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Oral Presentations

Diagnostics-Prognostics-Clinical

1

High and specific Humoral maternal-and-child response to NS1 correlates with long-term neurological effects during prenatal Zika virus exposure

Doris M. Salgado[1,2], José S. Cortés[1], William Mejía[1,2], Rocío Vega[1,2], Angela Ortíz[1,2], Irene Bosch[3,4], Isabel DeLaura[1,5], Lee Gehrke[6,7], **Carlos F. Narváez[1,2]**

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Humoral responses play a critical role in the protection and pathogenesis of the Zika virus (ZIKV) infection and its congenital consequences. To determine the pre-exposure to ZIKV in high-risk populations such as pregnant women and children through their virus-specific antibody response is crucial. Due to the high number of asymptomatic infections and cross-reactive responses among co-circulating Flaviviruses, screening for prior exposure is a challenge. Here, we analyzed the circulating ZIKV and dengue virus (DENV)-specific IgG, and the neurodevelopment in 104 symptomatic mothers admitted to the Hospital Universitario de Neiva, Colombia and the progeny exposed to ZIKV in utero, during the epidemic year. There were 17 newborns diagnosed with congenital zika syndrome (CZS) in this cohort. A NS1-based indirect ELISA was used to evaluate levels of ZIKV and DENV-specific IgG. A 12 months neurological follow-up including the specific Bayley III scale evaluating the motor, language, and cognitive aspects, was applied to all children. The ZIKV IgG NS1-based ELISA shows a low cross-reactivity with DENV when samples of children with confirmed primary or secondary DENV infection were tested. Mothers with ZIKV infections shown a significantly higher ratio of plasma ZIKV/DENV1-4 NS1 IgG than mothers without suspected ZIKV infection and IgG-ZIKV was higher in mothers that their children. When mothers and progeny were match, the levels of anti-NS1-ZIKV IgG was significantly higher in those that developed CZS that mothers exposed to ZIKV but children without CZS. We propose a transplacental movement of ZIKV NS1-IgG to explain the correlation found in virus-specific IgG between mothers and children. Levels of ZIKV NS1-IgG were transient as they fall to undetectable in children older 6 months. Neurological follow-up was done in 104 infants, including 17 patients with microcephaly, 10 with ocular abnormalities and 77 children without CZS. 16% of the children prenatally exposed to ZIKV without CZS presented other cognitive compromise, 15% motor and 6% of the language impairment. Thus, ZIKV prenatal infections induced a virus-specific humoral response with potential trans-placenta movement and correlated in magnitude with infection. Additionally, prenatal infection of ZIKV results in a high frequency of cognitive disorders that are uncover upon neurological follow-up.

2

Clinical and epidemiological characterization of orthobunyavirus associated with febrile disease in humans in the Peruvian Amazon

Crystyan Siles[1], *Stalin Vilcarromero[1], Helvio Astete[1], Carlos Alvarez[2], Amy C. Morrison[1,3], Carolina Guevara[1], Eugenio Abente[1], Julia S. Ampuero[1]*

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Introduction: Emerging infectious diseases can negatively impact medical readiness of the US military and continued surveillance is warranted to support Force Health Protection. Viruses from the genus Orthobunyavirus circulate globally in tropical, temperate and arctic ecological niches and include Oropouche (OROV) Guaroa (GROV) and viruses from the group C serogroup (GRCV). Objectives: To characterize the clinical and epidemiological aspects of orthobunyaviruses associated with febrile disease in humans. Methods: Subjects no less than 5 years old with fever no

less than 5 days in duration who sought medical attention in urban, peri-urban and rural health facilities in Iquitos, Peru were invited to participate in an IRB-approved clinic-based surveillance study. Acute and convalescent blood sample were collected. Samples were screened for evidence of viral infection by RT-PCR and/or viral isolation and/or a 4-fold increase in IgM antibodies as tested by ELISA in paired samples. Results: 2448/8395 samples collected between December 2010 and May 2016 were positive for an arbovirus: flaviviruses (n=2308), alphaviruses (n=94) and orthobunyaviruses (n=46). Among the orthobunyaviruses found, 20 were GROV, 17 were GRCV and 9 were OROV. Of the 46 positive-cases for an orthobunyavirus, 50% were women and the median age was 32.2 years (age range: 9-64). In all cases chills, malaise, and headache were reported. A comparison was made between each the clinical and epidemiological variables associated with an orthobunyavirus (OROV vs. GRCV vs. GROV). Three significant associations were identified in the univariate analysis: hospitalization and gastrointestinal bleeding with OROV ($p = 0.021$), pruritus with GROV ($p = 0.002$) and exposure (residence) to rural areas with GRCV ($p = 0.007$) and GROV ($p = 0.026$). Discussion and conclusion: Living in rural areas was identified as a risk factor for GRCV and GROV infections, highlighting the need for use of mosquito repellent strategies for military service members deployed to rural areas. Continued surveillance is necessary to characterize emerging pathogens and the risk they pose to civilian and military readiness.

3

Standard case definitions miss three-fourths of pediatric Zika cases as the clinical spectrum of Zika changes across pediatric age

Fausto Bustos-Carrillo[1,3], *Raquel Burger-Calderon*[1,2], *Lionel Gresh*[2], *Sergio Ojeda*[2], *Nery Sánchez*[2], *Miguel Plazaola*[2], *Leah Katzelnick*[1], *Brenda López-Mercado*[2], *Jairo Carey-Monterrey*[2], *Douglas Elizondo*[2], *Sonia Arguello*[2], *Andrea Nuñez*[2,4], *Aubree Gordon*[5], *Ángel Balmaseda*[2,4], *Guillermina Kuan*[6], *Eva Harris*[1]

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Introduction. While Zika in adults presents as a mild, dengue-like disease, pediatric Zika remains an understudied topic. The World Health Organization (WHO) and Pan American Health Organization (PAHO) Zika case definitions have not been assessed in children. Objective.We aimed to characterize clinical profiles and evaluate the diagnostic performance of the WHO and PAHO case definitions in a large cohort of pediatric Zika cases. Materials and methods. We prospectively followed a cohort of 3,700 children 2-14 years old in Managua, Nicaragua, from January 2016 to February 2017, encompassing the major 2016 Zika epidemic. We characterized acute clinical findings (signs, symptoms, and complete blood counts) and tested participants with a broad range of clinical profiles suspected of Zika using molecular and serological assays.Results. We analyzed 556 laboratory-confirmed Zika cases and 548 non-Zika cases. The WHO and PAHO case definitions captured 176 and 109 confirmed Zika cases, respectively, who presented with the most clinical findings and a dengue-like clinical profile. The remaining two-thirds of Zika cases, principally characterized by undifferentiated fever or afebrile rash, were missed. Among Zika cases, rash (n=440) –particularly generalized erythematous rash (n=334), fever (n=333), leukopenia (n=217), and headache (n=203) were most common and peaked within three days of illness onset. The most common Zika presentation over the first week of illness was rash only (n=80). The sensitivity of Zika case definitions increased across pediatric age (from 11% to 56% for the WHO case definition and from 6% to 37% for the PAHO case definition), as the prevalence of most clinical findings (particularly arthralgia) increased with age, irrespective of prior dengue virus infection. Consequently, Zika manifested differently across pediatric age; older Zika cases presented with a dengue-like clinical profile while younger Zika cases presented with undifferentiated fever or afebrile rash. Discussion. We provide the most thorough description of pediatric Zika to date. Most pediatric Zika cases go undetected under the WHO and PAHO case definitions, suggesting current standards for Zika case ascertainment require revision. Zika manifests with mild but differing clinical profiles across pediatric age, presenting major challenges to diagnosis, surveillance, and efforts to control future Zika epidemics.

4

Circulating microparticles deliver inflammatory signals in dengue

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Introduction: Dengue is the most prevalent human arbovirus disease worldwide. Dengue virus infection causes diseases from self-limited fever to severe dengue. Even though the mechanisms underlying severe dengue syndrome are not completely understood, is widely accepted that increased inflammatory response plays important roles in dengue pathogenesis. Microparticles are small vesicles actively shed from cellular membrane with recognized roles in cellular communication in immunity and inflammation. Objective: This study aimed to investigate the cellular source of circulating microparticles in dengue patients and their inflammatory content regarding different clinical presentations and degrees of severity. Methodology and results: Here we quantified microparticles in plasma from 65 dengue patients included according to institutional review board and identified their sources through flow cytometry. We observed increased levels of circulating microparticles in dengue-infected patients compared to healthy controls. Platelets were the main source of microparticles in both conditions. Dengue-infected patients presented lower percentages of platelet-microparticles alongside an enrichment in RBC-, lymphocyte- and endothelial-microparticles. Despite lower frequencies, platelet-microparticles' numbers were still higher in dengue patients than in control. Importantly, increased levels of RBC- and endothelial-microparticles associated with severe dengue syndrome, while microparticles from CD8+ lymphocytes and NK cells associated with mild dengue. To identify cytokines carried by microparticles, we performed multiplex analysis in whole plasma and paired microparticles-depleted plasma. The levels of the cytokines IL-1β, VEGF and PDGF but not TNF-α and IFN-γ, were completely blunted by microparticles-depletion in plasma from mild or severe dengue patients. Moreover, we demonstrated that the chemokines PF4/CXCL4, RANTES/CCL5, MIP-1β/CCL4 and MCP-1/CCL2, but not IL-8/CXCL8 and IP-10/CXCL10 circulate chiefly in microparticles. We confirmed through Western blot the presence of pro- and cleaved-IL-1β only in microparticles from dengue-infected patients, with higher IL-1β processing in severe dengue syndrome. Conclusion: These results indicate that differential sources and inflammatory content in circulating microparticles associate with severity of dengue.

5

The effect of prior Zika virus infection on markers of male fertility

Natalie Bowman[1], *Viviana Pinedo-Cancino*[2], *Filemón Bucardo*[3], *Jayrintzina Palacios*[3], *Hernán Vanegas-Solis*[3], *María Vásquez-Chasnamote*[2], *Rafael Saavedra-Langer*[2], *Saby Pinedo-Sotelo*[2], *Aravinda de Silva*[1], *Benjamin Leader*[4], *Matthew Coward*[1]

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Previously considered a mild febrile illness of little clinical importance, unusual characteristics of Zika virus (ZIKV) were newly identified during the 2015-2016 American pandemic – prolonged shedding in semen, sexual transmissibility, and congenital anomalies. In mouse models, ZIKV infection decreased testicular size and sperm production, resulting in significantly impaired fertility. It remains unknown if ZIKV has a similar effect in humans, as only two small studies to date have reported a decline in sperm count and increased anomalies after acute ZIKV infection that recovered after several weeks. Fever itself can impair sperm production, confounding interpretation of these results. To investigate the long-term effect of ZIKV infection on human male fertility, we conducted a cohort study of young healthy men in two sites (Peru and Nicaragua) affected by the ZIKV epidemic. Approximately half of the population at each site was exposed to ZIKV, providing internal cases and controls. Men aged 18-40 years were enrolled at the start of the arbovirus transmission season at each site. Demographic, clinical, and epidemiological data were collected at enrollment and updated quarterly. Men provided semen and blood samples quarterly for 6 months. Blood was tested for ZIKV EDIII IgG,

dengue IgG and IgM, testosterone, and inhibin B. Fresh semen analysis was performed on-site; frozen semen was shipped to Reprosource, Inc. for Advanced Semen ReportTM. Semen and serum markers of fertility averaged over visits were compared between ZIKV-seropositive and seronegative men. Samples collected after febrile episodes or incident dengue infections were analyzed separately. Data were analyzed using Student's t-test or Wilcoxon rank-sum test for continuous data and Chi-squared tests or generalized linear regression for categorical data. 110 men were enrolled (50 in Peru, 60 in Nicaragua). Mean age was 24 years. 96 completed at least two visits. Serological and Advance Semen ReportsTM are in process. Preliminary data from one site showed median sperm counts of 177-236 x 106, with 73% motility and 64-71% vitality. 4.1-6% of the sperm had normal morphology. This study will address the important unanswered question of ZIKV's effect on male fertility and distinguish between temporary, fever-related declines in fertility and long-term virus-mediated damage.

6

Association between Congenital Zika Virus Exposure and Growth and Neurodevelopmental Delays in Children Born Without Microcephaly

Nivison Nery Junior[1,2], *Juan Pablo Aguilar-Ticona*[1], *Claudia Sampaney*[3], *Millani Lessa*[1], *Bruno Freitas*[4], *Danielle Oliveira*[5], *Edison Durigon*[5], *Mitermayer Reis*[1,2], *Federico Costa*[1,2], *Albert Ko*[2,3]

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Introduction: The risk of developing growth and neurodevelopmental and neurosensory anomalies among children who were exposed to Zika virus (ZIKV) in utero but who did not have evidence of congenital Zika syndrome at birth has not been entirely characterized. Materials and Methods: We prospectively followed a cohort of81 mothers and their children who were born at a maternity reference hospital during the microcephaly outbreak, from October 2015 to January 2016, in Salvador, Brazil and did not have evidence of microcephaly or congenital Zika syndrome.We performed the plaque reduction neutralization test (PRNT) with ZIKV and DENV1-4on blood samples collected from mothers at the time of their infant's birth to evaluate congenital exposure of the child to ZIKV. Clinical evaluations were performed for the children with2 to 3 years of age which included anthropometric measurements, Bayley Scales of Infant and Toddler Development III (Bayley III) assessment, and neurologic, auditory and ophthalmologic evaluations. Results: ZIKV-exposed children had significantly lower median Z score in length and weight (-0.120 vs 0.166, $p<0.05$ and 0.230 vs 1.132, $p<0.05$, respectively) when compared to non-exposed children. ZIKV-exposed children were more likely to have neurodevelopment abnormalities during the Bayley III screening (15% vs. 0%, $p<0.4$) but this difference was not statistically significant. After performing the complete Bayley III scale assessment, we found differences in language, motor and cognitive skill scores between exposed and non-exposed children (9% vs. 4%; 4% vs. 0% and 2% vs. 0%, respectively) but these differences were also not statistically significant. Discussion/conclusion: We found that children who had evidence of ZIKV exposure but did not develop congenital ZIKV syndrome at birth had impaired anthropometric growth at 2 to 3 years of age. An increased proportion of these children had neurodevelopment impairments compared to children without congenital ZIKV exposure, however these differences were not statistically significant. These findings emphasize the importance of continued close monitoring for developmental delays amongst the large numbers of children who were born during the microcephaly outbreak but did not have overt ZIKV-associated birth defects.

Epi-Phylogenetics-Modeling-Burden

7

Population-based survey of Zika and chikungunya infections in a dengue hyperendemic city in Brazil

Cynthia Braga[1], *Celina Turchi-Martelli*[1], *Wayner Souza*[1], *Carlos Brito*[2], *Maria de Fatima Militão-Albuquerque*[1], *Clarice Lins-Morais*[1], *Carlos Frederico Albuquerque-Melo*[3], *Thomas Jaenisch*[4], *Ernesto Torres de Azevedo Marques*[1,5], *Isabelle Freire Tabosa Viana*[1]

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trict, Brazil; [4]University of Heidelberg, Heidelberg, Bade-Vurtemberga, Germany; [5]University of Pittsburgh, Pittsburgh, PA, USA

Introduction: In the last four decades, Dengue virus (DENV1-4) have circulated in endemic and epidemic cycles in the Northeast of Brazil. Zika (ZIKV) and Chikungunya (CHIKV) were recently introduced (2015/2016) in Recife, the epicenter of the Zika microcephaly epidemic. Chikungunya reported cases and deaths had an upward trend in this setting. Seroprevalence surveys of these arbovirus in urban settings are invaluable to understand the dynamic of transmission, surveillance and modelling purposes. Objective of the study: To estimate the prevalence of recent and past exposure of dengue, Zika and/or Chikungunya within diverse socioeconomic areas in the city of Recife. Materials and methods: We conducted a population-based survey in stratified sample of residents aged 5 to 65 years in the city of Recife from September 2018 to February 2019. We categorized the neighborhoods according to high, intermediate and low-income status (IBGE). We performed household interviews with samples collection (blood, saliva and urine). Molecular (qRT-PCR) and immunological (ELISA) assays are being performed at the Department of Virology, IAM-FIOCRUZ. In this analysis, we defined any molecular or serological positive result as viral exposure. Results: The survey included 2,079 residents from 887 randomly selected houses. Preliminary results - 2,079 (100%) participants were tested for anti-DENV and anti-ZIKV IgG, 1,754 (84.4%) for anti-CHIKV IgG, 1,597 for anti-CHIKV IgM and 1,654 (79.6%) for anti-Zika IgM. Of the serological samples screened for anti-ZIKV IgM, 10 (0.6%) were positive and 7 (0.4%) were undetermined. The prevalence of Zika (IgG) was 47.3% (95% CI: 42.8-51.9%), 50.3% (95% CI: 46.8-5.8%) and 50.8% (95% CI: 47.2-54.4%), while the prevalence of CHIKV (IgG and/or IgM) was 28.0% (95% CI: 23.2-33.1%), 41.8% (95% CI: 38.1-45.6%) and 42.6% (95% CI: 38.6-46.7%) in the upper, middle and low income neighborhoods, respectively. The overall prevalence of dengue infection was 88.4% (95%CI: 87.0-89.7). Discussion/conclusion: The preliminary results show the persistence of ZIKV transmission in the population. The analysis also showed a gradient in the seroprevalence of chikungunya in relation to the socioeconomic level of the area, with less exposure to the population living in upper income neighborhoods.

8

Arboviral genomic surveillance in Paraguay reveals high molecular diversity of DENV and CHIKV in recent outbreaks

Cynthia Vázquez[1], *Tiago Gräf*[2], *Marta Giovanetti*[3], *Fernanda Bruycker*[3], *Wagner Fonseca*[4], *Andrea Gómez de la Fuente*[1], *Shirley Villalba*[1], *Ana Maria Bispo de Filippis*[3], *Jairo Méndez*[5], *Luiz Carlos Junior Alcântara*[3],

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Paraguay is a South American inland country with a long history of DENV infections. Besides that, in 2015 the country has reported the first outbreak of CHIKV and the first infection cases of ZIKV. Here we present the results of a genomic surveillance study that analyzed a selected group of samples in 2018 in Paraguay. Aiming to investigate the CHIKV evolutionary history and current molecular diversity of DENV, 56 samples from eight departments collected between 2014 and 2018 were analyzed by real-time PCR and sequencing was attempted by using the portable MinION nanopore device. We were able to generate 33 genomes (17 CHIKV genomes and 16 DENV) that were submitted to phylogenetic analyses with phylogeographical reconstruction. Our results show that the CHIKV Asian genotype was introduced at least twice in Paraguay: in October 2014, most likely coming from Puerto Rico; and again in November 2015, with the most likely origin being estimated as Central America. Our analyses also identified the introduction of the East/Central/South African (ECSA) genotype in the Amambay department in December 2017, causing a CHIKV outbreak in this region after a period of very few CHIKV infections reported in the country. The origin this time was Brazil. Regarding DENV, all samples were from 2018 and we could detect the co-circulation of serotypes 1 (six genomes) and 4 (ten genomes). Brazil was reconstructed as the most likely origin for both DENV-1 and DENV-4 lineages circulating in the Paraguay in 2018, however, Argentina was also estimated as a plausible (posterior probability = 0.22) origin for DENV-1. In summary, our study reveals

a high arboviral molecular diversity in Paraguay, which within four years of observations saw the circulation of two CHIKV genotypes with multiple introductions, and two DENV serotypes. It also shows the valuable information that genomic surveillance applied to the investigation of arboviroses can generate, unveiling viral diffusion patterns that can be used to better predict future outbreaks.

9

Dengue and Zika viruses seroprevalence in a community-based cohort study in southern Puerto Rico

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Communities Organized forthe Prevention of Arboviruses (COPA) is a cohort study initiated in 2018 in Ponce, Puerto Rico, to measure arboviral disease risk and provide a platform to evaluate interventions. No current dengue virus (DENV) seroprevalence estimates are available for Puerto Rico. A newly available DENV vaccine can only be administered to individuals who had a previous DENV infection. Seroprevalence estimates are needed to inform the appropriateness of the vaccine in Puerto Rico and estimate its potential impact. The last DENV outbreak in Puerto Rico occurred in 2013, followed by a Chikungunya outbreak in 2014 and a Zika virus (ZIKV) outbreak occurred in 2016. We recruited participants aged 1–50 years (y) from households in 38 study clusters. Each participant completed an interview and provided a blood specimen, which was tested for the 4 DENV serotypes and ZIKV by plaque reduction neutralization assay (PRNT). Neutralizing antibody (PRNT50) titers positive at serum dilutions greater than 1:4 against DENV 1–4 or ZIKV were considered indicative of a previous infection. We used preliminary data to assess the seroprevalence of DENV and ZIKV by age. Updated results will be available in early 2020. During 2018–2019, 4,352 participants were enrolled and PRNT results are available for 184 participants ages 9-15 years. Overall, 57% (105) were seropositive for DENV, and seroprevalence varied from 53% (37/70) among 9–11 year-old children to 60% (68/114) among children aged 12–15 years. Overall, 45% (82) had neutralizing antibodies against more than 1 DENV serotype. A total of 34% were seropositive for ZIKV, 30% among 9–11-year-old children, and 37% among children aged 12–15 years. In this sample of 9–15-year-old children in Puerto Rico, more than half had been previously infected with a DENV and nearly one-third with ZIKV. These preliminary DENV seroprevalence data from an urban area of southern Puerto Rico suggest moderate transmission intensity as defined by WHO.

10

Yellow fever virus inside urban and periurban areas of Minas Gerais during major outbreaks in Brazil, 2017-2018

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The Brazilian 2016-2019 yellow fever (YF) epidemics have been considered the largest sylvatic YF outbreak in 70 years and was associated with a new lineage of the yellow fever virus (YFV). We investigated the YFV RNA in 771 NHP carcasses collected in urban, periurban, and rural areas of Minas Gerais (MG) state, from January/2017 to December/2018. Samples were analyzed according to the period of sampling, NHP genera, sampling areas,and sampling areas/NHP genera to compare the proportions of YFV-positive carcasses and YFV genomic loads. YFV was detected in the liver of 38.1% of 771 NHP. A total of 141of these NHP had brain samples tested, and YFV was detected in the brain from carcasses in which YFV was detected (n=36) and non-detected (n=21) in the liver, previously. YFV was detected in eight different species of the gen-

era Alouatta, Callicebus, Callithrix, and Sapajus, from rural, periurban and urban areas. YFV detection was positively associated with the rural environment and with epidemic periods, but with viral persistence during the non-epidemic dry season of 2017. Lower viral genomic loads were estimated in carcasses collected in urban areas. YFV detection and cycle quantification values in the liver were positively associated with YFV detection in the brain. The results confirmed the wide circulation of YFV in MG, including urban densely populated areas and new epizootics in 49 municipalities, where YF has not been previously reported during this period. The higher YFV dissemination in rural areas than in urban areas associated with lower genomic viral loads in urban NHP could have played a role in preventing urban transmission to humans. Although no human case was epidemiologically linked to urban transmission during the 2016-2019 outbreaks, the presence of YFV-infected NHP in urban areas together with high infestation by *Aedes aegypti* poses risks for YFV transmission and urbanization. Further studies should be conducted to better understand the YFV susceptibility and pathogenesis in the Brazilian NHP YF group: Érica Mello[2], Gabriela Oliveira[1], Pedro Alves[3], Vítor Mendonça[1], Rodolfo Stumpp[1], Alaine Prado[1], Adriano Paglia[1], Fernando Perini[1].

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Dengue seasonality and synchrony across the Americas

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Dengue is endemic throughout most of the Americas, but little is known about regional dynamics. We examined seasonal and multiannual dengue trends and possible underlying mechanisms across the Americas. We used monthly dengue cases from 241 provinces in 13 countries, ranging from 1985 to 2017 (6-22 years) to isolate seasonal (8-16 months) and multiannual (17-70 month) patterns using continuous wavelet transforms. We then analyzed coherence and differences in timing on these two scales between 57,840 province pairs. For each province, we estimated within-year seasonality and the peak magnitude for each season, a proxy for seasonal transmission intensity, using generalized linear models. Dengue is strongly seasonal across the region. Earlier seasonal peaks (February – April) occurred in the Southern Hemisphere and during later months (August – November) in the Northern Hemisphere. Brazilian provinces had the highest seasonal intensity, with heterogeneous intensity across other countries, including Mexico. Province's seasonal patterns showed high similarity within country, but also had variability from province-to-province, particularly in Colombia and Venezuela. Sub-regional and regional large-scale outbreaks occurred in 2005-2007, and 2009-2011. Comparing province pairs' dengue time-series by distance, close provinces had the strongest similarity in their seasonal (0.48 [95% Confidence Interval (CI): 0.14 to 0.75) and multiannual trends (0.46 [95% CI: 0.22 to 0.73]). These similarities decreased up to 1,500 Km, after which, average coherence remained relatively constant (0.46). The overall average time difference between seasonal patterns increased as distance increased. In contrast, average time lags of multiannual outbreaks increased up to 1,000 Km and then stabilized at a 6-month lag for greater distances, suggesting higher local synchrony for seasonal epidemics and a high degree of regional synchrony for multiannual epidemics. Our findings highlight the importance of regional epidemic dynamics. Seasonal peaks occur earlier in the Southern hemisphere and later in the Northern Hemisphere, likely due to climatic variation with some influence from neighboring provinces. Seasonal epidemics had decreased synchrony with distance whereas multiannual epidemics had high synchrony even at farther distances. Seasonal transmission showed a clear geographical pattern, but multiannual epidemics less so, suggesting that different factors are at play across these two time-scales.

Human Behavior & Community Engagement

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Risk for sexually transmitted ZIKV amongst a cohort of urban slum residents during the Zika outbreak

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Introduction: Zika virus (ZIKV) can be sexually transmitted but the contribution of this specific mode of transmission to overall transmission during the Zika outbreak in the Americas has not been fully elucidated.Method:We prospectively followed a cohort of 359adult and adolescent residents from an urban slum community in Salvador, Brazil who had age <13years and for whom sera was collected before (February 2015) and after (October 2015) the Zika outbreak.We used sero conversion in the ZIKV IgG3 NS1 ELISA to define ZIKV exposure. We interviewed residents to obtain information on sexual behavior, which included the number of sexual partners, casual sex and condom use, and information on signs and symptoms of ZIKV infection. We evaluated among participants living in the same household whether sexual relationships were more likely to be associated to concordant ZIKV exposure status than non-sexual relationships(mother-daughter, brother-sister, for example).Results: Among the 359 cohort participants,207(57.7%) had serologic evidence of ZIKV during the outbreak period. Sexual behavior was not associated to ZIKV infection in the general population.Among 79males and 128females, 38.2% and 61.8%, respectively, were exposed to ZIKV during the outbreak. Gender stratified analyses found that males who engaged in casual sexual relations were more likely to be ZIKV exposed than non-exposed(OR = 5.8; 95% CI 1.2 –28.4). We identified184 pairwise relationships containing at least one individual infected with ZIKV, of which, 16(8.7%)were pairs who had sexual relations and 168(91.3%)were pairs who did not have sexual relations. Pairs with sexual relations had similar proportion of concordant ZIKV exposure status as pairs without sexual relations(5.9 vs 11.6%, p = 0.24). Conclusion: In this prospective study of adults and adolescents during the ZIKV outbreak, we did not identify an association between sexual behaviors and increased risk of ZIKV transmission in the general population. However, males reporting casual sexual encounters had fivefold increased risk for acquiring ZIKV infection. These finding suggest that although sexual transmission may not have contributed significantly to overall transmission in a setting of high *Aedes aegypti* burden, specific groups may be at increased risk for sexual transmission.

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Measuring health related quality of life for dengue patients in Iquitos, Peru

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Previous studies measuring the health-related quality of life (HRQoL) of individuals with dengue focused on treatment seeking populations. However, the vast majority of global dengue cases are unlikely to be detected by health systems. Representative measurements of HRQoL should therefore include patients with disease not likely to trigger treatment-seeking behavior. This study based in Iquitos Peru used the Quality of Wellbeing Scale-Self Administered, a survey that enquires about physical, psychological and social wellbeing and rates HRQoL between 0 (death) and 1 (optimum function), to evaluate the impact of dengue on the HRQoL of those with the disease. In order to enroll treatment and non-treatment seeking participants three modalities of

participant recruitment were used. In addition to clinic and community- based febrile surveillance, a contact-cluster methodology was also employed to identify infected individuals less likely to seek treatment. We measured changes in HRQoL and identified common areas of health impairment in 73 virologically confirmed dengue cases at 3 time points during the participant's illness; the early-acute (days 0-6 post symptom onset), late-acute (days 7-20) and convalescent illness phases (days 20 +). Participants reported impairments in physical and psychological health and social wellbeing at significantly higher frequency during the early-acute versus convalescent illness phase (Fisher's exact: $P<0.01$).There was substantial heterogeneity in scores during each illness phase with median scores in the early-acute, late-acute and convalescent phases of 0.56 (IQR: 0.41-0.64), 0.70 (IQR: 0.57-0.94), and 1 (IQR: 0.80-1.00), respectively. This novel application of the Quality of Wellbeing Scale-Self Administered has demonstrated the potential for its use as a research tool, not only in dengue but also for other acute infectious diseases. Furthermore, these data illustrate the significant impact of dengue on HRQoL in a population predominantly recruited in the community, a previously understudied group.

Immunology-Vaccines

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Antibody Fc-effector functions as immune correlates of protection against symptomatic dengue virus infection

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Dengue is a mosquito-borne illness caused by one of four dengue virus serotypes (DENV1-4). Infections can be inapparent or present with a clinical spectrum ranging from classical dengue fever to severe dengue disease. Severe cases are often associated with a heterotypic secondary infection. In our long-standing pediatric cohort study of dengue in Nicaragua, we have shown that pre-infection DENV neutralizing antibodies play an important role in protection against symptomatic infection, while low pre-existing antibody titers can enhance dengue disease severity. Beyond antigen-specific antibody functions, antibodies can confer protection or mediate risk through multiple other mechanisms. The role of Fc effector function in dengue has not been systematically investigated and is a critical gap in knowledge. Here, we investigated novel immune correlates by exploring several features of the Fc region of anti-DENV antibodies. We selected pre-infection sera from 30 inapparent and 30 symptomatic DENV3 infections in our cohort study. We incubated these samples with DENV1-4 and ZIKV recombinant envelope protein (recE)- and non-structural protein 1 (NS1)-coupled beads. Biophysical features were assessed by Luminex. Effector functions were measured by incubating the immune complexes with THP-1 monocytic cells (antibody-dependent cellular phagocytosis), primary neutrophils (antibody-dependent neutrophil phagocytosis), primary NK cells (antibody-dependent cellular cytotoxicity) or guinea pig complement (antibody-dependent complement deposition). We then looked for features that were correlated with protection from a subsequent DENV3 symptomatic secondary infection. Our data suggest that total IgG and IgG4 levels against NS1 and recE, respectively, are higher in the pre-inapparent infection samples, indicating a potential role in protection. Binding to Fc receptors FcyRIIIA and FcyRIIB also appears to correlate with protection. Regarding effector function, multivariate analyses suggest two main modes of protection: one involving antibody-mediated phagocytosis and complement deposition and another involving NK cell-mediated degranulation. Several of these findings were observed with recombinant antigens from different DENV serotypes, demonstrating that these features are associated with cross-reactive antibodies. Thus, we found novel immune correlate candidates of protection against symptomatic DENV3 secondary infection associated with certain IgG isotypes and Fc receptor binding capacity as well as effector functions such as phagocytosis, complement activation, and cytotoxicity.

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HLA upregulation and NKG2D soluble ligands secretion as possible mechanisms of immune evasion during acute dengue infection.

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Natural killer (NK) cells are lymphocytes of the innate immune system whose activation during viral infection is dependent on the balance of inhibitory and activating signals they receive from their surface receptors. Upon activation, NK cells not only participate in the initial anti-viral response but also influence the adaptive immune response through cytokine secretion. Dengue virus (DENV) is the most prevalent arbovirus in the world. Prior studies have demonstrated that NK cells are activated during DENV infection. To identify the receptor-ligand interactions contributing to NK cell activation, we used mass cytometry (CyTOF) to profile the expression of NK cell receptors and their known ligands in a cohort of acute DENV patients. Our analysis showed increased NK cell expression of molecules associated with activation/killing potential, namely CD69, Fas-L, and perforin, in patients compared to healthy controls, also NKG2D, an activating NK receptor, in patients compared to healthy controls. Acute DENV infection is also associated with an increase inexpression of total HLA class I, HLA-E, MICA/B, and decreased expression of ULBP-1,2,5,6 on the surface of monocytes. As MICA/B and ULBPs are ligands of NKG2D, these data suggest a critical role for this receptor in the recognition of DENV-infected cells, particularly in light of prior data indicating that specific alleles of MICA and MICB are associated with disease progression. Finally, we show that DENV infection induces the soluble form of NKG2D ligands, sMICA and sMICB, but not soluble sULBP-1,2,3, in the sera of DENV-positive pediatric patients, as well as during in vitro DENV-infection of monocyte-derived immature dendritic cells (imDCs). Soluble ligands have been described in the context of cancer and have been shown to inhibit the NK cell response by competing with surface ligands and inducing the internalization of the NKG2D receptor. This suggests a potential escape mechanism by which DENV evades the NK cell response. Future studies using an inhibitor of ADAMs metalloproteinase, implicated in shedding of ligands, are necessary to determine the role of the soluble form of the NKG2D ligands in DENV-infected cells recognition.

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The enemy of my enemy: A novel insect-specific flavivirus offers a promising platform for safe and effective Flavivirus vaccines

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Introduction: Zika virus (ZIKV) is a re-emerging Flavivirus that has recently caused immense economic and health impacts throughout South and Central America. ZIKV's association with Guillain–Barré syndrome and congenital Zika syndrome are particularly concerning, as is ZIKV's capacity for alternative transmission routes, such as sexual, perinatal, and possibly blood-transfusion transmission. Objectives: Herein, we created a chimeric virus (ARPV/ZIKV) expressing ZIKV prM and E proteins on an Aripo virus (ARPV; an insect-specific flavivirus) backbone for evaluation as a ZIKV vaccine candidate. Materials and Methods: We explored a variety of in vitro and in vivo murine models to investigate the safety and efficacy of ARPV/ZIKV vaccination. The vaccine was compared to surrogate inactivated vaccine and live-attenuated vaccine controls. Results: In vitro safety studies demonstrated the absence of viral replication, translation, or cytopathic effects when mammalian cell lines were infected with ARPV/ZIKV. Additionally, blind serial passaging in vertebrate cells shows an inability to gain replication function, and retention of the desired host-restriction phenotype. Protective efficacy was evaluated by subcutaneous

vaccination of 4-week-old immune -competent and -compromised mice with ARPV/ZIKV, which produced robust neutralizing antibody responses within 1 week of immunization, and achieving PRNT80 titers of 267 and 800 respectively after 4 weeks. Vaccinated mice were completely protected against viremia, weight loss and death after ZIKV challenge. ARPV/ZIKV immunization also protects dams from disease and death, and completely prevents in utero ZIKV transmission in an IFN-$\alpha\beta$R-/- mouse model. Splenocytes harvested at 8- and 35-days post-vaccination derived from C57BL/6J vaccinated mice demonstrated strong ZIKV-specific CD4+ and CD8+ responses, and significant cytokine production. Discussion and Conclusion: Our studies show that a single dose of ARPV/ZIKV is exceptionally immunogenic in both immune-competent and -compromised murine models and offers complete protection from ZIKV-induced morbidity, in utero transmission and mortality. Strong neutralizing antibody responses were observed as early as seven days post-vaccination, indicating that protective efficacy is conferred within seven days. The inability for ARPV/ZIKV to replicate in vertebrate cells, and stable retention of the desired vertebrate host restriction promises outstanding safety for this vaccine platform.

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Efficacy of a Tetravalent Dengue Vaccine in Healthy Children and Adolescents

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Introduction: There is substantial unmet need for a safe and effective vaccine against dengue, particularly for dengue-naïve individuals and those under nine years of age. We present results from first two parts of an ongoing phase 3, randomized, double-blind trial of a tetravalent dengue vaccine candidate (TAK-003) in endemic regions of Asia and Latin America. Objective: The primary objective of the trial was to assess overall efficacy of TAK-003 to prevent virologically-confirmed dengue due to any serotype. The secondary objectives were to assess efficacy of TAK-003 by baseline serostatus, against individual dengue serotypes, to prevent hospitalized dengue, and the prevention of severe dengue. Methods: Between September 2016 and March 2017 we enrolled 20,099 healthy 4 to 16-year-old children and adolescents randomized 2:1 to receive two doses of TAK-003 or placebo three months apart. Participants presenting with febrile illness were tested for virologically-confirmed dengue (VCD) by serotype-specific RT-PCR. Results: Among the 19,021 (94.8%) participants included in the per protocol analysis, 27.7% were seronegative at baseline. The primary endpoint was met; overall vaccine efficacy was 80.2% (95% confidence interval [CI], 73.3, 85.3) in the 11-month period starting 30 days post second dose. Secondary efficacy endpoints were assessed in the 17-month period starting 30 days post second dose and efficacy was 76.1% (95% CI 68.5, 81.9) in baseline seropositives, 66.2% (49.1, 77.5) in baseline seronegatives, 90.4% (82.6, 94.7) against hospitalised dengue, and 85.9% (31.9, 97.1) against dengue haemorrhagic fever. Efficacies for individual serotypes were 69.8% (54.8, 79.9; DENV-1), 95.1% (89.9, 97.6; DENV-2), 48.9% (27.2, 64.1; DENV-3) and 51.0% (-69.4, 85.8; DENV-4). Cumulative rates of serious adverse events in both parts were similar in TAK-003 (4.0%) and placebo (4.8%) groups and were consistent with the expected medical disorders in this population. The vaccine was well tolerated, and no important safety risk was identified. Conclusion: TAK-003 was well tolerated and efficacious against symptomatic dengue in endemic countries regardless of baseline serostatus. The trial is ongoing, and the long-term data will be important in better defining the safety and efficacy profile of this vaccine.

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In-deep characterization of a novel live-attenuated Mayaro virus vaccine candidate by using an immunocompetent mouse model of Mayaro disease

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Mayaro virus (MAYV) is endemic in Central and South American countries where it is responsible

for sporadic outbreaks of acute febrile illness. The hallmark of MAYV infection is a highly debilitating and chronic polyarthralgia. Although emergence of MAYV infection is a potential threat, there are no specific therapies or licensed vaccine. Here, we developed a murine model of MAYV infection in immunocompetent mice that emulates many of the most relevant clinical features of MAYV infection in humans and evaluated the protective role of a novel live-attenuated MAYV vaccine against MAYV infection. Immunocompetent (BALB/c) or immunodeficient (A129-/-) mice were inoculated with MAYV WT or MAYV IRES strains via intraplantar route and several clinical (mortality and morbidity signs including mechanical hypernociception, a method to assess pain in animals), inflammatory (cytokines profile production, cell recruitment and activation, histopathological) and virological (viral loads, antibody titers) analyses performed. Inoculation of a WT strain of MAYV into immunocompetent mice induced persistent hypernociception, transient viral replication in target organs, systemic production of inflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α, VEGF, IL-17, IL-10) and chemokines CXCL-1, CCL2, CCL3, CCL4, CCL5, as well as specific humoral IgM and IgG responses. Inoculation of a live-attenuated MAYV vaccine candidate (MAYV IRES) in BALB/c mice induced strong specific cellular and humoral responses. Moreover, MAYV-IRES vaccination of immunocompetent and interferon receptor-defective mice resulted in protection of disease induced by the virulent MAYV WT strain. This study describes a novel model of MAYV infection in immunocompetent mice and highlights the potential role of an attenuated MAYV vaccine candidate in host's protection from disease induced by a virulent MAYV strain.

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Interrogating the dengue-specific memory B cell founder population in DENV-1 primary immune donors

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Dengue virus (DENV) is among the most important vector-borne pathogens of humans worldwide. Upon DENV infection, some naïve host B-cells expand, produce, and secrete DENV-specific antibodies (Abs) that recognize the viral proteins. Abs that recognize E, can neutralize the virus, while NS1 antibodies are thought to offer protection against NS1 mediated pathogenesis such as vascular leakage. After clearance, some of these B-cells become long-lived antibody-secreting cells (long-lived plasma cells) which secrete DENV-Abs into the serum, while others become DENV-specific memory B cells (MBCs) that remain in circulation, quiescent, but poised respond to future infections. It is this MBC population, the founder population, that we are interested in interrogating. Here we describe a strategy to identify and quantify DENV-specific MBCs in humans following a single DENV-1 infection. PBMCs from primary DENV-1 donors with times post infection ranging from from were used in two complimentary experimental approaches to quantify the DENV–specific MBC founder population. In the first approach, Limiting dilution assay (LDA),PBMCs are serially diluted and stimulated in vitro to become antibody-secreting cells. The resulting antibodies are assessed for DENV-specificity by ELISA using whole DENV virus (DENV1-4) and/or NS1. The second approach utilizes antigen-specific flow cytometry to quantify human MBCs (CD3-CD14-CD19+CD27+IgD-) that bind fluorescently labeled DENV-1. These DENV-specific MBCs are then single cell sorted and cultured along with cytokines and feeder cells to promote proliferation and antibody production. MBCs that secrete DENV-specific Abs undergo RT-PCR and cloning into human IgG expression plasmids for monoclonal antibody production. Using these approaches, we were able to identify DENV-whole virus and NS1-specific MBCs that remain in circulation decades after infection with varying frequencies. Single cell sorted DENV+ MBCs were stimulated in culture and screened for DENV-binding and neutralization activity.These experiments lay the foundation to characterize DENV-specific MBCs and functionally assess the antibodies they are programmed to secrete. The results of this project will provide insight into the MBC founder population following single DENV infection.

Vector Biology-Ecology-Control

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Exploring the role of thermal performance in dengue virus transmission by the mosquito, Aedes aegypti, under a changing climate.

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Mosquitoes transmit a diverse array of disease-causing viruses including Zika, West Nile, chikungunya and dengue. The global incidence of dengue fever alone has increased drastically within the last few decades, with approximately 390 million infections occurring annually. Dengue virus (DENV) is transmitted by the mosquito, *Aedes aegypti*, whose geographic range continues to expand. This expansion is in part due to increasing urbanization and changes in global climate. Temperature can have both beneficial and deleterious effects on mosquito physiology and also have impacts on human disease incidence. The effect of virus infection on mosquito health across a range of thermal regimes, however, has not been well studied. Using a novel physiological 'knockdown assay' aimed at measuring upper thermal limits (CTmax), we investigated the impact that DENV has on mosquito thermal tolerance, specifically relating to a history of heat exposure, evolutionary adaption and the involvement of stress disturbances. We found that infection substantially limits thermal performance in mosquito - contributing to a reduction in upper thermal limits. Our results also suggest that viral load within the mosquito directly correlates with performance in extreme thermal environments. Following exposure to a range of thermal regimes including varying base temperatures, and heat shock durations, we also show that prior thermal history during early development can have lasting effects on CTmax, acting as a buffer for individuals experiencing heat shock spikes later on in life. Our results suggest that mosquito populations exposed to viral infection will be less able to cope with changes in global temperature and the frequency and duration of extreme weather events. We suggest that virus-induced alterations of mosquito thermal performance should be taken into account when predicting virus transmission, and that current predictions for DENV transmission under global change may be overestimated.

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Estado de resistencia de Aedes aegypti a Malation en el Perú

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Introducción: En el Perú, el control químico del mosquito *Aedes aegypti*, se inició en 1990 con el uso del larvicida temefós y adulticidas piretroides. Sin embargo, en el año 2015, se detectó una amplia resistencia a piretroides y carbamatos. En consecuencia, se adoptó la Política Nacional del uso de malatión para el control del mosquito adulto *Ae. aegypti* y el uso del regulador de crecimiento (IGR) pyriproxyfen para el control de los estadios inmaduros. Objetivo: Determinar el estado de resistencia de *Aedes aegypti* a malatión en el Perú. Materiales y Métodos: Durante el 2018, se colectaron 15 poblaciones de *Ae. aegypti* empleando ovitrampas instaladas en los centros poblados Aguas Verdes, Pampa Grande, Corrales, Sagaro, Zarumilla, La Cruz, Cabuyal (Tumbes); Chulucanas, Máncora y San José (Piura); Puerto Maldonado (Madre de Dios); Belén, Punchana, Iquitos y San Juan Bautista (Loreto). A partir de huevos se obtuvieron progenies F1. Los bioensayos de susceptibilidad y/o resistencia se realizaron con hembras de 3 a 5 día de edad ante malatión 5%, bajo los lineamientos de la OMS. Resultados: Se evidenció a) Estado deResistenciaen las poblaciones de Aguas Verdes, Pampa Grande, Corrales, Zarumilla, La Cruz (Tumbes), Chulucanas, Máncora y San José (Piura); b) Estado deposible resistencia en las poblaciones de Cabuyal y Sagaro (Tumbes) y c) Estado de susceptibilidad en las poblaciones de Puerto Maldonado (Madre de Dios), Punchana, Belén, San Juan Bautista e Iquitos (Loreto), al insecticida malatión. Discusión/Conclusiones: Ante la evidencia de resistencia en 10 poblaciones de *Ae. aegypti* a malatión, es necesario realizar un uso

racional del insecticida y monitorear el desarrollo de la resistencia para asegurar la eficacia del control vectorial en la disminución de la transmisión de las arbovirosis en el Perú. Es importante resaltar el hecho del desarrollo de resistencia a malatión en menos de tres años en poblaciones que son resistentes a piretroides y carbamatos.

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Growing evidence that the World Mosquito Program's Wolbachia method reduces Aedes aegypti-borne viral transmission

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Introduction: The World Mosquito Program (WMP) is a global not-for-profit initiative that works to protect the global community from mosquito-borne diseases such as dengue, Zika and chikungunya. Known until recently as the Eliminate Dengue Program, the WMP uses naturally occurring bacteria called Wolbachia to reduce the ability of mosquitoes to transmit these diseases. Following years of laboratory research and field trials with promising results, the WMP has expanded to now be undertaking deployments in 12 countries around the world and has widespread support from communities, governments and regulators. Objective: To socialize the accumulated evidence on the impact on arbovirus transmission in countries where biological control is being evaluated with Wolbachia and the large clinical trials currently underway. Materials and methods: Data will be presented on the methodology used by the World Mosquito Program (WMP) for the implementation of biological control with Wolbachia and the large impact in many countries, including Australia, Indonesia, Vietnam, Brazil, Colombia and Mexico. Results: With the use of the Public Acceptance Model (MAP), the communities not only accept the release of mosquitoes with Wolbachia, but actively participate in the program. There is evidence of the reduction in the incidence of dengue in different countries greater than 95% in Australia in the last 7 years, greater 76% in Indonesia in the last two years, 86% in Vietnam in the last year and 50 to 79% in Brazil in less than a year. Conclusion: 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.

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Aedes aegypti (Diptera:Culicidae) persistence in Havana (Cuba) influenced by human behavior

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Introduction: Human and ecological factors create the conditions that determine the spatio temporal distribution and abundance of *Aedes aegypti* and the arbovirus transmission within urban environments. Objective: To determine the *Ae. aegypti* persistence in Havana, based on the interaction between human and ecological factors. Materials and Methods: The study was carried out in March/April 2019 in 4 health areas in 4 municipalities of Havana, based on the report of high Aedes infestation levels (Northern, Eastern, Southern and Western parts of town). In each site, 400 houses were selected, based on a multistage sampling approach, and inspected for presence of wet containers and of adult mosquitoes. Data were recorded on ubication of breeding site, type of containers, infestation level and characterization of houses. Results and Discussion: Aedes breeding sites were found in all areas, having 18.6% of all houses with at least one container positive for immature stages and 65.9% of these positive containers were found inside the houses. In 36% of the houses adult mosquitoes were collected. The house-type found to be most infested (64.4%), were small one-room houses called 'solares', which are connected through a narrow corridor with the main road. *Ae. aegypti* was found in 44 types of containers, but being concentrated (81% of all positives) in 12 of them. The water storage containers, well-known for Aedes breeding, represented 5.7% of the total containers revised and were positive for Aedes larval and pupal stages in 35.1% and 62.8% respectively. However it's important to highlight that *Ae. aegypti* larval and pupal stages were found in up to 50.7% and 15.2%, respectively, of the religious vases (representing 5% of the total containers revised). Conclusions: Although it is known that religious vases are breeding sites for *Ae. aegypti*, it's the first time that these con-

tribute so strongly to the maintenance of this mosquito in Havana city. As these are breeding sites that cannot be destructed, neither removed, communication messages towards the population need to be adapted.

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Possible non-sylvatic transmission of yellow fever between non-human primates in São Paulo city, Brazil, 2017-2018

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Yellow fever virus, a RNA virus of the Flavivirus genus, is endemic to tropical regions of Africa and South America. In South America, YFV transmission occurs through a "sylvatic cycle", involving non-human primates (NHP) and sylvatic mosquitoes (Haemagogus sp. and Sabethes sp.). Urban YFV transmission in Brazil was last reported in Brazil in 1942 and involved transmission between urban mosquitoes (*Aedes aegpyti*) and humans. Here, we describe positive YFV cases in NHP and mosquitoes collected by active opportunistic surveillance during confirmed epizootic events in São Paulo, capital municipality of São Paulo State, Brazil. Between October 2017 and December 2018, 542 NHP samples from different districts in São Paulo municipality were tested for YFV using RT-qPCR and immunohistochemistry. In addition, 2,886 mosquitoes were captured in areas with confirmed epizootics, divided into 437 pools and tested using RT-qPCR. A total of 162 NHP were positive for YFV (n=140 from *Alouatta clamitans*, median Cycle threshold = 11, min=7, max=38); and 22 Callithrix sp. (median CT=36, min = 33, max = 38). Three pools, 2 of *Haemagogus leucocelaenus* (Horto Florestal) and 1 of *Aedes scapularis* (Santo Amaro district) were found positive (CT values=18, 19 and 37, respectively). Among the Culicidae species collected, *Aedes scapularis* (n=1,625), *Ae. albopictus* (n=800), Ae. aegypti (n=73) and Hg. leucocelaenus (n=72) were most frequently detected. Aloutta epizootic events were observed in preserved forest regions where the sylvatic vectors are present. Notably, 19 Callithrix (86.4%) were found in urban areas and entomological collections executed in these areas did not detect the presence of strictly sylvatic mosquitoes (genera Haemagogus and Sabethes). Moreover, an isolated case of a free-living Alouatta monkey was positive in the city zoo, which has no connection to forest areas. Entomological collections performed were negative for Haemagogus genus. Genome sequencing and phylogenetic analyses suggests close genetic proximity of this strain to a strain recovered from an Alouatta from Piracaia (distance = 87Km). Our data suggests that non-sylvatic transmission of YFV in urban areas of São Paulo has been mediated by semi-domestic mosquitoes, and highlights the importance of joint entomological, epidemiological and genomic monitoring of YFV transmission in South America.

