antiviral and entomopathogenic bacteria. Therefore, we evaluated the larvicidal effect of 15 bacterial isolates obtained from *Ae. aegypti* mosquitoes collected in Botucatu, Brazil. For these bioassays, 10 early L4 *Ae. aegypti* larvae from the Rockefeller were incubated in triplicates with 1 ml of the test substance containing the challenging bacteria in 8 plates (12 well). Both treated and control larvae were maintained in standard insectary conditions at 28 °C, 80% relative and 12/12 h light/dark cycle, with mortality recorded at 24h and 48h post inoculation of either the test or mock substances. Our analyses revealed four isolates from the genera *Pseudomonas, Stenotrophomonas, Pantoea* and *Bacillus*, with mean mortality of around 90 ± 10%. We are currently testing the efficacy of these bacteria in different concentrations as well as other gut bacteria from our library.

Key words: *Aedes aegypti*, gut microbiota, bacteria, biological larvicides.
Sponsorship: FAPESP, CAPES and CNPQ

Neotropical Bats that Co-habit with Humans Function as Dead-End Hosts for Dengue Virus

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Introduction and objective: Several studies have shown Dengue Virus (DENV) nucleic acids and/or antibodies present in neotropical wildlife including bats, suggesting that some bat species may be susceptible to DENV infection. Here we aim to elucidate the role of house-roosting bats in the DENV transmission cycle.

Materials and methods: Bats were sampled in households located in high and low dengue incidence regions during rainy and dry seasons in Costa Rica. We captured 318 bats specimens from 12 different species in 29 households. Necropsies were performed in 205 bats to analyze virus presence in heart, lung, spleen, liver, intestine, kidney, and brain tissue. Histopathology, serology and entomological studies were also performed. Results: Histopathology studies from all organs showed no significant findings of disease or infection. Sera were analyzed by PRNT90 for a seroprevalence of 22% (53/241), and by PCR for 8.8% (28/318) positive bats for DENV RNA. From these 28 bats, 11 intestine samples were analyzed by RT-PCR. Two intestines were DENV RNA positive for the same dengue serotype detected in blood. Viral isolation from all positive organs or blood was unsuccessful. Additionally, viral load analysis in positive blood samples by qRT-PCR showed virus concentrations under the minimal dose required for mosquito infection. Simultaneously, 651 mosquitoes were collected using EVS-CO2 traps and analyzed for DENV and feeding preferences (bat cytochrome b). Only three mosquitoes were found DENV positive and none was positive for bat cytochrome b. Our results suggest an accidental presence of DENV in bats probably caused from oral ingestion of infected mosquitoes. Phylogenetic analyses suggest also a spillover event from humans to bats.

Conclusion: We conclude that bats in these urban environments do not sustain DENV amplification, they do not have a role as reservoirs, but function as epidemiological dead end hosts for this virus.

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Evaluation of an Autocidal Gravid Ovitrap intervention for the control of *Aedes aegypti* in the Lower Rio Grande Valley, Texas

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Introduction: Mosquito-borne viruses driven by *Aedes aegypti* continue to emerge and re-emerge globally. The Lower Rio Grande Valley (LRGV) has now experienced local transmission of Dengue, Chikungunya, and Zika virus. Our objective was to evaluate an Autocidal Gravid Ovitrap (AGO) intervention to identify strengths and limitations in South Texas for this form of mosquito control.

Methods: Eight communities from the LRGV region were selected for our study. Our intervention was carried out from August–December of 2017, concurrent with the recognized arboviral transmission season. Two middle-income and two low-income communities were randomly allocated into either control or intervention. The intervention communities had systematic recruitment of all available houses within those communities. Each recruited house had three AGO's placed in their premises, that were reset every two months. An average of five houses in each community served as our reference houses, weekly data was collected from indoor and outdoor sentinel AGO traps.

Results: For the intervention communities, we were able to recruit 50-75% of the houses. The most common cause for recruitment failure was absence of homeowners (35%), followed by abandoned houses (15%). For the October reset in the intervention communities we found an average of 80 mosquitoes per house, for both middle and low income communities. In the December reset we found an average of 50 mosquitoes per house. Mosquito densities between the control and intervention communities in either indoor and outdoor AGO reference traps were not statistically different.

Conclusion: Vector control programs for *Aedes* mosquitoes are urgently needed, especially within the Texas—Mexico border to fight off the introduction and establishment arboviral diseases such as Zika. The results of the first year of the AGO intervention yielded no evidence of reduced mosquito populations, likely due to the low recruitment of homes into the study. During year two, we plan to recruit more homes to achieve 80% of them having 3 AGO traps per house in a community.

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