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Metabolic basis of virus-host interactions between Arboviruses and their mosquito hosts

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Introduction: The cellular metabolome plays a key role in determining the outcome of Arbovirus replication in the mosquito vector. A fine balance between metabolic commensalism verses competition must occur for efficient viral entry, replication, virus assembly and dissemination within different mosquito tissues prior to transmission to a new host. These requirements are altered by the influence of endosymbiotic microbiota (Wolbachia), ecological conditions, insecticide resistance and the age of the vector. Objectives of the study: was to identify the specific metabolic pathways that are altered during Arbovirus infection of the *Ae. aegypti* mosquito vector and to determine how they influence viral replication, dissemination and transmission. Materials and Methods: We have used high resolution mass spectrometry-based metabolomics approaches combined with loss of function studies to understand how metabolic pathways are altered and utilized following dengue, Zika and

chikungunya infection of *Ae. aegypti*. Results: We found significant fluctuations in the expression of several lipid classes. Specifically, a temporal increase in the expression of phosphoglycerolipids, major components of cellular membranes, was coincident with peak viral replication in the midgut. Sphingolipids, which are bioactive effectors, were elevated in expression during early times post infection. In addition, evidence of disrupted mitochondrial function and possible diversion to glycolysis was identified. We discovered that the metabolic alterations caused by dengue, Zika and chikungunya viruses in *Ae. aegypti* were unique and were exacerbated with the age of the vector. Utilizing metabolomics approaches we are now analyzing the influence of Wolbachia on virus infection in these mosquito vectors and the influence of age and insecticides on mosquito metabolism. Discussion/conclusions: The metabolic rearrangements caused by arbovirus infection identified in this study provide significant insight into how these viruses interact with their host environment. Specifically, they help to identify requirements for viral replication versus those that are a host response to infection, provide insight into how the microbiome and aging of the vector might influence viral replication, dissemination and transmission and provide an avenue to design novel interference approaches. They also provide interesting insight into how different viruses influence the host metabolome such that co-infection without competition could be achieved.

Virology-Pathogenesis-Antivirals

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Flavivirus NS1-triggered endothelial hyperpermeability and vascular leak promote viral dissemination and enhance viral infectivity

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Flaviviruses such as dengue (DENV), Zika (ZIKV), West Nile (WNV), Japanese encephalitis (JEV), and yellow fever (YFV) viruses are pathogenic emerging arboviruses that cause millions of systemic or neurotropic-encephalitic pathologies in humans worldwide every year. Flavivirus pathogenesis leading to severe disease has been associated with exacerbated humoral and cell-mediated immune responses that may lead to dysfunction of biological barriers (e.g., blood brain barrier). In DENV infection, endothelial dysfunction and vascular leak are considered pathogenic hallmarks of severe disease. Recently, we found that DENV non-structural protein 1 (NS1) directly triggers vascular leak in vivo and endothelial hyperpermeability in vitro. We further determined that NS1 from multiple flaviviruses trigger vascular leak in a tissue-specific manner reflecting disease tropism. Although these studies provide insight into the role of NS1 in mediating flavivirus pathogenesis, if and how this NS1-triggered endothelial dysfunction benefits these flaviviruses during infection is unclear. Here, we demonstrate that NS1 mediates dissemination of multiple flaviviruses (DENV, ZIKV, and Kunjin virus, which is closely related to WNV) across endothelial cell monolayers in vitro and promotes the dissemination of DENV into tissues of infected mice in vivo. Dissemination of virus through endothelial monolayers is non-specific, as viral dissemination is promoted by both homologous and heterologous pairs of NS1 and virus. Intriguingly, we find increased viral infection of monocytes, placed beneath the endothelial cell monolayer, only when evaluating homologous pairs of NS1 and virus, suggesting that interaction between flaviviruses and their homologous NS1 protein enhances infectivity. We confirmed this by demonstrating interaction between NS1 and virions and showed that a single amino acid point mutation of NS1 ablates this interaction. This mutant NS1 protein retains its ability to trigger endothelial hyperpermeability but no longer enhances viral dissemination or infection in vitro. Thus, NS1-mediated viral dissemination appears to be a combination of non-specific diffusion through disrupted barriers as well as specific interaction(s) between NS1 and virion. These studies present a proviral explanation for the evolutionary conservation of NS1-mediated vascular dysfunction of multiple flaviviruses and highlight the interaction between the flavivirus virion and NS1 as a new target for antiviral therapeutics and vaccine design.

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JNJ-1802, a potent, pan-serotype dengue virus inhibitor exhibits unprecedented efficacy in dengue non-human primate model and dengue AG129 murine model

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Given the enormous and growing impact of dengue, highly potent pan-serotype antivirals against dengue virus are urgently needed. Here, we present a direct antiviral small molecule (JNJ-1802), which exerts pico- to nanomolar cellular activity against a panel of lab-adapted strains and clinical isolates covering all dengue virus (DENV) serotypes. After oral administration of JNJ-1802, a potent and dose-dependent efficacy against a DENV-2 challenge in a rhesus macaque (*Macaca mulatta*) model was observed. Once daily oral dosing (starting 1 day before viral challenge to 10 days post challenge) with either 0.93 or 3 mg/kg resulted in undetectable viral RNA and absence of IgG seroconversion through the end of the study (day 28 post inoculation), as compared with vehicle treated animals (mean peak viral load of 5.64 log10 copies/ml and seroconversion observed). Similarly, rhesus macaque (*Macaca mulatta*) challenged with a DENV-1 strain and orally dosed with JNJ-1802 at 6 mg/kg, q.d. (starting 3 days before viral challenge to 10 days post inoculation), showed undetectable DENV RNA and no IgG response throughout the study period, as compared with vehicle treated animals (mean peak viral load of 5.17 log10 copies/ml and seroconversion observed). To our knowledge, this is the first demonstration of pronounced efficacy of a dengue-specific antiviral small molecule against DENV infection in an NHP model. These results corroborated data obtained duringmouse (AG129) studies, where undetectable viral RNA was observed in DENV-2 infected animals (102 PFU/ml) dosed q.d. with 1 mg/kg of JNJ-1802 in a non-lethal challenge model. In addition, survival rates above 80% were observed in a lethal challenge model with DENV-2 (106 PFU/ml), for a dose range of 0.3 to 10 mg/kg, b.i.d., compared with vehicle treated animals (no survival). Furthermore, JNJ-1802 showed also a significant protection in a lethal challenge model utilizing AG129 mice against DENV-1, 3 and 4, demonstrating pan-serotype coverage in this pre-clinical in vivo mouse model. The molecule JNJ-1802 belongs to a chemical series of compounds that exerts their potent activity by preventing the interaction between DENV proteins NS3 and NS4B, a hitherto unknown antiviral mechanism. JNJ-1802 is currently in clinical evaluation in healthy volunteers.

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Role of host RNA-binding proteins during viral assembly and egress

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The single stranded positive-sense RNA genome from dengue virus (DENV) is used as mRNA for the translation of viral proteins and as template to generate additional genomes. Thus, RNA molecules are at the center of DENV replication cycle and as such, host RNA-binding proteins (RBPs) are critical determinants of infection. We recently identified that Y-box-binding protein 1 (YBX1) interacts with DENV RNA in infected cells and we described that transient silencing of YBX1 expression leads to a decrease in the production of DENV infectious particles. Here, we aim to dissect the molecular mechanism by which YBX1 mediates DENV infection. To this end, we studied different steps of DENV replication cycle

in CRISPR-Cas9-mediated YBX1 knock out (KO) cells. Our results show that YBX1 does not affect the intracellular accumulation of viral proteins or viral RNA. Nevertheless, significantly lower levels of DENV structural proteins and genomic RNA were detected in the supernatant of YBX1 KO cells, corresponding with an >80% reduction in infectious virus release. Interestingly, secretion of NS1 protein was also affected in the KO cells, suggesting that the secretion of virus particles and NS1 protein are interconnected processes regulated by YBX1. These data point towards a hitherto undescribed function of YBX1 during DENV assembly and/or egress and we are now investigating these steps in detail. Our research will open new avenues to understand how viral RNAs are sorted, packaged and secreted revealing novel functions of host RBPs during viral infection.

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Depletion of CD4-T cells overcome previous DENV-ZIKV immunity resulting in increased DENV-2 viremia and delayed neutralization in Rhesus macaques.

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ZIKV and DENV (1-4) share 52-57% total amino acid sequence identity, showing cross-reactivity in both humoral and cellular responses. Recent in vivo studies in mice have suggested a protective role for the cellular response during a DENV and ZIKV infection. The role of CD4-T cells in controlling a flavivirus infection has been demonstrated, specifically the generation of a polyfunctional CD4-T cell response during a ZIKV infection. In addition, recent data suggests that DENV-specific CD8-T cells are required to mediate this cross-protection against ZIKV infection and not reactive antibodies. However, little is known about cross-protection in the context of a heterologous flavivirus infection with different DENV serotypes having previous exposure to DENV-ZIKV. Furthermore, the roles of cellular and humoral immunity in mediating this cross-protection as well as virus control and clearance during a subsequent infection in a non-human primate (NHP) model have not been addressed before. Therefore, we aimed to determine the impact of DENV-ZIKV immunity on the outcome of a subsequent heterologous DENV infection and to identify the contribution of immune cell subsets in the virus control and clearance. To test this, we performed a CD4+, CD8+ and CD20+ depletion followed by a DENV-2 challenge on Rhesus macaques that were previously exposed to DENV 3 (N=4, 5 years), DENV 4 (N=4, 5 years) and ZIKV (N=8, 1.3 year). For the first time using a NHP model, our findings suggest that depletion of B cells results in DENV-2 viremia rebound as well as a longer viremia when compared to the control group. However, those cells were not essential for a robust neutralization. CD4-T cell depletion results in a delay of B cells activation during the peak of viremia and a delay in the expansion of DENV-2 neutralization titers. CD8-T cell depletion has no effect on B cells activation and have limited impact on viremia. Moreover, the effect of CD8-T cell depletion on DENV-2 neutralization titers is modest when compared to CD4-T cell depletion. Our results also suggest that CD8-T cells may play a role in increasing the viremia during a tertiary flavivirus infection.

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Structure of Flaviviruses

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Flaviviruses assemble at the endoplasmic reticulum in an immature, non-infectious state and particle maturation occurs when it enters the trans-Golgi network, where low pH induces a conformational rearrangement of the glycoproteins. This transition causes 60 prM-E glycoprotein trimers in the immature virus to rearrange into 90 M-E dimers in mature virus. We have hypothesized that flaviviruses do not have exact icosahedral symmetry, thus providing flexibility for the large conformational rearrangements that are required during the virus life cycle. When icosahedral symmetry constraints were excluded in the cryo-EM reconstruction ofi mmature Kunjin virus, it was found that the nucleocapsid core touched the inside of the viral lipid membrane at the "proximal

pole" and was asymmetrically positioned within the lipid bilayer envelope. The outer glycoprotein spikes on the "distal pole" were either distorted or missing. In the asymmetric reconstruction of mature Kunjin, the core was re-positioned, concentric with the glycoprotein shell and there remained a distortion of the glycoproteins on one pole of the virion. This implies that the glycoproteins have a geometric defect that perhaps facilitates the transitions that occur during maturation. This defect in number and arrangement of the glycoproteins may reflect the consequence of membrane budding. We have continued to probe the structure and composition of these viruses, particularly dengue virus, and their assembly intermediates. In particular, we have probed virus particle maturation examining the immature virus and the conversion of prM by cellular furin to the mature M protein. Antibody binding to the prM protein of virions has been examined and may reveal insights of maturation as well as entry of the virion into host cells. The ability to compare virus particles from multiple members of the flaviviruses in terms of structure and function serves as a powerful strategy to discern common processes and distinct features that contribute to virus biology.

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Suppression of PINK1-Parkin-dependent mitophagy by Zika virus and its critical role in virus replication and inflammation

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Recent evidence suggests that inflammasome activation by Zika virus (ZIKV) is tightly associated with virus pathogenesis in human fetal brain. Here, we identify a molecular mechanism whereby ZIKV suppresses the clearance of damaged mitochondria to promote virus replication and induce inflammation. The selective removal of damaged mitochondria by autophagy (termed mitophagy) is controlled by two proteins, PINK1 (kinase) and Parkin (E3 ubiquitin ligase), often mutated in familial Parkinson's disease (PD). By examining proteins that interact with ZIKV NS5, we have identified Ajuba as a novel protein required for PINK1-Parkin-dependent mitophagy. Following mitochondrial depolarization, Ajuba translocated to mitochondria where it co-localized with PINK1 and potentiated mitophagy, likely through relief of PINK1-autoinhibition. Importantly, mitophagy was reduced in Ajuba-/- MEFs and was associated with large increases in inflammatory signaling following RNA virus infection. Furthermore, ZIKV NS5 interacted with Ajuba to suppress mitophagy which was directly associated with increased replication, and a massive increase in inflammatory gene expression, including genes associated with the inflammasome-IL1b signaling pathway. These results suggest that suppression of mitophagy is required for efficient replication and simultaneously drives ZIKV-induced inflammation through release of mitochondrial danger-associated molecular patterns (DAMPs). This data also reveal a critical component of the PINK-Parkin pathway that may be a therapeutic target in mitophagy-linked diseases including PD.

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Disparate mechanisms of defective genome-induced inhibition of Zika virus in its two hosts.

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Flavivirus replication can give rise to degenerate forms of the viral genome called defective viral genomes (DGs), through a process known as recombination. While DGs have been previously described for other flaviviruses, their evolution, molecular characterization and biological role have been overlooked. In this work, we aimed to comprehend the diversity of DGs generated during flavivirus replication using Zika virus (ZIKV) as a model. To this end, we passaged ZIKV in vertebrate or invertebrate cells under high MOI conditions, to favor recombination. Using next-generation sequencing, we identified the population of DGs containing internal deletions in each passage. With the help of computational approaches, we show that a particular type of deletion is fit and maintained in vertebrate- and invertebrate-derived ZIKV populations. The DG lacks the region encoding the proteins PrME and a portion of NS1. Intriguingly, the open reading frame (ORF) following the deletion is maintained in 99.8% of the cases. Our work suggests that the DG is unable to replicate per se, but requires

wild-type virus NS1 for replication. Furthermore, we show that both, replication and packaging by wild-type virus rely on the conservation of the ORF following the deletion, providing an explanation as to why only in-frame DGs were identified. In vitro assays indicate that the DG can interfere with wild-type virus replication in vertebrate and invertebrate cell lines. In vertebrate cells, its inhibitory activity is highly dependent on the conservation of the ORF following the deletion and on interferon induction. Comparatively, in invertebrate cells, inhibition is independent of the conservation of the reading frame. Using Ago-2 knock-down cells, we show that inhibition in invertebrate cells is dependent on the RNAi response. Finally, in vivo studies show that the DG can lower viral loads in mouse sera and organs, and limit dissemination and transmission rates in mosquitoes. This work is the first of the kind characterizing flavivirus DGs and deciphering differences in their mechanism of action in vertebrate and invertebrate hosts. Ultimately, our work opens avenues for future arbovirus transmission control strategies based on DGs, which could be used in endemic settings to reduce global disease burden.

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Annexin A1-FPR2/ALX pro-resolving pathway in dengue disease: novel therapeutic target and potential marker of disease severity

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Dengue is a typical disease where immune mechanisms determine the pathogenesis and clinical severity. In most cases, Dengue virus (DENV) infection is asymptomatic or relatively mild. In certain individuals, however, the disease may be very severe and eventually lead to death. Severe dengue is characterized by a cytokine storm, intravascular coagulation disorder, and increased vascular permeability. However, host factors influencing disease severity remain unknown. Here, we hypothesized that inadequate engagement of inflammation resolution pathways may contribute to the excessive inflammatory response and disease severity in dengue. Accordantly, we identified reduced levels of the pro-resolving protein Annexin A1 (AnxA1) in the plasma of DENV-infected mice. Indeed, absence of AnxA1 or its cognate receptor FPR2/ALX aggravated hematological parameters in infected animals, as demonstrated by heightened thrombocytopenia, hemoconcentration, vascular permeability and levels of pro-inflammatory mediators in AnxA1 or FPR2/ALX knockout animals exposed to primary or secondary dengue infection. In contrast, pharmacological administration of the AnxA1 mimetic peptide Ac2-26 to DENV-infected mice ameliorated disease parameters and reduced the levels of circulating pro-inflammatory mediators in a FPR2/ALX-dependent manner. Infection of interferon α/β receptor knockout A129 mice demonstrated the ability of Ac2-26 to prevent weight loss, liver damage and to ameliorate other disease parameters, without affecting systemic viral burden. In vitro, Ac2-26 peptide partially prevented murine mast cell degranulation and human dendritic cell activation evoked by DENV-2. In accordance with our experimental data, circulating AnxA1 was greatly reduced in the plasma of dengue patients in comparison to healthy controls. After stratification, we identified even lower levels of AnxA1 in the plasma of severe dengue patients compared to the non-severe subgroup. Altogether, our results suggest that severe dengue is characterized by an inadequate engagement of the resolution circuit centered on AnxA1, which in turn contribute to driving disease severity. Therefore, AnxA1 could be explored as a potential risk stratifying marker as well as a therapeutic target to improve outcomes of severe dengue outcomes.

Highlighted Posters

Diagnostics-Prognostics-Clinical

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24-month neurodevelopmental outcomes of infants exposed to Zika in Leon, Nicaragua using Ages and Stages Questionnaire (ASQ) screening test.

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Introduction: The spectrum of neurodevelopmental sequelae in asymptomatic infants whose mothers were infected with Zika virus in pregnancy is unknown. Objective: To understand neurodevelopmental outcomes among infants who were exposed to Zika in utero, but asymptomatic at birth using the ASQ. The ASQ screens for delays in four domains: gross motor skills, fine motor skills, problem solving, and personal-social skills. The primary outcome was the ASQ score at 24 months. Material/methods: We enrolled 178 children born between February and October 2017 to mothers pregnant during the ZIKV epidemic in León, Nicaragua. Blood samples collected during routine pregnancy and cord-blood were tested by a neutralization assay (eFRNT) to confirm Zika exposure during pregnancy. Mothers (and thus infants) were classified as ZIKV-infected in pregnancy, ZIKV infected with ambiguous timing; and non ZIKV infected (naïve and pre-immune prior to pregnancy). The ASQ was administered in the homes of the children every three to six months by locally trained psychologists. Research nurses collected sociodemographic data, medical history, and infant anthropometric measurements at birth and when the ASQ was administered. Results: Of the 138 infants with exposure status, 33 (23.9%) were ZIKV-exposed during pregnancy, 18 (13.0%) had mothers with unknown ZIKV timing and 87 (63%) were non ZIKV-exposed during pregnancy. There were no differences in maternal age, education, mean birthweight, or gestational age at birth between the groups. The mean total ASQ scores differed between the three groups, were as follows, respectively: 12 months: mean, standard deviation (SD): 272 (SD= 29.6); 273 (SD =28.6); 282 (SD= 25.2); 24 months: 269 (SD= 27.6); 271 (SD= 32); and 277 (SD= 23). In a longitudinal mixed model controlling for potential confounders, having an incident infection corresponded to a 4.6 point decrease (95% CI: -12.0, 2.7; p = 0.225) in total ASQ score compared to the no ZIKV/pre-immune group. Discussion/conclusion: In our prospective cohort of infants, unaffected infants born to mothers with an incident ZIKV infection in pregnancy had lower total ASQ score at each time point that was not statistically significant. Correlation of ASQ with diagnostic neurodevelopmental tests is needed in this population.

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Clinical epidemiological study of pregnant women infected by zika and dengue viruses in a Brazilian endemic area

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Introduction: The possible association between Zika virus infection and malformations at birth

has gained global attention. However, the underlying mechanism has not been identified, so the real impact on the population has not been proven yet. São José do Rio Preto is an endemic area for DENV, and possibly ZIKV, being an important source of data for research on the topic. Objectives: This study aimed to evaluate the influence of DENV and ZIKV in pregnancy outcomes in a large retrospective sample of patients from a dengue-endemic area of Brazil. Methods: We evaluated 91.599 live birth records, 141.997 confirmed dengue cases and 1.374 notified ZIKV cases in São José do Rio Preto, Brazil, registered from January 2000 to December 2017. Data submitted to analysis included: 1-minute Apgar, 5-minute Apgar, newborn weight, pregnancy week, malformation at born, dengue incidence per year, Zika incidence per year and live births per year. Spearman's correlation coefficient and Mann Whitney test were used to study the effect of selected variables. Results:Considering the period from 2000 to 2015, we found a positive correlation between the incidence of dengue per year and birth rate (ρ=0,36; p<0,001), 1-minute Apgar(ρ=0,05; p<0,001) and 5-minute Apgar(ρ=0,01; p<0,001).Between 2016 and 2017, we found a negative correlation between the incidence of dengue per year and birth rate(ρ=-1,00; p<0,001), 1-minute Apgar(ρ=-0,02; p=0,01) and 5-minute Apgar(ρ=-0,01; p<0,001). The Mann-Whitney test comparing dengue incidence and malformations between 2000 e2015,resulted in insignificant association(p=0,20). However, the 2016 and 2017 analysis resulted insignificant association(p=0,01). Data for Zika incidence and malformations showed significant positive correlation in the analyzed periods.Discussion/Conclusion:As the incidence of dengue increased by year,and in the presence of ZIKV co-circulation,there was a reduction in the number of live births (high positive correlation), as well as in the 5 and 1-minute Apgar score (insignificant correlation).The correlation between malformation and dengue incidence gained significance when the period analyzed included Zika outbreak, supporting that the co-circulation can contribute to fetal complications.

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Late relapsing hepatitis after yellow fever

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Regardless of intense study, relatively little is known about yellow fever (YF) infection. YF is classically characterized by three periods: infection, remission, and intoxication. After acute phase, the duration of jaundice is unknown while weakness and fatigue may last several weeks, and slightly abnormal liver function may persist for 60 days or more. Here, we report a case of symptomatic late relapsing hepatitis after Yellow fever virus (YFV) infection. Two months after YF onset, one patient presented hyporexia, asthenia, adynamia, and jaundice, with laboratory tests indicating hepatic cytolysis rebounded by alanine and aspartate transaminases, total and direct bilirubin levels. The patient was followed up and monitored, and tests were run to investigate the case. Tests discarded autoimmune hepatitis, other inflammatory liver diseases, metabolic liver disease, or new infections caused by hepatotropic agents. IgM and neutralizing antibodies against YFV were detected, but no viremia. A liver biopsy was collected three months after onset of YF and tested positive for presence of wild-type YFV RNA (364 genomic copies/gram/liver) and of YFV antigens, besides findings of cell damage mostly in zones 2 and 3 of the hepatic acini. Transaminases and bilirubin levels remained elevated for five months, and the arresting of symptoms was reported for six months after the onset of YF. Neutralizing antibodies titers increased during late hepatitis syndrome and could be a reflection of the continuous immune stimulation caused by the persistence of YFV antigens or even viral particles in liver. Several serum

chemokines, cytokines, and growth factors were measured, and a similar immune response profile was observed when earlier phases of disease were compared (days 36 and 78 after symptoms onset), but more pronounced changes were observed in later stages (after 197 days of symptoms onset) at the same time aspartate and alanine transaminases turned normal. The results indicate viral persistence in liver and a persistent liver cell damage, three months after YF onset, and reinforce the need of extended follow up of YF patients, especially regarding hepatic function and further studies to investigate the role of a possible viral persistence and the immune response causing relapsing hepatitis following YF.

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Clinical and serological findings of Madariaga and Venezuelan equine encephalitis viral infections: A follow-up study five-years after an outbreak in Panama

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Background: Human cases of Madariaga virus (MADV) infection were first detected during an outbreak in 2010 in eastern Panama, where Venezuelan equine encephalitis virus (VEEV) also circulates. Little is known about the long-term consequences of either alphavirus. Methods: A follow up study of the 2010 outbreak was undertaken in 2015. Clinical information and blood samples were obtained from former patients and their household contacts. An additional survey was carried out two weeks after a separate 2017 alphavirus outbreak in a neighboring population in eastern Panama. Serological studies and statistical analysis were undertaken in both populations. Results: Seroconversions occurred in 6.2% and 12.3% of participants against MADV and VEEV, respectively. Seroreversion was only observed for MADV in 3.1%. Amongst individuals with MADV antibodies, 30.8% seroconverted to VEEV, compared to 11.1% amongst individuals who were alphavirus-negative at baseline. Memory loss, insomnia, irritability and seizures were reported at significantly higher frequencies in VEEV and/or MADV seropositive vs seronegative subjects. Conclusions: High rates of 5-year seroconversions to MADV and VEEV suggest continuous circulation of both viruses in Panama. Cross-protective immunity may be conferred by VEEV towards MADV, though prior exposure to MADV does not appear to protect against VEEV. We provide evidence of persistence of neurologic symptoms up to 5 years following MADV or VEEV exposure.

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Clinical Evaluation of Mast Cell Tryptase as a Candidate Biomarker for Severe Dengue Disease

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Dengue disease is endemic to over 100 tropical and sub-tropical countries worldwide, impacting nearly 400 million individuals annually. It is a mosquito-borne viral disease that progresses in some cases from expressing mild symptoms"with or without warning signs" (dengue fever(DF)) to life-threatening "severe dengue" (dengue hemorrhagic fever(DHF)/dengue shock syndrome(DSS)). Reports have indicated that elevated chymase, a mast cell (MC) protease, is highly predictive of DHF in adults and pediatric patients. Another MC protease, tryptase, which plays a role in regulating vascular leakage by cleaving PAR receptors,was investigated in this study to determine the extent of its association with vascular leakage complications of dengue, and if it could provide a detectable, early indicator of severe dengue. Here, we assessed whether there was a correlation between tryptase levels and signs of vascular leakage in prospectively recruited patients in Singapore. Plasma tryptase was quantified by ELISA. For statistical analysis, patients were divided into two groups,"bleeding" and "non-bleeding"based on displaying signs of bleeding during the clinical evaluation. The "bleeding" group showed average plasma tryptase concentrations of 385.31pg/mL, compared to 326.61 pg/mL for the "non-bleeding" group (t-value=1.86; p=0.035); demonstrating a statistically significant association between elevated tryptase levels and vascular complications of

dengue. Secondarily, an investigation into immune cell counts between the "bleeding" and "non-bleeding" groups showed that basophils were the only immune cell-type that was significantly higher (by t-test) in patients that experienced bleeding; however, regression analysis specified no significant correlation between basophil counts and serum tryptase levels. Although current conclusions are based on small sample size (with subject enrollment still ongoing), results so far indicate that higher serum tryptase levels and bleeding symptoms are correlated, making tryptase a promising biomarker for early detection of severe dengue disease.

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Creation of the national laboratory network for virological surveillance of the Dengue virus in Ecuador

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Dengue is a vector-borne disease caused by an arbovirus of the Flaviviridae family, of the genus Flavivirus. In Ecuador, the DENV-1, DENV-2, DENV-3, DENV-4 serotypes have circulated historically, especially in epidemic years. The Dengue virus represents a priority problem in public health in the country due to a large number of cases that occur each year. Since its resurgence at the end of 1988 several epidemic cycles have been recorded, is the tropical and subtropical areas of the country that are at risk of transmission of this Arbovirus, whose impact depends on the distribution and population density of *Aedes aegypti* and *Aedes albopictus*, as well as the circulating viral serotype. To strengthen the epidemiological surveillance of this disease, it was decided to form a national network of laboratories for dengue virological surveillance. The criteria for selection established were the epidemiological profile of an area of influence and to approve the quality certification process carried out by the Dengue national reference center. A total of 22 public laboratories were designated nationwide, performing ELISA NS1 tests for Dengue. To perform virological surveillance, designated hospitals must send 100% of the positive samples for ELISA NS1 of dengue cases with warning signs, severe dengue, patients with unusual clinical manifestations and deaths for serotyping by RT-qPCR. Currently, 13 of the 22 laboratories randomly select 10% of patients with Dengue without warning signs, to perform an Elisa NS1 test whose positive result is sent for serotyping. Based on the work done with the Laboratory Network in Ecuador during 2019, the circulation of the Dengue 1 virus in the provinces of Guayas, Esmeraldas, Loja, Napo, Pastaza, and Zamora was determined, while the presence of Dengue 2 in the provinces from Orellana and Napo. Through the creation of the Laboratory Network, the epidemiological surveillance of the Dengue Virus is strengthened and the contribution of the country's control measures is effective.

Epi-Phylogenetics-Modeling-Burden

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Genomic epidemiology reveals the resurgence of dengue virus post Zika in Brazil

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After ZIKV was introduced in the Americas in 2014, no large dengue outbreaks were reported until 2018, when many symptomatic cases arose. The aim of our study was to uncover the dynamics and origins of DENV-1 (genotype V) and DENV-2 (genotype AA) lineages causing outbreaks in northeast and southeast Brazil, from 2010 to 2019. To reconstruct these outbreaks, we used Illumina and Nanopore platforms to sequence 130 DENV genomes from human cases, which

were collected in the Brazilian states of Paraíba (n= 50) and São Paulo (n= 80), before and after the major Zika outbreaks. Using MAFFT, the new consensus genomes were aligned with existing ones from previous outbreaks in the Americas. By using BEAST v1.10.4, phylogeographic analyses were performed considering the following models: Yang96, relaxed molecular clock, and Bayesian SkyGrid tree prior. Our results show that the 2018 DENV-1 outbreak in Paraíba State was caused by viruses from southeast Brazil, introduced in a single event, most likely in early 2015. Remarkably, viral transfers from the southeast to the northeast region have shown to be a common pathway of DENV-1 spread. The DENV-2 outbreaks in São Paulo State were caused by two distinct lineages: (1) one introduced in early 2005, which has been circulating and causing recurrent outbreaks for more than a decade; and (2) another lineage, likely introduced in southeast Brazil in late 2013, responsible by a second outbreak in 2019. Our study revealed the dynamics of DENV before the major 2015-2016 Zika outbreaks in Brazil, and also uncovered the resurgence of DENV after the establishment of ZIKV. Importantly, we show that the 2019 DENV outbreaks in Paraíba and São Paulo were likely caused by viruses circulating in Brazil prior to (and despite) the Zika outbreaks, with significant DENV transmission during the period of low dengue incidence between Aug 2016 and May 2018. We hypothesize that the low incidence of dengue post Zika, prior to the recent dengue resurgence, could be due to waning cross-protection from severe dengue symptoms given pre-exposure to ZIKV, leading to a low proportion of reported cases despite significant transmission.

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Prior ZIKV infection increases risk of dengue disease in humans

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Protective and pathogenic immune interactions occur between the dengue viruses (DENV1-4). Whether Zika virus (ZIKV), an antigenically related flavivirus, participates in these interactions is unknown and of great interest. Here,we tested whether DENV and ZIKV immunity modulates risk of subsequent dengue and Zika disease in the Pediatric Dengue Cohort Study (2004-present), which has followed 3,800 children ages 2-16 in Managua, Nicaragua. We used relative risk regression (log-binomial model) to evaluate how prior DENV and ZIKV infection histories and antibody titers modify subsequent risk of symptomatic and severe (Dengue With Warning Signs and/or Severe Dengue) DENV infection. Models were adjusted for age and sex, and accounted for clustered data. Interestingly, we find that one prior ZIKV infection significantly elevates risk of symptomatic (Relative Risk [RR]: 3.71, 95%CI: 2.65-5.30) and severe (RR 4.66, 3.00-7.54) DENV2 infection compared to DENV-naïve children, similarly to those with one prior DENV infection (RR 3.15, 1.79-5.30). However, one prior DENV infection followed by ZIKV also significantly elevates risk of symptomatic DENV2 infection (RR 3.26, 2.17-4.91), unlike sequential DENV infections, which do not elevate risk. Further, children with intermediate titers of pre-existing anti-DENV antibodies, whether induced by prior DENV or ZIKV infections, are at highest risk of symptomatic and severe DENV2 infection. In a separate hospital study, we find that 88% of DENV2 cases in 2019 are secondary, and among a random subset tested (n=72), 86% had a history of prior ZIKV infection – significantly higher than expected based on ZIKV infection history in the cohort population (39%; test for proportions, p=2e-15). Importantly, prior DENV infection histories and pre-existing anti-DENV antibody titers increase risk of symptomatic DENV2 infection, but protect against symptomatic DENV1, DENV3, and ZIKV infections. Overall, we find that in some respects, ZIKV can interact with DENV like another serotype, but that dramatic differences exist among the serotypes: ZIKV and DENV immunity increase risk of disease caused by DENV2 but not by other serotypes or ZIKV. These findings highlight the importance of evalu-

ating whether future ZIKV vaccines could elevate dengue disease and provide insights into serotype-specific differences in efficacy and safety of existing dengue vaccines.

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Dengue virus genomic surveillance in the Americas

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Transmission of the four dengue virus serotypes (DENV-1-4) continues to expand globally, driven in part, by human travel and climate change that favors changes in vector geographic distribution. Despite the historical entrenchment of endemic DENV circulation in the Americas, a substantial reduction in case reporting was observed in the region in recent years and particularly during and after the Zika virus epidemic between 2015-2017. Dengue cases are now re-emerging throughout populations in Latin America with acquired immunity for ZIKV, presenting a distinct epidemiological scenario and opportunities for research in virus evolution, transmission diagnostics and pathogenesis. The rapidly changing epidemiology of DENV-1-4 in this region merits the implementation of robust molecular and genomic surveillance to detect, track and characterize current transmission of DENV-1-4. In 2016, the Centers for Disease Control and Prevention (CDC) collaborated with the Pan American Health Organization (PAHO) to initiate the Dengue Genomic Surveillance Project in the Americas (ViGenDA), promoting and expanding regional capacities to perform genomic surveillance in Latin America. The CDC developed the Dengue Virus E Gene Sequencing Assay, a standardized, serotype-specific laboratory tool for gene sequencing, using simple Sanger methods and genotyping virus directly from diagnostic serum specimens. Multiple bioinformatics tools were used to assemble consensus sequences and reconstruct phylogenetic trees using reference sequences. The assay was deployed to 14 national reference and public health laboratories of the network of arbovirus laboratories of the Americas (RELDA). We present phylogenetic analyses using Bayesian maximum credibility trees describing the recent spread and evolution of DENV-1-4 in the Americas. Our findings indicate that contemporary viruses are accumulating sufficient genomic changes to group separately, suggesting recent evolutionary divergence. Foreign introductions have been detected in several regions, on occasions linked to transient transmission and divergence of monophyletic groups. Relative phylogenetic diversity also seems to be increasing representing the recent re-emergence of DENV-1-4. These findings demonstrate the value of implementing genomic surveillance in the region. The CDC Dengue Branch and PAHO continue to support project ViGenDA and all public health programs with the objective of characterizing contemporary DENV-1-4 transmission and evolution across the Americas.

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Punta Toro serocomplex viruses and other arboviruses under dengue, Zika and chikungunya human febrile surveillance

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Dengue is the most prevalent arboviral disease in the Americas, it ranges of symptoms covers from a mild to a severe disease. With the recently introduction of chikungunya and Zika, the panorama of arboviral surveillance had changed and dengue-like diseases emerge as public health concern due the potential to cause important outbreaks. To determine if there are other arboviruses in circulation in Panama causing dengue-like syndrome, a retrospective study from 2008 to 2014 and a passive surveillance since 2014 were stablished by testing for other arboviruses in negative samples for dengue, Zika and chikungunya. A total of 12,600 negative sera from dengue-like acute patients from 2008 to 2018 were tested using end-time PCR with generic primers to detect viruses from the genus phlebovirus, flavivirus, alphavirus and orthobunyavirus. Positive amplicons were sequenced with Sanger method for viral identity. For PTV serocomplex, forty-one acute infection were detected under this surveillance. Forty of them were identified as Punta Toro virus, and one as Cocle virus. We also detected five alphaviruses identified as VEEV in 2009, a year before an important outbreak with the co-circulation of VEEV and Madariaga virus, in Darien endemic region.

Forty-one flavivirus were identified as DENV from 2009 (9), 29 (2010) and 3 (2011), showing that acute DENV surveillance before 2011 using viral isolation and indirect immunofluorescence, was less sensitive than the current molecular methods which may detect low viral load in sera samples. Punta Toro serocomplex viruses cause a dengue-like disease, with no severe cases reported yet. Its circulation under dengue, Zika and chikungunya umbrella can add a public heath burden to the actual complicated arboviral diagnosis situation. Our results show that Punta Toro serocomplex viruses are in dynamic circulation, our next step is determining the seroprevalence of these viruses in Panamanian population with the aim to know the diseases burden added mistakenly to dengue, Zika and chikungunya.

Human Behavior & Community Engagement

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Community-Based Dengue Prevention: implementing Camino Verde in Paraguay. Results from the first pilot, within cluster-based control trial

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OBJECTIVE: Test whether community mobilization reduces infestation levels in a vulnerable community of Asunción, Paraguay. DESIGN: Pragmatic controlled trial, with external surveys performed by public health institution. SETTING: Two homogeneous neighborhoods in Asunción, the capital of Paraguay, one in which community mobilization took place (Intervention) and one in which community mobilization did not take place (Control). PARTICIPANTS: Random sample of Houses from both neighborhoods, 4 external measurements performed, with community mobilization in the form of community-based entomological surveillance in between. External measurements were performed in the April 2018 (Nintervention= 222, Ncontrol = 218), in the dry month of July 2018 (Nintervention= 230, Ncontrol = 223), and again in the April 2019 (Nintervention= 243, Ncontrol = 275). An additional measurement was performed in May 2019 (Nintervention= 40, Ncontrol = 40).INTERVENTION: A community mobilization program began with community training. The program adapted the model of the Camino Verde pilot in Nicaragua. MAIN OUTCOME MEASURES: House index, defined as households with larvae or pupae/households examined. Other indices are also calculated, but not reported in this abstract. RESULTS: With neighborhood as the unit of analysis, fewer houses with larvae or pupae among houses visited (house index) were found in the intervention, or in case of increase, it was lessened in the intervention neighborhood, under the same stressful circumstances of flooding. The house indices in April 2018, the baseline, were set at 22.52% (intervention) and 15.59% (control). In July 2018, in dry season, the biggest decrease was observed in the intervention, with indices at 2.61% (intervention, -19.91%) and 9.87% (control, -5.73%). In April 2019, while the territories were half-flooded, both increased their house indices, but the increase was lessened in the intervention, resulting in indices at 26.92% (intervention, +4.4%) and 29.03% (control, +13.44%). At this point, volunteers of the community mobilization program decided to increase their visits. An additional measurement was performed in May 2019, resulting in indices at 17.50% (intervention, -5.02%) and 55% (control, +39.40%). CONCLUSIONS: Community mobilization substantially reduced house indices, or mitigated their increase on the face of stressing externalities.

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Evaluation of a community's perception of spatial emanators with metofluthrin for reducing the mosquito population in Yucatan, Mexico

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Introduction. In 2018 we conducted a field trial on the efficacy of a metofluthrin-treated emanator in Ticul, Yucatan, Mexico. Metofluthrin is a volatile pyrethroid that affects the survival and biting rates of mosquitoes. Objective. As part of our

evaluation we recorded the community's perception of the product. We enrolled 200 households (100 treated and 100 controls). Results. Main motives for acceptance were: the high number of mosquitoes in the home (17.45%), concern about mosquito-borne disease (MBD) in the community (11.41%), confirmed cases of MBD in families (10.74%), positive experiences with a previous project of our scientific team (10.74%). Three weeks after emanator placement, we assessed perceptions of efficacy. Most participants (85%) reported a significant reduction of mosquitoes inside homes. Almost all households (98%) were free of dengue, chikungunya or Zika symptoms (self report) and 85% considered that the emanator could form a complementary tool for Government programs on vector control. Overall, 83% liked the product because it 1) drove away mosquitoes and flies, 2) doesn't smell, 3) is safe, and 4) looks good aesthetically. There were reservations (12%) about the installation process (high humidity resulted in emanators detaching from their sticking points). Many householders (66%) stopped using other commercial products against mosquitoes mainly because the efficacy of the emanator, while 34% continued to use other control methods 1) as and when they saw mosquitoes, 2) from habit, 3) to kill other insects and 4) when they left their homes. Most (95%) thought that the trial should be scaled up to involve the rest of the community. Discussion and conclusions. These data demonstrate that a safe, unobtrusive emanator that does not smell, is easy to install, and effective in driving away mosquitoes is likely to be accepted by the community and could be adopted as another preventive measure against mosquito vectors of Dengue, Chikungunya and Zika virus.

Immunology-Vaccines

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T cell responses induced by attenuated flavivirus vaccination are specific and show limited cross-reactivity with other flavivirus species.

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Introduction: Flaviviruses share high amino acid sequence identity and are often geographically co-localized. Previous exposure to one flavivirus may affect the immune response to a subsequent infection or vaccination. Non-Structural (NS) and capsid (C) antigens are dominant targets of T cell responses, while the prM and envelope (E) proteins are a dominant target of neutralizing antibodies. Accordingly, several flaviviruses vaccine candidates utilize the prM and E proteins as the main immunogen, including the recently licensed Dengvaxia vaccine based on the delivery of the E and prM proteins by a YFV backbone suggesting that a cellular immunity will have to rely on YF/DENV T-cell cross-reactivity. Objective of the study: Define whether exposure or vaccination with one flavivirus is able to induce cross-reactive T cells with other flaviviruses and in particular to address to what extent DENV and YFV responses induced by vaccination are cross-reactive. Material and Methods: We tested pools of epitopes derived from the different flaviviruses in Intracellular Cytokine Staining (ICS) assays in individuals immunized with DENV or YF17D vaccines. Quantitative analyses at the level of single epitopes by generating epitope-specific short-term CD8 and CD4 T Cell Lines (TCLs) were also performed. Specifically, PBMCs from vaccinees were stimulated for 14 days with the homologous peptide and reactivity to homologous and heterologous corresponding sequences was determined by IFNγ ELISPOT assay. Results: CD8 T cell responses after DENV or YFV vaccination were able to cross-recognize epitopes from multiple flaviviruses. However, the magnitude of the cross-reactive responses was weaker than the one observed in the autologous epitope pools and cross-reactive cells expressed lower activation markers. TCL derived from DENV monovalent vaccinees induced CD8 and CD4 T cells that cross-reacted within the DENV serocomplex but were consistently associated with decreases in antigen sensitivity greater than 100-fold in the case of most other flaviviruses, with no cross-recognition of YFV derived peptides. Conclusions: Our data suggest limited cross-reactivity for both CD4 and CD8 T cell responses between flaviviruses and has implications for understanding immunity elicited by natural infection, and strategies to develop live attenuated vaccines against flaviviral species.

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FcγRIIIa Activation is Mediated by Crossreactive Antibodies in Acute and Convalescent Sera of DENV Patients

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Introduction: Dengue virus (DENV) is a serocomplex of four serotypes that belongs to the genus Flavivirus of the Flaviviridae family. Humoral immune response against DENV, mainly through IgG isotype, have a very fluctuating protective/pathogenic role that depends on multiple factors, including those mediated by recognition of the Fc domain by cell-membrane receptors. Activation of FcγRIIIa has been associated with both protection and disease severity. However, this interaction is not often contemplated when profiling the antibody properties against DENV due to cumbersome traditional techniques. Here, a simple assay for detecting antibodies triggering this activation was employed using a reporter cell line (BW5147) bearing a recombinant chimeric receptor of the human extracellular portion of FcγRIIIa with the murine CD3ζ intracellular domain. Objective of the study: In order to evaluate the quantity and importance of FcγRIIIA-activating antibodies within the human humoral immune response against DENV, activation of chimeric receptor FcγRIIIa-CD3ζ was evaluated using acute and convalescent sera from patients infected with DENV. Materials and methods: A total of 30 human serum samples from patients with acute and past DENV infections were used in this study. Humoral profile of the sera was determined by 1) neutralizing capability, 2) immune-enhancing potential (K562 cells Ab-mediated infection) and 3) FcγRIIIA-activation against the four serotypes. Results: Despite the lack of linear correlation between the neutralization titers and the FcγRIIIA activation against the four DENV serotypes, broad multitypic activating response was detected in serum samples having a high neutralization titer. A statistically significant correlation was observed between the enhancing activity and the FcγRIIIA activation. Furthermore, broader and stronger FcγRIIIA-activating response was observed in convalescent sera 40-120 days after infection, but no signal was detected in the same patient at later time points, e.g. 2 or more years after infection. Discussion and conclusions: In the present work, we successfully measured the effector function of antibodies present in human sera after DENV infection using a recombinant chimeric FcγRIIIA. The activation profile, mediated by cross-reactive antibodies, may have an important role in the heterotypic protection. This adds a novel tool to study protective/pathogenic balance of humoral immune responses in DENV pathogenesis.

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Molecular signatures of dengue virus-specific IL-10/IFN-γ co-producing CD4 T cells and their association with dengue disease

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Dengue virus (DENV) can cause diseases ranging from dengue fever (DF) to more severe dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Whether antiviral T cells contribute to the protection against or pathogenesis of severe disease is not well-defined. Here we identified antigen-specific IL-10+IFN-γ+ double positive (DP) CD4 T cells during acute DENV infection. While the transcriptomic signatures of DP cells partially overlapped with those of cytotoxic and type 1 regulatory CD4 T cells, the majority of them were non-cytotoxic/Tr1 and included IL21, IL22, CD109, and CCR1. Although we observed higher frequency of DP cells in DHF, the transcriptomic profile of DP cells was similar in DF and DHF, suggesting that DHF is not associated with altered phenotypic or functional attributes of DP cells. Overall, this study revealed a DENV-specific DP cell subset in patients with acute dengue disease and argue against altered DP cells as a determinant of DHF.

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Within-serotype genetic variation of Dengue virus 2 can modulate the neutralization activity of human antibodies

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Introduction: Dengue virus (DENV) infects an estimated 390 million people each year worldwide. While a DENV vaccine was recently approved for clinical use, the vaccine efficacy against the four serotypes is not uniform, with vaccine efficacy rates of 63% for DENV serotype 1 (DENV1), 75% for DENV3, 74% for DENV4 and 39% for DENV2 for Dengvaxia. However, the mechanisms underlying the observed poor vaccine efficacy for DENV serotype 2 remain unclear. Objective: To define the role of DENV2 genotypic variation on neutralizing antibody activity. Methods: We used reverse genetics to generate a DENV2 genotypic variant "global" virus panel that is representative of global genetic diversity. Both the precursor membrane (prM) and envelope (E) of distinct cotemporary circulating Asian I, Asian II, Asian-American, Cosmopolitan, Sylvatic African, and Sylvatic Asian genotype isolates were recombinantly produced in an isogenic DENV2 16803 WHO reference strain backbone. We evaluated virologic features of the distinct DENV2 variants. We tested the neutralization sensitivity of distinct DENV2 genotypic variants against human monoclonal and polyclonal sera in a Vero cell neutralization assay. Results: We evaluated the role of the genetic diversity within the prM and E of DENV2. Interestingly, the distinct DENV2 genotype variants had differences in growth kinetics and virion stability, suggesting heterogeneity within DENV2 strains. Moreover, the DENV2 genotypic variants were differentially neutralized by memory B cell and plasmablast-derived DENV2-specific IgG neutralizing monoclonal antibodies and polyclonal serum neutralizing antibodies from naturally infected and experimentally infected patients. Finally, the distinct DENV2 genotypic isolates were differentially neutralized by vaccine-elicited antibody responses from the experimental National Institutes of Health (NIH) DENV2 monovalent and DENV tetravalent vaccines. These findings suggest that DENV2 prM and E genetic diversity or maturation status modulates the neutralization activity of neutralizing antibodies, underlining a mechanism of Dengue virus immune evasion from host adaptive responses. Conclusion: Our findings suggest that DENV2 prM and E genetic diversity can have a major impact on the neutralization activity of human antibodies. This panel of isogenic DENV2 recombinant viruses will provide a template to study the role of natural variation in protective immunity, mechanisms of neutralization, maturation status, and vaccine performance.

Vector Biology-Ecology-Control

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Designing the optimal target zones: ecological genomics informs the control of Aedes aegypti

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Introduction: *Aedes aegypti*, the primary vector of dengue and other surging arboviral diseases, has proven difficult to control using conventional approaches. New methods for the suppression or replacement of local mosquito populations are being developed, and some have been tested in the field, including the release of mosquitoes carrying the symbiotic bacteria Wolbachia or a lethal transgene (RIDL). The WHO is also evaluating the use of CRISPR-based gene drive systems as the alternative tools in the following decades. One thing that all novel vector control strategies have in common is the need for a detailed understanding of local mosquito movement and breeding in order to (i) design safe and effective field trials, (ii) plan control strategy interventions, and (iii) address biosafety concerns. Objective: Entomologists are increasingly exploiting genomic data to implement effective and sustainable surveillance and management of mosquitovectors. Here I show how movement and breeding of *Ae. ae-*

gypti at a very fine spatial scale can be elucidated with high-resolution genotyping of geo-coded individuals. Materials and Methods: Individual adult mosquitoes are collected at different building floors in two highly urbanized sites, screened at thousands of SNPs using ddRAD-sequencing, and their relationship and spatial distance to all other mosquitoes is determined. Dispersal kernel is constructed using the spatial distribution of close-kin pairs (1st-3rd degree relatives), dispersal spread is estimated under the IBD framework, and genetic patch size is estimated via spatial autocorrelation analysis. Results: Close-kin and IBD methods give highly consistent estimates of average dispersal distance (85 m, 95% CI: 68.2-106.6 m), and genetic patch size (260-360 m), indicating a minimum target zone of 60 acres for a sustainable control campaign in this landscape. Conclusion: Information about the relatedness is combined with spatial data to determine the size of an area for the mosquito control campaign, to prevent quick re-invasion from the surrounding untreated areas in highly urbanized landscapes. The relatedness used as a genetic 'mark' provides a viable alternative to the traditional mark-release-recapture experiments.

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Blood and sugar feeding behavior of Aedes albopictus by host and flower availability

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Aedes albopictus is an invasive species of mosquito with an expanding global distribution. It is a competent vector of over 20 pathogens in the laboratory, including dengue, however the degree to which it contributes to transmission of these pathogens in different geographic and environmental contexts is not well known. One reason for the uncertainty around *Ae. albopictus* vectorial capacity is variation in reported feeding patterns and a lack of host availability measures in most studies. We conducted outdoor aspiration collections to investigate the blood and sugar feeding ecology of *Ae. albopictus* on Long Island, NY. We determined host sources of mosquito blood meals in the context of host availability as measured by household interviews and camera traps. We identified 92 blood meals, including 31 human, 22 cat, 16 horse, 12 possum, 5 dog, 2 goat, and 1 rabbit, rat, squirrel and raccoon. Host Feeding Index calculations based on abundance and time-weighted household interview data show that *Ae. albopictus* fed more often than expected on cats and dogs compared to humans. Forage ratio calculations based on camera trap data suggest slight preference for cats and avoidance of raccoons, squirrels and birds. One potential method of control is the deployment of attractive toxic sugar baits, however, information about the sugar feeding behavior of *Ae. albopictus* is limited, so it is unclear how effective this mechanism would be in Northeastern US. We determined the proportion of fructose-positive mosquitoes and framed this behavior in the context of temperature, humidity, and floral abundance.

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Optimizing the deployment of ultra-low volume and indoor residual spraying for dengue outbreak response in Iquitos

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Introduction: Recent years have seen rising incidence of dengue. In most settings, the primary intervention against dengue is vector control, such as indoor, ultra-low volume (ULV) spraying. Targeted indoor residual spraying (TIRS) has the potential to more effectively impact dengue incidence, but its implementation requires careful planning and evaluation. The optimal time to deploy these interventions and their relative epidemiological effects are not well understood, however. Objective and Methods: We used an agent-based model of dengue virus transmission calibrated to data from Iquitos, Peru to assess the epidemiological effects of these interventions under differing strategies for deploying them. Specifically, we compared strategies where spray application was initiated when incidence rose above a threshold based on incidence in recent years to strategies where spraying occurred at the same time(s) each year. Results: In the absence of spraying, the

model predicted 361,000 infections [inter-quartile range (IQR): 347,000 – 383,000] in the period 2000-2010. The ULV strategy with the fewest median infections was spraying twice yearly, in March and October, which led to a median of 172,000 infections [IQR: 158,000 – 183,000], a 52% reduction from baseline. Compared to spraying once yearly in September, the best threshold-based strategy utilizing ULV had fewer median infections (254,000 vs. 261,000), but required more spraying (351 vs. 274 days). For TIRS, the best strategy was threshold-based, which led to the fewest infections of all strategies tested (9,900; [IQR: 8,720 – 11,400], a 94% reduction), and required fewer days spraying than the equivalent ULV strategy (280). Discussion: Although spraying twice each year is likely to avert the most infections, our results indicate that a threshold-based strategy can become an alternative to better balance the translation of spraying effort into impact, particularly if used with a residual insecticide.

Virology-Pathogenesis-Antivirals

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The dengue virus non-structural protein 1 (NS1) uses the scavenger receptor B1 as cell receptor in hepatic and mosquito cells

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Dengue is the most common virus disease transmitted to humans by mosquitoes. The dengue virus (DENV) NS1 is a multifunctional protein that forms part of the replication complexes. In addition, NS1 is secreted, as a hexamer, to the extracellular milieu. Circulating or soluble NS1 have been associated with dengue pathogenesis by several different mechanisms. Cell binding and internalization of soluble NS1 results in increased permeability of endothelial cells and in increased cell susceptibility to DENV infection, in associated with a down regulation of the innate immune response. Thus, a better understanding of the DENV soluble NS1- cell interactions may result in strategies to combat DENV replication and pathogenesis. Here, we report that the HDL scavenger receptor B1 (SRB1) in human Huh-7 hepatic cells, and a scavenger receptor B1-like in mosquito (*Aedes albopictus*) C6/36 cells act as cell receptor for DENV soluble NS1. The presence of the SRB1 on the plasma membrane of C6/36 cells, as well as in Huh-7 cells, was demonstrated by confocal microcopy. Also, a putative gene for the SRB1, encoding a protein with an estimated molecular weight of 68.7 Kd, was found in the genome of *Aedes aegypti* (XP019931364.2). Internalization of NS1 in Huh-7 and C6/36 cells can be efficiently blocked by anti-SRB1 antibodies (Abcam) and previous incubation of those cells with HDL (Merck) significantly reduces NS1 internalization, as evaluated by confocal microscopy. In addition, the transient expression of the SRB1 in Vero cells, which lack the receptor, renders these cells fully susceptible to NS1 entry. Direct interaction between soluble NS1 and the SRB1 in Huh-7 and C6/36 cells was demonstrated in vivo by proximity ligation assays an in vitro by surface plasmon resonance using recombinant NS1 and human SRB1. Of note, the affinity of the NS1 and the HDL for the SRB1 did not differ significantly. Finally, data is presented indicating that the SRB1 also act as cell receptor for soluble zika virus NS1. These results demonstrate that DENV NS1, a bona fide lipoprotein, usurps the HDL receptor for cell binding and entry and offers explanations for the altered serum lipoprotein homeostasis observed in dengue patients.

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Dengue virus non-structural protein 1 (NS1) interacts with DIDO1 promoting flaviviral replication in mosquito cells

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The dengue virus (DENV) NS1 protein is a multifunctional protein essential for viral replication

which in addition, also act as an immunomodulator. Yet, increasing evidence indicate that some properties of NS1 differ between vertebrate and mosquito cells. In order to gain insight of DENV NS1 function in mosquito cells, the protein interactome of DENV NS1 in *Aedes albopictus* C6/36 cells was obtained using a proximity biotinylation system (BioID). This system is based on a plasmid that expresses the DENV NS1 protein fused to a promiscuous biotin-ligase (BirA) enzyme from *E. coli*, which will add biotin to proteins that potentially interacts with NS1. The biotinylated proteins are purified using streptavidin beads and identified by mass spectrometry. The results indicate an interactome of 817 proteins for NS1. Of these, near to 10% coincide with previous reports obtained in vertebrate cells, including ontology groups of the oligosaccharide transferase complex (OST), the chaperonin containing TCP-1 (CCT), nuclear import and export, vesicle localization and ribosomal proteins. Interestingly, other protein pathways such as epigenetic regulation, RNA silencing and apoptosis, not previously reported in vertebrate cells, were also found as part of the NS1 interactome in mosquito cells. The direct interaction between NS1 and 4 of the proteins observed in mosquito cells (Dido1, RPL26, Sec61A and GRP78) were validated in DENV infected C6/36 cells by colocalization and by proximity ligation assays. Due to the strong and novel, previously unreported, interaction observed between Dido1 (Death Inducer-Obliterator 1) and NS1, we further explore the role of Dido 1 in viral replication. Dido1 silencing in C6/36 and Aag2 (*Aedes aegypti*) cells results in a significant reduction in DENV and ZIKV progeny, evaluated by focus assay, suggesting that Dido 1 is a host factor necessary for flavivirus replication in the mosquito vector. The functions of Dido1 are related to its protein domains: PHD, TFIIS and SPOC, and are associated with RNA transcription and epigenetic regulation. We are currently attempting to elucidate the mode of action of Dido 1 in the DENV and ZIKV replication cycle in mosquito cells.

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Live-cell imaging reveals host modulations induced by ZIKV nonstructural proteins

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Introduction: Zika virus (ZIKV) is an enveloped, positive-strand RNA viruses of the family Flaviviridae and genus Flavivirus. Flavivirus genome replication occurs in close association with ER membranes within organelle-like structures originating from ER, which also provide innate immune evasion through shielding the double-stranded RNA replication intermediates. Objective: We are utilizing ZIKV as a model system to elucidate the complex networks of virus-host protein associations in the flavivirus life cycle. Our goal is to obtain a detailed understanding of virus-induced host modifications that facilitate flavivirus replication and assembly in live cells Materials and methods: Fluorescent-protein tagged (FP-tagged) ZIKV cDNA clones were generated after extensive insertional analysis using peptide tags to identify sites that tolerated insertions in ZIKV genome. Spatial and temporal studies of ZIKV infected cells expressing FP-tagged viral and host proteins were conducted using confocal and electron microscopy. ZIKV proteins that significantly modified ER morphology were selected for mutagenesis studies. Alanine-scanning mutagenesis was done using a full-length cDNA clone for NS1, NS2A, NS4A, and NS4B, and amino acids that affected the host modification were identified by live imaging. Pull-down analyses were performed to identify specific host proteins interacting with ZIKV proteins. Selected ER-resident proteins were used for live imaging to understand virus-host interactions. Results and discussion: Using live-cell imaging coupled with single-particle tracking of cells infected with FP-tagged viruses, host proteins, and viral proteins, we mapped the flavivirus-induced host modifications in real-time. Electron microscopy analyses further confirmed these host cell modifications. Using long-term live imaging of ZIKV infected cells, along with FP-tagged ER markers atlastin and Sec-61 beta and mitochondrial marker mito-BFP, we found that ZIKV causes extensive changes in ER and induces ER-mitochondria contacts. We analyzed the role of each viral replication protein in causing the changes in ER and hijacking host pathways for efficient virus replication. Conclusion: We provide evidence for extensive modification of the ER tubules and sheets induced by NS4A and NS4B. Molecular genetic analyses were performed to identify the specific ZIKV residues involved in the alterations of ER membranes, and their role in virus replication and assembly will be discussed.

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Identification of chemical compounds inhibiting Zika virus replication through a large-scale high-content screening approach

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Zika virus (ZIKV) is a human mosquito-borne positive-sense RNA virus, belonging to the Flaviviridae family. With 230,000 ZIKA cases confirmed in the Americas between 2015 and January 2018, World Health Organization (WHO) classified this virus as an emergency in 2016 and currently identifies Zika as a priority disease. Although symptoms are generally mild, a risk of neurologic complications including Guillain-Barré Syndrome, neuropathy and myelitis is associated with the infection in adults, while infection during pregnancy is responsible for microcephaly and other congenital malformations. Since no vaccine or commercialized antiviral targeting this virus are available, scientific efforts are currently focusing on the development of treatments to efficiently limit ZIKV spread. Prompted by this unmet medical need, we conducted a screen of 51,520 small chemical compounds using a high-content imaging cell-based assay, monitoring Zika virus replication within Huh-7.5 cells. Among the 99 candidates initially identified and validated, two compounds sharing a common structure presented a particularly promising antiviral activity with a selectivity index (IC50/CC50) greater than 30. This common chemical scaffold specifically inhibited ZIKV, displaying an antiviral activity against several strains of both African and Asian lineages but no effect on other Flaviviruses tested. Its antiviral activity was confirmed with similar efficacy in more relevant models for ZIKV infection, including human monocyte-derived dendritic cells (hMDDCs), human neural progenitor cells (hNPC) and the placenta-derived choriocarcinoma cell line JEG-3. Time of addition kinetics as well as specific replication assays highlighted an antiviral role during the RNA replication step. Selection for resistant mutant viruses allowed the identification of 5 mutations in the N-terminal region of NS4A. The insertion of each of these mutations into a ZIKV replicon conferred resistance to the drug. Current efforts are ongoing to identify the specific mechanism of action of the drug and better clarify the role of NS4A during the infection. In summary, using a cell-based large-scale high-content screening approach to identify small chemical compounds showing an antiviral activity against ZIKV, we identified a chemical scaffold specifically targeting this Flavivirus, inhibiting the RNA replication step and potentially contributing to a deeper understanding of the biology of the virus.

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Dengue RNA-protein interactions that modulate infection in mosquito and human hosts

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Introduction: Mosquito borne viruses such as Dengue (DENV) and Zika are emerging as severe threats to human health. DENV infections have increased by 30-fold in the last 50 years. Efforts to develop vaccines and vector control strategies that can limit viral spread are still underway. The RNA genome of DENV is involved in multiple RNA- protein interactions that help the virus to subvert biochemical machineries from both human and mosquito hosts for its own propagation. Understanding the mechanisms by which DENV co-opts host proteins for survival is critical to developing antiviral interventions. Objectives: The objectives of the study are to identify proteins that interact with the DENV RNA and compare RNA-binding protein signatures in mosquito and human hosts. Further we will test the functional role of identified proteins in the viral life cycle and host immunomodulation. Approach and results: In this study, we have employed a novel, highly sensitive biotinylation based approach (RaPID) to identify proteins that interact with DENV 3' untranslated region (3'UTR) in mosquito cells. We identified known and novel proteins which could either interact directly or indirectly as part of a ribonucleoprotein complex with the DENV RNA including RNA binding proteins, ER-associated proteins and translation regulators. We tested the impact of select proteins on DENV infection by dsRNA-based gene depletion. Among the tested proteins, partial knockdown of Sec61A1 and Loquacious (Loqs) proteins resulted in a significant decrease in DENV RNA levels implying a pro-viral role for these proteins in mosquito cells. Interestingly, we observed that human homologs of both Sec61A1 and Loqs are also essential for DENV replication in human cells, indicating that

some host factor dependencies are conserved between humans and mosquitoes. In addition to DENV, depletion of Sec61 and Loqs proteins also inhibited replication of other flaviviruses including Zika virus and Yellow fever virus but didn't affect Chikungunya virus which is a alphavirus. Conclusion: Thus, our screen is able to identify important host proteins that regulate arboviral infection in both human and mosquito hosts. The effects of these proteins will be further validated in infected mosquitoes and the underlying mechanisms will be studied.

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Unpicking the secrets of viral replication sites - African Swine Fever

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African swine fever virus (ASFV) is a highly contagious pathogen that causes a lethal hemorrhagic fever in domestic pigs and wild boar. So far, it is the only known DNA arbovirus and the causative agent of an ongoing outbreak in Europe and Asia that has resulted in the culling of millions of pigs and immense economic losses. ASFV replicates predominantly in the cytoplasm of infected cells after an initial nuclear phase. These complex perinuclear replication sites, known as viral factories, concentrate viral structural proteins and essential cellular factors to facilitate more efficient progeny virion assembly and protect against innate immune responses. This project aimed to facilitate a better understanding of the spatiotemporal relationship of replication processes during ASFV infection and to analyze the structure and organization of the virus factory. Super-resolution immunofluorescence studies of ASFV factories, in Vero cells and primary swine macrophages, were conducted by stimulated emission depletion (STED) microscopy. Moreover, DNA and protein synthesis were visualized in infected cells using Click chemistry. We revealed three distinct localization patterns of viral structural proteins inside the ASFV factory. Firstly, co-localization with viral DNA throughout the factory, secondly, a compact protein ribbon in the factory center and finally, mature as well as assembling virions aligned around the protein ribbon. Additionally, we discovered the accumulation of newly synthesized proteins in the viral factory during late replication stages and the overlapping of these accumulations with the ribbon-like structure. Virions appear to be assembled from newly synthesized proteins at the ribbon-edges. During late ASFV replication phases, global protein production seemed to be reduced while viral DNA synthesis appeared to be continuous. Furthermore, we found newly synthesized proteins concentrated in distinct nuclear sites early in the viral replication cycle. These findings suggest that ASFV factories are compartmentalized with distinct replication mechanisms occurring at specific sites and highlight a novel aspect of the early nuclear step of ASFV replication. Sub-viral structures were successfully resolved in ASFV infected cells using STED microscopy. Moreover, Click labelling of nascent proteins revealed to be a potent new system to study protein synthesis in viral infected cells.

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Inhibition of the enzymes indoleamine 2,3-dioxygenase and kynurenine 3-monooxygenase leads to neuroprotection during zika virus infection

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Zika virus (ZIKV) emerged as a global health threat due to its association with severe outcomes in humans, including microcephaly and other neurological complications. ZIKV replication and induction of neuronal death are considered key factors for severe ZIKV-induced disease. Understanding the pathogenic mechanisms induced by ZIKV infection is crucial to identify potential therapeutic targets that may prevent or at least minimize the consequences in early phases of disease and adulthood. Our aim was to evaluate the role of the enzymes Indoleamine 2,3-dioxygenase (IDO-1) and kynurenine 3-monooxygenase (KMO) during Zika virus (ZIKV) infection. Primary cultures of neurons from C57/BL6 mice and human cell line (neuroblastoma and astrocyte) were infected with ZIKV and treated with inhibitors of the enzymes [1-MT (IDO-1) and RO-61-8048 (KMO]. After 48 hours,

cell viability was assessed by MTT, LDH and live/dead assays, as well as viral loads by plaque assay in culture supernatants was evaluated. In addition, type I IFN$\alpha\beta$ receptor-deficient mice (A129) were infected with ZIKV via intravenous route and treated daily with both inhibitors, as a therapeutical scheme. Weight loss and survival rates were evaluated daily. On day 5th of infection, peak of disease, mice were euthanized and organs harvested for further analysis. Results revealed increased expression of IDO-1 and KMO enzymes upon ZIKV infection in vitro and in vivo (brain of ZIKV infected mice). Pharmacological inhibition of IDO-1 and KMO enzymes in vitro led to massive reduction of ZIKV-induced neuronal death without interfere with the ability of ZIKV to replicate in these cells. Furthermore, in vivo analysis showed that treatment with inhibitors attenuated the increase of intraocular pressure and resulted in massive amelioration of brain damage as shown by reduced microgliosis and astrogliosis in the brain of ZIKV-infected mice. Neuroinflammation, assessed by levels of cytokines and chemokines in the brain of ZIKV-infected mice was reduced by IDO-1 and KMO inhibitors. Similarly as in vitro, treatment was not able to reduce viral loads in optical nerve and brain of ZIKV-infected mice when compared to untreated ones. Overall, our results indicate that IDO-1 and KMO blockade provides potent neuroprotective effects against ZIKV-induced neurodegeneration.

Posters

Diagnostics-Prognostics-Clinical

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Algorithm DENGUE: correlation absorbances vs seroconvertion and steps infections

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Introduction: Exist more infections transmitted for vectors, specified mosquitos, very common in tropical zones in America and areas poor people. Into this infections in Panama find us: DENV, ZIKV, CHIKV, etc. In the last years close in the concept kmow as ARBOVIROSIS. In this work we will concentrated in the infection by Dengue Virus, and your vital cicle during interaction withg inmunological system and seroconvertion process y the correlation with the algoritm previusly established for organism worlwide. We tested the correlation betwen the algortim experimentally with algoritm teorical for can understand of better manner the seroconvertion of the antibodies and in the differents stages of infection through of results of absorbances of kits that help us to differentiated stages of infection by Dengue. Objetive: Stablish experimentally through of absorbances of our tests the stages of infection DENV and cicle of seroconvertion antibodies for understand the differences between other arbovirosis and possibles creation methods mor sensible and exactly. Matertials and Methods: Population: 152 Date of study: 2016 Years of Samples worked: 2015-2016 Kit PAnbio Dengue Capture for determine Ac IgM e IgG. Kit Panbio Dengue EARLY (NS1) Kit FOCUS Dengue Capture Detect IgG. Results: We can observe that results of absorbance of tests anlalyzed mantain correlation with stages of infection by DENV, and is possible determine the apparition sintoms in patients. Conclusion: Know the algoritm permite stablish how satge of seroconvertion of the antibodies is the patient and the type of test needed for diagnostics, vaccine preparations.

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Optimization of RT-qPCR Assay for Zika Virus Detection in Samples of Low Viral Concentration

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The Zika virus (ZIKV) outbreak of 2015 affected many countries in Latin America and highlighted a pressing need for sensitive molecular diagnostics

to detect the presence of ZIKV in various sample types at a range of concentrations. At the time, only two RT-qPCR assays, developed by Faye et al. and Lanciotti et al., were available to diagnose ZIKV infections. These assays useprimer sets that target either the NS5 or Envelope regions of the ZIKV genome, respectively,with enough specificity to serve as diagnostic tools that could differentiate ZIKV from other related arboviruses. Although the specificity of these assays was adequate, their sensitivity was low, resulting in false-negative diagnosis, particularly when ZIKV concentration was low (CT values around 30or higher). We have developed an improved RT-qPCR assay that uses optimized primer sets and cycling conditions in order to detect ZIKV with specificity and sensitivity, including at low concentrations previously considered undiagnosable (CT values up to 38).Wehave also created a positive control plasmid that includes both the NS5 and Envelope regions of the genome, allowing for side-by-side comparisons of the amplification efficiency of assays. Using this control plasmid,we estimated the limit of detection of the improved assay at 31 copies of the viral genome. The assay shows no cross-reactivity with other arboviruses tested, including all four serotypes of Dengue, Chikungunya, Mayaro, Oropuche, and Yellow Fever. We also tested the assay on samples derived from patients with Guillain-Barré Syndrome that did not have ZIKV symptoms, and did not identify ZIKV in them. In contrast, we used the assay to test a number of samples suspected of being ZIKV false-negatives,and found that about 14% of those retested were positive for ZIKV, with reproducible lower CTs when comparing against previous assays. Our results indicate that the improved ZIKV assay can help address the issue of false-negatives during ZIKV diagnosis with both specificity and sensitivity. As such, we expect it to be a useful tool for those interested in a fast, rapid, and sensitive diagnostic for ZIKV, particularly in samples of low viral concentration encountered during clinical evaluation or research.

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Peptide-based serologic platforms capable to differentially identify Dengue and Zika infections

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

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Evaluation of the sensitivity and specificity of a comercial NS1-targeted anti-Zika virus IgG enzyme-linked immunosorbent assay

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Currently, many countries have concomitant transmission of several arboviruses that cause exanthematic febrile illness with confounding symptoms. Because of the global spread of Zika virus and the expected cross-reactivity among anti-flaviviruses serological tests, accurate diagnostic immunoassays are needed. The commercial anti-ZIKV IgG and IgM ELISA from Euroimmun, (Lübek, Germany) are among the most widely used test to detect anti-ZIKV antibodies, and is reported to have high sensitivity and specificity for the serodiagnosis of ZIKV infections. In the context of the current flaviviruses circulation in Brazil, we evaluated the sensitivity and specificity of this anti IgG ELISA test by submit it against ZIKV, DENV and YFV positive samples. It was included 63 samples from patients with positive Real Time PCR for ZIKV in the acute phase of disease (13/63 (21%) of positivity among the acute samples and 58/63 (92%) reagents samples among the convalescent ones. The specificity of the test was evaluated using primary and secondary DENV and YFV serum samples.While no cross reaction was seen for primary dengue, 32/131 (24.4%) serum from secondary acute dengue presented unequivocal positivity in the anti-ZIKV IgG ELISA test. According to the serotype, 5.5% (1/18) presented DENV-1, 16.6% (8/48) DENV-4, 23.8% (5/21) DENV-3and astonishing 63.6% (28/44) had secondary DENV-2. No cross-reaction was observed in YFV post-vaccination samples. Crucially, such cross-reactivity for anti-ZIKV IgG in DENV acute samples, in particular from DENV-2, may be a problem in endemic regions for flaviviruses.

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Zika IgG Avidity in Dengue Immune Patients

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Introduction: Zika virus (ZIKV), a flavivirus closely related to dengue virus (DENV), emerged rapidly in the Western Hemisphere since its detection in Brazil in 2015. Congenital microcephaly and Guillain-Barré syndrome are two major complications of ZIKV disease. Diagnostics is confounded by the following: 1. ZIKV-RNA is temporary, 2. ZIKV IgM antibody levels are variable and temporary, 3. Cross-reactivity of antibodies to the envelope protein of flaviviruses. To aid in diagnosis we developed a microsphere immunofluorescence bead-based IgG avidity assay. Avidity of IgG antibodies is low early in infection and increases over time. Objective: The objective of the study was to characterize the change in IgG avidity index through time in sequential sera with known days post onset in ZIKV infected individuals with a history of previous dengue exposure. MATERIALS AND METHODS: Avidity assay was performed on sequential sera from three PCR-confirmed Zika patients with known days post onset. A mixture of six different microsphere beads were each coated with: Zika envelope, non-structural protein 1 and NS1 of all four serotypes of dengue and mixed with patient sera. Half the samples were treated with PBS while the other half with 8M urea solution. Avidity index expressed as a percentage was calculated as the ratio of the median fluorescence intensity (MFI) of the wells treated with urea to the MFI of the wells treated with PBS multiplied by 100. Results: Zika IgG avidity index (AI) demonstrated a linear increase for all three patients. ZIKV IgG antibodies were characterized by low avidity early in infection (Avg. AI 6%) and higher avidity 9-19 months post infection (Avg. AI 52%). Finally, DENV IgG antibodies in all three patients have had higher avidity indices compared to the ZIKV IgG avidity indices, confirming a more recent ZIKV and a past DENV infection. Conclusion: IgG avidity can potentially be used in identifying primary ZIKV infection in high risk patient population such as pregnant women, as the presence of low avidity ZIKV IgG antibodies suggest a more recent infection, whereas high avidity index antibodies suggest a past infection. Furthermore, avidity assay

can be a useful tool in vaccine studies.

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Sensitivity and specificity of Dengue IgM is dependent of age of patients in an endemic zone

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Brazilian epidemiologic surveillance services currently perform NS1 rapid test, IgM ELISA, and/or PCR assays for Dengue diagnostic. Serologic tests are widely utilized in the laboratory routine to aid the clinical diagnosis and, despite more sensitive molecular assays, the IgM test still is preconized by physicians. Additionally, due to the continental proportion of the country, serologic tests present a higher cost-benefit to the public health system. Variations in the detection limit of viral antigens or antibodies also depend on primary or secondary infections, taking from 4-5 days and 1-14 days to generate IgM and IgG anti-DENV antibodies, respectively. With a need for a detailed analysis of the effectiveness of these tests in an endemic context, the goal of this work was to evaluate the diagnostic performance of IgM assay in different age strata in an endemic zone. For this we select 1388 samples from patients with febrile symptoms during 2019' Dengue outbreak in the city of São José do Rio Preto, Brazil. The city is a hotspot for arboviruses infection, presenting a high infestation rate of the mosquito vector and in which dengue is prevalent without a break for more than 10 years with the occurrence of epidemics by the four serotypes. The samples were tested with RT-PCR and/or NS1, which were considered virologic confirmatory tests, and results were compared to those obtained with ELISA IgM. Information related to age present on the national database was utilized to correlate with the sensibility and specificity of the IgM test. It was observed that the population' IgM specificity is 67% and sensitivity is 71%, with a positive predictive value of 67%. Specificity was lower in children aged zero to 10 years (54%), young adults up to 30 years (68%) and increases with age, being higher in people over 61 years (78%). The sensitivity of IgM test was higher in children and young adults (from 71% to 87%) decreasing with age and being lower in people over 61 years, totalizing 56%. In conclusion, the sensitivity of the IgM test decreases with age and its specificity is higher in an endemic dengue population.

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Development of a New Molecular Diagnostic Tool for Differentiation of Lineages I and II of Dengue Virus Type 2 Circulating in Brazil

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DENV is an arbovirus belonging to the Flaviviridae family and the Flavivirus genus. It has an endemic characteristic in Brazil and is responsible for major epidemics and severe cases of the disease. Since the reemergence in 2007, a viral variant that, although belonging to the Southeast Asian genotype, has joined another monophyletic group, characterizing lineage II. DENV-2 has been the target of a number of studies that focus on better understanding these variants and the development of methodologies that act as virological surveillance tools. The objective of this study is to develop and standardize a "lineage typing" protocol for rapid and inexpensive differentiation of DENV-2 lineages, using the polymerase chain reaction (PCR) technique in real-time by analysis of the High-Resolution Melting (HRM) high-resolution dissociation curve tool. Methodology starts with the identification of the genome region where the single nucleotide polymorphism is located and primer design for amplification of this region followed by subsequent analysis of the high-resolution dissociation curve. Viral masses from different strains were extracted and quantified using Qubit Fluorometric Quantification and will be used as controls for assay standardization. Serum samples previously diagnosed as positive for DENV-2, containing genotype and lineage information will be used, as well as samples positive for DENV-2 without lineage information. Finally, Sanger sequencing will confirm the identity of the lineage characterized by PCR-HRM. Preliminary

results lead to the identification of optimal primers for the PCR. Experiments are still ongoing, and we expect that the melting curve analysis will be able to identify and differentiate the amplified fragments, according to the dissociation temperature inherent to each product. After validation, we expect this method to be incorporated as a new rapid and inexpensive tool for characterization and genomic surveillance of DENV that could be an alternative to the genomic sequencing approach.

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Symptomatic chikungunya disease and its relationship with IgG antibodies

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Chikungunya virus (CHIKV) outbreak (2014-2015) affected 294.831 people in Colombia. We have been conducting clinical and population-based studies in Santander, north-east of our country. In 2015, we observed a higher probability of positivity of the squeeze test per unit increase in IgG anti-CHIKV levels (OR=1.06, 95% CI:1.01-1.12) in cases from a chikungunya disease cohort conducted in Capitanejo, Colombia. There is experimental and epidemiological evidence suggesting that the levels of the convalescent antibodies reduce the individual probability of a symptomatic infection by Chikungunya. Then, we explored the relationship between the IgG anti-CHIKV antibodies and a symptomatic chikungunya disease in a cohort study performed from 2015 to 2017 in Piedecuesta, Colombia. This study included an active fever surveillance, as well as yearly visits in which a blood sample is collected. The antibodies IgG against chikungunya, dengue, and Zika virus were performed using a multiplex recombinant antigen-based microsphere immunoassay (ArboMIA). The measurements were done in all the serums collected in a subset of randomly selected participants. We evaluated MFI differences between groups using the Wilcoxon rank-sum test. We evaluated 511 subjects (mean age at baseline: 13.0 years; median follow-up: 1.9 years) of whom 173 (33.9%) had at least one symptomatic event. Medians of the highest ratios of MFI during follow-up for DENV, CHIKV and ZIKV were 30.7, 1.02, and 1.52, with higher levels observed among symptomatic than asymptomatic individuals for DENV (45.9 vs. 22.2, $p<0.001$), CHKV (1.2 vs. 1.0, $p=0.001$) and ZIKV (5.6 vs. 1.3, $p<0.001$). Once excluded individuals with positive ArboMIA tests for DENV and ZIKV at any time, those CHIKV (positive) were about twice more likely symptomatic than CHKV (negative): 19 (47.5%) and 37 (27.8%), $p=0.020$. Among CHKV (positive), maximum ratios of MFI were higher in symptomatic than asymptomatic subjects (136.6 vs. 81.0, $p=0.049$). We observed higher levels of antibodies among symptomatic cases for the three arboviruses. Moreover, a chikungunya positive result has twice as likely to have a symptomatic episode than those chikungunya negative results. These preliminary results will be validated in the same cohort taking account of the viral diagnosis (RT-PCR) in the symptomatic cases.

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Diagnosis of Dengue Fever by Rapid Test and Hematological Findings Guiding Treatment in Los Patios – Norte de Santander, Colombia.

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In the past two years, a significant increase in dengue cases has been recorded in the bordering areas of Colombia, mainly associated with forced migration. A significant number of infections with dengue virus (DENV) in rural areas are diagnosed by hematological findings because an ELISA or a PCR may not be available within appropriate time frame. Rapid tests are a less sensitive, yet timely way of detecting DENV infections. The purpose of this study was to determine whether there is an association between hematological findings and the probability for an NS1-based DENV rapid test to detect DENV NS1 antigen. In 2018, we collected serum samples from 161 patients with febrile syndrome admitted in Los Patios Hospital and 103 patients were positive for Xerion Dengue NS1 Antigen test. Hematological tests were also performed to each patient. Our preliminary results showed no significant differences in platelet levels between people with a positive or negative DENV rapid test. Although, "days of symptoms until diagnosis" was not significantly different between

groups, white blood cell count, hemoglobin, and hematocrit levels presented significant differences. Our results suggest that rapid tests are a good alternative for diagnosis in the absence of more precise diagnostic tools. We also believe that more studies addressing the clinical and hematologic findings in people with probable dengue infections are urgently needed to help physician in rural areas identify these patients better preventing severe forms of the disease.

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In silico screening of epitopes in the NS1 protein of flavivirus for diagnosis

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The nonstructural protein 1 (NS1) of Flavivirus is associated with the process of viral replication, immune system escape, viral tropism, and host cell modulation. It has been in recent years one of the main targets for the production of diagnostics, vaccines, and treatment. However, the structural similarities between the epitopes of these viruses, as well as the wide variability of existing polymorphisms for each species, make the development of efficient therapies difficult. The present study had a broad analysis of all DENV (1-4), Yellow Fever and Zika virus NS1 polypeptide sequences and three-dimensional structures found in Brazil and deposited in GenBank. The aiming was to compare epitopes already known for each of the species, taking into account, in a new way, the variability found in the country, as well as understanding how this variability can be used as an efficient virus trap against the immune system, and consequently against the serological diagnostic methods. A total of 352 polypeptide sequences were collected, and the optimal consensus for each species/serotype was made on the MergeAlign server. The determination of conserved blocks, as well as polymorphic residues, was done using MEGA and Discovery Studios software. Protein structural modeling for each virus was performed using the I-Tasser server (monomer) and Gramm-x to form the dimer. Structural refinement was done through the GalaxyWeb and ModRefiner servers. The quality of the models has been checked with the MolProbity server. NS1 antigenic epitopes already reported in the literature of DENV (1-4), Yellow Fever, and Zika were collected using the ViPR platform. The antigenicity of the epitopes was checked through the VaxiJen2.0 server. The identification of trap immunogenic regions was done by aligning the epitope sequences with NS1 variants. All trap regions were identified in the three-dimensional models as well as their physicochemical characteristics were described. The appointment of immunogenic regions with large polymorphic variation helps us understand the failures of the diagnosis methods of these viruses since the cross-reactivity commonly leads to false positives for these species and has been the major problem for epidemiological surveys.

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Evaluation of the dengue's severity cases after introduction of zika virus in a brazilian endemic area

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Introduction: Recently, besides dengue virus (DENV), another important arbovirus transmitted by Aedes the Zika(ZIKV),calls attention to a new pandemic.They share the same vector and have genetic similarities, DENV and ZIKV are closely linked, so sequential infections are common, and cross-immune responses are indeed possible. Objectives: This study aims to evaluate the severity of dengue cases after the introduction of Zika virus in a Brazilian endemic area.Methods:378 confirmed cases of Zika in Sao Jose do Rio Preto were evaluated between January 2016 and February 2018 besides confirmed cases of dengue in the same city between April 1998 and December 2006 and June 2016 and November 2018.The moment when there was no Zika circulation was called T1 and the moment after the introduction of Zika was called T2.Chi-square test was used to evaluate differences and association between variables in both periods.Binary Logistic Regression was also used to assess the risk associated with dengue hospitalization before and after the introduction of Zika in the same area.All analyzes

were performed using IBM SPSS software, version 19, at a significance level of 5%. Results: Among the variables analyzed for dengue inT1 and T2, is possible to conclude thatin both cases there was a predominance of number of cases in the female population (T1=59.3%/T2=53.9%) aged 15 to60 years (T1=80%/T2=72.9%).Other variables presented by most patients in both intervals, with statistical significance ($p<0.001$), but with a considerable decrease in the second period, were: fever (T1=96.6%/T2=66.8%), headache(T1=90.2%/T2=62.0%) and myalgia (T1=87.9%/T2=67.9%).It is also noted that some variables expressed statistical contrast between the periods: presence of retro ocular pain (T1=75.3%/T2=37.6%), vomiting (T1=60.5%/T2=15,4%), arthralgia (T1=70.7%/T2=12.7%) and abdominal pain (T1=37.7%/T2=99.9%), all with $p<0.001$.Discussion/Conclusion:Our findings suggest a change in the clinical presentation of dengue over the years. In fact, over the years several outbreaks of dengue occurred changing the epidemiological pattern of the region. Moreover, the introduction of Zika in 2016 added another issues in the equation. This change can be caused by a series of factors such as: i) the increase of secondary dengue infections; ii) the cross reaction of the antibodies raised to these closely related virus and phenomena like enhancement or protection; and iii) the mimicry of symptoms and misdiagnosis of these diseases.

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Development and performance of a sandwich Enzyme-Linked ImmunoSorbent Assay for detection of Zika virus NS1 in a hyperendemic area of Colombia

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Dengue (DENV) and the Zika virus (ZIKV) are two Flaviviruses important for public health. Nonstructural protein 1 (NS1) is a Flavivirus protein highly secreted by infected cells. Due to its high secretion, NS1 or antibodies anti-NS1 are important for the diagnosis of DENV and ZIKV infection. The development of an immunoassay for the detection of NS1-ZIKV based in specific monoclonal antibody (Mab) is necessary. Here, a sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) for the detection of NS1-ZIKV was performed using different specific Mab against this viral protein. A panel of murine Mab was tested as the coating of the assay and biotinylated Mab were used as detection antibodies. Both groups of Mab (coating and detection) were used at different concentrations to achieve the best conditions to perform the assay. ZIKV recombinant protein NS1 (rNS1, Native Antigens, UK) was used to the realization of these and subsequent experiments. Plasma samples from patients with confirmed ZIKV and DENV infection were used to evaluate the cross-reactivity. It was found that the pairs of monoclonal antibodies ideal for the detection of ZIKV-NS1 was the Mab680 and Mab644 both with a concentration of 2ug/mL that proved to be optimal. The saturating concentration was 200ng of rNS1. The limit detection of the assay was 7ng/ml. The ELISA was applied to 13 plasma samples that were IgM-ZIKV positive. 10 patients with confirmed DENV infection by RT-PCR and IgM were used as controls. The results of this trial were that it was possible to detect 3 positive patients for ZIKV-NS1 and 1 cross-reaction within the controls. The low ratio of detection for ZIKV infection can be explained by the sampling time, as those were included between 5-7 days after fever onset. In summary, we are developeing an ELISA with potential use in endemic areas.

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Development of a focus forming assay to identify infectious Zika viral particles

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The WHO declared ZIKV infection as a public health emergency. In Colombia at least 320 cases of congenital zika syndrome (CZS) were reported after the epidemic. To study the ZIKV in cell culture models, identify the absolute number of infectious viral particles in supernatant is a critical data. The focus forming assay (FFA) is a method for quantifying infectious particles using a monolayer of susceptible cells that are stained after infection. Specific monoclonal antibodies against the ZIKV non-structural (NS)-1 protein can be used to identify intracellularly, the absolute number of infected cells. We aimed to develop a protocol for the quantification of ZIKV

particles by FFA. We grew up Vero-76 cells in 96-well plates, the cells were infected with ZIKV. At each time, cell fixation and permeabilization were done by adding methanol. Then, we added sequentially the primary antibody, a biotin-labeled secondary antibody, the streptavidin-peroxidase, and finally, a non-soluble chromogen. Stained cells were counted with an inverted light microscope. First, we determined the optimal time of infection needed to detect the highest number of infected cells. We tried 6,12, 24 and 48 hours post infection (hpi). Then, we tested the confluence of the cell monolayer at the moment of the infection. We tried cell monolayers at 30%, 50%, and 70% of cell confluence. Next, we checked the best concentration of the primary antibody. We tried 0.3, 0.5,1 and 2 μg/ml of a panel of monoclonal antibody anti-NS1. At last, we added Brefeldin A after infection to see the effect of the inhibition of protein transport through Golgi in the count of infected cell and the quality of the staining. ZIKV infected cells could be better identified after 24hpi, the cell monolayers at 70% of confluence show better results. Neither using a concentration of $\geq$0.5 μg/ml of the primary antibody nor adding Brefeldin A after infection improved the staining quality of the infected cells. We developed a protocol to quantify infectious ZIKV particles using Vero-76 cells for FFA. ZIKV can now be locally studied using cell culture models.

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Development and evaluation of a novel highthroughput image-based fluorescent neutralization test for detection of arbovirus infection

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The Flavivirus genus comprises some of the most important public health viruses in the world nowadays, such as zika (ZIKV) dengue (DENV) and yellow fever virus (YFV). Serological diagnostic assays and populational serological-survey studies for these arboviruses proved to be a great challenge, due to the high cross-reactivity observed among them. In this scenario, the plaque reduction neutralization test (PRNT) is indicated to confirm positive samples for being more specific, however it is laborious intensive and time consuming. To overcome this limitation, we developed a high-throughput image-based fluorescent neutralization test for ZIKV, DENV and YFV, using the Operetta CLS High-Content Analysis System (PerkinElmer). For the ZIKV neutralization assay, we used 226 human specimens and showed that the new test presented higher throughput than traditional PRNT, maintaining the correlation between results. Furthermore, when tested with dengue virus samples, it showed 50.53% less cross reactivity than MAC-ELISA. The DENV fluorescent neutralization test was able to identify neutralization antibodies against the four serotypes in all DENV IgM positive samples tested. However, it was not possible to identify which DENV serotype was responsible for the current infection, indicating a probable secondary infection. The YFV neutralization test was standardized and validated with serum samples of healthy donors vaccinated for yellow fever. We tested 17 samples from different time points after vaccination, the test showed good correlation with PRNT. Results of samples before or up to a week after the vaccine were negative. With three weeks after vaccination, all samples showed neutralizing antibodies against YFV. These fluorescent neutralization tests could be used for clinical diagnosis confirmation of ZIKV, DENV or YFV infection, as well as for vaccine clinical trials and seroprevalence studies. Furthermore, this platform can be easily customizable to new viruses like chikungunya (CHIKV) and West Nile (WNV).

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A case series of Dengue/Zika co-infection cases in a cohort in Central Brazil

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Introduction: Dengue/Zika viruses co-infection has being described. However, understanding the clinical presentation of this co-infection is important to improve diagnostic accuracy. Objective: to describe the symptoms of Dengue/Zika coinfection cases in a cohort of children and adolescents, from 2015 to 2019 in Central Brazil. Methods: Fever were monitored weekly by telephone calls to participants' legal guardians and by direct notification to the project's physician.

Home visits were conducted by the physician for clinical evaluation. Results: Case 1: six years old, three days diffuse pruritic maculopapular rash, headache, myalgia, conjunctival hyperemia, mild sore throat, mild abdominal pain. Two low fever episodes. Leukocytes: 3,700 cells/mm3 and platelets: 173,000 cells/mm3. DENV NS1 antigen (ELISA) positive; ZIKV RT-PCR positive. Case 2: nine years old, three days diffuse pruritic morbilliform rash, headache, mild sore throat, nausea, vomiting, retroorbital pain, mild abdominal pain and diarrhea. One low fever episode. Leukocytes: 2,900 cells/mm3 and platelets: 173,000 cells/mm3. DENV NS1 antigen positive, ZIKV RT-PCR positive. Case 3: 13 years old, four days diffuse pruritic maculopapular rash, headache, conjunctival hyperemia, myalgia and nausea. No fever. Leukocytes: 4,400 cells/mm3 and platelets: 149,000 cells/mm3; DENV NS1 antigen (ELISA) positive; ZIKV RT-PCR positive. Case 4: 17 years old, three days of high fever, headache, retroorbital pain, myalgia, mild abdominal pain and late diffuse pruritic maculopapular rash. Leukocytes: 3,000 cells/mm3 and platelets: 157,000 cells/mm3. DENV/ZIKV RT-PCR positive. Case 5: 29 years old, four days diffuse pruritic maculopapular rash, retroorbital pain, myalgia, hand joint swelling, diarrhea and four low fever episodes. Leukocytes: 3,200 cells/mm3 and platelets: 172,000 cells/mm3. DENV NS1 antigen (ELISA) positive; DENV/ ZIKV RT-PCR positive. Case 6: 40 years old, three days diffuse pruritic maculopapular rash, headache, retroorbital pain, myalgia, arthralgia, hand joint swelling, nausea, mild abdominal pain and three low fever episodes. Leukocytes: 4,300 cells/mm3 and platelets: 120,000 cells/mm3. DENV NS1 antigen (ELISA) positive; ZIKV RT-PCR positive. Discussion/Conclusion: the clinical presentation of six cases of dengue/Zika co-infection differed, with predominance of clinical criteria for Zika in five and dengue in one. No unique characteristics can be identified as co-infection, emphasizing the importance of confirmatory laboratory tests for dengue and Zika.

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Clinical Presentation of Dengue and Zika Cases in a cohort of children and adolescents in Central Brazil

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Introduction: Dengue present a high burden in Brazil, with an endemic/epidemic pattern since the mid 80's. Zika virus is present in tropical and subtropical countries and the epidemic in 2016 in Brazil led to the recognition of the Congenital Zika Syndrome. The co-circulation of DENV and ZIKV infection often makes diagnosis and treatment a challenge for health professionals due to similar symptoms. Objective: To describe the clinical presentation of dengue and Zika cases among a cohort of children and adolescents, from 2015 to 2019 in Central Brazil. Methods: The cohort was established in 2015, with around 2,000 participants from 2 to 16 years of age. Fever episodes were monitored weekly by telephone calls to participants' legal guardians and/or by direct contact to the project's physician. For each fever episode, the physician performed a home visit for clinical evaluation. Results: 206 dengue cases were confirmed. 80,6% had usual clinical presentation and headache, myalgia, and retroorbital pain were the most common symptoms. Cold sweating and postural dizziness were the most frequent warning signs. 5.4% of the cases required venous hydration and there were no deaths. 61.5% had leukopenia and 48.8% thrombocytopenia. 19.4% of the confirmed dengue cases had an unusual presentation with absence of or low fever, short-term fever, and early diffuse skin rash. Headache, retroorbital pain and myalgia were the most common symptoms and no alarm signs were found. Among these cases, none required venous hydration. 55.0% had leukopenia and 22.5% thrombocytopenia. 37 cases of Zika were confirmed and skin rash was absent in 45.9% of the cases. Among the other 20 cases, rash was present early. Headache, retroorbital pain, myalgia and conjunctive hyperemia were the most common symptoms. 62.1% had leukopenia and 16.5% thrombocytopenia. Discussion: most of dengue cases had usual clinical presentation, but, mainly in 2019, 19.0% had unusual clinical presentation with milder general symptoms Approximately 46% of confirmed Zika cases did not meet the case definition criteria proposed by the Ministry of Health, with no skin rash as a guiding symptom. The clinical differentiation between dengue and Zika represents a major challenge for clinicians.

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Dengue prevalence in patients evaluated in the surveillance program in the Ica region during 2017

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Introduction: Dengue virus (DENV) represents a major public health problem in Peru. The epidemiology of this disease depends a lot on climate factors, among them, the presence of meteorological phenomena such as El Niño-Southern Oscillation, wich during 2017 generated a dramatic a increasing of DENV cases in regions where the prevalence was low or near to zero, as occurred in the department of Ica. Objective: To estimate the seroprevalence of dengue virus infectious in patients, we evaluated a surveillance program in the Ica region during the year 2017. Materials and methods: We conducted a cross-sectional population study in 2017 in the Ica region by which we used Enzyme-Linked ImmunoSorbent Assay test, to measure anti DENV IgG and IgM levels, and DENV NS1 antigen. Results: They captured a total of 2975 individuals with clinical manifestations of dengue from a community, under a passive and active epidemiological surveillance program, of which 56% were women and the average age was 31 years (95% CI 30.1 - 31.7 years). However, other studies have reported a greater number of dengue cases in the male population, and something similar with a higher probability of infection in the adult population; likewise, the highest number of those evaluated were from the province of Ica (46%), while the lowest was from Chincha (1.8%). The environmental conditions represented by temperature and relative humidity whose values were 24.6°C (95% CI 24.5 - 24.8) and 71.6% (95% CI 71.3 - 71.9). Thus, the total prevalence of dengue infection was 50.21% (95% CI 48.40 - 52.03%) with a prevalence of 48.1% in NS1 and 26.4% and 26.0% for IgM and IgG, evaluated respectively, confirming what many studies reported in which indicate a greater sensitivity of the NS1 test. Conclusion: We conclude that the prevalence of dengue in tha population captured under the active and passive surveillance program is very high and this reflects a possible influence of the as El Niño-Southern Oscillation within the epidemiology of the disease, so it is recommended to implement primary prevention measures in the region evaluated.

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Development of a molecular diagnostic test for dengue and zika viruses simultaneously

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Introduction: The co-circulation of arboviruses such as dengue and zika in the same geographic region, transmitted by the same vector and with overlapping symptoms, makes clinical management difficult due to the similarity of these diseases. Therefore, the present study aimed to develop a molecular diagnostic method for dengue and zika in a single reaction. Materials and Methods: Specific gene primers were designed using non-structural protein NS1 for DENV and envelope protein for ZIKV and analyzed in silico to rule out cross- reaction between them. The Duplex RT-PCR DZ reaction was developed and validated using serum samples collected from 2016-2017 in suspects of acute febrile syndrome (SFA) and primers described in the literature were used for validation. Results: Specific primers designed for DENV and ZIKV did not cross react with each other and amplified the positive controls. Samples of 42 suspected acute febrile syndrome were initially analyzed by RT-PCR for Flavivirus, dengue, and zika. For the Flavivirus genus of the 42 samples, 18 (42.8%) were positive, and of these, dengue RT-PCR was performed with 17 (94.4%) positive. And 1 dengue negative but Flavivirus positive sample was tested for ZIKV, being negative. Validation of D-RT-PCR DZ was performed with 18 Flavivirus positive samples, 18 (100%) samples were positive for DENV, and one sample was positive for DENV and ZIKV (5.5%). Discussion/ Conclusion: D-RT-PCR DZ was able to differentiate simultaneously the two major Flaviviruses (DENV and ZIKV) circulating in Brazil in a single reaction, faster and at lower cost. Therefore, investments for the development of diagnostic tests that facilitate and assist in the early detection of the differentiation of these viruses is extremely important for public health.

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Evaluation of eight serological commercially tests for diagnostic of Zika virus infection

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Zika virus (ZIKV) is an emerging mosquito-transmitted flavivirus currently causing large epidemics and represents a global public health. An available and sustainable surveillance for risk individual groups to ZIKV infection it is necessary and serological methods offer a good alternative. The study objective is to evaluate the clinical performance of eight commercially serologic assays for the detection of anti ZIKV antibodies.Two evaluations were developed, using a panel of 200 samples each one. One hundred positive and 100 negative sera to ZIKV infection were included, all characterized by molecular and serological standard diagnostic assays. A variety of immunoassay performances were useful, such as: indirect ELISA, Capture ELISA, immunoblot, chromatographic and immune-magnetic assays. Evaluated assays showed acceptable specificities (>80%) and variable sensitivities. The highest sensitivities for IgM detection were found for DiaPro-ELISA(87.8%) and Blusense (88.0%), StandartQ (97.0%) and Chembio (95.9%) rapid tests. For IgG detection only two tests were assessed (Blusense and Chembio), observing sensitivities of 51.1% and 68.1% respectively in samples with equivalent positive ZIKV IgM. The strongest agreements with the molecular reference (kappa index) were found by DiaPro-ELISA (0.87), Blusense (0.88), Standart Q (0.87) and Chembio (0.95) assays. IgM cross-reactivity in response to negative sera panel with positive immunity to other flaviviruses, were not found by Euroimmun, Diapro and Blusense. Chembio and Blusense showed similar behavior to ZIKV IgG antibody detection. Between the eight evaluated tests, DiaPro-ELISA, Blusense and Chembio provided the best-assessed parameters for IgM detection in sera collected between days 5 to 7 of onset of symptoms.

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Comparative Analysis of Vero-76, C6/36 and BHK-21 cell lines as substrate for Dengue Virus Titration by Fluorescent Focus Assay.

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Among research activities in Virology, robust and reliable viral detection and quantification assays are an essential part of the virologist's toolkit. In the case of Flaviviruses, these can be detected and quantified using a variety of virological methods such as Plaque Assays (PA), Reverse Transcription Polymerase Chain Reaction (RT-PCR), Transmission Electron Microscopy (TEM), Fluorescent Focus Assay (FFA) and 50% tissue culture infective dose (TCID50), each with its own limitations for the detection and quantification of viral genomic particles, viral proteins or intact infectious particles. FFA for flaviviruses it is a combination of plaque assay and immunofluorescence, where flaviviruses are inoculated at different dilutions on the cellular monolayer, then a cell incubation period is fixed to plaques with any organic solvent, and an immunofluorescence is carried out. Positive cells are observed with fluorescent foci that can be counted. In this study, we analyzed and compared three different Cell Lines as substrate (Vero-76, C6/36 and BHK-21) for Dengue Virus titration using a Fluorescent Focus Assay. We demonstrate another advantage of FFA which allow the use of C6/36 cells that are highly sensitive to detect dengue virus, and they cannot be used for the plaque assay as they do not form lytic plaques. One critical aspect of an FFA is the possibility of a secondary infection that could affect titration results. In our FFA, an overlay of CMC was used to prevent titer inaccuracy. The viscosity of the CMC prevents emerging viral particles from traveling beyond neighboring cells, thus preventing secondary foci. No statistically significant differences were found between the Vero-76 and BHK-21 cell lines. The C6/36 cell line proved to be more sensitive to infection by the flaviviruses used, achieving significantly greater titres than the other two cell lines.

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ARBOBIOS: A Brazilian cohort of dengue warning signs patients during 2019 epidemics

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It is known that 3.6 billion people worldwide live in areas that place them at risk of DENV infection, 400 million overall are exposed to DENV infection. Around 2 to 5% of infected individuals progress to Severe Dengue (SD). The mortality can be reduced to less than 1% if robust early predictor of progression to SD exists.To establish a warning signs cohort for identification and validation of prognostic biomarkers for the severe DENV infection.During the 2019 epidemic in Brazil, a prospective longitudinal cohort of warning signs (WS) DENV patients was constitute in the following cities: Araraquara, SP; Arcos, MG; Campo Grande, MS; Nova Serrana, MG; Palmas, TO and São José do Rio Preto, SP. Two following-up visits were programmed at days 7 and 14The presence of RNA DENV virus were done by inbrew qPCR after RNA extraction with EasyMag (bioMérieux) and serological responses were evaluate by IgM ELISA (PanBio) only for samples negative in qPCR. For this study, we used SMS data banking.A total of 1117 patients were enrolled and 28 refused to participate. 1089 were eligible and 1067 analyzed and from those 1016 were adults and follow-up were realized in 984 and 960 patients for days 7 and 14, respectively. The virus RNA was detected in 31% of the samples (370) and most of them were serotype 2, and out of 697 negative for virus RNA, 437 were reactive in the serological test(63%),it is assumed that 76%(807) of the participants presented DENV infection at the time of the study.A total of 1117 patients were enrolled and 28 refused to participate. 1089 were eligible and 1067 analyzed and from those 1016 were adults and follow-up were realized in 984 and 960 patients for days 7 and 14, respectively. The virus RNA was detected in 31% of the samples (370) and most of them were serotype 2, and out of 697 negative for virus RNA, 437 were reactive in the serological test (63%), it is assumed that 76% (807) of the participants presented DENV infection at the time of the study.

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24-month neurodevelopmental outcomes of infants exposed to Zika in Leon, Nicaragua

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Introduction: Neurodevelopmental sequelae in asymptomatic infants at birth whose mothers were infected with Zika virus in pregnancy is unknown. Objective: To characterize the neurodevelopmental outcomes of infants whose mothers were pregnant during the Zika epidemic using the Mullen Scales of Early Learning (MSEL). The primary outcome was early learning composite (ELC) score, which included fine motor, visual reception, expressive language, and receptive language scores. Material/methods: We enrolled 178 children born between February and October 2017 to

mothers pregnant during the ZIKV epidemic in León, Nicaragua. Blood samples collected during routine pregnancy and cord-blood were tested by a neutralization assay (eFRNT) to confirm Zika exposure during pregnancy. Mothers (and thus infants) were classified as ZIKV-infected in pregnancy, ZIKV infected with ambiguous timing, non ZIKV infected (naïve and pre-immune prior to pregnancy). The MSEL was administered in the homes of the children every three months by locally trained psychologists. Research nurses collected sociodemographic data, medical history, and infant anthropometric measurements at birth and when the MSEL was administered. Results: Of the 138 infants with exposure status, 33 (23.9%) were ZIKV-exposed during pregnancy, 18 (13.0%) had mothers with unknown ZIKV timing and 87 (63%) were non ZIKV-exposed during pregnancy. There were no differences in maternal age, education, mean birth-weight, or gestational age at birth between the groups. The ELC score mean and standard deviation (SD) at 12 months between the three groups were as follows: 91.6 (SD=13.5), 94.6 (SD=9.1), 96.0 (SD=11.5), respectively. At 24 months, the differences in the ELC score between the exposure statuses persisted and were as follows: 92.8 (SD=15.4), 90.7 (SD=16.8), 96.7 (SD=14.3). Having an incident infection corresponded to a 3.3 point decrease (95% CI: 0.5, 6.2; p = 0.026) in ELC compared to the no ZIKV/pre-immune group in a longitudinal mixed model. Discussion/conclusion: In our prospective cohort of infants, asymptomatic infants born to mothers with an incident ZIKV infection in pregnancy had lower ELC scores at 24 months. Children born to mothers with known ZIKV infection should be evaluated for neurocognitive delay in the first two years of life to identify delays and guide interventions.

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Molecular detection and viral isolation of Chikungunya virus from alternative clinical specimens, INOVACHIK study, Rio de Janeiro, Brazil

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Introduction: Chikungunya virus (CHIKV) is an arbovirus that causes an acute febrile illness characterized by severe and debilitating polyarthralgia and may have atypical manifestations such as neurological complications. For laboratory diagnosis, serum or blood samples taken during the first week after symptom onset is generally used. However, as with other arboviruses, alternative clinical specimens have been tested for diagnostic and viral persistence studies, such as saliva, urine, breast milk, vaginal secretion and semen. Objective: To investigate the use of alternative clinical specimens for virological diagnosis and detect infectious viruses of CHIKV these samples. Materials and methods: Here we describe viral RNA detection by reverse transcription technique followed by real-time polymerase chain reaction (Real time RT-PCR) and viral isolation in VERO cell culture from serum, urine, saliva and vaginal secretion samples collected from a 53 years old menopausal woman with 3 days of compatible symptoms for CHIKV, enrolled in an ongoing detection and persistence of CHIKV study in blody fluids (INOVACHIK study). Results: Real-time RT-PCR mean Ct (cycle threshold) values for serum, urine, saliva and vaginal secretion samples were 22, 34, 33 and 36, respectively. These samples were subjected to viral isolation and after two passages (after 7-10 days of inoculation), the Ct values obtained in real time RT-PCR were: serum (8), urine (27), saliva (33) and vaginal secretion (21). Discussion/conclusion: The large decrease in Cts values demonstrates the presence of viral replication in cell culture from serum, urine and vaginal secretion, where the viral titer was increased after cell culture passages. Although the Ct value in the isolate from saliva did not decrease, we believe there was also viral replication, as we obtained the same Ct value after the original sample was diluted and filtered for inoculation and subsequently passed into new cell cultures. If there was no viral replication, the result of cell culture would probably be negative. These results demonstrates that infectious viral particles can be found in serum, urine and vaginal secretion, and that other body fluids besides serum can be used for virological diagnosis of CHIKV in the acute phase of the disease and that studies on additional transmission routes of CHIKV are necessary.

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Performance of a MAC-ELISA that Differentiates Dengue and Zika Virus Infections

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The use of serology to diagnose Zika virus (ZIKV) infections in dengue virus (DENV) endemic countries proved to be a challenge due to the high cross-reactivity of these viruses in traditional assays. Confirmatory testing of ZIKV IgM positive results by Plaque Reduction Neutralization Tests (PRNT) provides clarification of few cases since most individuals infected with ZIKV had prior exposure to DENV and developed antibodies that contribute to ZIKV neutralization. The goal of this study was to evaluate the performance of a ZIKV/DENV DUO MAC-ELISA for discriminating between DENV and ZIKV infections. Our performance evaluation included acute, early and late convalescent specimens from patients with PCR-confirmed DENV or ZIKV from the Sentinel Enhanced Dengue Surveillance System (SEDSS) in Ponce, Puerto Rico. Based on DENV IgG testing for 0-5 days post-onset of illness (DPO), paired specimens were categorized as primary DENV, secondary DENV and ZIKV with approximately 80% of the ZIKV cases being probable secondary flavivirus infections. We also tested West Nile Virus (WNV)and Yellow Fever Virus (YFV) specimens in the assay and PCR-confirmed DENV specimens collected after a ZIKV outbreak in American Samoa. Testing of specimens during the optimal testing window (DPO 6-120) showed that the ZIKV/DENV DUO MAC-ELISA specificity was 100% for DENV ($n \geq 250$) and 98.4% for ZIKV($n \geq 450$). All ZIKV and DENV PCR-confirmed cases showed IgM seroconversion. The ZIKV/DENV DUO MAC-ELISA sensitivity was 100% compared to RT-PCR and approximately 90% compared to the CDC ZIKV MAC-ELISA and 96% compared to the InBios DENV Detect™ IgM Capture ELISA. No WNV or YF specimens tested positive for ZIKV in the ZIKV/DENV DUO MAC-ELISA and no YF specimens tested positive for DENV. Only 1/10 WNV specimens tested positive to DENV. No false positives were detected in any negative specimens. Our new ZIKV/DENV DUO MAC-ELISA was also able to distinguish ZIKV and DENV regardless of previous DENV exposure. We conclude this novel serologic diagnostic assay can accurately discriminate ZIKV and DENV infections. This can potentially replace the labor intensive and expensive PRNT assay for recent infections and aid diagnosis in areas that lack PRNT capacity, but experience circulation of both DENV and ZIKV.

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Highly sensitive ELISAs for the immune detection of emerging arboviruses in LATAM: dengue serotypes 1-4, Zika and Chikungunya

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Arbovirus infections pose one of the largest global risks to human health. The four dengue serotypes, Zika and Chikungunya initially present with similar symptoms. Early diagnosis and appropriate clinical management is critical and can be

achieved by detecting these viruses in serum during the acute phase. We developed and optimized enzyme-linked immunosorbent assay (ELISA) for the parallel detection of the main three mosquito-borne epidemics in the Americas: dengue, Zika and Chikungunya. To distinguish DENV-1-4 non-structural protein 1 (NS1) we selected serotype-specific monoclonal antibodies from immunized mice using individual NS1 proteins. For Zika, lymph node in addition to standard spleen derived hybridomas were harvested. For Chikungunya, we analyzed 10 different antibody clones and obtained best performing antibody pairs. Results: A total of 1046 DENV-immunized and 106 Zika-immunized mouse derived antibodies were harvested and screened for antigen binding to rNS1(s) or Chikungunya Virus Like Particles (VLP) using ELISAs. Pairs were selected for optimal binding. Validation of clinical samples consisted of 343 polymerase chain reaction (PCR)-confirmed dengue samples obtained from patients from Brazil and Honduras. The overall Sensitivity of the test for pan-DENV was 81.90% (281/343), and the de-aggregated Sensitivities for DENV-1, 2, 3 and 4 serotypes were 79.2% (38/48), 87.23% (41/47), 100% (45/45), and 79.6% (98/123), respectively. Specificity reached 94.07-100%. for all serotypes. For validation of Zika infections, we utilized 25 PCR confirmed samples from Colombia and Dominican Republic during the past Zika epidemic 2015-2016. The Sensitivity and Specificity were 84.00% (21/24) and 100%, respectively. For validation of Chikungunya infections, we utilized 100 samples of acutely infected patients from Honduras, previously diagnosed by PCR. The Sensitivity calculations covering a range of 4.5 log of viremia resulted in 88.80% (30/34) for higher viremias and 51.10% (31/51) for very low viremia (>30CT in their protocol), with overall Specificity range of 95 to 83%. Discussion: Our studies demonstrate that a robust mouse monoclonal antibody screening and pairing strategy enables the development of a serotype NS1-based ELISA for flaviviruses dengue (1-4) and Zika; as well as for VLP-based ELISA for E1/E2 Chikungunya detections. The immune assays were validated using acute clinical samples from Latin America, demonstrating future applicability due their excellent performance when compared to gold standard PCR.

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Long-term disability associated with dengue virus serotype 2 infection in Iquitos, Peru

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Introduction: It is estimated that there are 390 million dengue virus (DENV) infections per year worldwide. DENV poses a risk to US military because it is endemic in many regions where the US military travel to and/or are stationed. The etiology of serious DENV illness is not completely understood, but it is suspected to be due to immune enhancement and/or variation in virus virulence, and there have been reports of long-term disability due to infection. From 1990 to 2010 DENV in Peru reported low number of severe cases that required respiratory assistance. DENV serotype 2 (DENV-2) of the Asian-American lineage was introduced to the city of Iquitos in 2010 and there was a marked increase in severe cases with respiratory and neurological complications. Objective: To present a case report of a DENV-2 infection associated with long-term disability. Materials and Methods: The case was enrolled in a clinic based surveillance study approved by the NAMRU-6 IRB and RT-PCR was used to detect DENV. The level of disability was assessed using the Modified Rankin scale. Results: An 8 year old boy with normal psychomotor development reporting 3 days of illness was hospitalized in the intensive care unit (ICU) due to fever, abdominal pain, bleeding, vomiting, shock, dyspnea and respiratory distress. The clinical diagnosis at admission was acute respiratory distress syndrome and DENV-2 was identified by RT-PCR in a blood sample. The patient experienced cardio respiratory arrest and received advanced cardiopulmonary resuscitation. He was in the ICU for 38 days, before being discharged with mild respiratory difficulty, aphasia and deficiency of motor function in arms and legs, classified as moderately severe disability according to the Modified Rankin Scale. He is currently 16 years old, and after several years of rehabilitation and caring by his family, his functionality has reached a level of slight disability. Discussion and Conclusions: Our data provides insight on possible long-term disabilities

associated with DENV and this information can help guide Force Health Protection to understand the risks associated in dengue-endemic areas. Not only can DENV compromise readiness of military missions but long term disabilities may be associated with DENV infections, highlighting the need for DENV prophylaxis targeting naïve individuals and development of therapeutics.

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Association of Anti-DENV IgG antibodies with higher risk of dengue severity

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A rapid and potent plasmablast antigen-specific response is induced in dengue infection, predominantly with IgG-secreting cells, reaching maximum values between the 6th-7th days of the onset of patient's symptoms, a period that coincides with the development of the disease severity. In this study we determined DENV-specific IgG, IgG1 and IgG3 concentration in serum samples (n=65) from individuals with dengue distinct clinical manifestations, by enzyme-linked immunosorbent assay (ELISA), with in-house procedures. Serum samples were selected from two virologically- and/or serologically- well-characterized cohorts of DENV cases from health care units in Goiânia city, Gioás, Brazil, followed-up during two different DENV epidemics: October/2005-march/2006 and June/2012-july/2013, with the prevalence of DENV-3 (2005/2006) and DENV-1/ DENV-4 (2012/2013). DENV-specific IgG demonstrated a higher quantity in patients with dengue more severe clinical manifestation (p=0,001) and, by applying de ROC curve methodology, we observed an area under the curve of 0,6729 (p=0,049) when compared data from patients with dengue fever (DC) versus dengue hemorrhagic fever (DHF). We also estimated a risk of developing DHF in 5,5 (IC 1,2-23,9) in patients with IgG optical density values higher than 1,62. Analysis with DENV-specific IgG1 and IgG3 demonstrated no significant results. Our results suggest the participation of IgG antibodies in dengue pathogenesis and point for a future application of immunoenzymatic assays with DENV-specific IgG antibodies to monitor the disease severity in individuals with dengue from endemic areas.

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Performance of commercially available and in-house ELISA assays to detect Zika virus infections in a population highly exposed to flavivirus

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The current Zika virus (ZIKV) pandemic has been associated with unparalleled reports of neurological sequelae, urging the development of vaccines and diagnostic tools to mitigate infection spread. Detection of ZIKV infection is mostly based on the identification of IgM and IgG antibodies. However, cross-reactivity between Flaviviruses in serological assays is a main challenge for accurate discrimination of infections. Cross-reactivity is even more prominent in endemic areas, where repeatedly exposure to multiple Flavivirus outbreaks directly impacts the diagnostic value of currently used immunoassays. Therefore, ZIKV serological assays need to be validated in this context to ensure accurate diagnosis. Serum samples from a well-characterized panel were tested for the detection of anti-ZIKV IgG, IgM and IgM/A antibodies using commercial Euroimmun ELISAs, as well as IgG/M/A antibodies using commercial Native Antigen ELISA. Their diagnostic value is compared to an in-house assay based on the detection of IgG3 antibodies. The panel comprised of 219 serum samples from Brazil that were characterized by PRNT for ZIKV, Dengue virus 1-4 (DENV) and Yellow Fever virus (YFV) and subsequently divided into the following groups: ZIKV+, DENV+, YFV+, ZIKV+/DENV+/YFV-, ZIKV-/DENV-/YFV-. Our results revealed that detection of anti-ZIKV IgM antibodies using Euroimmun test showed remarkably low sensitivity (45.5%) while rendering high specificity (99.4%). The detection of IgM/A using Euroimmun test exhibited higher sensitivity (86.4%) with comparable specificity (92.6%). The detection of IgG/M/A antibodies by Native Antigen test presented the poorest diagnostic performance, with low sensitivity (48.0%) and specificity (60.0%). The identifi-

cation of recent ZIKV infections (up to 4 months) using our in-house ELISA, based on the detection of IgG3 antibodies, showed highest sensitivity (93.1%), compared to the commercial IgM assays, despite of the slightly lower specificity (81.8%). Detection of total IgG antibodies using the Euroimmun assay exhibited sensitivity of 85.7% and specificity of only 66.3%. Our findings highlight the need of thorough assessment of the available ZIKV immunoassays to ensure diagnostic accuracy and point out that our in-house detection of IgG3 antibodies followed by the detection of IgM/A antibodies by the Euroimmune test are most effective in discriminating flaviviral infections in actual populations from endemic areas.

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Case Report: Prenatal Dengue during the 2019 outbreak in Tegucigalpa, Honduras.

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Introduction: Dengue is an Arboviral disease commonly found in tropical areas, Honduras is one of the affected countries particularly hit hard by the outbreak during 2019. Dengue infection occurring in pregnancy could consequently be vertical transmitted, this event has rarely reported in the literature, and could occur from asymptomatic forms up to severe cases of illness, when incidence rates are high the perinatal and prenatal transmission should be considered in neonates. Objective: To report a case of Prenatal Dengue during an outbreak, that served as differentiating diagnosis of a neonate sepsis. Case Report: A newborn to a 15 years old mother, as a result of her first pregnancy. Three days prior to giving birth, the woman showed fever, headache, hematocrit of 51.3%, and platelet of up to 6,000/mm^3; she was diagnosed with a case of Dengue with alarming sign. On her fourth day of being ill, she was induced into labor. The newborn had a gestation age of 39 weeks, male, size and weight normal. The neonate at four days old had fever, tendency for bradycardia and upon further evaluation he has heart rate decrease, cold skin, cyanosis, capillary fillings of 28 sec, weak pulse, ascites, bilateral pleural effusion and polyserositis. In whole blood count the values hematocrit of 59.1% and platelet of up to 9,000/mm^3. As a result, he is checked into the Neonatology ward with diagnosis of severe Dengue with alarming sign. Blood samples were collected at sixth day of illness for mother and second day for neonate. Results: The mother and newborn samples analyzed by qRT-PCR (Johnson et al). Both tested positive for DENV2. The diagnosis of Prenatal Dengue in a newborn through vertical transmission from the mother during pregnancy was confirmed. Discussion: The transmission of Prenatal Dengue is rare, even in endemic regions; reports are relatively nonexistent, but possibility transmission to newborns through this route should be kept in consideration. The first signs and symptoms of Neonatal Dengue occurred within the fourth and sixth day life. There should be a focus on the diagnosis and management of pregnant women and in newborns during hyper endemic times.

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Re-emergence of dengue virus in San Juan Puerto Rico captured through enhanced surveillance for acute febrile illness

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Introduction: Dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses have historically caused large epidemics in the Americas, including Puerto Rico (PR). The last DENV outbreak in Puerto Rico occurred in 2012-13. The number of reported DENV cases remained low since, with no confirmed locally-acquired cases in 2018. Due to underreporting of patients with suspected arboviral disease to passive surveillance systems, in 2012 CDC and partners initiated the Sentinel Enhanced Dengue Surveillance System (SEDSS) at several facilities in southern PR. In November 2018, an additional SEDSS site was established in San Juan, PR to systematically identify and test patients with acute febrile illness (AFI). Methods: We enrolled patients into SEDSS with onset of fever within 7 days prior to presentation at the pediatric and adult emergency rooms at Hospital Auxilio Mutuo, a tertiary care referral hospital in San Juan. Serum speci-

mens were tested for evidence of DENV, ZIKV and CHIKV infection by RT-PCR and IgM ELISA. Naso-oropharyngeal swabs were tested for respiratory viruses including influenza A and B, human metapneumovirus, adenovirus and other respiratory viruses. Patients' medical records were abstracted to assess the clinical spectrum of disease, including frequency of hospitalization and manifestations of severe disease. Updated surveillance findings will be presented. Results: During November 2018–November 2019, a total of 1,542 patients were enrolled, of which 1,080 (70%) were pediatric patients. A pathogen was identified for 370 (24%) of participants. The most frequently identified pathogen was influenza A (200; 14%). Six (0.2%) patients had evidence of DENV-1 infection, all with illness onset during September–November 2019. All dengue patients resided in either of 2 neighboring municipalities in the San Juan metropolitan area, and none reported history of recent travel. All dengue patients were pediatric (age range: 3–15 years), and 1 (17%) was hospitalized due to presentation of dengue warning signs (abdominal pain) and co-infection with influenza A. Discussion and Conclusions: Enhanced surveillance for AFI in San Juan enabled confirmed detection of local DENV transmission in Puerto Rico during a period of low dengue transmission in 2019. Detection of continued DENV transmission may portend a pending dengue epidemic in the island.

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Perinatal Arboviral Transmission Study: a model for perinatal acute febrile illness (AFI) surveillance

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Introduction: Dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) are arboviruses of global importance capable of perinatal transmission during pregnancy. ZIKV infection during pregnancy can result in birth defects. Perinatal transmission of CHIKV has been associated to severe neonatal complications, including encephalitis, and perinatal transmission of DENV to a sepsis-like syndrome in newborns. Objectives: Establish a prospective cohort of women/infants with acute febrile illness (AFI) in the perinatal period to describe the etiology of AFI, the rate of perinatal transmission and the clinical outcomes related to each etiologic agent. Materials and Methods: As part of the Sentinel Enhanced Dengue Surveillance System in a tertiary care hospital in southern Puerto Rico, the study enrolls pregnant women with fever 7 days before, at time of, or 2 days after delivery and their newborns. Data on pregnancy, medical history and adverse outcomes are obtained from the birth record and during the first month of life by phone call follow-up. Participants' blood samples, cerebrospinal fluid from infants with CNS complications, and at time of birth, cord blood and placenta specimens are tested for DENV, ZIKV and CHIKV, and maternal NP/OP swab specimens are tested for respiratory viruses, including influenza A and B (FLU A, B), human metapneumovirus (HMPV), adenovirus (Adeno) and others. Results: Since April 2015, 28 mother-infant pairs have been recruited. Mean age for mothers was 26 years and 66% presented with fever of less than 3 days. We determined the etiology of fever among 6 (21%) women. One woman was positive for ZIKV by PCR and had an adverse outcome of fetal demise at 24 weeks gestation. Influenza A was present in 4 mothers and Influenza B in 1. Ten infants required NICU admission for observation due to suspected sepsis and 2 infants were born prematurely. Infants' diagnostic testing was negative. Discussion and Conclusions: Identifying arboviral disease as a cause of fever during the peripartum period in this population could help improve targeted interventions on the pregnant woman and her infant, such as the need for neurologic monitoring or fluid management. This practice may aid in avoiding unnecessary procedures or lengthy medical stays.

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Novel approach for molecular characterization of the 5' and 3' ends of the dengue virus genome

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Introduction: Dengue is a mosquito-borne disease of major public health importance in most tropical and subtropical regions around the world were

the vector is present. Sequencing of the 5' and 3' ends of the untranslated regions of dengue virus is important for comparative analyses of virus genetic background and the clinical outcome in the human host. Objective: To establish a protocol for molecular characterization of the 5' and 3' ends of dengue virus type-1 to -4. Methodology: The 5' and 3' ends of twenty-nine dengue virus isolates from confirmed dengue acute infections were PCR amplified through a modified protocol of the Rapid Amplification cDNA Ends approach. For the 5' end cDNA synthesis, specific anti-sense primers for each serotype were designed and used, followed by polyadenylation of the cDNA using a terminal transferase and subsequent PCR amplification using oligo(dT) and an internal specific reverse primer. At the 3' end of the positive-sense viral RNA, a poly-adenine tract was directly synthetized by using an *Escherichia coli* poly(A) polymerase, allowing subsequent hybridization of the oligo(dT) for cDNA synthesis. Results: The incorporation of the poly(A) tail at the 5' and 3' ends of the dengue virus cDNA and RNA, respectively, allowed the hybridization of primers and therefore the successful PCR amplification and direct sequencing of the ends of dengue virus 1 to 4. Discussion/Conclusion: The designed primers and probes, as well as the terminal transferase- and poly(a) polymerase-based approach allowed the successful characterization of dengue virus genome ends, an important complement to the genome sequencing through direct and next-generation methods. The completion of dengue virus genomes is crucial for comparative genomics in unraveling the virus genome contribution to severe dengue pathogenesis.

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Tracking the clinical and cultural characteristics of Chikungunya and Dengue in Nariño (Colombia)

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Dengue is an important arboviral disease with more than 390 million people infected and around 20.000 annual deaths. Chikungunya caused an epidemic in the Americas with more than 2.500 million cases until 2017. In Colombia the report of dengue cases during the period 2014-2017 was around 100.000 and for chikungunya there was an epidemic peak in 359.281 cases. In Nariño most of the cases occur in the Pacific coast, where cultural aspects determine their dynamics. Objective: To present a clinical characterization of cases of Dengue and Chikungunya (D/C) reported in Nariño (Colombia) during the period 2014-2017 and to analyze cultural aspects that have influence on their diagnostic, prognostic and treatment. Methods: An observational, descriptive, cross-sectional study was carried out, with a retrospective review of clinical records; besides, health personnel and traditional healers were interviewed in Tumaco (Nariño) to identify their experience with patients diagnosed with D/C and their limitations for the diagnosis and treatment. Results: There were 2514 hospitalizations for dengue and 460 for chikungunya. Twenty-two cases of severe dengue were identified, with one death by 2017. A review of the records of 1735 patients with complete information revealed that the most frequent clinical manifestations of dengue were: fever (100%), headache (84.6%) and myalgias (83.7%) as the most frequent symptoms, followed by arthralgia, rash and abdominal pain. Dengue was more prevalent in men (56.8%) and Chikungunya in women (52.0%). For the age, both diseases were more frequent in the population over 40 years old with 24.5% and 27.2%, respectively. The greatest burden of disease in the Pacific subregions, Cordillera, Telembí, Sanquianga and Abades lies in the diversity of epidemiological scenarios that are presented for the transmission of vector-borne diseases, which constitute a perfect enclave for the occurrence of Infectious diseases. Interviews with two traditional healers and four health professionals revealed the need for training on transmission, signs, symptoms and complications; laboratories in the Pacific coast of Nariño need to be improved. Conclusion: More studies are needed to identify clinical characteristics of Chikungunya and Dengue according to the epidemiologic and cultural context, this may lead to implement better intervention programs.

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No evidence of Chikungunya transmission during the massive 2017 El Niño Dengue outbreak in Piura?

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The Peruvian north-coast reports annually one of the highest incidences of dengue virus (DENV) cases in the territory, principally in Piura. The higher temperatures, rain seasons, and poor sanitary conditions are determinants for vector development in those regions. From 2015-2018, Piura reported a considerable increase in DENV and Chikungunya (CHIKV) cases. In 2017, Piura reported the highest frequency of CHIKV cases. Since the outbreak of Zika virus in Latin America and their serious effects on children's development, the main attention has been given to pregnant women. We evaluated the seroprevalence of CHIKV and DENV IgG in 498 serum samples from pregnant women. The participants were enrolled from 2015 to 2018 at the Santa Rosa Hospital located in Piura-Perú. We used a commercial ELISA IgG Kit for DENV and CHIKV (Euroimmun, Germany) and followed the manufacturer instructions. We found CHIKV IgG antibodies in 1 of 191 (0.5%) from samples collected in 2015, 1 of 122 (0.8%) in 2016, 0 of 63 (0.0%) in 2017 and 3 of 120 (2.5%) in 2018. DENV IgG were detected in 38 of 191 in 2015 (18.9%), 25 of 122 (20.3%) in 2016, 12 of 63 (19.1%) in 2017 and 53 of 121 (43.8%) in 2018. One sample presented both DENV and CHIKV IgG antibodies. The highest frequencies reported for CHIKV IgG and DENV antibodies were in 2018 (2.5% and 43.8%, respectively), explained by the Niño phenomenon from 2017. This phenomenon occasioned a DENV and CHIKV outbreak caused probably by an increase in arboviral vector circulation and a lack of immunity. The reported frequencies of CHIKV and DENV IgG confirmed the co-circulation and human exposure to arboviruses in Piura. The presence of the vector and huge population at risk are the principal contributors to the increase of these viral illnesses that represents a major health concern.

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Factores asociados con el compromiso osteomuscular persistente por virus Chikungunya en población colombiana. 2015- 2016

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Introducción: la infección por el virus Chikungunya es una enfermedad emergente en las Américas y en Colombia. Los síntomas más característicos son fiebre, dolor muscular intenso y artralgias de aparición aguda. El compromiso osteomuscular puede durar un largo tiempo y comprometer de manera importante la salud y calidad de vida de los pacientes. Objetivo: determinar la incidencia y factores asociados al compromiso osteomuscular persistente, posterior a la infección por virus Chikungunya en una población de los departamentos de Antioquia, Tolima y Meta durante los años 2015 y 2016. Metodología: estudio observacional, de seguimiento a una cohorte retrospectiva, correspondiente a 111 pacientes que tuvieron infección por virus Chikungunya, confirmada serológicamente y procedentes de los departamentos ya descritos. La variable de interés fue la persistencia de compromiso osteomuscular. En el análisis bivariado, se estimaron razones de riesgo crudas con intervalos de confianza y en el multivariado las razones de riesgo ajustadas a través de regresión logística binaria. Resultados: la incidencia del compromiso osteomuscular persistente posterior a la infección por virus Chikungunya fue del 75,5%. Las personas que experimentaron grado de dolor articular de tipo severo en fase aguda de la infección (RR = 7,6; IC de 95%, 1,3–44,3) y quienes presentaron un compromiso de tipo inflamatorio (artritis) (RRa = 11,0; IC de 95%, 2,4–49,9) tuvieron un riesgo significativamente mayor de padecer compromiso osteomuscular persistente tres meses después de la infección aguda por virus Chikungunya. Conclusión y discusión: se encontró una alta frecuencia de compromiso osteomuscular persistente. Es de los primeros estudios en Colombia que incluye confirmación de la infección a través de anticuerpos de tipo IgG específicos y que abarca tres poblaciones de estudio. Los presentes hallazgos pueden ser aplicables en la atención médica con el fin de clasificar el riesgo de compromiso

crónico posterior a la infección por Chikungunya e identificar a los pacientes que se beneficiarían clínicamente de la adecuada intervención

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Frequency of Dengue virus serotypes and co-infections during Dengue Outbreak in Honduras, 2018- 2019

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Introduction: Dengue virus (DENV) has four different, but closely related serotypes; all are present in the Americas since 1970 and during 2019, the circulation of all have been reported. This year Honduras declared epidemiological alert at national-level, reporting the highest incidence rates in the region. During this outbreak, children under the age of 15 are mostly affected. Objective: To report the frequency of different DENV serotypes circulating and the occurrence of coinfections of DENVs and others such as Influenza, CHIKV, ZIKV during the Dengue outbreak in Tegucigalpa, Honduras 2018-19. Materials and methods: 289 samples were collected from cases with suspected Arboviral infections during 2018 and from January to July 2019. Plasma samples were tested using qRT-PCR (DENV-Johnson et al, DENV, ZIKV, and CHIKV by Lanciotti et al). Pharyngeal swabs were taken in a subgroup of patients who also had respiratory symptoms and were tested by FilmArray® Respiratory panel. Results: The overall positivity by DENV was 41% (119/289); three serotypes were identified (DENV-1, DENV-2, and DENV-3). We found 2.5% DENV-1/DENV-3 (n=2) and DENV-1 /Flu A (n=1) coinfections. Furthermore 90% (n=107) were only DENV-2 positive and 7.5% (n= 9) Denv-3. There were no co-infections found with ZIKV and CHIKV and respiratory agents. Besides DENV-1/FLU A case who reported respiratory symptoms also had epistaxis, intense abdominal pain, gingivorrhagia, vomiting. The three reported cases with co-infections were classified as Dengue with warning signs. Discussion/Conclusion: As arboviruses continue to re-emerge, the occurrence of coinfection could increase as well, especially during large outbreaks, but we know a little about it. Patients with coinfection showed warning signs. Many questions continued open such as if coinfections worsen the clinical presentation and recovery in the affected patients, due to this, it is necessary to continue studying the presence of co-infections in DENV patients, to understand their clinical, virological and epidemiological impact.

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Dengue Cerebellitis in an Adult Male - A Case Report

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Introduction: Purely cerebellar syndromes complicating dengue fever in an adult patient with risk factors for stroke are rare. Our literature review identified only 5 other similar cases, all from tropical countries. Case: This is a case of a 36-year old hypertensive and dyslipidemic Filipino male treated as a case of dengue fever. On the fourth day of his illness, he suddenly presented with cerebellar symptoms. Neuroimaging done was negative. (see Figure 1) His dyslipidemia and hypertension were managed accordingly with medications. His dengue was managed with IV fluid hydration and serial full blood count monitoring. All of his neurologic symptoms resolved spontaneously within 2 weeks. Discussion: Dengue fever can manifest with neurological features ranging from 0.5% to 21% of in-hospital cases. In multiple case reports,patients with dengue cerebellar syndrome all recover spontaneously without permanent neurological sequelae. Five out of the six known cases, including that of our patient had unremarkable neuroimaging findings. (see table 1) The exact pathology of neurological syndromes in dengue fever are yet to be established. However, due to the positive serum Immunoglobulin M (IgM) of the subjects, we can conclude that this may be immune-mediated. Another possible pathology is the direct invasion of the virus. However, the predilection for the cerebellum is not yet known. Physicians should be made aware of such complications as dengue is epidemic in our setting. Since dengue causes a hyper-coagulable state with a higher risk for stroke, stroke should still be ruled out by neuroimaging.

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Use of IgM antibodies against Dengue and Zika in GBS cases during Zika outbreak in Honduras

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Introduction: When Zika emerged in Latin America, an explosion of Guillain-Barré cases (GBS) was observed in the affected regions. As GBS manifestations are post-infectious, the laboratory diagnosis of Zika by molecular means is challenging, the introduction of Zika where other Arboviruses are endemic brought other issues for the laboratory diagnosis due to the cross reactivity with related Flaviviruses, such as Dengue, specifically using serological testing. Several efforts have been done to develop laboratory tests to detect Zika-IgM specific antibodies, although its utility in clinical cases like GBS, should be determined. Objective: We determine the frequency of seropositive GBS cases for Zika and Dengue IgM Antibodies with CDC-MAC ELISAs (EIA) and compare their ODs in both tests. Material and Methods: Plasma samples from 115 GBS cases were tested for IgM antibodies for Zika & Dengue with CDC-MAC-ELISAs. All samples were tested by Zika qRT-PCR methods (Lanciotti et al-2008/Waggoner et al-2016). Results: Out of 115 GBS cases; 41% (n=47) were Zika IgM positive and 59% negative (n=68). From the 47 Zika IgM-Ab positive cases, a 25.6% (n=12) were positive only for Zika IgM-Ab, but not for Dengue-Ab. A 74.4% (n=35/47) were positive for both Zika and DN-Abs. In the samples seropositives in both, the EIA ODs from Zika IgM-Ab were three times higher than EIA ODs from Dengue positives (Median-ODs: Zika 1.767; DN: 0.496). Out of 68 samples negative for Zika Abs, 14.7% (n=10) were only positive for Dengue IgM-Ab and 85.3% (n=58) negative for both Zika and Dengue antibodies. From all these GBS cases 7.8% were positive for Zika by qRT-PCR. Discussion: It was possible to detect that a 25.6% GBS were Zika seropositive and 7.8% by molecular testing, suggesting that serological testing could contribute an added value to associate Zika infection in GBS. In those GBS cases from the Zika outbreak with positive serological results for Zika and Dengue, it was observed ODs three times higher for Zika than Dengue which could also be considered at the time to perform serological screening in GBS cases. One have kept in mind the OD values in geographical areas where other Flaviviruses are endemic such as Dengue.

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Detección del virus de la Fiebre Amarilla, a propósito de un caso. Venezuela 2019

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Introducción: El virus de la fiebre amarilla (FA) pertenece al género Flavivirus y se encuentra relacionado a otros virus del mismo género como los del Dengue, Zika, encefalitis japonesa y encefalitis del Nilo Occidental. Puede ser transmitido al humano principalmente por vectores selváticos. La sospecha diagnóstica de fiebre amarilla se basa en los antecedentes clínicos epidemiológicos del paciente con énfasis en las actividades y la historia epidemiológica del lugar donde posiblemente ocurrió la infección. El diagnóstico específico y la confirmación de casos requieren el análisis de laboratorio. Objetivo: Detectar el virus de Fiebre Amarilla, como parte de la vigilancia de síndromes ictero hemorrágicos. Materiales y métodos: Se recibió una muestra de suero de un paciente masculino de 46 años perteneciente a la etnia Pemón, Estado Bolívar ubicado al sur de Venezuela, frontera con Brasil. La muestra fue tomada 13 días posterior al inicio de los síntomas. El algoritmo diagnóstico llevado a cabo fue realizado para la detección de anticuerpos IgM contra el virus Dengue y Fiebre Amarilla, mediante la prueba de MAC-ELISA, respectivamente. Se realizó la técnica de inhibición de la hemaglutinación (HI) para determinar el título de anticuerpos. Para la detección del ARN del virus FA, se llevaron a cabo métodos moleculares como la PCR en tiempo real, mediante el uso de cebadores y sondas específicos. Resultados: Se encontró una reactividad cruzada para la detección serológica de anticuerpos tipo IgM contra el virus Dengue y FA. Los resultados obtenidos en la técnica de HI mostraron un título $\geq$ de 10250 para Fiebre Amarilla y 320 para

virus Dengue. Se obtuvo un resultado positivo para la detección del ARN del virus FA. Discusión: Debido a la reactividad cruzada hallada en ambas pruebas serológicas, y dados los antecedentes clínico epidemiológicos del paciente, es importante considerar el uso de pruebas moleculares que permitan la confirmación del caso y establecer un algoritmo de trabajo en casos sospechosos para la rápida identificación de casos cuando exista reactividad cruzada. Conclusión: Es importante el fortalecimiento de la vigilancia epidemiológica, en especial en áreas fronterizas y el envío oportuno de muestras hasta el centro de referencia.

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Prospective Cohort Study of Primary Dengue Infection in University Students of Medellin (Colombia)

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Introduction: Dengue is a viral disease transmitted by mosquitoes of the Aedes genus. In Colombia, during the last ten years, there has been a significant increase in the number of cases, with epidemic waves every 3-4 years. Even so, there is a lack of comprehensive data that reflects the real burden of the disease, which would allow decisions to be made to evaluate the implementation of a new treatment for Dengue and the introduction of vaccines. Objective: Determine the incidence of primary Dengue virus (DENV) infection (symptomatic and asymptomatic) among students of Antioquia University in Medellin. Materials and methods: Through a prospective cohort study, a serological survey was conducted in students for two years through Panbio® indirect IgG, with semiannual sample collections, to determine the incidence of the disease, as well as, an active uptake of febrile patients. Results and Discussion: During 2018, 1800 volunteers were approached for the cohort, of which 38% (678) showed antibodies against the virus. The 1122 seronegative volunteers were followed from 'starting February 2018 until February 2020 (5 samples). The average age of the evaluated patients was estimated at 20(SD +/-3.66), 714/1800 (39,66%) were men and 1086/1800 (60,33%) were women. Preliminary results indicated a seroconversion rate of 0.98% (11/1122), and of these volunteers, none showed symptoms. this study will continue with a second cohort of another 1056 students spread over 3 universities, that will be followed for 2 years as well. Conclusion: Medellin is considered a hyperendemic city for Dengue. The results of this study provide epidemiological evidence of asymptomatic dengue cases, their immunological behavior, and knowledge of circulating serotypes. This facilitates the modeling of future studies for testing new interventions against dengue.

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Dengue and zika occurrence in children and teenagers in a high endemicity area

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For three decades dengue has been endemic in Brazil and in the last three years, circulated simultaneously with Zika in an extensive epidemic. Dengue (DENV) and Zika (ZIKV) are flavivirus with similar clinical manifestations, implying the need of a differential diagnosis. In this study we investigated Dengue and Zika infection in a cohort of children and adolescents with febrile cases in Goiânia, Goiás, Brazil Midwest, from 2015 to 2019. 947 subjects had serum and urine samples collected between the 1st- 5th day of disease. 59.3% subjects aged between two and nine years and 50.2% were female. Real time polymerase chain reaction followed by reverse transcription (RT-PCR) and serological techniques for NS1 antigen (NS1Ag) and antidengue and antizika IgM and IgG antibodies were performed for laboratory confirmation at BioTec Laboratory, Pharmacy Faculty- UFG. Results demonstrated that 135/947 (14.2%) samples were positive for dengue virus detected by one or more tests used. 75 (55, 5%) samples had Dengue IgG positive demonstrating secondary infection. Viral RNA was detected in 68 (50.4%) samples (Sensibility: 48,89%: 40,2-57,6; specificity: 100% 99,5-100, 95% CI), 120 (88,9%) were positive for NS1Ag (Sensibility:46,1%: 37,4-55,1; specificity: 99,2%

98,3-99,8, 95% CI), 72 (53.3%) for antidengue IgM (Sensibility: 46,15%: 37,4- 55,1; specificity: 99,27%: 98,3-99,8, 95% CI). 80 (59,2%) samples were Dengue IgM positive without zika diagnosis confirmation. 37/947 (3.9%) samples were positive for ZIKV, which the viral RNA was detected in 24 (64.9%) serum samples (Sensibility: 66,67%: 49 – 81,4; specificity: 100% 99,6-100, 95% CI). Coinfection in Zika and Dengue cases was evidenced in seven cases which has RT-PCR Zika results with NS1Ag and Dengue IgM positives. The antibody cross-reaction in Zika IgM and Dengue IgM was observed in four cases. Regions with DENV and ZIKV co-circulation the use of techniques with viral detection is enable differential diagnosis. Brazil Midwest is a region with high incidence resulting in significant epidemics, emphasizing the importance of understanding these diseases.

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Microneutralization as a tool for detection of neutralizing antibodies against DENV-2

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Introduction: The microneutralization test (MNT) makes possible to determine the presence of neutralizing antibodies.It processes a larger number of serum samples, saving materials and time compared to the plate reduction neutralization test (PRNT). However, the usage of the MNT has complications due to a weak cytopathic effect of dengue serotype 2 (DENV-2) wild-type virus and some prototype strains. Objective of the study: the present study aims to optimize the MNT for the detection of neutralizing antibodies against DENV-2 in VERO-76 cell line. Materials and methods:A wild type DENV-2 isolated in Peru was confirmed by PCR and propagated by 5 passages in the VERO-76 cell line. For the MNT, a monolayer method and acell suspension method were evaluated with 3 cell concentrations (0.6×105; $0.9 \times 105$and 1.2×105cell / mL).3 percentages of fetal bovine serum (2, 3 and 4%)and the plaques were dyed at days8, 9 and 10. Once the technique was standardized, 5 sera negative to DENV-2 and yellow fever virus (YFV), 5 sera negative to DENV-2 and sera positive to YFV and 5 sera positive to DENV-2 determined by the PRNT were subsequently evaluated by the MNT. Results: The optimal method for the MNT was the cell suspension one using 0.9×105cell / mL as cell concentration with 2% fetal bovine serum, which was dyed on day 10. The only minimal dilution used to differentiate a positive serum to DENV-2 and a negative serum to DENV-2 was 1:40. In addition, the MNT for DENV-2 does not cross-react with YFV since 1:40 dilution. Spearman's correlation index of 15 serum samples titers obtained by PRNT and MNT for DENV-2 was $p<0.05$ with an $R = 0.887$. Discussion/Conclusion:The MNT can be used to titer and to evaluate the presence of neutralizing antibodies to DENV-2 becoming an extremely useful tool for serological studies in our country.

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Caracterización clínica de Dengue, Chikungunya y Zika durante el primocontacto de 2016 en Veracruz, México

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Introducción: Con la llegada de los arbovirus Chikungunya y Zika, el diagnóstico clínico entre estos y Dengue se torna aún más complicado ya que comparten similitudes en sus cuadros clínicos, destacando su importancia clínica y epidemiológica como causales de complicaciones secundarias, como cuadros de síndrome de Guillain-Barré entre otros. Métodos: Se estudian los casos de arbovirosis transmitidas por vector presentados en la delegación Veracruz Norte durante el 2016, clasificándolos acorde a las definiciones operacionales de caso sospechoso de Dengue, Zika y Chikungunya. Resultados: Se registraron un total de 10,327 casos de arbovirosis, de las cuales 5,388 casos cumplieron la definición operacional de Dengue no grave (52.2%), 3,529 casos para Zika (34.1%) y 1,410 casos para Chikungunya (13.6%). Respecto de la sintomatología, los principales síntomas y signos presentados en los casos de Dengue fueron : fiebre, cefalea, artralgias, exantema y nauseas/vómito; para los casos de Zika : exantema, adenomegalias, cefalea, artralgias y conjuntivitis; para los casos de Chikungunya : exantema, fiebre, artralgias, cefalea y nauseas/vómito. Por su alta incidencia los principales

síntomas se agruparon en triadas que tienen particularidades, la triada de Dengue esta integrada por fiebre, cefalea y artralgias; la triada de Zika por exantema, adenomegalias y artralgias; y la de Chikungunya por exantema, fiebre y artritis. Conclusión: Fue posible caracterizar cada caso acorde a las definiciones de caso sospechoso y se integraron triadas con particularidades que pueden emplearse como auxiliar de diagnóstico clínico.

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Guillain-Barré Syndrome in Arbovirus Outbreak in Veracruz, Mexico. Follow-up to 3 years of the pandemic

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Background: From the arrival of Zika to America in 2015, and the increase in cases of Guillain-Barré syndrome in South America apparently associated with acute viral infection, Mexico had its first contact in 2016, with an increase in the incidence of cases of the syndrome, initiating a protocol study to look for the causal association of the Zika virus. Methods: We conducted a descriptive, prospective and longitudinal study in Veracruz, Mexico, where follow-up of cases of Guillain Barre Syndrome (GBS) ocurred during 2016 to 2018. The central point of the study is to look for the etiological association of GBS with the presence of acute zika infection. Secondarily, other know neutropic agents, both viral and bacterial were searched. The diagnosis techniques used were PCR-RT (blood and urine) and IgM/IgG for Zika; serum PCR-RT and IgM/IgG for Dengue and Chikungunya; IgM/IgG for TORCH; PCR-RT in CSF for Herpes and Enterovirus; serological panel of Hepatitis B and C; PCR-RT in rectal swab for Campylobacter. Results: A cohort of 39 patients has been formed over 3 years of study. 38 patients met the operational definition of a suspected case of Zika, of wich only 2 cases were identified by PCR-RT in urine; During the search protocol for infectious agents, others were identified such as: Dengue, Chikungunya, Enterovirus, Herpes and Hepatitis B, however the identification of Campylobacter was even more remarkable, also highlighting that only four patients had diarrhea . Regarding the treatment, 37 patients received IVIG, 1 patient received plasmapheresis and 1 patient received both. The prognosis was good in 34 patients (basal Hugues from 4-5 to 2), 5 had poor prognosis and died. Conclusion: The incidence of Zika as a cause of GBS is relatively low (5%), so the etiological association could not be demonstrated; other neurotropic viral agents were identified, however the presence of Campylobacter cases was more notable.

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Manifestaciones oftálmicas de las infecciones arbovirales en adultos

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Introducción y Objetivos: Las infecciones por arbovirus emergentes tienen síntomas clásicos, como fiebre, artralgias o rash; dado que algunas tienen síntomas/signos oftálmicos, el objetivo central es evaluar si estos ayudan a esclarecer el diagnóstico clínico. Material y Métodos: Estudio descriptivo y retrospectivo, se analizan los casos de adultos que acudieron a evaluación en 2016, cumpliendo la definición de caso de dengue, zika y chikungunya; se analizan las sintomatologías general y oftálmica. Resultados: Se registró un total de 10.327 casos de arbovirosis, de los cuales 5.388 fueron dengue (52,2%), 3.529 zika (34,1%) y 1.410 chikungunya (13,6%). Los principales síntomas y signos de dengue fueron: fiebre, cefalea/dolor retroorbitario, artralgias, exantema y náuseas/vómito; para los casos de zika: exantema, adenomegalias, cefalea, artralgias y conjuntivitis; para los casos de chikungunya: exantema, fiebre, artritis, cefalea y náuseas/vómito. El grupo con más signos/síntomas oftálmicos es el de zika, predominando conjuntivitis no purulenta y dolor retroorbitario, epífora, epiescleritis, uveítis anterior, hasta síndromes neurológicos, como parálisis aisladas de pares craneales (iii y iv) o síndrome de Miller-Fisher. Conclusiones: Los signos/síntomas oftálmicos de la infección por zika pueden ayudar al diagnóstico clínico entre estas arbovirosis.

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Socio-emotional development after prenatal zika exposure from a cohort study in puerto rico

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Introduction: A causal link has been established between in utero Zika Virus (ZIKV) infection and birth defects, the most severe characterized as the Congenital Zika Syndrome (CZS) with microcephaly, brain defects and malformations. A broader spectrum of developmental and behavioral abnormalities has been described in children with congenital Zika, including socio-emotional alterations similar to those in the Autism Spectrum Disorder (ASD). Persistent socio-emotional and behavioral problems impede positive social interactions and may lead to more severe behavioral disturbances. Early identification can reduce morbidity and improve the wellbeing of children and families. Objective: The Pediatric Outcomes of Prenatal Zika Exposure study aims to characterize the spectrum of neurologic, sensory, developmental and behavioral outcomes of exposed children from birth to the age of school readiness at 60 months. Methods: Infants born to mothers with confirmed (positive Real Time-Polymerase Chain Reaction (RT-PCR)) or probable (positive Immunoglobulin MEnzyme-linked Immunosorbent Assay) ZIKV infection were enrolled. Microcephaly was defined as head circumference z score >-2 at birth and percentile <3rd at 18 months. Follow-up at 18 months included the Ages and Stages Socio-Emotional Questionnaires-2(ASQ-SE-2)that provide scores on social, emotional and behavioral competence to counsel parents and for monitoring or referral for more complex evaluations. Results: Descriptive analysis revealed that of 49 participants, 22 (45%) were born to RT-PCR positive mothers. Thirty (61%) were female, two (4%) had head circumference consistent with microcephaly at birth that normalized before 18 months, and one (2.0%) has CZS. ASQ-SE-2 scores identified 6 (13%) infants needing monitoring and 9 (20.0%) requiring a referral. Higher risk scores were reported in the behavioral areas of self-regulation, social communication, interaction and adaptive functioning.The mothers identified "difficult to handle" temperament in 19 (41%) infants. Discussion: Socio-emotional high risk scores were indentified in 31% of infants after prenatal Zika exposure. This information is useful to counsel and support families, can be used to facilitate early intervention and to make timely referrals for evaluation when ASD is suspected. A comparison group of neurologically-typical unexposed children is required to further assess the risks for autism and behavioral problems after prenatal ZIKV exposure.

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Impact of zika virus infection during pregnancy. The Cienfuegos experience

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Introduction: Once ZIKV transmission was demonstrated in the country, a strong surveillance was established to early identify the infection in pregnant women. Additionally, a periodic follow up was implemented.During the epidemic, Cienfuegos province reported a high number of infected pregnant women. Objective: to evaluate the causal relationship between ZIKV infection in pregnancy and congenital malformations. Material and methods:Pregnant women (prospective study) were recruited and follow up during pregnancy. Kinetic serum and urine including samples at delivery (both from the mother & child) were collected and tested for ZIKV infection. Additionally, children born from recruited pregnant women as well as children born from zika positive women on 2017 (retrospective study) were also recruited. Mother and child general and clinical data were introduced at CRF. Informed consent was obtained from all participants.Results and Discussion: A total of 320 pregnant women and 280children were recruited (from this, 100born from ZIKV positive mothers). More than 1400 samples from pregnant women and more than 120 from children have been collected and tested for ZIKV determination. Pregnant women recruitment was con-

cluded. Children recruitment is still ongoing. A preliminary analysis of children information suggest a positive impact of ZIKV infection in the children development. Here we show results of this analysis. This study is part of Zikalliance multicountry project.

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Usefulness of serum and urine samples in the molecular diagnosis of Zika, Cuba 2016-2017

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Introduction: From the establishment of the special surveillance for the detection of the introduction of Zika in Cuba in February 2016 until December 2017, 1107 cases of Zika were confirmed in the country by molecular diagnosis. Objective: In order to determine which of these two clinical samples was the most useful, a probabilistic study was carried out taking into account that both samples had been received simultaneously and that these had been positive. Material and Methods: To perform the molecular diagnosis, the IPK Arbovirus National Reference Laboratory (NRL) performed the purification of the RNA from the samples using the commercial QIAmp viral RNA kit automatically in a Qiacube extractor. The RT-PCR was performed using the Lanciotti et al. protocol recommended by the Arbovirus Laboratory Network of the American region. From these patients serum and urine samples were collected in the first eight of the date of onset of symptoms(DOS).Results and Discussion: Of all the samples studied, 62.9% of urine samples and 55.6% of serum samples were positive. Days 2 and 3of the DOS were those with the highest detection of the viral genome for both samples. During the first three days there was a greater probability of detecting the virus in the serum samples studied while for the remaining days the possibility was higher for urine samples. When positive symptomatic pregnant women were studied separately, the positivity in these samples was greater in serum, which could be related to prolonged periods of viremia in this risk group. Our results suggest that the collection of both samples is important for the diagnosis of Zika in the first 5 days of the DOS and in the case of symptomatic pregnant women should be collected up to 8 days of the DOS. These results have a direct impact on epidemiological-molecular surveillance and constitute an alert to public health authorities.

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Differential diagnostic of Chikungunya Fever by serological and molecular tests in Mexico during 2018-2019

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Abstract: Since the past century, arbovirus infectius disease has ben a public healt problen an around the world because of lager number of cases presents in several regions in the world. The reemergency in the Americas of Chikungunya virus in 2013 presented clinical patterns that generated complications in the diagnostic. With it today, it´s clinically difficult to differentiate it from other arbovirus infectius disease. Only in the two last years it has incrased the number of cases associated to some arbovirus, with a percentage around to 5% with suspicius clinic for CHIKV, which were not confirmed by diagnostic methodologies. Thus, this proyect seeks to make a diferenttial diagnosis of suspicius cases for Chikungunya fever with a negative result by RT-qPCR or serology. Two study groups were formed: the first, 75 serums with suspicius clinic to CHIK and the second as control group, 25 serums without suspicius clinic by CHIK. RT-qPCR were applied individually for Chikungunya to confirm the result from the coming laboratory and RT-qPCR for Mayaro as part of the differential diagnosis. In other side, MAC ELISA test was done for antibodies IgM for Chikungunya, Dengue, Zika and Mayaro. As results, in the first group we had 2% of serums with IgM for CHIK, 24% with IgM for DEN and/or ZIK and a possible co-infection between ZIK and CHIK. For the second group we had 4% of serums with IgM for CHIK, 40% with IgM´s for DEN and/or ZIK and one possible multi-infection with IgM's for DEN, CHIK and ZIK. As a conclusion, we found that the polyartralgias are being produced mostly by flavivirus and not so much by alphavirus, also we found that the antibodies IgM

presents on 1st, 2nd or 3th day, so the diagnosis by PCR can not detect the infection in every cases.

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IgG-ELISA based on NS1 recombinant protein multieptitopo for the diagnosis of Dengue cases

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Introduction: Dengue virus (DENV) is associated with explosive epidemics and has become a major public health problem worldwide. The diagnosis of dengue can be made using various approaches, however, specific and sensitive assays to diagnose initial phase of the fever are desirable. Previous studies have shown the role of NS1 antigen capture tests for diagnosis However, a significantly lower sensitivity was observed in cases of DENV-4. The use of antigen recombinant can eliminate the problems associated with the standardization of DENV antigen prepared in mouse brain or cell culture and avoids the laborious procedures and variable quality associated with these methods. The cost of most kits for the diagnosis is prohibitive for many dengue endemic countries and the internal production of recombinant polypeptides could provide a valuable and safe resource for the serological diagnosis. Objective: Here, we evaluated a recombinant NS1 multiepitope antigen from DENVs, produced in baculovirus / insect cell as an expression system, being potentially useful for the early diagnosis of dengue. Methods: It served as antigen in a recombinant antigen-based ELISA to detect IgG antibodies (REC IgG-ELISA) in human serum. Sera from 80 patients with clinically characterized infections by DENV (20 seropositive sera from each serotype), 10 sera from patients with antibodies against yellow fever virus, 10 sera reactive for ZIKV, 10 positive sera of CHIKV and 50 seros negatives were investigated for anti-DENV antibodies. Results: The recombinant multiepitope antigen showed 45.5% sensitivities to 90.9% considering the different sera analyzed, with DENV-4 being the one with the lowest sensitivity. Specificity of 96.5% was identified, based on the analysis of sera from dengue negative individuals. No cross-reactivity was observed with sera from patients infected with yellow fever. However, cross-reactivity was shown with a positive case for ZIKV. Conclusion: The immunoassay provides an alternative to traditional ELISAs that detect antibodies against DENV using mice.

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Evaluating the validity of dengue clinical-epidemiological criteria for diagnosis in patients residing in a Brazilian endemic area

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Introduction: In the absence of laboratory testing or in epidemic situations, clinical diagnosis of dengue can be undertaken based on specific clinical criteria, following WHO 2009 guidelines. In these situations, clinical epidemiological criteria are considered as confirmatory evidence of dengue and are sufficient for immediate diagnosis, disease notification, and treatment. Objectives: This study aimed to evaluate the validity of clinical diagnosis compared to laboratory diagnosis of dengue in a large retrospective sample of patients from a dengue-endemic area of Brazil. Methods: We evaluated 148,299 reported dengue cases in São José do Rio Preto, Brazil. Of these, 83,506 (56.3%) were diagnosed based exclusively on clinical-epidemiological criteria, and 64,793 (43.7%) also received laboratory confirmation. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of patients' demographic and clinical characteristics were analyzed, and whether thrombocytopenia was present, compared to a laboratory-based dengue diagnosis. We measured the association between these variables and dengue-positive laboratory tests. Logistic regression was undertaken to evaluate the probability of dengue-related signs and symptoms being present in clinical and laboratory diagnosis, compared to clinical diagnosis.

Results: We found variability in sensitivity to signs and symptoms (ranging from 0.8 to 81.1 (hematuria and fever, respectively), and in specificity, ranging from 21.5 to 99.6 (fever and metrorrhagia, respectively). Thrombocytopenia exhibited a higher PPV (92.0) and a lower NPV (42.2), and was the only variable showing some agreement with a specific laboratory diagnosis of dengue (φ = 0.38). The presence of exanthema led to a greater likelihood of concordant clinical and laboratory diagnoses (odds ratio (OR): 4.23; 95% confidence interval (CI), 2.09–8.57), as did thrombocytopenia (OR: 4.02; 95% CI, 1.32–12.27), when using multivariate logistic regression. Discussion/Conclusion: Many studies support the use of laboratory confirmation of dengue fever as a standard procedure for diagnosis, and caution is necessary when relying on clinical and epidemiological criteria for diagnosis. A clinical epidemiological diagnosis of dengue may increase the number of misreported dengue cases. We found substantial variation in sensitivity, specificity, PPVs, and NPVs, concerning clinical-epidemiologically determined dengue signs and symptoms.

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Challenges in zika virus sorological diagnosis in aregion with flavivirus co-circulation

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Dengue (DENV 1-4) and Zika(ZIKV) are flaviviruses, family Flaviviridae that co-circulate in Brazil since 2015. These viruses clinical diagnosis have been a challenge due to antibody cross- reaction in serologic tests. In this study, we analyzed the immune response in pregnant women with suspected ZIKV infection during a Zika/Dengue epidemic in Goiania city, Goias, Brazil Midwest, from 2016 to 2017. The study population was composed with women with age varying from 16 to 37 years, in differential gestational period,with more than five days since the disease onset of symptoms. Serum samples were collected for antibody antidengue and antizika(MAC-ELISA) detection, followed by plaque reduction neutralization test (PRNT), preliminarily with DENV-1, DENV-2 and ZIKV. In MAC-ELISA assay, 7 samples were ZIKV positive and 19 demonstrated cross-reactivity between DENV and ZIKV. When we apply PRNT50, 15 samples presented cross-reactivity, making impossible the determination of the infection etiologic agent. In PRNT90, 22 (n=26) samples confirmed ZIKV infection and 4 (n=26) confirmed flavivirus infection. The majority of positive samples for both, DENV and ZIKV, in MAC-ELISA (n=19), 84% were ZIKV positive in PRNT90 and, all MAC-ELISA ZIKV positive(100%) maintained the same result in PRNT90. Flaviviruses diagnosis in samples with cross-reaction was possible when applied the PRNT90 criteria. More rigorous titles in PRNT90 are useful in DENV endemic areas for diagnostic studies, decreasing flaviviruses serum background cross-reaction. This study proves the challenges for ZIKV and DENV diagnosis in patients with history of previous flavivirus infection, shows that PRNT is the serological technique that enables the differential diagnosis of DENV and ZIKV in a scenario of co-circulation of these flaviviruses. The use of stricter PRNT titers for diagnosis enables the reduction of cross-reactivity of these viruses.

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Zika virus infection in a stillborn with Cyclopia during Zika outbreak in Honduras

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Introduction: After Zika virus (ZIKV) introduction in Latin America, an unexpected increase in fetal microcephaly was reported in the affected countries, this became of worldwide concern and one of the main reasons of the international emergency declared by WHO. Experts alerted that birth defects detected at that time, were only the tip of the problems for the viral infection during pregnancy. Now it has been observed other types of fetal damage (i.e. exencephaly and schizencephaly), and also neurodevelopmental problems after birth in those children in with no evident signs of abnormalities were detected. Objective: To describe a case of cyclopia in a stillborn in whom it was detected ZIKV RNA from his blood sample and her pregnant mother exhibited, previously during pregnancy symptoms associated to Zika infection. Material and methods: A 39-year-

old pregnant woman from Tegucigalpa Honduras, was suspected to be infected with ZIKV presenting; headache, myalgia, arthralgia, rash and retro-orbital pain at first trimester of pregnancy, during Zika outbreak in 2016. At the first-trimester of gestation, the ultrasonography was normal. Between 26 to 36 weeks, ultrasounds revealed signs of cerebral malformation in the fetus with ventriculomegaly, signs of head circumference lower than the normal range, intrauterine growth restriction. The fetal death was confirmed at 36 weeks of gestation with congenital malformations as arthogryposis and cyclopia. Blood sample collected from the fetus at the moment meaning, time of delivery, the sample was tested positive for ZIKV RNA by qRT-PCR (Lanciotti et al 2008). Results and discussion: Although the complete spectrum of congenital abnormalities caused by Zika virus is not well known, the severe defects as cyclopia in this fetus and his death was attributed to the in utero transmission. To our knowledge this constitutes the first report of cyclopia associated to Zika infection, and the findings strengthen the evidence reported in literature of the capability of ZIKV to cause neurological damage in fetus, also highlights the value of molecular testing and radiological findings in the diagnosis of Congenital Zika Syndrome.

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Hospitalization among Pediatric and Adult Dengue Cases with Warnign Signs from an Enhanced Surveillance System in Ponce, Puerto Rico: 2012-2018.

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Puerto Rico (PR), a Commonwealth of the USA, has the largest US population in a dengue virus (DENV) endemic area. The Sentinel Enhanced Dengue Surveillance System was established in a tertiary hospital at the southern region of the island, to conduct surveillance for DENV and other acute febrile illnesses. Our goal was to identify and describe dengue warning signs (WS) used for clinical decision-making in pediatric and adult patients and to determine the frequency of hospitalization as proxy for severity among patients with one versus two WS at triage. We enrolled patients who arrived at the emergency department with fever or history of fever

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Detection of Yellow fever virus in cerebrospinal fluid of natural infected patients

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Wild-type Yellow fever (YF) has been related to viscerotropic and hemorrhagic disease, while the vaccine virus 17DD has also been associated with neurological manifestations. Recently, members of Flavivirus genus, DENV, ZIKV and others, have been associated with neurologic syndrome. This work aimed at the investigation of YFV in cerebrospinal fluid (CSF) from YF patients. Our laboratory received CSF from 14 fatal YF cases in 2018. Total RNA was extracted, and samples were tested by RT-qPCR targeting YFV-5'-UTR. Among 14 samples, four were YFV RNA positive. Patient 1 (P1) was 23 years old and presented mental confusion, headache, convulsion, AST=14,060U/L, ALT=5,681U/L, INR=3,99. P2 was 61 years old and presented asthenia, AST=410U/L, ALT=3,800U/L, INR=4,82. P3 was 44 years old and presented asthenia, headache, myalgia, AST=7,491U/L, ALT=5,029U/L, INR=2,39. P4 was 41 years old and presented headache, myalgia, AST=17,000U/L, ALT=13, 544U/L, INR=9,27. All patients presented fever and vomit. The CSF was collected with 10, 5, 7 and 10 days after onset YF symptoms, respectively. We also performed a quantitative RT-qPCR using Bio-Gene-Research-Febre-Amarela kit (Bioclin), and the genomic load in CSF ranged from 1.7 to $5.5x10^3$ RNA copies/mL. Although patients did not present neurological symptoms, YFV RNA viral loads in CSF and liver injury markers (AST,ALT and INR) were highly elevated. Nucleotide sequencing fol-

lowed by phylogenetic analysis (based on partial NS5 sequence) from P2 and P4 CSF indicated the virus clustering within wild-type South-American I genotype, demonstrating the presence of wild-type YFV RNA. IgM and IgG anti-YFV antibodies were investigated by immunochromatography tests (EcoDiagnóstica). P1 and P3 CSF were IgM anti-YFV positive. The patients were tested for the presence of YFV neutralizing antibodies in CSF, and virus neutralization above 50% was observed in P1 (up to 1:160 dilution) and P3 (up to 1:640 dilution). These findings could be related to YFV multiplication in the CNS. The anti-genome analysis and viral isolation will be carried out to have more evidence on viral replication. YFV did not have a known neurotropism. However, the possible acute liver failure and encephalopathy could be facilitated YFV entry into the CNS, since this picture may compromise the permeability of the blood-brain-barrier. Further studies to investigate neurotropism need to be done.

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Serological analysis of serum samples stored on filter paper during Dengue, Chikungunya and Zika outbreaks in Mexico.

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Introduction: Dried blood samples have been used for serological and molecular testing in viral infections, specially in low resource settings. In Mexico, we propose the use of dried serum samples (DSS) to store and ship samples to the reference diagnostic centers. Objective: To compare the anti-Dengue (DENV), anti-Zika (ZIKV) and anti-Chikungunya virus (CHIKV) IgG reactivity in paired serum and DSS obtained in Mexico during 2013, 2015 and 2016 outbreaks. Materials and Methods: A total of 159 paired serum and DSS samples were evaluated: 49 samples collected in DENV outbreak during 2013, 88 during CHIKV outbreak in 2015 (Veracruz), and 22 during ZIKV outbreak in 2016 (Oaxaca). The antibodies from the DSS were eluted in PBS and the DENV-specific IgG, ZIKV-specific IgG and CHIKV-specific IgM were detected in paired samples by using commercial ELISA kits (Euroimmun and DRG). We also analyzed the samples collected in 2013 and 2015 with the ZIKV-specific IgG ELISA kit. Results: The concordance of results from DSS and serum samples was high for all the assays (95-100%). The sensitivity for DENV-specific IgG, ZIKV-specific IgG and CHIKV-specific IgM was: 89%, 95% and 75% respectively. When samples collected in 2013 and 2015 were analyzed for ZIKV-specific IgG, we found 7.4 and 20.5% positive samples. Conclusions: The results showed that dried serum samples were useful to evaluate IgG reactivity against DENV, ZIKV and CHIKV. The stored samples in filter paper could be an excellent option for retrospective and prospective epidemiological studies. Besides, the antibodies stored in dried samples could be used to analyze cross reactivity among flavivirus in specific populations or geographical areas.

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Standardization of Fluorescent Focus Assay and Comparison with the "Gold Standard" Plaque Assay for Dengue, Yellow Fever and Zika Virus Titration using Vero-76 Cell Line as substrate.

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Flavivirus is a genus of arthropod-borne viruses belonging to the family Flaviviridae. The flavivirus genome consists of nonsegmented single-stranded positive-sense ribonucleic acid and have enveloped and spherical virus particles that are between 40 and 60 nm in diameter. Flavivirus that emerge globally and cause significant human diseases like encephalitis or hemorrhagic fever. At laboratory level, Plaque Assay is a "Gold Standard" method for Flavivirus titer quantification, which is based on the formation of plaques in a cell monolayer after viral infection. In this investigation a related technique was proposed, the Fluorescent Focus Assay which is performed very similar to Plaque Assay, and is based on the detection of viral proteins expressed by infected cells through fluorescent-labeled antibodies, which does not require agar overlap, and uses

only 24-72 hours of infection time to generate results. Three Flavivirus (Dengue-2; Yellow Fever and Zika) were selected for titration assays using the Vero-76 cell line as substrate. Thawing, propagation and maintenance of virus and Vero-76 cell line was successfully carried out obtaining optimal viral seeds for the quantification tests. Quantitative comparisons between FFA and PA were performed after standardization. FFA for flaviviruses was validated for accuracy, precision, specificity, and robustness of the assay.

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Recruitment Strategies in Cohort Studies: Experience with Dengue-Seronegative University Students in Medellin, Colombia

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Introduction: A challenge that prospective studies face, is the difficulty of recruiting the required number of study participants within a prespecified time frame. Other experiences concluded that success depends on the cooperation and commitment of volunteers. Therefore, in the Prospective Cohort Study of Primary Dengue Infection in University Students of Medellín (Colombia), a recruitment strategy based on an ethnographic study was implemented. It focused on identifying aspects that would promote the decision making of future participants to participate, resulting in increased enrollment rates. Objective: Within the context of prospective cohort study of dengue primary infection in university students in Medellin, develop and implement an innovative recruitment strategy, beyond the traditional (flyer, poster) approach to increase overall recruitment efficiency. Materials and methods: An ethnographic study with students was developed. With the results, a recruitment strategy culturally adapted and adjusted to the study's requirements was set up. Results and Discussion: Twenty-five students participated in the ethnographic study. Based on the results, a strategy consisting of a) posters and flyers, b) student meetings, c) solidarity volunteering: Refiamigos, d) Roulette (game) was developed. The strategy was executed between December 2017 and March 2018 (cohort I) and February and March 2019 (cohort II). It was identified that traditional actions (posters and flyers) only inform, whereas on the other hand, active strategies, such as Refiamigos, were able to encourage participation. In the first cohort, 315 volunteers called 766 participants/Refiamigos. In the second, 297 called 729. This represented a total saving of 5.3 weeks of fieldwork. The Refiamigos accounted for 42.5% of the sample in cohort I and 40.5% in cohort II. Conclusion: Recruitment strategies that involve volunteers and give them a leading role in the recruitment of other participants are more successful than traditional ones (posters, flyers). They generate significant savings of time and resources.

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Report of the first cases of Mayaro virus in Ecuador

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Mayaro virus (MAYV) of the genus Alphavirus and family Togaviridae, is a single-stranded positive RNA virus that was first isolated in Trinidad and Tobago in 1954. It is enzootic for South America and endemic in rural areas, its life cycle It seems the yellow fever virus and its symptoms are similar to dengue in the first few days and Chikungunya virus from the first week. In 2010, the Pan American Health Organization / World Health Organization (PAHO / WHO) announced an epidemiological alert describing several outbreaks of mayaro virus in the Amazon region of Peru, Bolivia and Brazil. Migration flows in recent years have caused the rapid spread of the virus to neighboring regions, making it necessary to develop and maintain the capacity for detection and laboratory diagnosis of this disease. The objective of the study was to establish an extended surveillance of arbovirus by laboratory, for this purpose, 10% of the samples of serum or cerebrospinal fluid between 1 and 5 days of symptoms and negatives for viruses circulating in Ecuador (Dengue, Zika, Chikungunya, Yellow Fever) by the RT-qPCR technique will be processed following the protocol recommended and delivered

by PAHO with primers and probes developed by Kanya C. and collaborators (2011). Viral genome amplification was obtained in 5 of the analyzed samples which belong to the provinces of Portoviejo, Guayas, Santo Domingo and Babahoyo, following the working algorithm, for its confirmation, viral isolation was performed, and the samples were inoculated in C6 / 36 cells and the isolation was confirmed by RT-qPCR. Amplification curves were obtained with cycles among 32-39 in the samples, confirming the first cases of infection by Mayaro virus in Ecuador. The results were communicated to the Ministry of Health for national and international notification. In the future, will expect analize the results of the nucleotide sequencing of the positive samples as well as to implement the serological test for detection of antibodies against mayaro, with the support of the WHO/PAHO, for the strengthening of the epidemiological surveillance of this arbovirosis.

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Predictors for Dengue fever and Rickettsiosis in a hospital-based surveillance in the Amazonian city of Yurimaguas, Peru

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Introduction: Dengue fever and Rickettsiosis are a significant cause of acute febrile illness in tropical regions of low- and middle-income countries. However, the differentiation between these two diseases on clinical grounds is difficult, and pathogen-specific laboratory diagnostics are not uniformly or rapidly available. We explored the utility of the clinical and some laboratory parameters in distinguishing dengue fever from rickettsiosis. Method: We analyzed data from one year (2006) of a febrile surveillance program at the Public Hospital of Yurimaguas, a city in the Peruvian Amazon. Leukocytes and platelet counts were performed on all study patients on the first or second day of enrollment by the hospital's laboratory staff. Acute and convalescent serum samples were tested for dengue virus (virus isolation, PCR, ELISA IgM) and Rickettsia infection (indirect immunofluorescence assay to detect immunoglobulins G and total immunoglobulins) at the laboratory of the U.S. Naval Medical Research Unit No. 6 in Lima, Peru and, the Instituto Nacional de Salud (INS) in Lima, Peru, respectively. Result: 280 (84.27%) out of a total 337 patients were included in this analyze, of whom 51 (18%) were confirmed to have dengue fever (serotype 3) and 24 (15%) Rickettsiosis. 215 (75.7%) were 18 years old (mean 27.79 , 226 (79.6%) were recruited 4 days from onset and 159 (56%) were male. In the logistic regression analyzes, prostration(0.001, OR=0.27), paleness (0.002, OR=3.6), leucocytes (3,500-4,000, p=0.011, OR=4.3) and platelet count (100,000-150,000, p=0.000, OR=3.8) were associated to dengue fever, but no association were found for rickettsiosis or co-infections. In the univariate analyze, adenopathy (p=0.006), arthralgia (p=0.028), platelet count (50,000-100,000) and leukocytes (4,500-5,000) were associated to rickettsiosis, while facial erythema (p=0.007) and vomiting (p=0.002) were associated to co-infection respectively. Conclusion: We conclude that paleness, prostration, mild leukocytopenia and mild thrombocytopenia are important predictor for dengue fever, while normal leucocytes and moderate thrombocytopenia may suggest Rickettsiosis. These laboratory parameters are readily available in most hospitals, including in low- and middle-income countries, and may serve as valuable tools and predictor for dengue and suggest rickettsiosis.

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Development and evaluation of an antigen detection assay for Dengue and Zika diagnosis

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Dengue (DENV) and Zika (ZIKV) viruses represent a threat to public health and military forces deployed in endemic areas including South American countries. Infection with these viruses is marked by fever, body aches and may include a rash, moreover each virus can result in more severe disease manifestations. Viral isolation followed by a direct viral detection technique is still the most sensitive approach to diagnose infection. ZIKV and DENV can be directly detected

by PCR or immunofluorescence (DIF); however, these techniques require specialized equipment that might not be available in laboratories with limited resources. Here, we report the development and evaluation of an antigen detection assay (ADA) for DENV and ZIKV using basic laboratory equipment with viral culture. We conducted in vitro infection using Vero76 cells with DENV and ZIKV inoculums. Supernatant samples were collected daily until the harvest day, defined as when cells displayed a cytopathic effect of >75%. At the harvest day, cell pellets were also collected. Briefly, for the ADA, 50uL of supernatant or cell pellet was dried overnight into coat microplates and then fixed with 70% ethanol. Viral antigens bound to plates were first recognized by hyperimmune mouse ascitic fluid against DENV or ZIKV and then visualized with anti-mouse peroxidase-labeled antibodies. The ADA was developed utilizing standard methods and the optical densities (OD) were recorded. Scatter and fractional-polynomial predictions plots were built to evaluate ADA's limit of detection. As a quantitative control DENV and ZIKV viral loads using virus-specific RT PCR and plaque assays were used to calculate the viral load based on cycle threshold (Ct) and plaque forming units (PFU/mL), respectively. The ADA displays good performance for DENV detection in supernatants with Ct2.8e5 PFU/mL, and for ZIKV in supernatants with Ct1.4e4 PFU/mL. DENV and ZIKV screening by ADA using cell pellets correlated adequately to DIF results when they were dichotomously analyzed, but the correlation reduced when DIF lectures were compared against OD values. Our findings suggest that the ADA could be used as a reliable, low cost diagnostic assay for dengue and Zika cases.

Epi-Phylogenetics-Modeling-Burden

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Detection and whole genome characterization of Ilheus and Iguape virus strains in historical mosquitoes samples from Southeastern region, Brazil, 1994

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Introduction: In Brazil, flaviviruses have been caused massive outbreaks. Surveillance programs designed to monitor virus activity in vectors provides a system for mapping disease distribution and to identify vector species for targeted control. Objectives: The aim of the present study was to describe the detection and the whole genome characterization of Ilheus virus (ILHV) and Iguape virus (IGUV) strains obtained from historical mosquito's samples collected in the Southeastern region of Brazil. Phylogenetic analysis was also conducted. Methods. Based on sample availability and status of conservation, a total of 12 historic mosquitoes' pools specimens (inoculated and stored in neonate mouse brain) collected in the state of São Paulo, Brazil, during 1993, 1994 and 1997 seasons were selected to investigation. Viral RNA was extracted from triturated brains supernatants and tested by qRT-PCR with Flavivirus genus-specific primers. Positive samples were sequenced, and phylogenetic analyses were performed. Results. Flavivirus was detected in 50% (6/12) of the samples by qRT-PCR. Positive samples were successfully Sanger sequenced. Three *Anopheles cruzii* pools collected in 1994 were positive for IGUV. One Culex sp. pool, one *Anopheles triannulatus* pool, and one *Coquillettidia juxtamansonia* pool, both collected 1994, were positive for ILHV. Metagenomic sequencing successfully characterize one ILHV and four IGUV full genomes. High homology was observed between the Brazilian ILHV and IGUV strains and other isolates available in GenBank. Phylogenetic analysis of partial ILHV NS5 gene revealed three distinct lineages (clades), an indication of genetic heterogeneity in circulating strains in Brazil. Nucleotide insertions and high-level of nucleotide diversity were observed in NS1 protein and capsid region of IGUV strains, respectively. Discussion/Conclusions. Detection of ILHV and IGUV in local mosquitoes confirms the historical circulation of these viruses in Southeastern Brazil. This is the first evidence of ILHV in *Anopheles triannulatus* and a potential importance of *Anopheles cruzii* in the IGUV transmission cycle in the region could be speculated. Genomic and phylogenetic analysis of these viruses provided further insights into their diversity and evolution. In-depth studies

of ILHV and IGUV including vector competence and molecular studies are needed to shed light on their epidemiology and potential risk of future emergence.

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Reemergence of Dengue virus 2 due the introduction of a new lineage in São José do Rio Preto, SP, Brazil

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Over the years DENV-2 has been an endemic virus in our city, circulating every year since our monitoring started but has not been a major agent of epidemics. The endemic virus was an BR3 lineage belongs to American/Asian genotype strain of DENV-2 described before. In late 2018 a rapid and intense increase of cases of DENV-2 was detected in the city that ultimately was responsible for the biggest DENV outbreak in the history. In order to understand the mechanism of this DENV emergence we performed the phylogenetic analysis of DENV-2 for four years, including endemic and epidemic periods. A total of 3925 serum samples were analyzed and 21.48% were positive for DENV and DENV-2 was the serotype prevalent in 95.3% of the cases. Samples were sequenced in E gene (n=34) by Sanger methods and complete genome (n=34) by NGS (Illumina and Minion Nanopore Methods). The phylogenetic reconstruction was performed by Neighboor-Joining and Maximum Likelihood Methods. The phylogenetic reconstruction showed the circulation of two lineages belonging to American/Asian genotype of DENV-2: the BR3 that already circulated in previous years in the municipality; and a new strain (called BR4) that was recently introduced. Some genetic and amino acids variations were observed when comparing the strains and in some positions the new strain BR4 presented amino acids existing in ancient strains of BR3 that caused previous epidemics in the country. The more recent BR3 and new BR4 co-circulated in final of the year of 2018 and in 2019 the BR4 showed a higher frequency. These data allow us to associate the introduction of the new strain with the outbreak in 2019 (January) and this might be explained by the differences in viral and epidemiological fitness between these lineages. This work demonstrates the importance of constant molecular surveillance and phylogenetic studies to improve the knowledge about the evolution and spread of DENV in human population over the years and space.

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Phylogenetic Analysis of dengue virus type 2 and 4 from Brazil, 2002 - 2015

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Dengue is an arthropod-borne viral disease that is widely spread in the tropical regions of the world. The disease is caused by dengue virus (DENV), member of the Flaviviridae family, genus Flavivirus and transmitted between humans by Aedes mosquitoes. DENV has four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3 e o DENV-4). The objective of the present study was to perform the molecular characterization and phylogenetic analysis, using the E gene, of DENV-2 and DENV-4 viral samples isolated at Arbovirology and Hemorrhagic Fever Section of the Evandro Chagas Institute between 2002 and 2015, seeking to identify the presence of amino acids changes that may be related to virulence markers of E protein. In the study, 32 DENV isolates were selected, 11 of DENV-2 and 21 of DENV-4, which were submitted to the nucleotide sequencing reaction, using the chain termination Sanger method. For assembly, alignment and homology analysis of sequences, the program package Unipro UGENE v. 1.32.0 was used, and for the phylogenetic analysis the programs used were Mega 7.0 and RAxML 8.0. The nucleotide sequences obtained for the DENV samples used in this study were compared to each other and to sequences of known strains available in the GenBank database. In this study it was possible to conclude that DENV-2 Southeast Asian/American genotype and DENV-4 II genotype are prevalent in Brazil and the isolates showed significant changes of biochemical character. Furthermore, it was possible to identify fre-

quent nonsynonymous substitutions in the DENV-2 (E61, E129, E131, E170, E203, E340 e E380) and DENV-4 (E201) isolates.

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Molecular surveillance of Dengue, Zika and Chikungunya in symptomatic patients from São José do Rio Preto, SP, Brazil

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Aiming to detect patterns and mechanisms of viral circulation, various surveillance actions have been intensified, as well as molecular surveillance in different regions of the country. The objective of this study was to analyze the presence of Dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus (ZIKV), their subtypes and associated genotypes in clinical samples of patients with fever who sought the Health Services of the Municipality of São José do Rio Preto in an active epidemiological surveillance system in the last four years. For the investigation of these arboviruses specific primers for the E gene (ZIKV), NsP1 (CHIKV) and NS5 (DENV) were used through real-time RT-PCR. Between February/2016 and May/2019, 3925 serum samples were analyzed. Two hundred and twenty three samples (5.68%) were confirmed as positive for ZIKV, 843 (21.48%) for DENV and three (0.076%) for CHIKV. The serotypes of DENV found were: 95.3% (803/843) DENV-2, 4.5% (38/843) DENV-1 and 0.2% (2/843) DENV-4. It is important to note that eight cases of DENV-2/ZIKV co-infection (2016 and 2018), three cases DENV-1/ZIKV (2018) and eight cases of DENV-1/DENV-2 co-infection (2019/outbreak) were observed. Samples of the DENV were sequenced in the E gene (DENV-1: 07; DENV-2: 34) and complete genome (DENV-1: 04; DENV-2: 01) sequencing and were used for phylogenetic reconstruction. The analyses showed the circulation of genotype V of DENV-1 and genotype III (also known as Asian-American genotype) of DENV-2 in the municipality. In the four years these three arboviruses circulated in the municipality in different frequencies but recently our data showed DENV-2 as the predominant serotype and responsible by outbreak started in the final of 2018 with a exacerbation in number of the cases in January/2019. This work demonstrates the importance of university-health system integration through molecular studies to better the understanding about the epidemiology of the infectious diseases, emphasizing the importance of using them as a tool to predict epidemics through of continues epidemiological surveillance programs.

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Genotyping with the Denv-1 and 2 Envelope protein in 2012 and 2013, in Mexico.

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Serum samples were analyzed in the acute phase, with a positive result at NS1, serotype confirmation by RT-qPCR Fourplex (Santiago et al. 2013). The envelope amplification consisted of 8 fragments by RT-PCR (ViGenDA. CDC. 2016). Capillary sequencing was performed in the Molecular Biology department of InDRE. The quality of the fragments was measured based on homology percentages by invoking BLAST with reference sequences (GenBank). The phylogenetic approach proved that the genetic diversity of DENV had no changes at the genotype level in the years of study, which were grouped in genotype V for DENV-1 and Asian/American genotype for DENV-2. The genotyping of DENV-1 differs with the results of Carrillo et al. in 2010, since they identified genotype III as the predominant until 2007, however in the review carried out by Ramos et al. in 2017, which covers more years (until 2013), the presence of genotype V is corroborated, this difference can be associated with the evolutionary pattern of genotype displacement described by Carrillo, it is possible that both genotypes were circulating simultaneously and at some point after 2007 the Genotype V will begin to displace genotype III. In the case of DENV-2, its genotyping corresponds to both authors, where the Asian / American genotype is mostly established, even two different lineages were identified circulating in Mexico, of whom their common ancestor appeared in the

90's. When performing a more detailed analysis of the final and initial part of domains I and II of the Envelope protein, corresponding to fragment 2 of the ViGenDA protocol, it was demonstrated that the variation at the nucleotide level in DENV-1 and DENV-2 is low, corroborating with the described by Chunlin in 2005, where the low genetic diversity of DENV-1 was associated with a prevalence shared with DENV-2. In Mexico, however, the introduction of new Arboviruses generated an increase in the prevalence of DENV-1 in later years (Secretary of Health. 2016-2017). The protocols for genotyping from fragment 2 of the ViGenDA protocol can be made more efficient, since said fragment presented homologies greater than 96%, in this way the costs of its implementation would be reduced.

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Endemic and epidemic human alphavirus infections in Eastern Panama; An Analysis of Population-based Cross-Sectional Surveys

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Background: Madariaga virus (MADV), has recently been associated with severe human disease in Panama, where the closely related Venezuelan equine encephalitis virus (VEEV) also circulates. In June, 2017, a fatal MADV infection was confirmed in a community of Darien province. Methods: We conducted a cross-sectional and outbreak investigations. By applying a catalytic, force-of-infection mathematical model to two serosurveys from Darien province in 2012 and 2017, we investigated whether endemic or epidemic alphavirus transmission occurred historically. Results: In 2017, MADV and VEEV IgM response was 1.6% and 4.4%, respectively; IgG antibody prevalences were MADV: 13.2%; VEEV: 16.8%; Una virus (UNAV): 16.0%; and Mayaro virus (MAYV): 1.1%. Active viral circulation was not detected.No additional encephalitis cases were found. Force-of-infection analyses suggest endemic alphavirus transmission historically, with recent increased human exposure to MADV and VEEV in some regions. Conclusions: The lack of additional neurological cases suggest that severe MADV and VEEV infections occur only rarely. Our results indicate that, over the past five decades, alphavirus infections have occurred at low levels in eastern Panama, but that MADV and VEEV infections have recently increased — potentially during the past decade. Endemic infections and outbreaks of MADV and VEEV appear to differ spatially.

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Was Puerto Rico a hub for Zika virus spread? A phylogeographic and travel-based analysis

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The rapid spread of Zika virus (ZIKV) throughout the Americas was a cause for international concern due to links to severe neurological sequelae including Congenital Zika Syndrome and Guillain-Barré Syndrome. ZIKV was first detected in Puerto Rico in December 2015, and was quickly followed by an epidemic with over 39,000 cases confirmed by the through the beginning of 2017. Puerto Rico recently experienced other epidemics of emerging arboviral diseases, including chikungunya virus in 2014-2015 and dengue virus (serotypes 1-4) since introduction in the early 20th century, underscoring the threat posed by emerging arboviral diseases to Puerto Rico and raise the possibility that Puerto Rico is a hub for arboviral disease transmission. Here, we tested the hypothesis that San Juan, the capital city of Puerto Rico with a high volume of international travel, was a source for dissemination within Puerto Rico and to other areas in the Americas. To infer the patterns of

spread, we analyzed ZIKV genomes from >80 clinical samples from Puerto Rico (see Santiago et al presentation), spanning the epidemic period. Genomic, spatial, and temporal data from these samples, as well as from a curated set of ZIKV genomes from throughout the Americas, were used to build a Bayesian phylogeographic model of intra-national and international spread. We cataloged all origin-destination spread events using PastView, and compared known ZIKV spread events to the patterns and volumes of flights and cruise ships through Puerto Rico (while correcting for sampling bias). We also used genomic, epidemiological (PAHO and CDC), and travel data (IATA and US Census Bureau) from within Puerto Rico to characterize local spread. Our models bridge the gap between the genomic epidemiology of the local Puerto Rico ZIKV epidemic and the continent-wide emergence event. Finally, all of our phylogeographic and phylodynamic results will become available online for open visualization and interaction using a Nextstrain community build. Overall, our results will help us determine the role played by travel to and from Puerto Rico in the rapid dissemination of ZIKV during the epidemic.

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First report of Aedes albopictus infected by Dengue and Zika virus in a rural outbreak in Brazil

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Introduction: In Brazil, Dengue (DENV) and Zika (ZIKV) viruses are reported as being transmitted exclusively by *Aedes aegypti* in urban settings. There, *Aedes albopictus* has never been associated with DENV and ZIKV transmission, despite being an efficient vector with a broad dispersion in the Brazilian territory. Therefore, an unusual outbreak of dengue-like illness reported in a rural area of Espírito Santo state, Brazil, in March 2019 required further investigation. Objective: Establish the vectors and viruses involved in an arbovirus outbreak in a rural area of Espírito Santo state, Brazil. Materials and Methods: Mosquitoes were collected during the outbreak using an entomological net and insect aspirator in intradomicile, peridomicile and in plantations around the area. The mosquitoes were morphologically identified using identification keys and, then, they were divided in pools and submitted to molecular analysis for arboviruses detection. Phylogenetic reconstruction was performed for the viral sequence obtained. Results: All 393 mosquitoes were identified as *Aedes albopictus*. DENV-1 genotype V was present in one pool and another pool was positive for ZIKV. Discussion/Conclusion: This is the first report of *A. albopictus* infected by DENV and ZIKV during an outbreak in a rural area in Brazil, indicating its involvement in arboviral transmission. The strain from the study setting is closely related to viruses that have circulated previously in large urban centers of three different regions in Brazil (Southeast, Northeast, and Midwest), showing the capacity of the virus dispersion from urban to rural areas. In addition, it demonstrates that the strain found in the *Ae. albopictus* was not new in Brazil, being involved previously in epidemics related to *Ae. aegypti*, suggesting the potential to *Ae. albopictus* in transmitting viruses already circulating in the Brazilian population. the study findings are relevant to the adoption of actions for prevention and control. *Ae. albopictus* must be considered in areas with arbovirus risk transmission and should be included in public health programs, especially with a focus on epidemiological and entomological surveillance, inclusive in rural areas.

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Time series analysis of Dengue in seven provinces of the Peruvian Amazon

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Dengue is the mosquito-borne disease with the fastest spread and incidence increase in the world in recent times. In Peru, during 2018, cases were reported in 19 out of 25 departments, and the departments of the Northern Coast and the Amazon rainforest were the most affected. The environmental conditions in the Peruvian Amazon are suitable for the survival of dengue vectors (Aedes Aegypti in urban areas and Ae. Albopictus in rural areas) throughout the year, which makes it a reservoir of dengue for the country and region. This project aimed to fit a forecasting model using a seasonal autoregressive integrated moving average (SARIMA) model to the dengue incidence (cases per 1000 inhabitants) with environmental variables as external regressors, gathered from weather forecasting and satellite imagery in the seven provinces of the department of Loreto in the Peruvian Amazon. Monthly data were collected from 2003 to 2013 on eleven environmental variables and dengue cases in each of the provinces of the department of Loreto (Amazon region). In addition, the environmental information was collected in a 2.5 km radius from the villages to only account for direct impacts on human populated areas. The most recurrent SARIMA model was SARIMA(0,0,1)(0,1,1)12 such as those observed in the provinces of Alto Amazonas, Loreto, Maynas and Ucayali with a mean absolute error (MAE) of 0.42, 0.11, 1.04 and 0.11, respectively. In the provinces of Datem del Marañon and Mariscal Ramon Castilla the model with the lowest MAE was SARIMA(0,0,2)(0,1,1)12 with 0.26 and 0.11, respectively. Finally, in the province of Requena the model with the lowest MAE was SARIMA(1,0,0)(0,1,2)12 with 0.05. High and low transmission areas were identified in the provinces of Alto Amazonas, Maynas, Loreto and Datem del Marañon reporting an incidence rate greater than 10; and the provinces of Ucayali, Requena and Mariscal Ramon Castilla with incidence less than 10. The environmental parameters such as Enhanced vegetation index (EVI), Land surface temperature (LST), Runoff and Normalized difference water index (NDWI) were the most suitable in the final models to predict dengue incidence.

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Epidemiological and Clinical Characteristics of a Dengue/Zika Outbreak in the Caribbean Region of Costa Rica 2017-2018

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Introduction: Dengue (DENV) and Zika virus (ZIKV) are mosquito-borne Flaviviruses circulating in the Americas. Since the first Costa Rican ZIKV autochthonous case in 2016, several ZIKV outbreaks have been reported in DENV hyperendemic regions, posing difficulties in clinically-differentiating both infections. Nevertheless, healthcare facilities from the Huetar-Caribbean region from Costa Rica reported more than 2900 suspected DENV cases and 1800 ZIKV cases, respectively during 2017-2018. Objective: To characterize from the epidemiological, laboratory and clinical aspects a Dengue/Zika Outbreak in the Caribbean Region of Costa Rica during 2017-2018. Materials and Methods: Healthcare facility-based surveillance was performed in this region by obtaining acute-phase serum or urine of a total of 398 patients with a presumptive diagnosis of dengue-like illness. Real-time PCR (RT-PCR) to confirm DENV, ZIKV, and/or Chikungunya (CHIKV) infection, associated clinical manifestations, laboratory findings, and virus phylogenetics were investigated. Results: Samples from 36 (9%) patients were positive for DENV, 109 (27.4%) for ZIKV, and 6 (1.5%) were positive for both DENV and ZIKV simultaneously. No sample was positive for CHIKV. Phylogenetic analysis showed DENV-2 American/Asian as the circu-

lating DENV genotype in this outbreak. Overall symptoms and laboratory reports were similar between DENV and ZIKV cases. Nevertheless, patients with confirmed ZIKV infection were more likely to report rash at the day of healthcare facility visit. Patients with DENV and ZIKV co-infection presented laboratory findings more similar to ZIKV-laboratory confirmed cases than those presented after confirmation of DENV-infection alone. DENV-2 isolates were distributed between two clades with one clade presenting statistically significant more thrombocytopenia and leukopenia. Whole genome analysis of these two clades are in progress. Furthermore, phylogenetic analysis revealed that the circulating strains of ZIKV belong to the Asian lineage and were closely related to other strains circulating in the region. Discussion and Conclusions: After suspecting dengue-like illness, only less than 40% were successfully confirmed in the laboratory. Given the diversity and overlapping symptoms of these arboviruses, 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 and which other infectious agents are causing these overlapping symptoms.

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Tracking Dengue Virus 1 During Epidemic and Non-Epidemic Periods in Puerto Rico Using Next Generation Sequencing, 2010-2019

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DENV-1 has been circulating on the island since the early '80s and caused epidemics in 1998, 2010 and 2012. Previous phylogenetic studies, using the envelope gene sequences, identified that the epidemics caused by DENV-1 in 1998 and 2010 are associated with the African-American genotype V and a clade replacement event occurred sometime between 1998 and 2010.No previous studies described the most recent epidemic in 2012, its recent decline during and after the Zika epidemic and its current re-emergence. Our objective is to describe the micro-evolution of DENV-1 in Puerto Rico and its role in epidemic and non-epidemic transmission periods from 2010 to 2019. We developed a universal amplicon-based next generation sequencing protocol for Illumina MiSeq using Primal Scheme, and we implemented this procedure to sequence DENV-1 from more than 50clinical samples. For genome assembly, we used the iVAR bioinformatics pipeline adapted to DENV-1. The phylogenetic analysis incorporated additional, publicly available,whole-genome sequences .We conducted maximum likelihood and Bayesian analysis to reconstruct the DENV-1 transmission in Puerto Rico and its contribution to global spread. Our analysis reveals distinct clades between epidemics in 2010, 2012 and the reemergence in 2019. Lastly, our results will help to identify potential sequences associated with the epidemic, non-epidemic periods, the decline in transmission and the recent re-emergence of DENV-1. This study provides a useful method for the whole genome sequencing of DENV-1 and a detailed evolutionary analysis of the virus transmission in Puerto Rico and in the global context.

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Peruvian dengue serotype 2 viruses carry signature mutation in the E-domain III

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In Peru, 25 million people is at risk of infection by dengue virus (DENV) due to the dispersion of its main vector *Aedes aegypti*, present in 20 out of the 24 departments. The four dengue serotypes have been reported in Peru, however; since 2000 the American (AM)/Asian (AS) genotype from serotype 2 (DENV-2) has become the most prevalent. During the outbreak in Yurimaguas in 2018, more severe Dengue cases were observed then normal and we investigated whether viral genetic traits could be related to this observation. We

collected 170 DENV cases and 69 were confirmed and serotyped as DENV-2 using RT-PCR. The envelope (E) gene (1485 bp) was then amplified and sequenced from 10 viral isolates as well as directly from 24 patient samples. Phylogenetic analysis shows that the sequences from the current outbreak form a monophyletic group within other, older Peruvian sequences belonging to the DENV-2 AM/AS genotype lineage 2. Additionally, the DENV-2-E amino acid aligment of AM/AS strains confirmed the high conservation of E protein, with single amino acid polymorphisms at positions 91, 129, 131, 170, 203, 340, and 380, relevant for phylogenetic classification. A new non-conservative substitution from isoleucine to threonine at position 379 and a conservative substitution at position 484 from valine to isoleucine were found in the sequences from the current outbreak. I379T is located in the β-strand F of the domain III. Mutations in DIII are postulated to have consequences for host range, tropism and virulence due to the presence of putative receptor-binding sites. V484I is located in the transmembrane region of the E-protein, which is highly conserved among flaviviruses. This substitution was present in all sequences from this study and in a strain from Iquitos reported in 2010 as well as in the Asian I and American genotypes. Unfortunately, our study is limited by the scarcity of Peruvian sequences publicly available from 2011 onwards. Functional studies and long-term follow-up are therefore needed to characterize these specific mutations and their possible implications on viral fitness and pathogenicity of the dengue disease dynamics in Yurimaguas.

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Multiple virus introductions and complex molecular evolution sprung the Zika epidemic in Puerto Rico (2016-2017)

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The emergence of Zika virus (ZIKV) in the Americas was initially documented in northern Brazil in 2015 and the virus spread rapidly throughout the continent, with approximately 48 American countries reporting transmission, underscoring the alarming public health concern associated to congenital malformations. Consequently, Puerto Rico (PR) experienced a substantial epidemic with over 39,000 confirmed cases between late 2015 and early 2017. Our objectives are to reconstruct the emergence, genomic complexity, and evolutionary dynamics of ZIKV in PR and understand its contribution to the overall genomic epidemiology of Zika in the Americas. We conducted targeted next-generation sequencing directly on 80 serum samples collected from locations that represent the epidemic geo-temporally. Phylogenetic analyses were performed using maximum likelihood analyses on these new sequences and over 500 reported from Asia, the Pacific and the Americas. Our findings indicate that the island experienced at least two foreign introductions that sustained in situ transmission evidenced by two distinct monophyletic lineages. The most preponderant of these two PR lineages is related to sequences from South America, and spread broadly through the island, further diverging into two clades and multiple subgroups. Molecular clock analyses to estimate the evolutionary history of the epidemic suggests that the introduction of thislineage could have occurred at least one year prior to the initial clinical report, concurring with recent reports of ZIKV emergence in the Americas. The second PR lineage, introduced during the epidemic, is related to sequences from Central America and transmission was more circumscribed to the west of the island. In addition, a small number of PR genomes grouped with sequences from the Dominican Republic, USA-Florida, Honduras and Colombia suggesting additional introductions that did not spread further. Our results demonstrate that the intense ZIKV epidemic in Puerto Rico was propelled by complex evolutionary patterns including multiple introductions. This is the first comprehensive sequencing and genomic epidemiology study of the Zika epidemic in Puerto Rico. Our study will progress to understand the factors that drove these epidemic dynamics in the context of transmission in the Americas.

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Epidemiological benefits and cost-effectiveness of dengue vaccination with CYD-TDV vaccine in Puerto Rico

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The first dengue vaccine (CYD-TDV) was licensed in 2015. However, recent analyses showed an increased risk of severe dengue upon subsequent natural infection among vaccinees without previ-

ous exposure to dengue virus. The WHO recommends pre-vaccination screening to ensure that only those with previous exposure to DENV are vaccinated. However, rapid diagnostic tests with high sensitivity and specificity are not currently available. Hence, it is important to estimate the potential benefits or risks of a pre-vaccination screening strategy with CYD-TDV. We evaluated the impact of this strategy in Puerto Rico with an agent-based model of dengue virus transmission. We estimated the impact of this intervention by evaluating the epidemiological benefits and cost-effectiveness of a vaccination program over a 10-year time-frame in a setting representative of Puerto Rico. Assuming moderate transmission and a highly specific (0.95) and sensitive (0.8) screening test, we found epidemiological benefits of vaccination. Around 6% of hospitalizations in 9-year olds were averted over 10 years, and around 20 hospitalizations were averted for each additional hospitalization in children without previous exposure to dengue virus. We also found that the Incremental Cost-Effectiveness Ratio of the strategy was around $7,000 for the baseline scenario. Our results suggest that prioritizing the specificity of screening tests over sensitivity would improve the benefits of vaccination.

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Serotyping and population structure analysis of Dengue virus in the north coast of Peru, 2015 - 2017

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Dengue represents a threat to public health around the world with increasingly large outbreaks. Population genetics, genetic diversity and phylogenetic relationships studies allows to design better molecular surveillance strategies and achieve a more successful containment of vector dispersion. Dengue virus (DENV) structuring are determined by the vector population structure, as well as viral intrinsic factors such as gene variability, geographic distribution, gene flow and episodic positive selection. While the inference of DENV structuring is based on genomes, different gene areas evolve at different rates and exhibit "hot spots" of high mutation rates. This occurs with the envelope gene (gene E), which is also a widely used marker to analyze phylogenetic relationships between different DENV strains, showing its potential as a candidate to infer population structure. The aims of the study were to identify the circulating serotypes of DENV and to analyze its population structure between and within the serotypes found in samples from the north coast of Peru during the 2015-2017 period, by analyzing a region of gene E. We conducted a descriptive-retrospective study that included samples from 100 patients diagnosed serologically with Dengue at the Regional Hospital of Lambayeque and Regional Hospital II-2 Jamo Tumbes, both in the North Peruvian coast separated by 353 kilometres. We identified the DENV serotypes using a conventional PCR that targeted a conserved region of the capsid. The four DENV serotypes were found in Tumbes, while only DENV2 and DENV3 were found in Lambayeque. An in silico analysis with GenBank sequences was performed to infer population structure from genomes and gene E. We extract 818 and 129 informative sites from the genome and genes, respectively, as input for the Structure v2.3.4 program. Using the Evanno method we determined the best K, which means the most probable number of populations, and for both genetics regions we obtained the same value of the best K. Therefore, specific primers designed for partial regions of gene E will be used for population structure inference and phylogenetic analysis. The results could lead to further investigations and greater understanding of genetic diversity and a better design of molecular surveillance strategies.

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Genetic diversity between primary isolates of dengue virus serotype 3 (DENV-3) sampled in states from Colombia

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Introduction: In Colombia, dengue virus serotype

3 (DENV-3) genotype III was detected by the first time in 2001 in the northeast region; in 2002, the virus was detected in the western and southern regions. Causing a large epidemic, the virus also was the most prevalent in the 2009-10 epidemic but became the second least prevalent since 2014. Knowledge about the genetic differentiation between viruses is required. Objective: To determine genetic diversity and differentiation, based on E gene in DENV-3 isolates sampled in states of Colombia. Methods: Full-length nucleotide sequences of E-gene from DENV-3 sampled in Colombia were obtained in this study and other sequences were downloaded from GenBank. The final filtered dataset included 127 sequences from 11 states. ML methods (RAxML v.8.2 software) support the phylogenetic analyses. Analysis of molecular variance (AMOVA) and neutrality test were performed (ARLEQUIN v.3.5.2.2 software) to assess the genetic variation between DENV-3, and MJ network of haplotypes was constructed using Network (V.5.0.1.1 software). Results: The ML analysis recovered two lineages of DENV-3. Data (π=0.0172; Hd=0,9974±0,0011; Tajima's D=-0.51967, p=0.57213) suggest virus population expansion. A total of 108 haplotypes forming six haplogroups were recovered in the MJ network. The AMOVA analysis shows high genetic differentiation between viruses sampled from different Colombian states (Fst=0.27). DENV-3 from Santander State (northeast Colombia) formed five haplogroups, two of them were also present in the virus population from Valle del Cauca State (southeastern) whereas the remaining haplogroup was exclusive. Valle del Cauca and Santander viruses exhibited value of migrants per generation (Nm>1) with eight of the 11 states, opposite to Sucre State viruses showed Nm values <1.0 with all the other states. Discussion and conclusion: The analyses suggest demographic expansion and high genetic diversity of DENV-3 from Colombia, which could be related to geographic origin and therefore distinct routes of dispersal across the country. Comprehensive studies are needed to better understand the role of genetic diversity in the dynamic of DENV-3.

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Investigation of a Dengue Outbreak in a Remote Rural Community in Northern Coastal Ecuador

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Introduction: Small, remote communities are often thought to be unable to sustain dengue transmission. The community of Santa Maria was formed through the union of two settlements, one Afro-Ecuadorian and one Indigenous Chachi. It is separated by 2-3 hours travel, by boat, from the nearest population center. In 2019, the community experienced a dengue outbreak. Objective: To identify spatial risk factors for symptomatic dengue in Santa Maria, Ecuador. Materials and methods: We paired unmanned aerial vehicle (UAV) mapping with epidemiologic and entomological surveys to characterize the Santa Maria dengue outbreak. Epidemiologic surveys included a community census and georeferencing of all households. Active surveillance was then used to identify febrile cases. Visible water containers in UAV images were mapped, and the green-red vegetation index (GRVI) was calculated. Summary variables characterized water containers within 40 meters of each household and proximity to public spaces like schools and meeting areas. Individual risk factors were also considered including age, sex, ethnicity, and livelihood. Mixed-effect logistic regression models accounted for clustering by household. Results: Forty-five cases, from 30 households (8% of the population), were identified between May and September 2019. All PCR-confirmed cases were Dengue virus type 1. Most cases occurred among 15- to 30-year-olds. Significant risk factors were Afro-Ecuadorian (versus Indigenous) ethnicity (OR=2.8, 95% CI: 1.1, 7.2), and living within 40m of the football field (OR: 6.5, 95% 1.9, 22.1). There was no association between case status and sex, livelihood, local household GRVI, or history of travel. The overall Breteau index (BI) was 19.6. Among Afro-Ecuadorian households, located mostly on the eastern side of the community, the BI was 20.0. On the western, Indigenous side of the community, the BI was 4.5. Chachi households had more members than Afro-Ecuadorian households (mean 5.7 versus 3.3, p<0.0001), and fewer containers

in a 40m radius (12.6 versus 16.4, p=0.0304). They were more likely to report that their primary source of water was the nearby river, rather than rainwater (23.4% versus 11.1% of households). Discussion: UAV mapping enabled us to identify, with high resolution, variations in the community environment related to disease risk.

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Physical Growth and Neurodevelopment after 3.5 year of follow-up of children born from mothers with gestational Zika in Risaralda, Colombia.

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Introduction: After Zika virus (ZIKV) epidemics microcephaly and other structural neurologic abnormalities were the main concern after gestational exposure. However, data suggest that neurodevelopment and growth could be impaired. Objective:To describe the physical growth and neurodevelopmentof a cohort of childrenborn from mother with confirmed ZIKV infection during pregnancy. Methods:We conducted a follow-up study of children from mothers with RT-PCR confirmed ZIKV infection during pregnancy in two hospitals in Risaralda, Colombia. We assessed anthropometric measures according to WHO-standards and neurodevelopment with the neurodevelopmental abbreviated scale validated in Colombia. Results:Sixteen cases were followed up to 11 times during 3.5 years of life. The IQR of ZIKV diagnosis on mothers was 13-31 gestational weeks. During follow-up, two of them born with Head Circumference (HC) <33 cms (13%), and one of them stayed with HC ¡-2SD in the next two controls. Three of them (19%) felt into post-natal microcephaly. Only one (6%) remained with microcephaly until the follow-up end-point. Surprisingly, six presented HC >+2SD (macrocephaly) during follow-up (38%),andtwo of them remained with macrocephaly until the follow-up end-point. Weight-for-age(WFA),or length/height-for-age(LHFA) were above +2SD in four and in six patients respectively. LHFA was below-2SD in two patients. We found two patients with macrocephaly and high BMI-for-age, two with macrocephaly plus high WFA and LHFA, and two with macrocephaly and high BMI-for-age and WFA. Neurodevelopment was appropriate for age in each patient.Conclusions: As expected congenital and postnatal microcephaly was present.Remarkably, an important proportion of children presented macrocephaly without other apparent etiologies and no significant neurodevelopment impairment. Although this requires further analyses, the full spectrum of the head structure and growth alterations would be broader than reported.

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Incidence and mortality estimates of chikungunya virus infection in children, Colombia, 2014-2017

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Introduction: After the widespread chikungunya virus (CHIKV) outbreak during 2014-2015 in Colombia, concerns were raised because of its chronic sequalae and the report of atypical and fatal cases. Cohorts started but the pediatric population has been underrepresented. Objective: To describe the incidence and mortality rates of CHIKV pediatric infection in Colombia during 2014-2017 Methods: Observational, retrospective study in which crude and adjusted incidence rates of chikungunya were estimated (cases/100,000 population) using information derived from the

National Surveillance System in Colombia (SIVIGILA) and population data extracted from the official population estimates of National Statistics Department (DANE) for children between 0 to 14 years-old.Results:A total of 77,608 cases were reported during the studied timeframe in the general population, of which 9,852 cases (12.69%) were reported in children between 0 to 14 years-old (cumulative incidence 93.31cases/100,000pop), with 61.77% of the cases reported during 2015During this timeframe the median incidence rate was 4.13 cases/100,000pop. The highest incidence were reported among children 10 to 14 years old in Vichada during 2015 (442.96 cases/100,000pop). Remarkably, a total of 14 deaths associated to CHIKV infection in the pediatric population were reported, three of them (21.4%) reported in 2015 in Tolima, and a case fatality rate of 0.14%. Conclusions:The cases of CHIKV in children in Colombia reached incidences has high as 442.96 cases/100,000pop, and even lethal cases were reported. However, little is still known about the clinical spectrum of the disease in this population and their risk of evolution to chronic disease.

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Burden of disease derived from dengue infection in children, Colombia, 2012-2017: Assessing age as risk factor for severe dengue

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Introduction: Dengue is a major burden of disease in children in tropical countries, where they are in higher risk of complications and death. Observational studies have shown a bimodal distribution of severe dengue among children and antibody dependent enhancement has been proposed as explanation. However, evidence is still controversial and data from epidemiologic surveillance could provide insights into the role of age as risk factor for complications. Objective:To compare severe dengue incidence, mortality and case fatality rates (CFR) among children from Colombia using routine epidemiologic surveillance and burden of disease analysis. Methods: Burden of disease study assessing incidence rates of cases reported to the National Surveillance System (SIVIGILA) between 2012 and 2017, and population information from the Colombian Administrative Department of Statistics (DANE) for children between 0 and 14 years-old. The burden of disease was estimated through Years Lived with Disability (YLD), Years of Life Lost (YLL) and Disability-adjusted life years (DALYs) using Disability Weights and expansion factors previously published. Mortality and CFR, as well as adjusted DALYs were compared across different age groups, including adults. Results: A total of 526,827 cases were reported in the general population (cumulative incidence: 1099.05cases/100.000pop), with 39.22% of cases in children. The departments with the highest cumulative burden were Meta (75215.58 DALYs/100,000pop), Tolima (67735.80 DALYs /100,000pop) and Huila (64128.74 DALYs/100,000pop). The children represented the 53.1% of the severe cases, and the 41.0% of the lethal cases, with 22,423.85 YLL due to premature death, and 3,045,395.18 DALYs lost. The highest mortality was found among children between five to nine years-old (3.63 deaths/100.000pop). The departments with the highest cumulative incidence of deaths were Meta (11.64 deaths/100,000pop), Huila (8.85 deaths/100,000pop) and Tolima (8.00 deaths/100,000pop). Lethal cases had a bimodal distribution with two peaks in children 3 to 4 y-old and 6 to 7 y-old. CFR were steady among children but in adults 65y-old CFR ranged from 9.67 to 100. Conclusions: Children bear the 40% of the dengue burden in Colombia. CFR were steady among them, but it increased in the elderly, supporting WHO current recommendation of include >65y-old as patients in greater risk of complications.

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Arboviral infections in elderly patients from an endemic area in Risaralda, Colombia: Clinical and laboratory characteristics

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Introduction: Arbovirus infection (ARBV) endemic areas in Latin-America face the epidemiologic consequences of aging and increased morbidity in elderly patients. Elderly patients represent nearly the 5% of ARBV cases in Colombia. And age has been proposed as a risk factor for severe infection. However, little is known about the clinical course of ARBV in elderly patients. Objective:To describe the clinical and laboratory characteristics of ARBV in a cohort of elderly patients from Risaralda, Colombia. Methods: We conducted a retrospective cohort study in of patients older than 65y-old with suspected ARBV. We compared clinical and laboratory characteristics among two main groups: 1Ṗatients with acute undifferentiated febrile illness (AUFI) and 2Ṗatients with suspected or confirmed ARBV. The last group was composed of three subgroups (ZIKV, DENV, and CHIKV). Qualitative and quantitative variables were summarized with proportions, means or medians with their respective confidence interval (CI), interquartile rank (IQR) or standard deviation (SD). Hypothesis were tested with parametric or non-parametric test when appropriate, with a significance of 5%. A multiple logistic regression was performed for confounder adjustment Results:A total of 71 patients were included. Among them 53 patients were suspected ARBV and 18 AUFI. Median age was 70 y-old, 52.11% were women, 76.06% reported at least one comorbidity and 18.30% polypharmacy. Confirmatory IgM ELISA was performed in 17 cases (23.94%), PCR in 5 cases (7.01%), and both in 3 cases (4.22%). There were not ZIKV cases. The mean duration of fever was 3.6 days (SD $\pm$2.03 days). Eleven patients were hospitalized and four presented with hemorrhagic manifestations, without reported lethal or atypical cases. After adjustment by confounders, adynamia (OR 17.40 CI95% 1.67 to 182.61, p = 0.017) bleeding (OR 48.39 CI95% 2.37 to 988.29, p = 0.012) and lymphocyte count (mean $9.82x10^3$cells/mL CI 4.15 to 15.4, p=0.03) were associated with confirmed ARBV. Conclusions:Clinical picture of ARBV in the elderly had a similar clinical course for patients in other age groups. We found some clinical and laboratory parameters associated with confirmed ARBV and DENV. We did not find lethal or atypical cases.

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Immunity and susceptibility to endemic flaviviruses in pregnant women in Risaralda, Colombia.

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Introduction: Zika virus (ZIKV) emerged in 2015 as a major global problem affecting maternal and child health due to adverse fetal outcomes (AFO) caused by ZIKV infection of pregnant women. Nevertheless, key questions remain, including how previous infection by related flaviviruses such as dengue (DENV) may mediate risk for maternal/congenital ZIKV infection and AFO. Objective:To define flavivirus immune profile and susceptibility to Zika infection in a cohort of pregnant patients from Risaralda, Colombia.Methods: A cross-sectional cohort of pregnant patients was established in Pereira, Colombia, recruiting adult women upon presentation to Labor and Delivery from 2017 to 2019Ṡerologic specimens were obtained during admission. Flavivirus seroprevalence was determined by antigen capture ELISA for IgG. Neutralizing antibody titers against ZIKV and DENV1-4 were approximated by a four-dilution

(1:20-1:1280) focus reduction neutralization test (eFRNT). Results:A total of 115 pregnant women (median 25.1 y-old, IQR 21.06-29.32) were included in this analysis, 77% of them live in urban areas in Risaralda: 66% were housewives, 74.8% have never visited another country, 72.2% never used a mosquito net, 13.0% used repellent, and 41.7% received the yellow fever vaccine. Ten patients (8.7%) reported history of dengue, three (2.6%) of ZIKV, and four (3.5%) of fever during 6-months prior to enrollment. Flavivirus seroprevalence was 75.7% (IgG+). Most subjects (n = 63, 54.8%) had neutralization testing consistent with multiple prior flavivirus infections, whereas fewer (n = 33, 28.7%) had high titers to a single virus (primary flavivirus infection). Two subjects (1.7%) had clear evidence of ZIKV infection without immunity to DENV, and 66.9% of pregnant women did not exhibit neutralizing activity against ZIKV. Discussion: Our results suggest most pregnant women (70%) remain susceptible to ZIKV despite a high rate of DENV seropositivity, making future outbreaks a high concern in this population. This ongoing study represents an opportunity to increase flavivirus surveillance efforts locally and conduct translational research to understand determinants of flavivirus disease and protection in pregnancy.

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Early identification of Dengue virus lineage replacement in Brazil using portable genomic surveillance

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INTRODUCTION: Despite efforts to mitigate the impact of DENV epidemics, the virus remains a public health problem in tropical and subtropical regions around the world. Most DENV cases in the Americas between January and July 2019 were reported in Brazil. São Paulo state in the southeast of Brazil has reported nearly half of all DENV infections in the country. OBJECTIVES OF THE STUDY: To understand the origin and dynamics of the 2019 DENV outbreak. BRIEF MATERIAL AND METHODS: Here using portable nanopore sequencing we generated 20 new DENV genome sequences from viremicpatients with suspected dengue infection residing in two of the most-affected municipalities of São Paulo state, Araraquara and São José do Rio Preto. We conducted a comprehensive phylogenetic analysis with 1,630 global DENV strains to better understand the evolutionary history of the DENV lineages that currently circulate in the region. RESULTS: The new outbreak strains were classified as DENV2 genotype III (American/Asian genotype). Our analysis shows that the 2019 outbreak is the result of a novel DENV lineage that was recently introduced to Brazil from the Caribbean region. Dating phylogeographic analysis suggests that DENV2-III BR-4 was introduced to Brazil in or around early 2014, possibly from the Caribbean region. DISCUSSION/CONCLUSION: We report genomic epidemiological findings from our surveillance of two municipalities in the São Paulo state, Araraquara (ARA) and São José do Rio Preto (SJRP), between early June 2017 and the end of April 2019. To date, 3 genetic lineages of DENV2 genotype III (DENV2-III) have been reported in Brazil named as lineages 1-3 or BR1-BR3. Our analysis strongly supports (approximate likelihood ratio test = 1.00) the clustering of the 2019 DENV2 cases from Brazil (18 of the 19 sequences collected in 2019) into a single monophyletic group (named here as DENV2-III BR-4), which is the result of a new and recent introduction of DENV2-III from outside of Brazil. Our study describes the early detection of a newly introduced and rapidly-expanding DENV2 virus lineage in Brazil.

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Dengue deaths in Brazil, 2000 to 2019

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Introduction: Since 2000, dengue fever severe cases have been increasing in Brazil, especially in the past decade. This trend reflected the shifts in

the predominant dengue virus serotype over time. In this scenario, a detailed analysis of the deaths due to dengue may help prepare for future outbreaks. Objective: To characterize dengue deaths in Brazil. Methods: A cross-sectional descriptive observational study based on secondary data recorded by the National Dengue Surveillance System (NDSS) between 2000-2019 in Brazil. All confirmed dengue deaths from January 1st, 2000 to October 31st, 2019 were analyzed. Records with inconsistent data were excluded. Deaths were characterized according to age, gender, comorbidities, predominant serotype, final classification, interval between the onset of symptoms and death. Results: During the study period, 7,612 deaths were confirmed by the NDSS. No predominance was observed according to sex. The median age (in years) of deaths ranged from 29 in 2007 to 60 in 2014 and 2019. During the outbreaks of DENV2 in 2007/2008, more than 25% of deaths were observed in the age group bellow 15 years of age. Around 50% of the deaths occurred between 3 to 7 days after the onset of symptoms. However, this interval increased according to the age groups, with a higher proportion of deaths after 10 days of the onset among patients over 60 years of age. Comorbidities were observed in 46,7% of the deaths, ranging from 10% to 67,5% in those below 15 and above 60 years of age, respectively. Discussion: A death caused by dengue was a rare event until 2001. After the introduction of DENV3, more severe cases and deaths started to occur. A shift in severe cases/deaths towards children was observed with DEN2 and since 2009 severe cases/deaths in the elderly became more frequent. As expected, most of the deaths occurred around the 5/6th days after the onset of symptoms stressing hypovolemic shock. However, among the elderly, deaths around or after 10days of the onset were observed, suggesting a different pattern on those with comorbidities. Further discussions on how to classify dengue deaths may be needed.

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Entomovirological surveillance in mosquitos from the District of Santa Marta, Colombia

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Introduction: Multiple cities and municipalities in Colombia meet the requirements for mosquito reproduction at high densities and the potential arbovirus transmission. The District of Santa Marta is one important city in Colombia with around 500.000 national and international visitors to the natural parks Tayrona and Sierra Nevada de Santa Marta during 2018. This intense flow of people has profound impact in the arbovirus dynamics. Objective: Describe the molecular detection and characterization of circulating arbovirus in the District of Santa Marta, Colombia. Methodology: Mosquitoes were collected during the period 2018-2019 in sylvatic, rural and urban areas of Santa Marta, Colombia. Morphotypes were pooled and stored in liquid nitrogen for RNA extraction using the RNeasy mini kit (Qiagen Inc.). Generic and specific RT-PCR assays for the detection of alphaviruses and flaviviruses were performed. Specific genes were amplified and sequenced for phylogenetic inference. Results: Multiple mosquito genera were identified through the gradient from sylvatic to urban areas, including Aedes (Ae.), Anopheles, Coquillettidia, Culex, Deinocerites, Haemagogus, Lutzomyia, Mansonia (Ma.), Ochlerotatus, Psorophora (Ps.), Sabethes (Sa.), Trichoprosopon, Uranotaenia and Wyeomya; the species *Ae. aegypti*, *Ae. taeniorrinchus*, *Ma. titilans*, *Ps. ciliata*, *Ps. ferox*, *Sa. cyaneus*; and 14 morphotypes are being processed. A total of 210 mosquito pools (2331 specimens) were processed for molecular detection. Dengue virus (DENV), Zika virus (ZIKV) and Culex flavivirus were detected in 6, 1 and 1 pool, respectively, for a Minimal Infection Rate of 2.6, 0.4 and 0.4 infected mosquitos per 1000 processed mosquitoes. Two strains of DENV-1 genotype V (American/African) with different recent evolutionary origin were identified. ZIKV and DENV-1 were simultaneously detected in a single pool. A flavivirus integration in the *Ae. aegypti* genome was detected. Conclusions: DENV-1 was detected, in agreement with its predominant circulation in the whole country during the 2018-2019 epidemic. Our findings demonstrate the maintenance of DENV and ZIKV in the vector population in the urban area of the District of Santa Marta, despite ZIKV not being correlated to the disease epidemiology. The cryptic circulation of ZIKV justifies an intensified post-epidemic surveillance in endemic areas were the co-circulation with other arboviruses limits the clinical diagnosis.

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Multiple Chikungunya virus introductions in Mexico

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Introduction: Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that causes Chikungunya fever. Multiple CHIKV introductions were previously reported in Nicaragua during the Caribbean outbreak. Objective: Identify different CHIKV introductions in Mexico during the Caribbean outbreak. Material and 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. Viruses were isolated and the whole genome was sequenced. Phylogeny reconstruction was inferred using maximum likelihood approach and maximum clade credibility. In the analysis, we included all the Asian genomes reported in GenBank. Results: We obtained the whole genome of five CHIKV isolated from patients. The five genomes grouped in the Asian lineage, specifically with the Caribbean strains. Two of the isolated viruses grouped in the subclade CO1.3 with viruses from Nicaragua. The rest of the isolated viruses grouped within clade CO1. Viruses from the rest of Mexico, isolated in the sates of Chiapas (2014) and Tamaulipas (2015), grouped in the subclade CO1.4 with viruses from Guatemala and Nicaragua. Other viruses from Chiapas state isolated in 2014 grouped in the clade CO4 with viruses from British Virgin Islands and Saint Martin. The virus from an imported CHIKV case in Jalisco state grouped in an unspecified clade composed of viruses isolated from Caribbean islands. All the aforementioned clades and subclades had high posterior probability values. The time to the most recent common ancestor (TMRCA) of the CO1.3 and CO1.4 subclades are May 2014 and February 2014, respectively. The TMRCA of the CO4 and unspecified clade are both July 2013. Conclusion: We report at least three CHIKV introductions in Mexico; first from the Caribbean Islands, and posteriorly from Guatemala and Nicaragua.

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Spatial distribution and risk factors for infection with chikungunya and Zika viruses in a community-based cohort study in southern Puerto Rico

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Chikungunya virus (CHIKV) caused a large outbreak in Puerto Rico in 2014, followed by a Zika virus (ZIKV) outbreak in 2016. We assessed risk factors for previous CHIKV infection and recent ZIKV infection among a community cohort in southern Puerto Rico, Communities Organized for the Prevention of Arboviruses (COPA).Participants aged 1–50 years (y) were recruited from households in18study communities. Each participant completed an interview and provided a blood specimen, which was tested by anti-ZIKV IgM MAC-ELISA assay and anti-CHIKV IgG ELISA assay. We assessed the distribution of CHIKV and ZIKV infections within communities using Ripley's K function, and evaluated factors associated with a positive result for CHIKV or ZIKV after adjusting for confounders. During 2018–2019, 4,352 participants were enrolled in COPA; 60% were female and median age was 29y (IQR 17–41).Sixteen percentof participants had a positive result for ZIKV by IgM, and 31% had a positive result for CHIKV IgG. Clustering of ZIKV and CHIKV infections was identified in 17% and 50% of study communities, respectively. Cross-clustering of ZIKV and CHIKV infections were identified in 50% of study communities.Increased risk of ZIKV positive results were associated with male sex (OR1.4; 95% CI 1.1–1.6), older age (OR 1.7; 95% CI 1.4–2.1), lower income (OR 1.5; 95% CI 1.2–1.9), and rat sightings in the community (OR 1.3; 95% CI 1.0–1.5). Lower risk for ZIKV was associated with screens (OR 0.7; 95%

CI 0.5–0.8) and air conditioning (OR0.7; 95% CI 0.6–0.9) in the home. For CHIKV,lower educational attainment(OR 1.4; 95% CI 1.2–1.7), rat sightings, (OR 1.3; 95% CI 1.1–1.5), and public insurance versus private (OR 1.6; 95% CI 1.1–2.2) were associated with a positive result; lower risk was associated with screens (OR 0.5; 95% CI 0.4–0.6) and air conditioning (OR 0.7; 95% CI 0.6–0.8) in the home. Clustering of CHIKV and ZIKV infections were identified within communities. The presence of screens and air conditioning in the home reduced risk of a positive result for both ZIKV IgM and CHIKV IgG.

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Epidemiology of Dengue in Guatemala 2000 to 2016

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Introduction: Dengue fever is endemic worldwide and about 1% of cases progress to severe haemorrhage and shock, little is known regarding the burden of dengue across space and time. We explored the epidemiological trends of dengue in Guatemala using official records from the period 2000 to 2016. Objective: We analysed 17 years of country-wide dengue surveillance data in Guatemala, to describe epidemiological trends from 2000 to 2016. Methods: Data from the national dengue surveillance database was analysed to describe dengue serotype frequency, seasonality and outbreaks. We used poisson regression models to compare the number of cases in a given year with subsequent years and to estimate incidence ratios within serotype adjusted by age and gender. Results: 91,554 samples were tested. Dengue was confirmed by RT-qPCR, culture or NS1-ELISA in 7097 (7.8%) cases and was IgM ELISA-positive in 19,290 (21.1%) cases. DENV1, DENV2, DENV3 and DENV4 were detected in 2218 (39.5%), 2580 (45.9%), 591 (10.5%) and 230 (4.1%) cases. DENV1 and DENV2 were the predominant serotypes but all serotypes caused epidemics. The largest outbreak occurred in 2010 with 1080 DENV2 cases reported. The incidence was higher among adults during epidemic years, with significant increases in the 2005, 2007 and 2013 DENV1 outbreaks, the 2010 DENV2 and the 2003 DENV3 outbreaks. Interpretation: Dengue fever is hyperendemic in Guatemala, with the circulation of the four serotypes and an significant increase in the second decade of this century. Adults had a lower incidence immediately after epidemics, which is likely linked to increased immunity.

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Human Mobility Assessment in a Community Cohort in Puerto Rico

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Participants ages 1–50 years (y) are recruited from randomly selected households. At the initial visit, participants are asked how many daytime (6am–8pm) hours (h) they spent at home and provide a serum sample, which is tested by anti-Zika virus (ZIKV) IgM MAC-ELISA assay and anti-chikungunya (CHIKV) IgG ELISA assay to detect prior infection. We evaluated the relationship between hours spent at home and individual demographics and arboviral seropositivity. Additional data on the location and characteristics of places where participants spend $\geq$5h per week is currently being collected. Among the 4,377 participants in the initial study visit, a median of 6 hours per day (IQR 1–14) during weekdays and 14 hours per day (IQR 1–14) during weekends were spent at home. Participants 21y and older spent more time at home per week (mean = 63, range: 4–98) compared to participants younger than 21y (mean = 59, range: 3.5–98) (p Spending more time at home was also associated with increased likelihood of prior CHIKV infection with 24% (46/190, reference) seropositivity among participants spending 1–24h, 29% (661/2,287, p-value = 0.1688) among those spending 25-60h, and 35% (638/1,812, p-value = 0.0024) among those spending 61–98h at home. There were no significant differences in time spent at home by sex or ZIKV seropositivity.Our results suggest that Wolbachia suppression and other spatially limited vector control interventions will have the greatest impact on reducing arbovirus infection among individuals that spend many daytime hours at home. More detailed mobility data will be valuable in better defining how distances and patterns

of movement impact arbovirus infection risk and vector control effectiveness.

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Nanopore-based genome sequencing technology for temporal and epidemiological investigation of Brazilian 2018-2019 dengue outbreak: results from the Workshop with training, research, surveillance and scientific dissemination.

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Dengue Fever is a tropical mosquito-borne viral disease present in more than 110 countries and a current threat to half of the world population. Brazil over the years has been facing several outbreaks caused by different serotypes of the virus. Authorities in Brazil have reported by the end of 2018 an explosion in the number of cases of dengue fever as increasingly extreme weather patterns fuel the spread of the potentially lethal, mosquito-borne disease. Using a combination of portable whole genome sequencing and phylogenetic analyses, we generate 225 complete genomes sequences from DENV-1 (55) and DENV-2 (170) obtained from infected patients sampled in 7 distinct Brazilian states. To investigate the diversity and the origins of the ongoing DENV-1 and DENV-2 outbreaks in Brazil, we performed phylogenetic analysis using all publicly available complete or partial genome sequences from these two serotypes. Our Maximum Likelihood (ML) phylogenetic reconstruction of the DENV-1 outbreak shows that the virus genomes recovered from the 2019 cases all belong to genotype V and highlights the presence of tree main clades, suggesting three possible independent introductions in different Brazilian states. In addition, our ML phylogeny on the DENV-2 dataset shows that the new viral genomes belong to genotype III, and formed a single monophyletic group, which appear to be the result of a new introduction of DENV2-III, hereafter labelled as Brazilian lineage IV (BR4). Our estimates further suggest that the DENV-2 epidemic was caused by a single introduction from the Caribbean region to Southern, midwestern and northeastern Brazilian states of São Paulo, Mato Grosso, Mato Grosso do Sul, Minas Gerais and Bahia. These findings reinforce the co-circulation of DENV-1 and DENV-2 serotypes in Brazil and future time-measured phylogenetic analyses will help to understand arbovirus epidemics, which might help to attenuate public health impact of infectious diseases.

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Modeling Dengue immune responses mediated by antibodies: insights on the immunopathogenesis of severe disease

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Introduction: Epidemic modeling became important to understand infectious disease dynamics, to predict its spread, and finally to develop effective decision making tools for disease prevention and control. Dengue fever dynamics shows large fluctuations in disease incidence, and several mathematical models describing the transmission of dengue viruses have been proposed to explain the irregular behavior of dengue epidemics, contributing to public health authorities' capacity to implement the available intervention measures to control disease transmission. High quality data combined with the correct interpretation are essential for the development of realistic and accurate epidemic models. However, for immunological systems high quality data are often limited by measurements constrains and therefore a model able to provide insights on missing immunological information allowing hypothesis testing is of an urgent need. Objectives: To understand the in-

terplay between IgM and IgG antibodies in primary and secondary dengue infections, including the mathematical explanation of antibody dependent enhancement (ADE) and severe disease. Methods: We develop an intra-host model, a system of ordinary differential equations, to understand the dynamics of primary and recurrent dengue infections and the path leading to severe disease due to (ADE). The model is analyzed with dynamical systems theory and is calibrated using immunological data. Numerical analysis and simulation techniques are used. Results: Our model shows qualitatively good agreement with the expected pattern of disease evolution, from primary to secondary infection, as well as ADE described recently in cohort studies. We we able to describe: i) viral load and clearance via IgM in primary infection; ii) disease protection in secondary infection with the same virus and iii) early response via the pre-existing IgG, leading to a higher viral load, correlated with disease severity, in secondary infection with a different virus. Conclusions: The used methodology allows the mechanistic understanding and testing of hypotheses regarding the immunology of disease response, bridging gaps between different microbiological observations which are normally not directly linked. This model can be used as a guiding tool for the complete understanding of dengue pathogenesis, allowing insights into missing laboratory measurements needed for various studies, from sever disease early detection to vaccine performance.

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Introduction patterns of Chikungunya virus from Panama during 2014 outbreak using complete genome analysis

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Chikungunya virus (CHIKV) is a positive sense RNA alphavirus that belongs to the family Togaviridae, and is considered a public health disease. Chikungunya emerged in the Americas in 2013 from the islands in the Caribbean and then spread throughout Central, South and North America. In Panama, the firsts cases were reported in May of 2014, but these cases were imported from travelers from Haiti and Dominican Republic. It was until August 2014 that we had the firsts autochthonous cases. The chikungunya outbreak was very limited, and the circulation of the virus has been decreasing over the years. Here we evaluated the introduction patterns of CHIKV in the 2014 Panama outbreak using next generation sequencing. Since the first case reported in May 2014 until December 2014, Panama had 350 suspected cases of them 68 were confirmed by laboratory. Acute positive samples confirmed by Real Time RT-PCR from August to December 2014 were isolated in Vero cells, the RNA was extracted and amplified using complete genome specific primers for CHIKV (Joshua Quick, Nathan D Grubaugh et al.) and sequenced with Illumina MiSeq. Obtained reads were merged, filtered base on quality and length with PeAR and aligned with reference using Bowtie2. We obtained 14 CHIKV complete genomes. Phylogenetic and geographic analysis comparing sequences published elsewhere and Panamanian sequences were performed with nextstrain pipeline. Our analysis shows two points of CHIKV introduction during 2014. They support the introduction origin of CHIKV from the Caribbean as previously published for the Americas, with a second introduction point in Panama from Central America. This helps to better understand the movement of CHIKV and emergent viruses during an outbreak in a new territory, data highly valuable for creating models of outbreak prediction and thus design data-based further control measures for the public health system.

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Temporal-spatial model to predict the activity of Aedes aegypti and Dengue from climatic variability in Cuba

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Introduction: The climatic variability, as primary expression of the climate change, is the most significant environmental problem that humanity will face in the next years. On the other hand, arboviruses such as dengue are considered today one of the most important infectious diseases in terms

of morbidity and mortality. Cuba, a Caribbean country is also affected by both situations.The association of vector density and dengue circulation and various climatic elements have been previously published in other settings. Objective: to study the influence of the climatic variability on vector density and dengue cases and to propose a model to predict the future of the vector indexes and dengue cases. Material and methods: The data of the climatic variables (atmospheric pressure, relative humidity, temperature, rainfall, wind speed and direction, UV radiation, and point of dew)were obtained from the climate station network of the Meteorology Institute in the period 1981-2010 for the baseline and 2010-2015 for the current conditions. *Aedes aegypti* data as well as the number of confirmed dengue cases were obtained from the Ministry of Public Health. Results and Discussion: A positive correlation was observed among House and Breteau indexes and Bulto complex indexes. Months with a low, medium and high transmission were identified that correlated to climatic variables. An accumulative effect of the climatic variables on Aedes indexes and virus transmission was observed. A model for the simulation and prediction of vector indexes and dengue cases in spatial and temporal scale from the climatic variability were proposed using of the Bulto complex indexes, which allow to alert and support the arbovirus surveillance. Here we show obtained results.

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Dengue in Colombia: fluctuations in the predominance of virus serotypes linked to the incidence of dengue.

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Introduction: Co-circulation of all four dengue virus serotypes (DENV-1-4) in a particular location may result in complex patterns of competition, leading to fluctuations in the predominant serotype that may result in changes in number dengue cases. All four DENV serotypes are co-circulating in Colombia since 2001. Objective: To investigate the link between the predominant fluctuations of DENV serotypes and dengue incidence in the metropolitan area of Bucaramanga from Santander State, and to provide insight into the genetic diversity of viruses. Materials and Methods: We used both the monthly number of notified dengue cases and DENV isolates for the 2007–2010 and 2014–2017 periods, in the time-series analysis (MATLAB, Mathworks Inc., Natick, MA), to determine whether fluctuations in the prevalence of DENV serotypes and dengue cases were correlated. Full E-gene sequences from isolates belonging to each virus serotype were compared to examine the genetic diversity by using the maximum likelihood phylogeny method (PhyML v.3.1 software). Results: DENV-1 was the dominant serotype followed by DENV-3 or DENV-2 depending on the period and DENV-4 was the least prevalent virus in both periods. Cross-correlation analyses suggest a temporal relation between fluctuations in the DENV serotypes prevalence, which were almost simultaneous (lag= 0) or related to recent past fluctuations (lag>1.0) in the number of dengue cases. Local viruses were grouped into Genotype V, Asia/American III and II for DENV-1, -2, -3 and -4, respectively; intra-genotypic diversity was detected. Discussion/Conclusion: In Santander State, Colombia, a sustained dominance of DENV-1 could have played an important role in the dengue incidence between 2001 and 2017; an increase in the DENV-4 prevalence could be linked to 2009–2010 outbreak; and a switch in the predominant serotype from DENV-3 to DENV-2 could be linked to 2013–2014 outbreak. Passive laboratory-based disease surveillance studies allow an initial understanding of the link between fluctuations in serotypes prevalence and incidence of dengue. The present work highlights the need for comprehensive studies on the dynamics of DENV in Colombia to understand the transmission of dengue and evaluate the effectiveness of a vaccination program.

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Portable DNA sequencing in Brazil: impacting the response to arboviral diseases on the ground, providing a snapshot of their complex dynamic's evolution

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Mosquito-borne viral diseases are infections transmitted by the bite of infected mosquitoes. The burden of these diseases is highest in tropical and subtropical areas and they disproportionately affect the poorest populations. Since 2014, major outbreaks of dengue, chikungunya, yellow fever and zika have afflicted populations and overwhelmed health systems in many countries. Distribution of mosquito-borne diseases is determined by complex demographic, environmental and social factors, causing diseases to emerge in countries where they were previously unknown. Coupling genomic diagnostics and epidemiology to innovative digital disease detection platforms raises the possibility of an open, global, digital pathogen surveillance system. Real-time sequencing, bioinformatics tools and the combination of genomic and epidemiological data from viral infections can give essential information for understanding the past and the future of an epidemic, making possible to establish an effective surveillance framework on tracking the spread of infections to other geographic regions. To understand the molecular epidemiology of the arboviral upsurge, we performed, a mobile nanopore sequencing mission in Brazil. Genomic data and phylogenetic reconstructions were communicated immediately to the Brazilian Ministry of Health authorities to inform the public health response. Real-time analysis of 200 complete genomes from zika, chikungunya and yellow fever, sequenced in the country of origin revealed extensive diversity and phylogenetic intermingling with strains from previous years, suggesting independent and cross border transmission within South American countries allaying concerns of the re-emergence and spread of arboviral diseases in other geographic regions.

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Entomo-virological surveillance of human arboviruses in Aedes aegypti populations

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Entomo-virological surveillance (EVS) of Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV) viruses detects viral RNA or live virus in adult females of *Aedes aegypti* under field conditions. EVS can be valuable to detect DENV and ZIKV circulation because most human infections are asymptomatic. Because human arboviruses such as the above circulate only between people and mosquitoes in urban areas, detecting the virus in mosquitoes that do not fly very far indicates the presence of infectious people around capture sites. Conducting EVS is facilitated because RNA of DENV, CHIKV, and ZIKV can be detected in dead mosquitoes exposed to field conditions for one week. This time interval allows using passive gravid traps at weekly intervals or less. Using passive mosquito traps that do not require power facilitates the deployment of many more traps. Trapping gravid *Ae. aegypti* females also enhances the likelihood of detecting the virus because these mosquitoes had taken at least one blood meal. We show how EVS in *Ae. aegypti* captured in Autocidal Gravid Ovitraps (AGO traps) has allowed us tracking local circulation of urban arboviruses during outbreaks, evaluating the impact of vector control on arbovirus circulation, and assessing the risk for outbreaks following severe storms in Puerto Rico. Next steps include 1- validation and use of mosquito super-pools to significantly decrease the number of tests, 2- explore the use of portable and less expensive emerging RT-PCR tools that can be used by field personnel without extensive laboratory training, and 3- explore if EVS in mosquitoes can be used as a valid proxy for human infections, which would simplify the experimental demonstration of the epidemiological impact of disease control approaches.

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Higher risk for Zika virus seropositivity among sexual partners of index patients from Recife, Brazil

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In 2015-2016, we prospectively identified patients from Pernambuco, Brazil with symptoms of arboviral disease and who tested positive for either Zika or chikungunya virus (ZIKV, CHIKV) through qRTPCR. In 2017, we followed-up with these index patients (IP) and analyzed their seroreactivity to both viruses, as well as the seroreactivity of their household members (HM). Serology data on anti-ZIKV or anti-CHIKV IgG showed that among IP households, there were significantly higher odds for HM also being seropositive for the same virus of the IP (PIP CHIKV+ odds ratio (OR)=3.8 [2.1-6.7], HMIP ZIKV+OR=2.7[1.3-5.6]), reflecting the efficiency of household transmission of both these mosquito (Aedes spp.)-transmitted viruses in an endemic area. To disentangle the relative contribution of sexual and mosquito-borne ZIKV transmission, we then examined the odds of seropositivity of these same HM when they were sub-grouped as sexual partners or non-sexual partners of the IP, and used CHIKV as a control because it is mosquito-transmitted but not sexually-transmitted. As expected, both sexual partner (SP) and non-sexual partner (NSP) HMIP CHIKV+had significantly higher odds of also being CHIKV seropositive (PIP CHIKV+ OR=3.3 [1.3-8.1]; NSP-HMIP CHIKV+OR=4.1 [2.0-8.6]). However, only the sexual partners of IPZIKV+had significantly higher odds of being ZIKV seropositive (SP-HMIP ZIKV+OR=5.3 [1.5-20.4]; P=0.01), while there were no increased odds of ZIKV seropositivity in the non-sexual partners (NSP-HMIP ZIKV+OR=1.9 [0.8-4.6]; P=0.15). Very importantly, ZIKV plaque-reduction neutralizing antibody titers (PRNTs) showed the exact trend as the anti-ZIKV IgG data. These surprising results suggest either that sexual ZIKV transmission is much more efficient than mosquito-borne ZIKV-transmission, or that sexual exposure to ZIKV particles facilitates higher rates of seropositivity to the virus. Furthermore, PRNTs against ZIKV in IP sera dropped from 3.7 Log_{10} in the acute phase of the disease to 2.7 Log_{10} 2 years after infection. Overall, these serological data are important for epidemiological and vaccine research, and indicate that sexual transmission or exposure of ZIKV may be more epidemiologically relevant than previously thought.

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Relationships between dengue effective reproduction number (rt) and incidence with climatic variables are further explained through correlation with additional climatic variables across colombian municipalities

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Climatic variables have been shown to correlate and have potential to predict dengue incidence. However, studies have also demonstrated that the dengue-weather relationship is highly variable across geographies, and even among nearby foci such as municipalities. For this reason, we wanted to investigate the variability of such relationships across 25 Colombian municipalities with continuous presence of dengue cases during years 2011 to 2017. After calculating the effective reproduction number (Rt) across those years, we used Spearman correlation analyses to determine how the relationship between dengue incidence and Rt with several climatic variables – temperature, daily difference of temperature, precipitation, and relative humidity – behaves across Colombian municipalities. We found general patterns between climatic variables with Rt and dengue incidence, such as a trend towards negative correlation between temperature as Rt, as well as precipitation and Rt. However, some municipalities didn't follow the general trend, having a positive correlation between Rt and temperature or precipitation. When

we evaluated the relationship, we noted that the relationship between temperature and dengue incidence was most consistently influenced by minimum temperature and precipitation, and that the calculated Rt relationship was most often correlated with maximum temperature. When we observed these trends with data from Colombian outbreaks and weather data from nearby weather stations, we were able to validate that Rt, while most often driven by precipitation and relative humidity, is significantly positively influenced by maximum temperature when it is above 32°C. On another hand, dengue incidence was also negatively correlated with the minimum temperature when that variable was around 24.3°C. This work reveals that the relationship between dengue incidence, Rt, and climatic variables is not monotonic, and that to capture the totality of the climate-dengue relationship, more than univariate relationships are likely needed.

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The basic reproduction number (R0) of chikungunya in Colombia during 2014-2016 and its correlation with eco-environmental factors

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Chikungunya virus arrived to Colombia in 2014 into a presumed fully susceptible population. This resulted in a quick and intense spread across numerous municipalities, resulting in an epidemic that affected an estimated of 450,000 people. We wanted to analyze the eco-environmental factors associated with the spread of CHIKV that produced significant outbreaks in different municipalities. To do this, we estimated the basic reproduction number (R0) in 85 municipalities, which jointly were responsible of the 65.6% of reported cases in Colombia. At first, we divided municipalities into higher and lower R0 and compared both groups across 13 different environmental and ecological variables like those related to temperature, demographic, and geographical variables. These variables were analyzed by correlation analyses to confirm their association with R0. We found that temperature-related variables are significantly related to higher R0 while other variables like duration of outbreak and size of the urban area are inversely related to R0. We conclude that those municipalities with high R0 associated with high temperatures had fast growth of cases in a shorter time period (with faster cessation of outbreak transmission), resulting in fewer cases than when transmission was associated with lower (but still >1) values of R0 where transmission was slow and steady, resulting in a higher cumulative number of cases. We propose, then, that transmission may follow a tortoise-hare model such as proposed for viremia-based transmission by Althouse and Hanely (2015). That is, slow-and-steady wins the race.

Human Behavior & Community Engagement

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Dengue knowledge and preventative practices in Villa el Salvador, Lima, Perú

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Introduction: Since 2000, the Peruvian Ministry of Health has recorded the presence of *Aedes aegypti* in 41 out of 43 districts in the province of Lima and a number of dengue outbreaks have occurred. As global dengue transmission is predicted to spread, Lima could expect increased outbreaks in the future. Therefore, understanding the population's dengue knowledge, attitudes and practices (KAP) would provide useful information to help target public health campaigns. Objectives: To describe and quantify the dengue related KAP of residents in an urban shantytown in Lima's southern district of Villa El Salvador. Methods: We performed a cross-sectional survey of adults between 18 and 80 years. The survey included knowledge of dengue symptoms, transmission, prevention and current mosquito control practices. Results: Although most of the 240 respondents (97.5%) had heard of dengue only 54.1% knew it was transmitted by mosquitos. The most commonly known dengue prevention practice was covering water containers (65.4%). Fe-

male sex (OR:4.5, CI:2.19-9.61), education level (OR:1.17/yr, CI:1.08-1.26) and the presence of a child

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Dengue characterization in Cartagena de Indias D.T during and epidemic year, 2019

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Introduction: Dengue is a systemic and dynamic infectious disease caused by an arbovirus (DENV), which represents a problem for public health because it has been increasing in incidence and despite the fact that global mortality is less than 1%, During epidemiological weeks 01 to 39 of 2019, 93,533 cases of dengue and 1012 cases of severe dengue have been registered for a total of 94,545 cases, of which 2130 correspond to Bolivar department and specifically in Cartagena were 957 cases recorded. The national incidence of dengue is 252.3 cases per 100,000 inhabitants. Since the epidemiological week 08 cases behavior has been presented nationwide, compared to the behavior in the past years (2011-2018); what puts the country in an epidemic situation. The objective of this study is record the risk factors that could contribute to the development of DENV infection and describe the previous and new knowledge about dengue in population at risk of the disease in Cartagena de Indias D.T during the epidemic in 2019 according to the epidemic pattern of DENV worldwide specially in tropical areas where is endemic. Materials-Methods: This is a cross-sectional descriptive study where KAP type surveys are conducted, administered by researchers to the population that voluntarily decide to participate and whose inclusion criteria is to have presented the DENV infection, taking as a guide the frequency data of the pathology reported by the national and territorial control entities for 2019. Results: We found lack knowledge, attitude and practice in more than 50% of the population surveyed. Especially when the patient already has the symptoms but approximately 70% of the patients assured that they put DENV diagnosis in last place, but when they had heard about a case close their neighborhood the concern increase. Discussion-Conclusion: People living in DENV areas where Dengue has been always endemic has lack of knowledge of strategies of prevention and even thinking they have enough information to prevent factors related with the vector (*Ae. aegypti*) even all the conditions that allow the persistence of the vector in charge of the spread of the virus, but the study demonstrated the opposite.

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Anxiety and depression in pregnant women after a virus Zika sprout in a public hospital

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Introduction: Zika virus infection caused a great epidemic that spread throughout South America and Central America. Congenital malformations in newborns after a pregnancy affected by Zika virus infection caused great concern. Meanwhile, the media generated alarm in the population, especially in pregnant women. The levels of anxiety and depression may have increased after the Zika virus epidemic occurred in 2016. With the objective of assessing the level of knowledge about Zika and determining the levels of depressive symptoms and anxiety in pregnant women attending control. Prenatal at the Regional Hospital of Loreto. Methodology: An analytical cross-sectional study was carried out, with the participation of 178 pregnant women who came to their prenatal control, who were invited to participate in the study responding to a socio-demographic questionnaire and obstetric background, as well as a depression test (Beck) and another Anxiety (STAI). For the analysis, relative frequencies and percentages were used and to find association the chi-square test with a level of significance <0.05. Results: It was found that the average age of the participants was 27.29 years, of urban origin (90.4%), with secondary / higher education level (92.7%). 79.78% were in the third trimester of pregnancy and 72.4% had a previous delivery. 65.31% of pregnant women think that Zika does cause disease and is responsible for miscarriage in 79.59%, and that the fetus does not grow or develop in 68.03%, that baby is born died in 40.14%, develop microcephaly in 57.82% or present some disability in 69.39%. The prevalence of depression found was 14, 12% and that of anxiety was

71.18%. An association was found between the degree of instruction and depression ($p = 0.029$), as well as with miscarriage and Zika anxiety ($p = 0.045$). Conclusions: High levels of anxiety were found more than depression in pregnant women who reached their prenatal control. These values are higher than those reported in other studies and deserve immediate preventive actions, such as greater education and psychological management.

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Social evaluation on an integrated intervention for the prevention of Zika and other Aedes-borne diseases in pregnant women in Mexico

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Introduction. An integrated intervention model for the prevention for Zika and other Aedes-borne diseases (ABD) in 200 pregnant women was conducted in Merida, Mexico (2017-2018). Objective. To make a social assessment this integrated intervention model for the prevention of Zika. Methods and materials. Participants were provided with: Insecticide-Treated-Screens (fixed on doors /windows of their houses), repellent, larvicide, an educational brochure, a thermometer, a Carnet (for laboratory tests), condoms, and access to a 01800-0-ZIKA call center to report cases. A study on social acceptance and perceived efficacy of the intervention was carried-out on a subsample of 30 women. Results. Main acceptance reasons included: worries of ZIK infecting their babies (41.86%); concerns about multiple Aedes-borne diseases circulation (20.93%), that they were pregnant at that time (18.60%), recommendation from relatives (13.95%); and having a relative infected with ZIK (4.65%). The majority of respondents (96.55%) reported effective vector reductions inside their homes, mainly because ITS. Overall, 83.33% of participants reported the use of the topic repellent. Educative information on ZIKA was perceived as good; 53% confirmed that Zika can be transmitted sexually and 43% disagreed. Discussion and conclusions. All participants recommended the scaling-up of the intervention, and considered Zika infection as a dangerous illness to mothers and newborn. Results show that pregnant women can be provided with low-cost integrated methods of known efficacy and educative strategies to enhance maternal-child health for Zika and other ABD.

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Community knowledge, attitudes and practices related to Dengue and its vectors; its relation with the presence of vector's breeding places

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Dengue is the most prevalent arboviral disease. Its main vectors are *Aedes aegypti* and *Ae. albopictus*, that can transmit other arboviral diseases including Zika, Chikungunya and Yellow Fever. Thus an efficient vector control program should have an impact on all these arbovirosis. The government vector control programs and the communication campaigns for behaviour change have not been sufficient to decrease the presence of vectors to a low enough level to permit the decrease in dengue cases. Previous behavioral studies in the Americas have shown that, even if the population has some knowlegde on Dengue and its vectors, there is a low prevalence of good prevention practices and attitudes. Our main objective was to determine the current state of knowledge, attitudes and practices of the population in three jurisdictions of Panama city, where arboviral febrile cases have been high in the last ten years. 900 houses were selected randomly, from these, 305 participated in the study and completed the survey during house to house visits. The survey collected sociodemographic and socioeconomic data, housing characteristics, knowledge on Dengue and other arboviral diseases, attitude on dengue disease and practices for preven-

tion. In order to identify whether there is a significant correlation ($p<0.05$) between knowledge, attitudes and practices, and the presence or absence of vector breedding places in the house and its surroundings, 260 of the surveyed households also agreed to vector inspection. This consisted in identifying breeding places, collecting larva and puppa, as well as aspirating inside the house looking for adult mosquitoes. The quantitative and qualitative analysis of the survey response, and consequent correlation analysis with the collection of vectors, as well as detection of main arboviruses in these samples are currently underway.The psicometric validation of the instrument will be shared. We expect the results of this study will help understand the current knowledge, attitudes and practices, including the motivations, barriers and beliefs associated with the adoption of prevention measures at home. The findings can inform the design of public health intervention and vector control programs, taking into account a deeper understanding of community context and participation, to maximaze efficiency.

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Factors associated with protective practices against mosquito-borne diseases: a survey in Ponce, Puerto Rico

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Introduction: Dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV) viruses transmitted by *Ae. aegypti* mosquitoes present a growing public health challenge. Nearly 53% of people in the world live in areas that are suitable for arbovirus transmission. Puerto Rico (PR) experienced major arboviral epidemics of dengue in 2010 and 2012, chikungunya in 2014, and Zika in 2016Without effective vaccines or therapeutics, vector control represents a necessary measure to prevent arboviral infections. Objective: The purpose of this study was to examine differences in mosquito bite prevention strategies among a representative household sample in the municipality of Ponce, PR. Methods: The Communities Organized to Prevent Arboviruses (COPA) project is a study designed to evaluate the epidemiological impact of a novel vector control intervention. We recruited participants aged 1–50 years from randomly selected households in 38 study clusters. Each participant completed an interview to assess personal protective behaviors and provided a blood specimen. Two dichotomous outcomes were defined to examine the respondents reported avoidance strategies: 1) use of mosquito repellent in the past 30 days and 2) use of three or more of the following mosquito avoidance strategies: screens, exterminator, insecticide, bed net, repellent, eliminating stagnant water, and cleaning debris around the home. Results: Among 3,862 participants, 53% reported the use of repellent, 63% reported the use of three or more mosquito bite avoidance strategies in the past 30 days, and 70% indicated that mosquitoes represent a problem in their communities. Participants using repellent in the past 30 days were more likely to be of older age, female sex, have a higher level of education and income, and report perceiving an increased risk of arbovirus infection (all p

Immunology-Vaccines

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Dengue protein-based immunogen composed by gold nanorods generates high humoral, cellular, and memory responses in challenged mice

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Dengue is one of the most important infectious diseases in the world in terms of epidemiological impact. Consequently, the development of an effective vaccine has been considered a high priority. Although a vaccine is currently licensed in many countries all over the world, doubts about its global efficacy have suggested that it is prudent to keep other anti-dengue vaccine strategies on the pipelines. Nanotechnology is a field of interdisciplinary research involving chemistry, engineering, biology, and medicine, and potential applications include the development of detection/diagnosis' methods and treatments for an array of different diseases. Gold Nanorods (GNR) are of particular interest, especially considering their optical properties, the chemistry of their surfaces and their low toxicity in biological systems. We have designed and tested a new immunogen against the Dengue virus (DENV) employing GNRs covalently functionalized with recombinant DENV3 envelope protein (GNRpE). The construction of the GNRpE immunogen was confirmed by UV-visible spectroscopy, transmission electron microscopy, and atomic force microscopy. Upon mice immunization in a prime-boost-boost protocol with the experimental immunogen, high levels of anti-DENV IgGs and neutralizing antibodies were detected, as well as robust cellular response, with the secretion of important cytokines such as IFN-γ, IL-10, and IL-17a after in vitro stimulation of splenocytes from immunized animals. Immunized mice were challenged with the DENV-3 virus (1x107 PFU/mL) and clinical and pathophysiological aspects were analyzed 48 hours after infection using an acute challenge model infection. Unimmunized and challenged mice had increased hematocrit and vascular permeability, and low platelet counts. Contrarily, animals immunized with the GNRpE immunogen showed no signs of infection, similarly to the non-challenged animals. Also, a post-challenge cytokine profile analysis showed the same results as in vitro stimulation. Finally, post-challenge analyses revealed an accumulation of effector memory CD8 T cells in the spleen of GNRpE immunized mice. Our results indicate that the use of GNRs as immunogen carriers are a viable and interesting alternative in the quest for a still elusive efficient anti-dengue vaccine.

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Prior flavivirus exposure impacts virus-specific and cross-reactive CD8 T cell populations

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More than half of the world is at risk of being infected with a flavivirus; flaviviruses including Zika virus (ZIKV), the four serotypes of dengue virus (DENV), and yellow fever virus (YFV) circulate in many the same geographic regions, making it incredibly likely that a person living in those regions will be exposed to multiple flaviviruses throughout their lifetime. It has been appreciated for some time that flaviviruses share a substantial degree of genetic similarity and consequently antigenic overlap so that specific protective immune responses generated against one flavivirus often are often cross-reactive. Therefore the flavivirus immune response generated following sequential encounters with related co-circulating flaviviruses is predicted to be different compared to an individual expose to only a single flavivirus infection. What is unclear is whether the consequences of cross-reactive immune responses are protective or pathological for subsequent flavivirus exposures. In investigating this question we have hypothesized that cross-reactive CD8 T cell responses are preferentially expanded during subsequent heterologous flavivirus exposure. To test this hypothesis we have used a mouse model comparing primary and secondary flavivirus exposures with either ZIKV followed by DENV or the converse infections. Using standard immunological assays, we noted a preferential expansion of ZIKV specific cross-reactive CD8 T cells in mice that had received DENV followed by ZIKV infection. These ZIKV-specific cross-reactive T cells were functionally superior to CD8 T cells responding to the same ZIKV-antigen but had not been exposed to heterologous dengue viral challenge. Notably this enhanced cross-reactive response came at a cost, as the non cross-reactive ZIKV-specific CD8 T cell population was significantly diminished in mice exposed to heterologous flaviviruses, DENV and ZIKV, as compared to mice that had only encountered a ZIKV flavivirus multiple times. Our study

would therefore suggest that cross-reactive CD8 T cells are more functional then non cross-reactive T cells responding to the same antigen. However this increased functionality associated with cross-reactivity may also cause a narrowing the diversity of the immune response, restricting the number of different flavivirus-specific antigens the CD8 T cell can respond against. These finding have significant implications for the development of a pan-flavivirus vaccine, which could capitalizes on the antigenic similarity between flaviviruses but may restrict the diversity of responses to individual flaviviruses.

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Influence of the application of revised 2009 World Health Organization dengue guidelines in pediatric immunological studies

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Dengue virus (DENV) is responsible for 390 million infections annually worldwide. In 2009 the world health organization (WHO) recommended the use of a new classification for disease caused by DENV infection dividing clinically the dengue cases in dengue without warning signs (DNS), dengue with warning signs (DWS), and severe dengue (SD). As the clinical criteria play a key role in the revised classification, the effect of its use in immunological studies such as those assessing the circulating proinflammatory cytokines is not completely clear. In this study, we analyzed 120 children with confirmed DENV infection clinically ranged from mild to severe and classified them by the 1997 and the 2009 dengue guidelines. Then, we assessed the levels of Interleukin (IL)-6 and IL-8 in plasma and compared the changes in their respective median (range) per group when both classifications are used. When the 1997 classification is used a significant difference between dengue fever and dengue hemorrhagic fever was found. Furthermore, this difference was maintained when dengue fever and dengue hemorrhagic fever were compared with dengue shock syndrome. For the 2009 revised classification, circulating IL-6 and IL-8 were higher in children with SD when compared with DNS. However, in children with DWS constituting the bulk of the children hospitalized by DENV infection, no differences in plasma IL-6 and IL-8 were found when compared to DNS or SD. Thus, the type of classification used to analyze the children with dengue could affect the levels of the cytokines reported.

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A Humanized Mouse Model for Dengue Virus Infection and Vaccination

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Dengue pathogenesis is influenced by viral and host factors and remains incompletely understood. The limitations in our current understanding of dengue pathogenesis may in part be attributed to the lack of an ideal animal model. To address this, we have utilized an immune-compromised mouse strain which is transgenic with human HLA and is reconstituted with a human immune system. The DRAGA (HLA-DR4.HLA-A2.Rag1KO.IL2RgcKO.NOD) mouse model is advantageous as it repopulates the peripheral lymphoid organs with human T and B cells. We investigated if DRAGA mice can support dengue virus (DENV) replication/infection, develop clinical signs of disease, and elicit humoral/cellular immune responses to DENV-1 infection. We found that mice infected with DENV-1 through both intraperitoneal and intravenous routes developed viremia and displayed clinical signs of disease. Infectious DENV from bone marrow and sera from infected DRAGA was propagated in Vero and DC-SIGN-Raji cells ex vivo. Humoral responses included the production of human anti-DENV specific IgM and human cytokines. These data suggest that the DRAGA mouse model has the potential to be a useful small animal model for the testing of experimental vaccines and for the advancement of candidate dengue vaccines to human trials. Ongoing studies are focused on the characterization of tissue tropism and histopathology of the DRAGA mice after DENV infection and on the implementation of the DRAGA model for vaccine evaluation.

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Age- and demography- based avidity assessment of anti-dengue virus antibody response elicited by a live attenuated tetravalent dengue vaccine

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Antibody affinity maturation is a key aspect of an effective immune response to vaccination. Previously, we described a novel assay employing bio-layer interferometry (BLI) and dengue virus-like particles to measure the avidity of antibody responses to Takeda's live attenuated tetravalent dengue vaccine candidate (TAK-003). TAK-003 is comprised of structural proteins from each serotype in an attenuated dengue virus type 2 (DENV-2) genomic backbone. Here, we describe our findings based on age cohort analysis of serum samples from a phase 2 clinical trial, obtained pre- (day 0) and up to one-year (day 360) post-vaccination with TAK-003. Analysis of three avidity parameters (response, dissociation rate-koff and avidity index) revealed a decrease in koff and an increase in both response and avidity index for up to one-year post vaccination in all participants. Sera from baseline seronegative participants aged 1.5-45 years old were divided into four age-ascending groups for assessment. The younger vaccine recipients demonstrated lower koff and higher magnitude of response and avidity index, compared with older participants. These data suggest that antibody maturation elicited by TAK-003 could be activated and sustained for up to one-year post-immunization in younger vaccine recipients from areas of high dengue endemicity. We are now conducting demographic, endemic versus non-endemic, and age cohort-based analyses for avidity parameters in children and adults, to assess antibody affinity maturation profiles elicited by TAK-003.

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CYD-TDV Dengue Vaccine: Alternative Vaccination Schedules with Fewer Doses in Healthy 9- to 50-Year-olds in Latin America and Asia Pacific

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A 3-dose vaccination schedule of CYD-TDV dengue vaccine (Dengvaxia®) demonstrated efficacy in 2 Phase III trials. Efficacy was correlated with neutralizing antibody (NAb) levels elicited by the vaccine. This observer-blind, randomized, Phase II non-inferiority study among subjects 9-50 years of age is being conducted in 2 stages. Stage I, presented herein, evaluates the 28-day and 1-year NAb response (PRNT50) after a 2-dose schedule compared to after a 3-dose schedule. The NAb response after a single dose of CYD-TDV is also described. There were 1048 subjects included (523 in Colombia, 525 in Philippines) and randomized 1:1:1 to receive 3, 2 or 1-doses of CYD-TDV. Of these, 993 subjects were vaccinated and 860 were seropositive before receiving the first dose (mean age 31.1 years). 28 days and 1 year after the last injection of primary series, pre-specified non-inferiority criteria (lower limit of the two-sided 95% CI greater than >0.5 for Geometric Mean Ratios [GMRs]) were met for the 4 serotypes (ST) with the 2-dose vaccination schedule compared to 3 doses in baseline seropositive vaccinees for both the 28-day and 1-year timepoints. The GMRs between a 2-dose and a 3-dose schedule in baseline seropositive individuals at 28 days for the 4 STs were: ST1 1.09 (0.862; 1.39), ST2 0.993 (0.820; 1.20), ST3 0.983 (0.816; 1.18), ST4 0.960 (0.809; 1.14). At 1 year GMRs where: ST1 1.03 (0.757; 1.40), ST2 0.897 (0.705; 1.14),

ST3 0.917 (0.724; 1.16), 0.884 (0.720; 1.09). As a secondary objective, the GMRs 28 days and 1 year after last injection between the 1-dose and 3-dose schedules in baseline seropositive subjects were: at 28 days, ST1 1.44 (1.14; 1.82), ST2 1.19 (0.987; 1.44), ST3 1.12 (0.927; 1.35), ST4 1.10 (0.918; 1.32); at 1 year, ST1 1.24 (0.924; 1.66), ST2 0.979 (0.783; 1.22), ST3 0.818 (0.661; 1.01), ST4 0.862 (0.700; 1.06). 60 Serious Adverse Events (SAEs) were reported up to 1 year after any injection, none were related to vaccination. No dengue cases were reported. This suggests that 2 doses are non-inferior to 3 doses; similar NAb levels could be reached after one dose.

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New Protective Human Antibody Epitopes on Dengue Virus Serotype 3

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Four serotypes (1-4) of dengue virus (DENV) circulate in human populations, and immunity to one serotype does not confer long-lasting immunity to the others. Rather, pre-existing DENV immunity may actually increase the risk of severe dengue after exposure to a second serotype. The possibility of antibody-mediated enhancement has complicated vaccine development because of the need to induce robust immunity to all 4 serotypes simultaneously. After a primary infection, type-specific (TS) antibodies to individual serotypes of DENV is thought to be associated with robust, life-long homotypic protection, but the full repertoire of primary neutralizing antibody epitopes in each DENV serotype remains incomplete. Currently, the only DENV3 TS neutralizing human monoclonal antibody (mAb) is 5J7, which recognizes a complex quaternary epitope spanning 3 protomers of the envelope (E) glycoprotein. Importantly, several studies in natural DENV-infected cohorts suggest only a fraction of the polyclonal response targets the 5J7 epitope and there are additional neutralizing epitopes. To test this hypothesis, we immortalized memory B cells from DENV3 infected individuals from a cohort in Nicaragua. New DENV3 TS neutralizing mAbs were identified that do not use the 5J7 epitope to neutralize DENV3. We designed panels of chimeric DENV3/1 viruses containing increasingly larger transplants of the DENV1-specific 1F4 and 14c10 epitopes into the DENV3 E protein along with chimeric DENV1/3 viruses containing increasing portions of domain I of DENV3 transplanted into DENV1. We tested the genotypic breath of the 15 DENV3 mAbs and used the differences in a susceptible DENV3 genotype versus a resistant DENV3 genotype to construct additional panels of chimeric DENV3 viruses. Using these panels of DENV3/1, DENV1/3 and genotype chimeras, we mapped 15 new human mAbs to 4 distinct areas of the E protein. Thirteen mAbs were mapped by gain-of-function focus neutralization assays. When tested in mice, some of the new mAbs were highly protective of challenge with DENV3. These findings provide new insights into the mechanism of DENV3 neutralization and will lead to assays for defining the primary neutralizing epitopes associated with DENV3 protective immunity following natural infection or vaccination.

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Transcriptome-wide analysis of immunity to vaccination with a live-attenuated tetravalent dengue virus vaccine

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Innate immunity has an essential role in the development of adaptive immune responses to immunization. However, the mechanisms by which distinct or particular combinations of innate immune receptors influence the strength and quality of the protective immune response are poorly understood. We performed transcriptional profiling of whole-blood from healthy adults after vaccination with a replication-competent tetravalent dengue vaccine (TDV), with significant differences in follow-up geometric mean neutralizing antibody titers at one month. Analyses of differential gene expression, splicing, and transcript isoform expression revealed significantly different expression patterns according to the distribution of titers. Transcript isoform expression alterations showed the largest enrichments and effect size in

response to vaccination. Coexpression networks of immune cell subtype-, cell cycle-, and interferon-response-related gene modules positively enriched in the blood two days after vaccination, were strongly associated with the later antibody response. A core signature of blood transcription modules whose positive enrichment was significantly altered by vaccination is shared with the potent live-attenuated yellow fever virus vaccine, YF-17D. Together, our results show that defined sets of temporally expressed genes induced in the blood days after vaccination are predictive signatures of TDV immunogenicity. These observations highlight transcriptional signatures induced in the blood days after vaccination and provide insight into the tunable regulation of adaptive immune responses by the innate immune system.

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The ability of Zika virus intravenous immunoglobulin to protect and enhance Zika virus disease

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The closely related flaviviruses, dengue and Zika cause significant human disease throughout world. The flavivirus specific polyclonal antibody response has the capacity to potentiate disease, or mediate protection upon exposure to a secondary flavivirus infection; although the exact factors responsible for this dichotomy within polyclonal sera are poorly understood. Here we use human flavivirus-intravenous immunoglobulin (IVIG) preparations to understand how the human polyclonal antibody response can protect against, as well as potentiate disease in the context of dengue and Zika virus infection in a mouse model. We evaluated the ability of three IVIGs (ZIKV-IG, FLU-Ig and Gamunex) to neutralize and/or enhance Zika and dengue 2 and 3 viruses in vitro to understand the balance between virus neutralization and enhancement. Next we use this data to predict the IVIG concentrations, which could protect or enhance disease both homologous and heterologous infection in vivo. Results from our in vivo studies with Zika and dengue 2 and 3 demonstrate that a human polyclonal antibody response is cable of driving enhanced disease, and that disease potential occurs when the concentration of IVIG is no longer capable of completely neutralizing virus in vitro. This study demonstrates that using in vitro neutralization and enhancement assays we are able to predict the concentration of antibody capable of driving both Zika and dengue ADE in vivo, and that polyclonal antibody response can drive ADE once antibody neutralization is not complete.

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Ex vivo profiling of innate immune responses to NIAID LATV DENV vaccine candidate in primary human cells

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With nearly half of the world's population at risk of infection, dengue virus (DENV) is the arthropod borne virus of greatest human significance. Annually roughly 2.5 billion people are at risk for DENV infection and the incidence of infection has increased 30-fold since its discovery in the 1950's. At the present time, there are no globally licensed antiviral treatments or vaccines that protect against all four of the DENV serotypes (DENV 1-4). The NIAID LATV vaccine candidate is composed entirely of attenuated variants of the four DENV serotypes with a Δ30 deletion in the 3' untranslated region (UTR). The vaccine candidate encodes all the nonstructural DENV proteins which could be of critical importance in the presentation of DENV specific viral epitopes in a manner that facilitates antigen presentation and confer higher protection to patients. All experiments were carried out in MDDCs. Our lab and others have established that MDDCS can be effectively infected with all four serotypes of DENV. To characterize the infection kinetics of the DENV NIAID vaccine strains we infected MDDCs at 6, 12, 24, 48 hpi and assess for infectivity via qRT-PCR. Quantification of percent infection is quantified via flow cytometry using a 4G2 antibody that binds to flavivirus E pro-

tein. Innate immune activation was measured by secretion of cytokines and chemokines via multiplex ELISA.We hypothesized that the vaccine candidates present in the NIH/NIAID LATV are attenuated not only in their replication potential, but also in their ability to manipulate human innate immune responses, resulting in the generation of robust adaptive immune responses to the vaccines. Recently gathered data indicates that while the NIAID vaccine strains have the same replication kinetics as their wild type counterparts, they show later peaks of replication while also showing increased expression of cytokines like IP-10, MCP1 AND IL-6. Current and future experiments will continue to characterize the innate immune signatures induced by the NIAID LATV vaccines in human primary MDDCs and correlate these signatures to the induction of a robust adaptive immune response in vaccines in an effort to identify markers that have the potential to be reliable predictive measures of vaccine immunogenicity and efficacy.

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A protective Zika virus E-dimer-based subunit vaccine engineered to abrogate antibody-dependent enhancement of dengue infection

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Introduction.Infections with dengue virus (DENV) and Zika virus (ZIKV) can induce cross-reactive antibody responses. Two immuno-dominant epitopes -one to precursor membrane protein (PrM) and one to the fusion loop epitope (FLE) on envelope (E) protein- are recognized by poorly neutralizing cross-reactive antibodies that can promote increased viral replication and disease severity via antibody-dependent enhancement (ADE), a significant concern for both ZIKV and DENV vaccines. In this context, the development of safe vaccine candidates, specifically design to maximize the response against highly neutralizing epitopes while preventing the induction of ADE-prone antibodies is a high priority. Objectives:We aimed to design and produce covalently stabilized ZIKV E-dimers and study their potential as vaccine candidates to target the antibody immune response to the highly cross-neutralizing EDE epitope while minimizing the cross-reactive response to FLE. Materials and methods:ELISAs, FRNT and ADE tests, combined with domain-specific antibody depletion techniques, were used to perform a detailed characterization of the antibody response elicited by ZIKV E-monomers and E-dimers in BALB/c and CD1 mice; while vaccine protection to viral infection was assessed in challenge and pregnancy models using C57BL/6J mice. Results:Our data shows that immunization of mice with ZIKV E dimers, which lack PrM and do not expose the immuno-dominant FLE, induces dimer-specific antibodies, which protect against ZIKV challenge during pregnancy. Importantly, in contrast with the antibodies raised by ZIKV E-monomers, the ZIKV E-dimer-induced response does not cross-react with DENV or induce ADE of DENV infection. Conclusions:Stable ZIKV E dimers are immunogenic, and protect against ZIKV challenge and infection of the placenta and fetus in pregnant mice. As responses to the FLE are minimized, cross-reactivity to DENV is limited, which markedly reduces the ability of ZIKV E-dimer vaccination to prime for ADE of DENV. While we did not find evidence that ZIKV E-dimer immunization could induce quaternary E-dimer-specific antibodies that cross-react to the EDE on DENV, our future experiments will test the feasibility of generating such DENV/ZIKV EDE-specific antibodies.

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Chikungunya virus infection in human monocytes and monocyte-derived macrophages induces TLR expression and both inflammatory and antiviral responses

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Introduction: Chikungunya virus (CHIKV) is a zoonotic arthropod-borne virus which during the last 50 years has caused several outbreaks in tropical and subtropical areas worldwide. Monocytes and macrophages are phagocytic cells of the innate immune system that have been implicated in some aspects of CHIKV pathogenesis. Objetive of study: The aim of this study was to determine the modulation of Toll-like receptors (TLRs) expression, cytokines production and antiviral factors in monocytes and monocyte-derived macrophages (MDM) infected with CHIKV. Materials and methods: Human monocytes were enriched from PBMCs by adherence to the plastic. Then, cultures were incubated overnight to obtain monocytes or for 6 days to obtain MDM. Both cultures were infected with CHIKV and viral replication was measured by plaque assay. Levels of IL-1β, IL-6, IL-8, IL-10 and TNF-α in culture supernatants were measured by CBA. The mRNA of TLRs (TLR2, 3, 4, 7/8), IFN-I, 2',5'-oligoadenylate synthetase 2 (OAS2) and double-stranded RNA-activated protein kinase R (PKR) was quantified by qPCR. Results and Discussion: We reported that human monocytes and MDM are target cells of CHIKV replication in vitro. In addition, we found that CHIKV infection induces TLRs expression and pro-inflammatory cytokines production during the first hours of infection in monocytes and MDM. Furthermore, we correlated an increase in mRNA of IFN-I, OAS2 and PKR with the control of CHIKV replication in monocytes and MDM late in infection.Although additional studies are required to confirm the function of TLRs in monocytes andMDM, our data provide evidence of CHIKV infection activating the TLRs pathway in primary monocytes and MDM, which could be engaged in CHIKV pathogenesis or host defense.

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Down regulation of IL-1β secretion by TGF-β1 in macrophages infected with dengue virus

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Introduction: Several pathogenic mechanisms have been linked to the severity of dengue virus infection, like viral cytotoxicity, underlying host genetics and comorbidities such as diabetes and dyslipidemia. It has been observed that patients with severe manifestations develop an uncontrolled immune response, with an increase in pro-inflammatory cytokines such as TNF, IL-1β, IL-8, IL-6 and chemokines that damage the human microvascular endothelium, and also in anti-inflammatory cytokines IL-4, IL-10 and TGF-β1. The role of TGF-β1 on dengue is not clear; few studies have been published, and most of them from patient sera data, with both protective and pathological roles have described. Aim: The aim of this study was to evaluate the ability of TGF-β1 to regulate the secretion of IL-1β in macrophages infected by DENV. Methodology: THP-1 cells, differentiated with 10 nM PMA for 72 h, were treated with recombinant TGF-β1 before or after DENV infection (MOI=1). Il1B expression and secretion changes were determinate by RT-PCR and ELISA, respectively, after 24 hours of infection. Results: By RT-PCR we did not observe a difference in IL-1β expression between infected cells pretreated with TGF-β1 and those that were not. However, secretion of IL-1β was reduced only in cells stimulated with TGF-β1 before infection, and not in those treated 2 hours post-infection. TGF-β1 receptor blockage with SB505124 inhibitor, prior to the addition of TGF-β1 and infection, abrogated the inhibitory effect of TGF-β1. Conclusion: Our results suggest that DENV could regulate the function of TGF-β1 on macrophages. This negative regulation of the TGF-β1 pathway could be used by DENV to evade the immune response and could contribute to the immunopathology.

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A Phase I Study to Evaluate the Safety, Tolerability, and Immunogenicity of a Live, Attenuated Tetravalent Dengue Vaccine (V181)

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Introduction: V181 is a live, attenuated tetravalent dengue vaccine (LATV) comprised of three full-length homotypic viral components of DENV1, 3 and 4, and a chimeric virus of DENV2 PrM and E proteins on the DENV4 backbone. The LATV was in-licensed from the NIH and manufactured in two formulations (TV003 and TV005) from viral seed stock generated by Merck & Co., Inc., Kenilworth, NJ, USA. TV003 and TV005 are identical in formulation with the exception that the DENV2 component is given at a 10-fold higher dose in TV005. The safety, tolerability and immunogenicity of V181 in flavivirus-naïve and flavivirus-experienced healthy adults was evaluated. Methods: In this Phase I double-blind, placebo-controlled study, 200 healthy adults were randomized 2:2:1 to receive TV003, TV005 or placebo as a subcutaneous injection administered at Day 1 and Month 6. The study enrollment targeted 50% flavivirus-naïve and 50% flavivirus-experienced participants. Standard methods were used to assess safety and tolerability and the immunogenicity was measured using a Virus Reduction Neutralization Test (VRNT) at Day 1 (baseline); 28 days, 56 days, and 6 months postdose 1; and 28 days postdose 2. Results: A total of 200 subjects were enrolled in the study. 100% of the participants received the first vaccination and 91.3%, 91.3% and 90.0% of the participants received the second immunization of TV003, TV005 or placebo, respectively. There were no discontinuations of the study due to adverse experiences in any vaccination group and no serious adverse events reported in Days 1-28 postvaccination. Both vaccine formulations induced strong dengue-neutralizing antibody responses. Responses induced by TV003 were generally similar or higher than those induced by TV005. In the group immunized with TV003, 100% of flavivirus-experienced and 92.6% of flavivirus naïve participants demonstrated seropositivity to three or four dengue serotypes (tri or tetravalent) postdose 1. Minimal boosting was observed in either group following the second immunization. Conclusions: The vaccine formulations were generally safe and well tolerated in healthy adults with balanced tetravalent immunogenicity through 6 months postdose 1. These data support the continued development of V181 for the prevention of dengue disease.

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Inapparent dengue infection induce higher frequencies of polyfunctional central memory CD8+ T Cells

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It has been shown that T cell responses influences strongly the protection against the infection with dengue virus (DENV), however, little is known about the differences in the cell-mediated immunity between individuals who had clinical symptoms or not. The aim of this work was to describe phenotypic and functional differences of memory CD8+ T cell response between DENV immune individuals with previous symptomatic or non-symptomatic disease. Peripheral blood lymphocytes from 35 healthy donors with previous symptomatic or inapparent DENV infection were stimulated using 11 peptides in two different pools. The pool A contained peptides restricted to alleles A*02 and A*24 ($NS3_{(34-44)}$, $NS3_{(259-267)}$, $NS3_{(299-316)}$, $NS4b_{(351-359)}$, $NS4b_{(389-397)}$, $NS5_{(435.443)}$) and the pool B contained peptides restricted to B*07 and B*35 ($NS3_{(34-44)}$, $NS3_{(54-63)}$, $NS3_{(203-211)}$, $NS3_{(245-254)}$, $NS4b_{(342-350)}$,$NS5_{(522-531)}$). The frequency of virus-specific CD8+ memory T-cells and its ability to express cytokines were analyzed by flow cytometry. Independently of the stimulus, frequencies of CD8+ memory T-

cells producing IFNg were similar in these two group of donors. However, after stimulation with pool B, donors with a previous inapparent DENV infection had significantly higher median frequencies of IFNg+IL-2+TNFa+ (0.03% vs 0.008%; p=0.047), IFNg+IL-2-TNFa+ (0.02% vs 0.006%; p=0.031) and IFNg+IL-2-TNFa- (0.11% vs 0.018%; p=0.011) CD8+TCM cells. Additionally, median frequencies of CX3CR1+GrB-IFNg+ CD8+TCM cells were also higher (0.061% vs 0.011%; p=0.0043) in these donors. Interestingly, when the CD8+TEMRA subset was analyzed, donors with previous symptomatic DENV infection had higher median frequencies of CX3CR1+GrB+IFNg+ (0,092% vs 0,024%; p=0.005), CX3CR1-GrB+IFNg+ (0.050% vs 0.007%; p=0,043) and CX3CR1-GrB+IFNg- (0.32% vs 0.03%; p=0.048) CD8+TEMRA cells. These results suggest that polyfunctional DENV-specific CD8+ TCM cells could be involved in long-lived protection against symptomatic infection. Therefore, future vaccine candidates should aim to increase central memory CD8+ T cell polyfunctionality.

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Neutralizing antibody titration against Yellow Fever in immunized persons without a history of arbovirosis.

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Introduction: Forty-seven countries in Africa and America have Yellow Fever-endemic zones (YF). In 2016, Peru reported 61 YF cases, the greatest number of cases since last 5 years. 80-100% of people are immunized with a YF vaccine within 10 days and more than 99% of people are immunized within 30 days. Neutralizing antibodies have high specificity and provide long-term protection, even for life. Some studies reveal the lack of immunity in less than 10 years in people previously immunized, and the factors that can influence this response are not clear. Objective of the study: The present study aimed at determining the neutralizing antibody titer for people being immunized against YF. Materials and methods: 30 participants, residents in the city of Lima (Peru) aged between 19 and 37 years, without history of arbovirosis and negative for neutralizing antibodies against YF, were selected and immunized to determine their neutralizing antibody titers on days 10 and 40 post immunization by the plaque reduction neutralization test (PRNT80) in VERO-76 cells. Results: 100% of the participants showed seroconversion neutralizing antibodies against YF (PRNT80 $\geq$ 1:10) from day 10 post immunization. The geometric means [95% CI] of the neutralizing antibody titers expressed in Log10 were 2,46 [2,31-2,48] and 3,38 [3,25-3,51] on days 10 and 40 post immunization respectively; the minimum and maximum value of the neutralizing antibody titers expressed in Log10 were (1,15-3,24) and (2,97-4,52) on days 10 and 40 post immunization respectively. No significant statistical influence of sex and age was found on the results obtained on day 10 post immunization. Discussion/Conclusion: Such results suggested the existence of populations with similar or lower immune responses that could be at risk of contracting this virus, so it is necessary to continue an epidemiological surveillance of immunized population against YF and the surveillance on the immunity levels achieved after immunization.

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Large scale HLA-tetramer tracking of T-cells throughout dengue infection reveals broad acute activation and differentiation into two memory cell fates

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Introduction: Dengue virus (DENV) is the cause of the most prevalent mosquito-borne viral disease afflicting humans with an estimated 390 million infections per year resulting in 500,000 severe cases and 20,000 deaths. There is no specific therapeutic for dengue and the only licensed dengue vaccine has demonstrated only partial protective efficacy and fails to elicit a dengue-specific T cell response in dengue-naïve individuals. T-cells play important multi-faceted roles during dengue infection and understanding the T cell response during dengue infection is important for defining corre-

lates of protective immunity and identifying effective vaccine antigens. Objective of Study: The goal of this study is to provide an in-depth definition of HLA-restricted CD8+ T cell responses during the course of a natural dengue infection and analyze the impact of epitope immunodominance and HLA type on dengue-specific T cell phenotypes. Materials and Methods: Using mass cytometry and a highly-multiplexed peptide-human leukocyte antigen (HLA) tetramer staining strategy, we probed T-cells from Singapore dengue patients during acute, post-febrile and convalescent infection for a total of 430 dengue and control candidate epitopes together with key markers of activation, trafficking, and differentiation. To understand the interplay between CD8+ T-cells and the wider immune context, we also analyzed by CyTOF a second complementary panel which includes markers for diverse immune subsets and performed Luminex analysis for cytokines in longitudinal plasma samples. Results/discussion: Acute dengue infection causes broad activation of CD4+T, CD8+T-, Vd1+/Vd2+ gd T-cells, mucosal associated invariant T (MAIT) cells, B cells, CXCR5– B cells, plasmablasts, and NK cells. In-depth analysis of dengue-specific CD8+ T-cells using peptide-HLA tetramers shows expression of a unique profile of activation and trafficking receptors that distinguished them from other virus-specific T-cells. During convalescence, dengue-specific T-cells differentiated into two major cell fates, CD57+ CD127- resembling terminally differentiated senescent memory cells and CD127+ CD57- resembling proliferation-capable memory cells. Validation in an independent cohort of patients showed that these subsets remained at elevated frequencies up to one year after infection. These analyses aid our understanding of the generation of T cell memory in dengue infection or vaccination.

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T cell cross-reactivity during heterologous flavivirus infection and its potential impact on viral population dynamics

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In addition to circulating in the same geographic regions, flaviviruses share a substantial degree of genetic, and consequently antigenic similarity. This begs the question: In areas of endemic flavivirus circulation, how does immunity to one flavivirus shape immunity to the next?. Moreover, how does this divergent immune restriction alter the viral swarms that replicate within the host?. Our research efforts are to define how exposure to a heterologous flavivirus impacts the T cell response to ZIKV in a mouse model of infection, and how those T cells in turn impact viral populations within the host. We have shown previously that CD8+ T cells play a critical protective role in a mouse model of ZIKV infection and were able to identify specific epitopes to which the responses are directed in this model. We have also generated data showing that CD8+ T cells expanded during infection with DENV1-4, YFV, Usutu virus, Kunjin virus or WNV functionally cross-react with at least one of these epitopes. We have shown that ZIKV infection in the context of prior heterologous flavivirus exposure results in robust expansion of these cross-reactive T cells at the expense of the ZIKV-specific T cells. Moreover, during heterologous infection, these cross-reactive T cells display distinct phenotypes compared to cross-reactive cells derived from a homologous ZIKV infection, including elevated granzyme B expression and enhanced cytolytic potential. This indicates that heterologous infection results in a unique immunological environment during ZIKV infection, placing distinct selective pressures on the virus as it replicates and disseminates. This led us to investigate how this alternative selective pressure impacts viral intrahost diversity, dissemination, and fitness. This is particularly important due to the neuroinvasive nature of the virus, as previous studies in poliovirus have linked viral intrahost diversity to its ability to disseminate to the CNS. We are currently investigating this question using amplicon-based deep sequencing to identify specific nucleotide variants that emerge during heterologous infection. These studies fully integrate immunology, virology, and evolution to link immune restricting phenotypes and distinct outcomes for the virus, leading to a more thorough understanding of immune cross-reactivity and its impact on viral emergence.

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Identification of Novel Yellow Fever Class II Epitopes in YF17D Vaccinees

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Yellow fever virus (YFV) is a mosquito-borne member of the genus Flavivirus, which includes other important human-pathogenic viruses such as dengue, Japanese encephalitis, and Zika. Herein we report the identification of 129 YFV Class II epitopes in donors vaccinated with the live attenuated YFV vaccine. A total of 1156 peptides predicted to bind 17 different common HLA-DRB1 allelic variants were tested using IFNgELISPOT assays in vitro re-stimlulated PBMC from twenty-six vaccinees. Overall, we detected responses against 215 YFV epitopes. We found that the capsid and envelope proteins as well as the non-structural protein 3 (NS3) and NS5 were the most targeted proteins by CD4 T cells from YF-VAX vaccinated donor. In addition, we designed and validated by flow cytometry a CD4 mega pool composed of structural and non-structural peptides in an independent cohort of vaccinated donors. Overall this study provides a comprehensive prediction and validation of both structural and non-structural YFV epitopes in a cohort of YF17D vaccinated individuals. With the design of a CD4 epitope MP we further provide a useful tool to detect ex vivo responses of YFV-specific CD4 T cells in small sample volumes.

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Detailed characterization of immune responses to a live-attenuated tetravalent dengue vaccine

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Dengue viruses cause a global disease burden of approximately 100 million apparent infections annually, and a safe, effective vaccine continues to be a global health priority. The quantitative immune parameters commonly employed for functional measurement of immune responses to viral vaccines include antiviral antibodies and viral neutralization titers in post-vaccination serum. While neutralizing antibodies correlate with protection against several flavivirus infections, studies of immunity to Yellow Fever virus vaccine (YFV 17D), one of the most efficacious vaccines, have demonstrated that a broad range of immune responses elicited after vaccination may contribute to protective immunity. Takeda's live attenuated tetravalent dengue vaccine candidate (TAK-003) has structural proteins from each serotype on the dengue virus type 2 (DENV-2) genomic backbone. We conducted exploratory immunology studies to assess several attributes of the neutralizing antibody response, including breadth of neutralization against diverse DENV genotypes, specificity and antibody avidity, as well as humoral and cellular responses directed against the viral structural and nonstructural proteins. Randomly selected serum samples from baseline seropositive and seronegative vaccine recipients in phase 2 clinical trials conducted in areas of high dengue endemicity were used in these assessments. To study vaccine coverage, a panel of genetically diverse DENV strains representative of historical genotypes isolated in Asia and Latin America were tested against post-vaccination serum samples. Neutralization was observed across all genotypes and serotypes tested. Avidity analyses indicated that TAK-003 drives affinity maturation of the neutralizing antibody response. Vaccination with TAK-003 was found to significantly increase binding IgG responses to the virus components in the vaccine, and elicit functional DENV-2 NS1-specific antibodies that were cross-reactive with NS1 from DENV-1, 3, and 4. Vaccination also elicited cellular immune responses directed to epitopes across the DENV proteome. The characterization of immune responses elicited by TAK-003 in phase 3 clinical trials is currently underway, to address questions such as similarities and differences in immune response between seropositive and seronegative vaccine recipients, between tetravalent vaccination and primary, secondary or post-secondary natural infections, and the relationship between specific immune response parameters and outcome or severity of subsequent infection.

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Combination of E- and NS1-derived DNA vaccines: the immune response and protection elicited in mice against DENV2

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The occurrence of dengue disease has increased radically in recent decades, causing millions of infections worldwide every year. Currently, the only licensed vaccine against DENV, Dengvaxia (Sanofi Pasteur), has safety issues that restrict its administration to dengue immune individuals and children older than nine years old. In this context, our group constructed the pE1D2 and pcTPANS1 DNA vaccines that encode the DENV2 envelope (E) and the non-structural 1 (NS1) proteins, respectively, and induce protective immune responses in mice. As an approach to decrease the number of plasmids in a tetravalent formulation, we also constructed a DNA vaccine, pNS1/E/D2, that encodes both E and NS1 proteins concomitantly. In the present work, we evaluated the ability of this new pNS1/E/D2 vaccine to mediate expression of the recombinant proteins, as well as to induce a protective immune response in mice. Immunofluorescence analysis of BHK-21 cells transfected with the pNS1/E/D2 showed cells expressing both E and NS1 proteins concomitantly, as well as cells expressing only one of these proteins. Apparently, expression of NS1 was inferior than that of the E protein, probably due to the different promoter regions controlling each expression. To evaluate the immune response elicited by the DNA vaccines, we immunized BALB/c mice with pNS1/E/D2, pE1D2, or pcTPANS1 individually or with a mixture of the two plasmids pcTPANS1 + pE1D2. Animals immunized with pNS1/E/D2, pE1D2 or with the plamid mixture presented significant anti-E and neutralizing antibodies, detected by ELISA and PRNT50 assays. In contrast, antibodies against the NS1 were only observed in mice inoculated with the pcTPANS1 or the mixture of pcTPANS1 + pE1D2 plasmids. We also analyzed the cellular immune response elicited by these DNA vaccines by IFN-gamma ELISPOT assays. Splenocytes from pNS1/E/D2-immunized animals responded to both NS1- and E-derived synthetic peptides, as well as the pcTPANS1 + pE1D2-vaccinated group. All the DNA vaccines generated protection in mice challenged with DENV2, although the combination pE1D2 + pcTPANS1 seemed to be more protective, with no morbidity appearance, followed by the pNS1/E/D2 and pE1D2, with 100% survival and 10-13% morbidity.

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CyTOF profiling of Zika and dengue virus infected human peripheral blood mononuclear cells identifies phenotypic signatures of monotype subsets

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Zika and dengue virus (ZIKV and DENV) are two flaviviruses responsible for important vector borne emerging infectious diseases. While there have been multiple DENV or Zika epidemics in the last decades, our current knowledge about the biology of ZIKV, the disease, and the immune responses in humans is limited because there are no good animal or human models of DENV or ZIKA disease yet.Here, we test human peripheral blood mononuclear cells (PBMCs) from healthy donors infected ex-vivo by ZIKV and DENV as a human model of ZIKA and DENV infection. We used mass cytometry (CyTOF) to perform a detailed characterization of the innate immune responses induced by PBMCs after ZIKA and DENV infection. We found that ZIKV and DENV exposure of human PBMCs induces global phenotypic changes in myeloid cells, characterized mainly by upregulation of co-stimulatory molecules (CD86 and CD40), CD38, and the type I interferon inducible protein CD169, a marker for phagocytic function and cross-priming potential in myeloid cells. Also, we found that ZIKV induces expansion of non-classical monocytes in cell culture. The analysis of the phenotype of the three monocyte subtypes (classical, intermediate and non-classical) at the single cell level identified differences in their expression of CD86, CD38, CXCL8 and CXCL10 during ZIKV and DENV infection. Overall, using CyTOF, we found that ex-vivo infections of PBMCs with ZIKV and DENV reproduced many aspects of the profile found in blood from patients, which highlights the suitability of this system for the study of the human host responses to these viruses.

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Enhanced immunogenicity and reduced viremia in DENV-2-challenged mice conferred by a vaccine formulation based on DIII-C and NS3 proteins

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Introduction: The DENV2-NS3 protein is considered the main target of cytotoxic T cells, mechanism effector of the antiviral immune response. Therefore, the inclusion of the recombinant NS3 protein in the Cuban vaccine formulation, DIII-C-2, might significantly improve its efficacy and provide long-term protection against dengue disease. We previously reported that the NS3 protein expressed in Escherichia coli preserved structural and antigenic determinants of the native virus relevant for dengue vaccine design. In addition, the recombinant protein showed high levels of IFNgamma-secreting cells after stimulation with DENV2 in mice. Objective of the study: In the present study, we evaluated the immune response and protective capacity induced by the DIII-C-2 vaccine formulation containing the recombinant NS3 protein in mice. Materials and Methods: Groups of mice were immunized intraperitoneally with the DIII-C-2 formulation combined with the recombinant NS3 protein, the proteins NS3 and DIII-C-2 alone, and the controls on days 0, 15 and 30. One month after the last dose, the response of anti-viral and neutralizing antibodies, as well as the subclasses of immunoglobulins generated, were characterized. The protective capacity was determined by quantifying the viral load in the mice's brain seven days after the viral challenge. Besides, the levels of cytokine genes expression after the homologous viral challenge were determined by Real Time RT-PCR. Results: The inclusion of the NS3 protein increases the response of Acs IgG in the combined formulation DIII-C-2/NS3 with a subclass balance of Th1/Th2 immunoglobulins. In general, a significant decrease of the viral load was obtained in the group of mice immunized with the combined formulation respect to placebo group, indicating protection in mice. The cytokine gene-expression pattern showed that the combined formulation DIII-C-2/NS3 induced a regulatory response. Conclusions: The DIII-C-2/NS3 formulation induces an immune response efficient in the control of viral infection and reduces the immunopathogenic effect of the antiviral proinflammatory response. This study demonstrates the enhancer function of the NS3 protein on the immune response generated by the DIII-C-2 formulation, as part of the most advanced Cuban vaccine candidate against DENV.

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Codon-optimization of the DENV2 E and NS1 genes for DNA vaccines tested in mice: is it worth it?

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The development of an effective tetravalent vaccine against dengue virus (DENV) remains a priority. The envelope (E) and non-structural 1(NS1) proteins are considered promising antigens in the development of vaccines against dengue because they induce a robust immune response. The protein E acts on adsorption events of the virus to target cells and antibodies against it can be neutralizing. The NS1 is essential for DENV viability and is involved in the early stages of viral replication. Previously, our group constructed plasmids encoding the DENV2 E (pE1D2) and NS1 (pcTPANS1) proteins. These DNA vaccines were able to induce specific immune responses and protection in mice. In attempt to increase the in vivo protein expression and, therefore, to improve the immune response elicited by these DNA vaccines, in the present work we constructed new plasmids containing the E (pEotmD2) and NS1 (pNS1otmD2) genes codon-optimized for expression in mouse and human cells. Human (Huh7) and murine (3T3) cells were transfected with the original and codon-optimized plasmids in order to evaluate the in vitro expression of the recombinant proteins by immunofluorescence and flow cytometry. Results indicated that the pE1D2 was more efficient than pEotmD2 for expression of the E protein, whereas the pNS1otmD2 led to higher expression of the NS1 comparing to the pcTPANS1. Thereafter, BALB/c mice were immunized with one of these DNA vaccines and challenged with a lethal dose of DENV2. ELISA and

IFN-γ ELISPOT essays were carried out to evaluate the humoral and cellular immune responses elicited in these animals. The pEotmD2 was less efficient than the pE1D2 regarding the antibody response, by inducing lower anti-E and neutralizing titers, while the cellular response induced by these two vaccines was similar. In accordance to the antibody response, protection after virus challenge was higher in pE1D2-immunized mice than in animals inoculated with the pEotmD2. Regarding the NS1, the pcTPANS1 and pNS1otmD2 plasmids induced comparable levels of antibody and cellular immune response. The protective effect of these two NS1-based vaccines were also similar. In conclusion, optimization of the E and NS1 genes did not increase the protective immune response elicited in mice.

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Isolation of a ZIKV-neutralizing IgM from a pregnant woman in Brazil as a candidate antibody-based prophylaxis during pregnancy

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Introduction: Congenital transmission of Zika virus (ZIKV) in 7-14% of infants born to ZIKV-infected mothers may lead to lifelong morbidity with symptoms like microcephaly, neurodevelopmental defects, visual impairment, and motor dysfunction. Without licensed vaccines, passive administration of immunoglobulin to pregnant women may be a valuable prophylactic option. Objective: We sought to isolate affinity matured neutralizing antibodies from ZIKV-specific memory B cells in a pregnant Brazilian woman with prolonged viremia. Methods: PBMCs were collected 30 days after viremia cleared. ZIKV-specific memory B cells (CD14-/CD16-/CD3-/CD19-/IgD-) were sorted using fluorescently-labelled UV-inactivated ZIKV, stimulated with EBV and plated at limiting dilution in presence of CD40L-expressing MS40L cells, ODN2006, IL-21 and CHK2-inhibitor for 14 days. A B cell line was derived from a ZIKV+/IgM+ B cell clone, and purified monoclonal antibody (mAb) was tested for ZIKV and dengue virus (DENV) serotypes 1-4 binding and neutralization. An IgG isotype mAb with the same variable regions (DH1017.IgG) was recombinantly produced. Results: Subject B1_0037 had high titers of ZIKV and DENV1-4 neutralizing antibody. Six of 14 Ig+ culture supernatants (5 IgG and 1 IgM) confirmed reactivity with ZIKV (OD450>1). From one immortalized ZIKV+/IgM+ B cell line, we isolated mAb DH1017.IgM. This DH1017.IgM demonstrated enhanced ZIKV neutralization and binding potency as compared to DH1017.IgG (DH1017.IgM FRNT50= 24.7 ng/mL and ED50=474 pM; DH1017.IgG FRNT50= 149.5 ng/mL and ED50=3000 pM). DH1017.IgM bound E protein dimer (E80=228 ng/ml) and weakly to E monomer (E80=934 ng/ml), but not to E domains I and III. DH1017.IgM and DH1017.IgG did not bind or neutralize DENV1-4. Conclusions: From a memory B cell, we isolated a ZIKV type-specific and strongly neutralizing mAb DH1017.IgM, which appears to bind to a quaternary structure epitope displayed on the E dimer. Dh1017.IgM is more potent than DH1017.IgG with similar antigen binding regions. Since IgM cannot be transferred across the placenta, this mAb would not facilitate fetal disease by transcytosis of ZIKV, nor increase risk of severe DENV infection for the newborn. These characteristics suggest a role of IgM memory B cells in protection against ZIKV infection and posit DH1017.IgM as a suitable candidate for a prophylactic ZIKV intervention during pregnancy.

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Evaluación de la infección por los virus DENGUE y ZIKA en el modelo murino

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El DEN (DEN) y el ZIK (ZIK) son flavivirus antigénicamente relacionados transmitidos al hombre por mosquitos *Aedes aegypti*. A pesar de la importancia de estos como patógenos humanos, aún no existe tratamiento específico o vacuna eficaz para su control. Resulta de gran interés evaluar la respuesta inmune cruzada

que estos generan por su posible impacto en la patogenia de las enfermedades que ellos causan, conocimiento que tiene aplicación directa en el diseño y evaluación de candidatos vacunales. El presente trabajo se propuso caracterizar la respuesta inmune inducida por la infección por estos flavivirus en el modelo murino. Para esto se determinaron los niveles de los anticuerpos inducidos en ratones Balb/c inoculados con estosvirus. Se evaluó la respuesta inmune celular específica y de reactividad cruzada frente a antígenos virales, identificando las subpoblaciones. Los resultados mostraron la inducción de respuesta humoral y celular específica pero también cruzada entre estos flavivirus, con diferencias de acuerdo a la vía de inoculación empleada. Se indujeron anticuerpos IgG por ambos virus, siendo los mayores niveles frente al DEN y a predominio de la subclase IgG2a, mientras que en el ZIK predominó la IgG1. Tras el cultivo de las células del bazo de los ratones con el virus DEN se indujeron los mayores niveles de TNF, predominando el IFN en la respuesta cruzada frente al ZIK en las células TCD4+. Por su parte, en los ratones inoculados con ZIK los mayores niveles de IFN se produjeron frente a este virus en respuesta específica por células TCD8+. Los resultados de la presente investigación tienen implicaciones en el diseño y evaluación de vacunas frente a estos flavivirus e imponen evaluar el impacto de la respuesta inmune inducida en el control o patogenia de estas infecciones

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Immunogenicity and Safety of Takeda's Dengue Tetravalent Vaccine in Dengue-endemic Countries: 48-Month Data from a Large Phase 2 Pediatric Trial

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Background: Dengue is the fastest spreading arboviral disease and is one of the World Health Organization's top ten threats to global health in 2019. To assist the global effort to overcome the massive burden of dengue, Takeda has developed a dengue tetravalent vaccine candidate, TAK-003, based on a live-attenuated dengue serotype 2 virus, which provides the genetic "backbone" for all four vaccine viruses. Previous phase 1 and 2 data showed that TAK-003 was immunogenic in both seropositive and seronegative participants, and the vaccine was generally safe and well tolerated. Recently, TAK-003 showed efficacy in preventing dengue fever in an on-going phase 3 pivotal trial (ClinialTrials.gov NCT02747927). Methods & Materials: Here, we present the final 48-month results from a phase 2 randomized placebo-controlled trial (NCT02302066) which assessed the immunogenicity and safety of three dosing schedules (one-dose, two-dose and one-dose plus a booster at 12 months). The trial was conducted in 1,800 participants aged from 2 to <18 years resident in Panama, the Dominican Republic and the Philippines. Immunogenicity and non-serious adverse events were assessed in a subset of 600 participants. Serious adverse events and febrile cases of dengue etiology were assessed in all subjects. Results: The results from the primary and secondary immunogenicity endpoint showed good maintenance of the neutralizing antibody responses elicited by TAK-003 with titers maintained up to 48 months with little or no decline. There were no marked differences by 4 years in terms of immune persistence between the two-dose regimen and one-dose plus a booster at 12 months. There were no related SAEs or any severe dengue cases among trial participants. The febrile illness surveillance indicated significant reduction in virologically-confirmed dengue over the 4 years of trial duration in participants vaccinated with TAK-003 compared with placebo recipients. Conclusion: These results make major and important contributions to the long-term assessment of the immunogenicity and safety profiles of TAK-003.

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Rational development of safe and effective vaccines against Zika virus

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Zika virus(ZIKV) has been reported to cause congenital Zika syndrome in fetuses and infants in the recent outbreaks in the Americas and Caribbean. Currently, there are no licensed human vaccines for ZIKV. Live attenuated vaccines (LAVs) are one of the most important strategies to control flavivirus diseases. The nonstructural (NS) 4B proteins is a critical component of both the virus replication complex and evasion of host innate immunity. We have used site-directed mutagenesis of residues in the highly conserved N-terminal and central hydrophobic regions of ZIKV NS4B protein to identify candidate attenuating mutations. Three single-site mutants were generated of which the NS4B-C100S mutant was more attenuated than the other two mutants (NS4B-C100A, and NS4B-P36A) in the mouse models of fatal ZIKV disease. Mice immunized with the NS4B-C100S were all protected from subsequent wild-type ZIKV challenge. Interestingly, NS4B-C100S induces stronger anti-viral immune responses than wild-type ZIKV. Similar to the NS4B-C100S mutant, ZIKV NS4B-P36A is highly immunogenic and confers host protection against ZIKV infection in mice. Prior work showed that ZIKVE-N154Q mutant, which lacks the envelope (E) glycosylation is highly attenuated in animals. Mice immunized witht his mutant virus developed a robust neutralizing antibody response and were completely protected from wild-type ZIKV challenge. To develop safe and genetically stable LAVs, we next constructed ZIKV mutants with substitutions in NS4B-C100 or NS4B-P36 in combination with the glycosylation mutation in the E protein. We found that the NS4B-P36A/E-N154Q double mutant has signicantly reduced neuroinvasiveness and neurovirulence in mice. The NS4B-P36A double mutant was genetically stable following serial passages in Verocells. Furthermore, the NS4B-P36A/E-N154Q double mutant retains strong immunogenicity compared to the NS4B-P36A mutant and protects mice from wild-type ZIKV challenge. In summary, our results suggest that the ZIKV NS4B/E double mutant serves as a candidate ZIKV LAV due to its attenuated phenotype, high genetic stability, and strong immunogenicity.

Vector Biology-Ecology-Control

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Uso de peces para el control larvario de mosquitos y factores asociados en localidades rurales de Acapulco, México: estudio transversal

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Introducción: Los criaderos más productivos son recipientes convencionales de almacenamiento de agua. Las acciones con pesticidas no han reducido el problema. Existen métodos de control biológico con empleo de peces que disminuyen la densidad larvaria, como fue evidenciado en el ensayo de Camino Verde. Objetivo: conocer la ocurrencia de uso de peces para el control larvario de mosquitos y los factores asociados en conglomerados rurales del municipio de Acapulco, Guerrero. Material y métodos: realizamos un estudio transversal en cinco conglomerados rurales de Acapulco, entre abril y mayo de 2018. Previo consentimiento informado encuestamos 1474 hogares, obtuvimos información sobre variables sociodemográficas, conocimientos del vector, abastecimiento de agua y pertenencia al programa Prospera. Así como acciones de control y uso de peces larvívoros. Realizamos análisis bivariado y multivariado con el procedimiento de Mantel-Haenszel ajustado por clúster para identificar los factores asociados a uso de peces. Estimamos intervalos de confianza de 95% según Cornfield. Resultados: la ocurrencia de uso de peces larvívoros fue 20% (300/1474). Los factores que resultaron asociados al uso de peces para el control larvario de mosquitos fueron: tener pilas y tambos (OR 7.17; IC95%acl 1.81-28.45), hogar con seis personas o más (OR 1.53; IC95%acl 1.10-2.12) y ocupación remunerada del jefe de familia (OR 1.52; IC95%acl 1.04-2.22).

Conclusiones: la alta ocurrencia de uso de peces larvívoros en localidades rurales, como se mostró en el ensayo Camino Verde refleja la iniciativa de las comunidades para buscar estrategias de control del vector Aedes aegypti. Los resultados muestran que la inclusión de peces nativos podría contribuir en el fortalecimiento del programa de prevención y control de vectores.

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Vector competence of Stegomyia (Aedes) albopicta S. (Diptera: Culicidae) for dengue-2 virus circulating in Colombia

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Dengue fever is one of the most priority events in the public health of Colombia because there are continuous virus circulation and transmission during the year. In 2019 have been reported more than 96.000 cases of dengue. The introduction and dispersion of *St. albopicta* in part of the territory and its antecedents as a vector in other countries, lead to suspect that this species may contribute to the maintenance of dengue virus (DENV) transmission in Colombia. There are no official reports about the entomological relevance or participation as a vector of *St. albopicta* in the territory. For that reason, the aim of this project is to evaluate the vector competence of wild St.albopicta for DENV-2 circulating in Colombia. For this, was carried out the collection of *St. albopicta* in the surroundings of the Universidad Icesi (Cali, Colombia), using ovitraps in an transect of 300m. The eggs were taken to CIDEIM, for their breeding and maintenance. The DENV-2 was supplied by the Virology Laboratory of the Instituto Nacional de Salud-Colombia, amplified in C6/36HT cells, and used in the artificial infection of adult mosquitoes through a system of membrane-coated glass feeders. Fifteen days post-infection, we preserved for each mosquito: midgut, wings and legs, and head. The presence/absence of the virus in these samples was determined by nested RT-PCR, in order to calculate the infection, spread, and transmission rates. The results allowed us to postulate the hypothesis that local *St. albopicta* mosquitoes are competent to transmit the DENV-2 (Transmission rate 0.10; Spread rate 0.40 and Infection rate 0.64). This preliminary and innovative study allows the establishment of the species as a potential vector and its role in the maintenance of the transmission and circulation of the DENV-2 in Colombia. This have important repercussions in different fields of action as in the area of public health with the design of surveillance and vector control strategies and in the field of natural sciences in the generation of new projects that investigate the different vector-pathogen interactions.

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Effectiveness of the formulated Delta 5% SC (Deltamethrin), Permex 25 CE (Cypermethrin) and Mafar 57 CE (Malation), from FARMEX SA / PERU, in Aedes aegypti , in the Ramón Martinez Health Area, Cárdenas Municipality, Matanzas, Cuba

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Introduction: *Aedes aegypti* is an insect adapted to the domestic life of man, a vector of diseases such as Dengue, Chikunguya, Zika and Yellow Fever. Among the chemicals used for its control are the molecules of malathion, cypermethrin and deltamethrin. Study area: In the Ramón Martínez Health Area of Cárdenas Municipality, Varadero and Santa Marta localities were selected. With Delta 5 SC, 13 blocks by residual treatment were treated with 1450 dwellings at a dose of 12.5 / liter of water, applied with the Guarany Hand Compression Spray with flat nozzle 80-02, with Permex 25 CE, 7 blocks were treated with 320 homes, at the dose of 10ml/liter of diesel and with Mafar 57 CE, 21 blocks were treated with 1052 dwellings at the dose of 35 ml/liter of diesel, both applied with the Igeba TF 34 thermospray. Results: Blocks treated with Delta 5 SC, in Santa Marta had an HI (Home index) of 2.19 and BI (Bretaux Index) of 4.15 and in Varadero the HI was 3.55 and the BI of 5.52, 6 months prior to treatment, six months after treatment they were zero in both locations. With Permex 25 EC the HI and BI in Santa Marta was 2.91 and 4.05 and in Varadero it was 4.01 and 5.99; 3 months later it was zero in both locations and with the Mafar

57 EC the HI and BI were in Santa Marta of 2.92 and 4.56 and in Varadero they were 4.38 and 5.70 respectively and 4 months later they were zero. Conclusions: The Delta 5% SC, Permex 25 CE and Mafar 57 CE formulation produced a significant reduction in the infestation indexes by *Aedes aegypti* in the blocks treated

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Evaluation of K-Othrine PolyZone 62.5 SC, in the control of residual infestations of Aedes aegypti, in Cárdenas Municipality, Matanzas, Cuba

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The reduction of *Aedes aegypti* infestation is a priority task of vector control programs throughout the country. Objective: to determine the insecticidal efficacy of K-Othrine PolyZone® SC 62.5 applied at resting sites inside and outside dwellings against adults of *Aedes aegypti* mosquito Methods: The Popular Council Heroes of Moncada belonging to the municipality of Cárdenas, Matanzas, which during the last 6 months of 2017 had an index of 0.27 and a Bretau index of 0.38 was the selected area. Susceptibility to deltamethrin was determined before the study started with insecticide-impregnated papers supplied by the WHO. The density of larval and adult infestation in the study area before treatment was determined with Ovitraps. Cone bioassays were performed on the surfaces of the wallsbefore being treated. In addition, the Aedes population after treatment will be controlled by Ovitraps, during the 12 weeks after treatment. The residual evaluation activity was measured once a week after treatment by cone bioassays. The residual treatment with K-Othrine Polizone was carried out at the dose of 10 ml/liter of water in the resting places inside the houses and outside by means of a compression sprayer with a flat fan nozzle. (Spray 30-50 ml/m2 or to point of run-off, in order to left 20-25mg active ingredient/m^2. Results: The strain evaluated behaved as susceptible as the Rockefeller with 100% mortality. Mortality in cone bioassays before treatment showed that there were no residues from other chemical treatments. Before treatments, the ovitraps percentage positive infested with Aedes larvae was between 22 and 33, after treatment between 10 and 15. The mortality percentage is greater than 90 in the cone bioassays up to week 12. The blocks treated (56) showed a significant reduction of infestation rates (House and Breteau index) of 0.47 and 0.66 before treatments to 0.19 and 0.33 after seven months and one year then to 0,21 and 0,26 ; in the 14 blocks with internal and external treatments of 1 and 1.7, at much lower values, five of the months with values of zero and one year after to 0,06 and 0,06. Conclusion: This shows that K-Othrine Polizone 62.5 SC is effective in *Aedes aegypti* control

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Association of vgsc mutations V410L, V1016I and F1534C with phenotypic pyrethroid resistance in Aedes agypti populations of Central America

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Aedes aegypti (Linneaus, 1762, Diptera: Cuilicidae) is the main vector of dengue and other arboviruses (eg. Zika, Chikungunya) in Latin America. Outbreaks of Zika (2016) and more recently Dengue (2019) in Central America reflects the importance of vector control for this mosquito. The main strategy for the control and prevention of these Aedes-borne diseases is based on insecticide vector control, specifically pyrethroids. There is a gap of information about the status of insecticide resistance in Central American countries where a high percentage of the population is susceptible to these diseases. Therefore, we genotyped *Aedes aegypti* populations from sentinel sites in Guatemala, Nicaragua, Costa Rica and Panama to detect the vgscV410L, V1016I and F1534C mutations and associate its presence to their phenotypic resistance status. We found co-occurrence of this three mutations in all populations. The V410L mutation was present in all sites analyzed, representing a wide-spread presence and the first report of this mutation in field populations of Central America. Overall, we observed an association of the V1016I mutation to deltamethrin and permethrin resistance. The F1534C mutation was found to be fixed in all resistant mosquitoes and phenotype/genotype association analysis is still pending for the V410L mutation. Additionally, two distinct haplotypes and evidence of genetic sweep due to external selection pressures were found analyzing the kdr region where the

1016 codon is located. Molecular mechanisms are important to better understand insecticide resistance in *Aedes aegypti* and this study provides new found information to start elucidating the role of vgscmutations in insecticide resistance found in the Central American region.

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Factores asociados a la presencia de criaderos de mosquitos en el hogar, municipio de San Marcos, Guerrero, México: estudio transversal

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Introducción: el vector *Aedes aegypti* se reproduce principalmente en recipientes artificiales con agua. Transmite el virus de dengue, zika, chikungunya y fiebre amarilla. Objetivo: identificar los factores asociados a presencia de criaderos de mosquitos en hogares. Material y métodos: realizamos un estudio transversal en cinco conglomerados del municipio de San Marcos, Guerrero, México. Previo consentimiento informado encuestamos 2589 hogares. Medimos variables sociodemográficas, servicio de agua, saneamiento básico, programa Prospera y conocimiento del vector. Además, realizamos revisión entomológica, registramos el número de recipientes con agua y presencia de larvas y pupas. Calculamos índices entomológicos. Hicimos análisis bivariado y multivariado con el procedimiento de Mantel-Haenszel ajustado por clúster para identificar los factores asociados a presencia de criaderos de mosquitos en el hogar. Resultados: el 50% (1303/2589) de los hogares tuvieron criaderos de mosquitos. El índice de recipiente fue 15% (2081/13511) y Breteau 80% (2081/2589). Los factores asociados a hogar positivo a criadero de mosquito fueron no tener conocimiento sobre el vector (OR 1.91; IC95%acl 1.06-3.46), tener $\geq$5 recipientes con agua (OR 1.84; IC95%acl 1.33-2.55), tiempo de colocación de larvicida $>$2 meses (OR 1.69; IC95%acl 1.15-2.48), usar productos antimosquitos (OR 1.42; IC95%acl 1.15-1.77) y escolaridad $\leq$6 primaria (OR 1.27; IC95%acl 1.01-1.61). Conclusión: la alta frecuencia de criaderos de mosquitos en hogares y la falta de conocimiento del ciclo de vida del vector, muestran que se requieren estrategias que incluyan el diálogo informado con la comunidad para motivar la participación en la prevención y control de criaderos.

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Current situation of arboviruses transmitted by Aedes in Brazil, difficulties and new methodologies in integrated management of vectors under test.

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The presentation of the entomological and epidemiological situation of the presence of Aedes mosquitoes and of the arboviruses transmitted by them in Brazil, together with the macrodeterminant factors that facilitate their infestation and distribution in the national territory. An approach to the 115 years of struggle against the vector and the changes that have occurred in the control programs in the face of the housing and population development of cities, the particularity of actions in favelas with problems of drug trafficking and public safety. Rational use of old and new control methodologies and technologies based on specific epidemiological and entomological situations, new entomovirological surveillance technologies, hotspots, traps, dissemination stations and resistance to insecticides. In summary, a current overview of control in a developing country with climate and urbanism favorable to the dispersal of arboviruses, an experience to assist in the global prevention against mosquito-borne diseases. And Bônus: We have a new scientific film that talks about the interaction mosquito virus produced by Fiocruz with the support of the Pan American Health Organization. Knowing the Aedes mosquitoes, and their interaction with the Arboviruses. Knowing Aedes mosquitoes - arbovirus transmitters Produced in 2018 Duration: 41 minutes The aim of this video is to disseminate knowledge about mosquitoes of the species *Aedes aegypti*, *Aedes albopictus* and *Aedes polynesiensis*, presenting these arthropods as the vectors of Dengue, Zika and Chikungunya, which in recent years have caused serious problems of public health throughout the world. The documentary is permeated by real and virtual images that portray the morphological characteristics, external and internal, of mosquitoes, as well as the evolutionary process of viral infection in the tissues and organs of these arthropods, es-

pecially the extrinsic incubation period, that is, time mosquito, who became infected with one of these types of virus, by stinging a person who was contaminated, becomes able to contaminate new people. The cinematographic originality of this work comes from the strategies used in the construction of the scenes that show unpublished details of the life of the mosquitoes of the genus Aedes. The images were obtained by shooting with HD and high speed cameras, as well as by 3D virtual modeling and animation. The film was produced in Portuguese, English and Spanish. we will have some free copies to offer to the participants of the event.

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Frequency of kdr alleles in Aedes aegypti populations from coastal and high jungle areas in Peru

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The use of chemical insecticides for *Aedes aegypti* control has led to the development of resistance. Pyrethroid resistance has been associated with the co-occurrence of two knockdown resistance (kdr) mutations (V1016I and F1534C) of the voltage gated sodium channel (VGSC) gene. We characterized and quantified kdr mutations in Peruvian *Ae. aegypti*, vector of dengue, Zika and Chikungunya viruses, collected during 2017-2019 from coastal communities in Arequipa, Piura and Tumbes (10) and high jungle communities in Amazonas and San Martin (3). Mosquitoes were genotyped using standard melt curve analysis for the 1016 and 1534 SNPs; ORL1952 and Puerto Rico strains were used as susceptible and resistant strains, respectively. Genotype frequencies were calculated. Amazonas, Piura and Tumbes populations were fixed or nearly fixed ($\geq$95%) for 1534CC, unlike Arequipa and San Martin where 1534FF mosquitoes were found (50-100%). Moderate levels of 1016II were found in populations from Tumbes (31-74%) and Piura (20-39%); very low or no 1016II were found in Arequipa (17%), Amazonas (6%) and San Martin (0-8%). Seven genotypes were observed (IICC, IIFC, VICC, VIFC, VVCC, VVFC, VVFF) with resistant genotypes (IICC, VICC), indicative of higher resistance (20X-60X) recorded in Tumbes and Piura, and less resistant genotypes (VVCC, VVFC, VVFF) indicative of lower resistance (4X) in Arequipa, Amazonas and San Martin. Only the wildtype (VVFF) was found in one San Martin population. Results suggest that pyrethroid resistance genetic markers are widely present in Peruvian *Ae. aegypti*, yet resistance levels vary across regions with a higher frequency of kdr mutations in mosquitoes from Tumbes and Piura, high dengue transmission areas. This may explain why pyrethroid insecticides are not being effective against *Ae. aegypti* in coastal areas in northern Peru. Further sampling and analysis is needed to confirm these results. This study provides insights into pyrethroid resistance mechanisms and may guide mosquito control operations.

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Investigation of the viral circulation of Saint Louis encephalitis viruses and nest west in Rio de Janeiro state

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Epidemics and epizootics caused by arboviroses are reportes wordwide. The Saint Louis encephalitis virus (SLEV) and West Nile virus (WNV) are rboviruses medical and veterinary magnitude in the americas. In Brazil, WNV was first sorology detected in horses from Mato Grosso do Sul in 2011. A case of neurological human disorder was diagnosed in 2015 in Piauí. Detection occurred in equine neurvous tissue that died of neurological syndrome in 2018 in the Espírito Santo state and in 2019 in the Ceará state.SLEV was evidenced in viral meningitis in 2006 in São Paulo and in an equine brain with encephalitis in Minas Gerais. Although the state of Rio de Janeiro is a large herd of horses, there are only few studies on the circulation of these viruses. In this study, through a serological epitope-blocking enzyme-linked immunosorbent assay (blocking ELISA), to perform these two antigens WNV and SLEV were used an investigation for the circulation of WNV and SLEV in healthy horses and with neurological disorders of different regions of RJ. From a total of 435 serum samples submitted to the blocking ELISA, 38 (8.7%) were monotypic reactions to SLEV and 89 (20.5%) presented monotypic reactions to WNV. The highest number and frequency of flavivirus positive animals were in the North (65.8%) and Northwest Fluminense

(67.10%) mesoregions. Detection of positive samples in the epitope- blocking ELISA suggests that RJ horses have been exposed to WNV and SLEV.

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Detection of dengue virus natural infection in Aedes aegypti from northern Peru by portable qRT- PCR and next generation sequencing

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Aedes aegypti, the yellow fever vector, which also transmits Dengue virus (DENV), Chikungunya virus (CHKV), Zika virus (ZIKV) and Mayaro virus (MAYV), is one of the most important vector threats globally. There are different methods for arbovirus detection in *Ae. aegypti* with reverse transcription polymerase chain reaction (RT-PCR) and quantitative real time PCR (qRT-PCR) offering a reduced sample processing time and comparable or better sensitivity than virus isolation. We compared two approaches for DENV detection in field collected *Ae. aegypti*, an optimized Flavivirus RT-PCR and a field-deployable qRT-PCR (Biomeme Dengue Virus subtyping panel) combined with MinION sequencing confirmation. A total of 689 *Ae. aegypti* (74 pools) collected in 2016-2019 from 12 Peruvian military bases (294 mosquitoes, 34 pools) and 22 civilian communities (395 mosquitoes, 40 pools) located in coastal and jungle regions in northern Peru were tested. Lab-reared *Ae. aegypti* experimentally infected with DENV-2 were included as positive controls in addition to standard positive controls. Four and eight DENV positive pools (Ct = 8.9 - 23.19) were detected by FU1/cFD2 RT-PCR and Biomeme qRT-PCR, respectively. All four Flavivirus RT-PCR positive pools corresponded to Biomeme DENV positive pools with Ct higher than 18. RT-PCR amplicons (250 bp) were sequenced; BLASTn analysis showed 97-99% identity with DENV-3 (accession KJ643590.1). Biomeme DENV positive pools were sequenced using MinION; 3 mosquito pools were negative and 5 mosquito pools were positive for DENV-3. MinION sequencing results coincide with FU1/cFD2 amplicon sequencing for 4 mosquito pools. These 4 DENV-3 mosquito positive pools belong to collections performed in 2017 from military and civilian communities in Piura coinciding with a dengue outbreak in this state during the coastal "El Niño". Our results indicate that our RT-PCR/amplicon sequencing approach and the combined portable Biomeme qRT-PCR/MinION sequencing provide comparable results for DENV natural infection detection in field-collected *Ae. aegypti*.

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Evaluation of "Caserotek" a low cost and effective artificial mosquito feeding device

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Laboratory colonies of mosquito vectors are essential for basic biology, vector competence, insecticide resistance, and mark-release-recapture studies. We have developed a low-cost blood feeding apparatus not requiring an external power source (as do Hemotek, or glass feeder systems) to maintain blood at a temperature consistent with warm blooded animals. Our device uses commonly available materials found in low resource environments and can be easily scaled to large mosquito production facilities. We describe and evaluate our artesanal feeding system called "Caserotek" compared to Hemotek and traditional glass/membrane feeding systems that are more expensive and require a power source. We conducted blood feeding experiments simultaneously using three methods: "Caserotek", Hemotek, glass membrane feeder. We compared blood feeding rates (engorged mosquitoes), 30-d survival rates, egg production, egg hatch rates, and adult emergence rates between the 3 methods. Chicken blood was used in the three apparatus, under standardized laboratory conditions: temperature 26-28°C, relative humidity 62-83% (ambient indoor temperature) and photoperiod 12hr daylight/dark in Iquitos, Peru. For each feeding method, three groups of 100 3-5 d starved (water/sugar withheld) female mosquitoes (3 methods x 3 replicates) offered chicken blood

one time per week for 21 days. Feeding was carried out for 30 minutes for all feeding methods and replicates simultaneously. Caserotek had a significantly higher engorgement rate (91.1%) compared to Hemotek (47.7%), and glass feeder (29.3%) ($P<0.0001$) 30d survival were similar among the feeding devices, ranging from 86 to 90%. The average number of eggs laid per surviving engorged female was 59, 36, and 28 for the glass, hemotek, and Caserotek feeders, respectively. There were no statistically significant differences observed in either survival, egg production, or adult eclosion rates between the 3 methods. Our new artificial feeding system had significantly higher blood feeding rates than more expensive artificial systems and equivalent in other fitness parameter. Our system uses a Styrofoam liner to maintain the temperature of the blood which allows easy scalability without requiring an external power source, only the ability to boil water. It can be easily used under austere conditions where mosquito colony maintenance is required.

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Preliminary results of vector competence to dengue-2 and Zika viruses in wild strains of Aedes aegypti with Kdr resistance mechanism

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Aedes aegypti is the principal vector for dengue and Zika viruses in Colombia, where the spraying with pyrethroid insecticides, as lambdacialotrin, is a frequent strategy for mosquitoes' control. Recurrent use of these insecticides increases the resistance by Kdr mechanism, associated to Ile1016Ile and Cys1534Cys mutations in the VGSC gene. Different studies show that these mutations could be related with an increase of vector competence of mosquitoes for dengue and Zika viruses. These study pretend to evaluate the vector competence to dengue-2 and Zika viruses in strains of *Ae. aegypti* with Kdr mechanism for insecticides resistance. *Ae. aegypti* mosquitoes were collected in different areas from Colombia. The resistance to lambdacialotrin was evaluated using OMS assays. Presence of mutations in Val1016Ile and Phe1534Cys loci was verified by allele-specific PCR. Three strains were chosen to feed artificially with dengue-2 and Zika viruses isolated from patients. Viruses were detected in midgut and salivary glands using RT-PCR. Infection and transmission rates were calculated. Sixteen artificial feedings were made, for 501 mosquitoes feeding. The Nunchía and Villavicencio strains were selected by to be resistant to lambdacialotrin (mortality of 43% and 38%). And the Cali-S laboratory strain was used as infection control by its susceptibility to dengue-2 (New Guinea-C). Additionally Cali-S strain was susceptible to insecticide lambdacialotrin. The infection and transmission rates for Nunchía strain were 0,60 and 0,21, and for Cali-S were 0,61 and 0,33, respectively. In these strains, the frequency of the mutant genotype for locus Cys1534Cys was 0,88 for Nunchía strain and one for Cali-S strain, the frequency of heterozygotes in both cases was less than 0,1. However, for locus Val1016Ile the frequencies of the mutant Ile1016Ile and heterozygous Val1016Ile genotype in Nunchia strain were 0,48 and 0,21. Cali-S strain the mutant genotype is absent and the wild genotype frequency is 0,99. Experiments are being done to measure the effect of mutations on the vector competence for Zika virus in the chosen strains. The Cys1534Cys mutation is fixed in both strains and the rates of infection and transmission of dengue-2 virus were variable, so this condition may not influence the vector competence.

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Effectiveness of Actellic® 300 CS (Pirimiphos-methyl 28.9%), a potential ULV formulation against household Aedes aegypti mosquitoes at Monterrey, Mexico

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Spreading of pyrethroids resistance in Latin American countries and elsewhere is moving control programs to newly developed insecticide groups, or to safer and well-known organophosphate compounds. Actellic® 300 CS is a capsule suspension formulation designed to prolong residuality of active ingredient when acting by contact during indoor residual spraying (IRS). However, Actellic® 300 CS field research to assess the impact of its airborne properties, to control endophagic mosquito females has been poorly documented. In addition, considering the advantages of being an organophosphate product along with its very low dermal and oral toxicity (>5000 mg a.i.); we conducted a small scale ULV treatment upon 10-households in Monterrey city, NE Mexico. Our aim was only to explore airborne activity of the capsule formulation in the house environment against resting mosquitoes. Groups of 1-d old female *Aedes aegypti* were placed in 15 × 10 × 30 cm cages distributing two at room indoors, and one at outdoor house backyards. Two control houses were used concurrently. Actellic® 300 CS were prepared mixing a 833-ml commercial bottle up to complete 10 L of water. Average volume of households were 175 m^3, thus we calculated that, a dose of 0.02 g of a.i./m^3 or 20 g/1000 m^3 were sprayed. There are not reports ULV indoor dose, so we decided to use it for our cold fogging trial.Flow rate was 150 ml/min while mean diameter of microdroplet were 23.7 microns. Results: Indoor and outdoor 24-h mortality resulted in 100% for both house areas, respectively. Females *Ae. aegypti* were knocked-down 1-h after spraying as high as 99.2% and 97.5%, indoor and outdoor, respectively. Results shows an optimistic potential of recommending Actellic® 300 CS as a house ULV treatment during severe Dengue outbreaks in Latin America.

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Advances in the biological control of Dengue through the release of Ae.aegypti with Wolbachia in Bello and Medellin; Colombia

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Introduction: Wolbachia is an obligate intracellular insect bacterium, present in approximately 60% of the insect species; it is transmitted in a transovarial manner; and in the *Ae.aegypti* prevents the replication of Dengue, Zika, Chikungunya, Yellow fever, and Mayaro. Therefore, it is a good alternative as a control measure of these viral diseases. The World Mosquito Program-WMP started in Australia, where they have managed to control the transmission of Dengue with the use of this bacterium. In Colombia, it started in 2014 and is currently being used in twelve countries of the world. Objective: Assess the acceptance of the community about the release of *Ae.aegypti* with Wolbachia, the persistence of infection in the native *Ae.aegypti* population, and its efficacy for Dengue control. Materials and methods: After implementing the Public Acceptance Model and receiving approval of most of the population, approximately 120 Aedes with Wolbachia were released sequentially per week for 20 weeks every 2500m^2 in the municipalities of Medellín and Bello (100Km^2). Results and discussion: The establishment of the infection and its persistence in wild Aedes has been achieved for four years. 90% of the population accepts the prevention measure, and the endemic channels of Dengue in both cities show a significant decrease in expected cases. Conclusion: Biological control with Wolbachia can be regarded as the most economical, environmentally friendly, and effective control measure with strong international evidence of effectiveness.

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Spatial and Temporal Population Genetics of Aedes aegypti pyrethroid resistance in Iquitos, Peru

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Pyrethroids target the voltage-gated sodium channel gene (VGSCG) in arthropods and have been an effective method of controlling mosquito populations for several decades. However, resistance to pyrethroids has evolved in multiple mosquito species, including the dengue vector, *Aedes aegypti*. A number of mutations in the VGSCG gene have been identified and at least two single nucleotide polymorphisms (SNPs), F1534C and V1016I, have been shown to be important for pyrethroid resistance in Central and South Amer-

ica. In addition, emerging technologies to examine genome-wide SNPs are available to answer more comprehensive population genetic questions. This study explores the spatial and temporal population genetics of resistance in *Aedes aegypti* in Iquitos, Peru across an 18-year period by examining kdr haplotypes and genome-wide SNPs genotyped from field-collected individuals. The results provide valuable information that may improve understanding of insecticide resistance evolution. Further, evidence is provided that identifies significant heterogeneity in the fine-scale population structure of *Aedes aegypti* in Iquitos. Together these data provide crucial information for improving mosquito control programs and for delaying widespread insecticide resistance. Additionally, these data improve the accuracy of empirical evidence used to model emerging genetically-based mosquito control techniques.

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Arbovirus surveillance, vector-host preferences and genomic characterization of five novel Orthobunyaviruses from Panama

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Decrypted the enzootic arbovirus maintenance in nature is a crucial step for epidemics response and control. A zoonotic alphavirus, Madariaga virus caused its first documented outbreak in Panama, during 2010. We aimed to actively search for vector-host prefences and viral isolation in a MADV endemic region using a modified mosquitoes Trinidad No. 17 baited with different sources (rodents, hamsters, chickens and frogs). We describe the performance of the modified Trinidad trap and the arboviral surveillance. The study was conducted in the community of Aruza, in Darién during the outbreak season (July 2017) and not outbreak (July 2018). In 2017, 5 traps baited with white mice were placed for 3 days. In 2018, there were 12 traps for 5 days (5 with hamsters, 5 with chickens and 2 with toads). Mosquitoes were collected and identified at the species level in the Gorgas Memorial Institute. Animals with evidence of disease were sacrificed and their tissues preserved. The mosquito poles and tissue suspensions were inoculated into vero cells and observed until the appearance of cytopathic effect. The proportion of individuals by species was estimated, using Simpson (D) and Shannon-Wiener (H) indices, by year and type of trap. A complete genome of five viral isolates were generated. A total of 11,276 mosquitoes were collected, represented in 9 genera and 30 species. The most effective sentinel animal were hamsters (9,078 mosquitoes). The species *Cx.(Mel.) Spissipeswas* the most abundant 34.61%, followed by *Mansonia amazonensis* 24.49%, *Coquillettidia venezuelensis* 21.13% and *Cx.(Mel.) pedroi* 8.58%. Among the most abundant species per bait are *Cx.(Mel) spissipes* in mice and hamsters, in chickens it was *Coquillettidia venezuelensis* and *Mansonia amazonensis* in toads. 2017 presented greater diversity: (H = 2,136) and (D = 0.149), against a (H = 1,628) and (D = 0.252) in 2018. Preliminary phylogenetic analysis suggest that isolated viruses represent five new species within the Orthobunyavirus group. In periods of outbreaks the diversity of vector species was greater in 2017, compared to 2018, where no cases were reported by MADV and VEEV in humans. Trinidad traps-17 show efficiency to detect active arbovirus circulation.

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Reversal of resistance to the organophosphate temephos in an Aedes aegypti laboratory strain from Cuba

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Objective: The purpose of this study was to investigate whether resistance to temephos developed in a laboratory strain of Aedes aegyptifrom Cuba could be reversed. Methods: The resistant laboratory strain of *Ae. aegypti*, named SANF6, was left without temephos selection pressure for 12 generations. The level of temephos resistance was determined using WHO bioassays and mechanisms of metabolic resistance were determined based on enzyme activity levels detected by biochemical assays. Bioassays andbiochemical

assays were conducted on the SAN-F6 parental strain and every three reversal generations (SANRevF3, SANRevF6, SANRevF9, and SANRevF12) without temephos selection pressure. Results: After 19 years of keeping the SAN-F6 strain under selection pressure with the LC90of temefos, the resistance ratio (RR50) was 51.5x. Biochemical assays indicated that esterasas,mixed-function oxidase and glutathione s-transferase are still responsible for temephos resistance in this strain. Experiments on resistance reversal showed that temephos susceptibility could be recovered as metabolic enzyme activity levels decreased. Conclusions: The SAN-F6 strain has provided an essential basis for studies of temephos resistance in Cuba. It was demonstrated that temephosresistance in *Ae. aegypti* from this Cuban lab strain is a reversible phenomenon, which suggests that similar outcomes might be expected in field populations. As such, the use of temephos alternated with other larvicides recommended by WHO such as Btior pyriproxyfen is recommended to achieve more effective *Ae. aegypti* control.

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Dynamics spatiotemporal of the transmission of Dengue in Mexican endemic populations during 2014 - 2016

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Introduction: The Mexican towns, Tepalcingo and Axochiapan, are endemic dengue areas because environmental, biological and sociodemographic conditions converge. Knowing the behavior and the distribution of dengue infections (asymptomatic or symptomatic) allows to detect spatial patterns and conglomerates of cases. This information could be useful to detect areas of high transmission risk and to perform intensive surveillance and control activities in these areas. Objective: To describe the spatiotemporal dynamics of dengue transmission in the towns of Tepalcingo and Axochiapan, in the state of Morelos, Mexico, during 2014-2016. Methodology: A Prospective Cohort study was conducted in these towns between 2014 and 2016. A secondary analysis of information collected from subjects older than five years old, evaluated every six months (five surveys and blood samplings), was performed. Serological diagnosis of recent DENV infection was done (Panbio IgM ELISA and capture IgG). Each household was georeferenced. Spatial distribution maps were constructed using Arcgis 10.5 software. Results: An average of 505 people were evaluated at each follow-up, and 175 recent DENV infections were identified throughout the period. 50% of infections were detected at the first follow-up (August-November 2014) and 21.7% at the fifth follow-up (August-November 2016). The spatial distribution and local grouping maps in Tepalcingo and Axochiapan showed a higher frequency of recent DENV infection in the Guadalupe, San Francisco and Buenos Aires (Tepalcingo) neighborhoods, and in Bugambilias and Del Carmen (Axochiapan). The highest frequencies of DENV infection ($>$33%) were located in the northern part of Axochiapan, while the San Francisco and Buenos Aires neighborhoods of Tepalcingo had 100% recent infection of those subjects evaluated in some polygons. We are conducting a multiple analysis for finding asociated factors to conglomerate of Denv transmission. Conclusions: Two neighborhoods in both towns concentrated most of the DENV recent infections, representing most of the dengue transmission in the studied area. These findings evidence the focal nature of the infection in these Mexican towns. Knowing characteristics of the population at risk of DENV infection and detecting spatial patterns, as conglomerates of infections, allows focusing control measures.

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Geographical ecology of Aedes-transmitted viruses: significant variables in Coast, Andes and Amazon

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The dengue virus, Zika and chikungunya, have a similar ecoepidemiology product of sharing the same vector (*Aedes aegypti*) and its ecology in urban and rural areas. The objective was to compare three large biogeographic regions of Ecuador in terms of the limiting or triggering variables for the success of the vector and the incidence and prevalence of these viruses, know-

ing the marked differences in the ecology of the areas and socio-environmental aspects. Eight (18 subvariable) orographic, climatic hydrology, socioeconomic and public services, demographic, epidemiological and entomological variables were evaluated through geospatial analyzes and remote sensors, epidemiological data, vector sampling and vector and historical population census reports. Variables such as orography, slopes and altitude and its association with minimum temperatures and daily temperature intervals limit the success/presence of the vector above 1,700m on both slopes of the Andean mountain range. Annual precipitation patterns and accumulated precipitation, as well as storage patterns due to deficiencies in the supply of drinking water through pipelines, deficiencies in solid waste collection and disposal services define differences in population density and persistence of the vector on the coast and amazon; and demographics such as population density and types of human mobility (mobilization, migration and displacement) determine the incidence rates of dengue and the pattern of entry and spread of Zika and chikungunya. The interrelation and synergy of these variables in a differential and particular way in the three regions of Ecuador, determine the incidence and prevalence of dengue (and outbreaks of Zika and Chikungunya), in a west-east geographical order from highest to lowest in sequence: Coast Pacific> Sierra Pacific slope (less than 1,700 m)> Low Amazon> Amazon mountain range (less than 1,700m)> High Andean highlands (no transmission, greater than 1,700m), and geographical latitudinal north¿ south of Ecuador for epidemic expansion, which is evidenced in a regional pattern segmented by quadrants of the ecology of the disease.

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Precipitaciones acumuladas y el riesgo de la ocurrencia de los brotes de dengue en el Perú, 2000-2017

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Las precipitaciones extienden el hábitat del vector, lo cual posteriormente puede incrementar el número de casos de dengue. Sin embargo, un estudio en el Perú no encontró asociación entre las precipitaciones y la mayor incidencia de dengue. La transmisión del dengue puede ser diferente entre la costa norte con lluvias escasas y estacionales en comparación con la selva con lluvias más frecuentes. El objetivo es determinar la asociación entre el volumen de precipitaciones acumuladas y el riesgo de ocurrencia del brote de dengue en estos dos contextos ecológicos. Estudio observacional, analítico, longitudinal y retrospectivo a nivel de distrito - temporada. La variable dependiente es el tiempo a la ocurrencia de un brote, tiempo desde el inicio de la temporada de precipitaciones hasta el inicio del brote en distrito - temporada, 8 semanas de tiempo de seguimiento. La variable independiente es terciles de volumen de precipitaciones acumuladas totales semanales durante la temporada de precipitaciones. Para determinar la temporada de precipitaciones en la costa norte se empleó como umbral 7mm. de precipitación acumulada semanal, mientras que en la selva fue de 14mm. Los distritos presentaron al menos un brote identificado en 41.7% (83/199) y 20.7% (50/241) en costa y selva respectivamente (p=0.000). El promedio de precipitaciones acumuladas semanal en los distrito de la costa norte es diferente a la selva (10.1±17.7; 35.9±31.8; p=0.0000). En la costa norte el tercil superior de volumen de precipitaciones tiene 3.4 veces el hazard de la ocurrencia del brote con respecto al tercil inferior, ajustado por temperatura y humedad relativa (p=0.000), mientras que en la selva es 1.4 veces el hazard (p=0.089). En la costa norte a un mayor volumen de precipitación habrá un mayor riesgo de ocurrencia de brote de dengue, mientras que en la selva no se aprecia esta relación. Grandes volúmenes de precipitaciones en la selva pueden tener un efecto de eliminar los criaderos del vector.

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Insecticide resistance mechanisms in Aedes aegypti (Diptera: Culicidae) from Boyeros municipality, Cuba

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Background: Since the beginning of the *Aedes aegypti* eradication campaign in Cuba, in 1981, the organophosphate temefos and pirethroids have been the most insecticides used for *Ae. aegypti* control. Among the operational factors that may be affecting the effective *Ae. aegypti* control, is the development of insecticides resistance. We investigated the levels of temephos and pyrethroid resistance and the mechanisms in-

volved in five *Ae. aegypti* field colonies, collected from Boyeros municipality, Havana city. Methodology: Larvae Bioassays were performed according to World Health Organization guidelines and resistance in adults was evaluated through standard bottle assays. The activities of metabolic enzymes were assayed through biochemical tests. Individual mosquitoes were genotyped for 1016 and 1534 sites, based in allele-specific PCR. Larval bioassay indicated high levels of temephos resistance, mainly associated with high α esterase and GST activities and less associated with β esterase and multifunction oxidase activities. Electrophoresis results showed a strong band named EST-A4 in the field colonies but not in Rockefeller strain. Adult mosquitoes showed that all the field colonies were resistant to deltamethrin, but no to lambdacyhalothrin and cypermethrin. The presence of two mutations V1016I and F1534C, "Kdr gene" in adult mosquitoes, responsible for pyrethroid resistance was detected in *Ae. aegypti* field populations. Conclusions: Our results showed that temephos and pyrethroids resistance are emerging at the studied sites, and two metabolic enzymes and two mutations associated with the Kdr gene are present at high frequency, so alternative insecticides should be considered to preserve the effectiveness of the insecticides available in public health for vector control in Cuba.

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Vectors presence and community knowledge about yellow fever as factors for disease transmission in La Macarena (Meta), Colombia

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La Macarena is a Colombian municipality cataloged as one with the highest risk for yellow fever virus (YFV), because of the presence of previous cases, presence of vectors and its ecological, environmental and socio-economic conditions. The aim of this study was to characterize some aspects of vectors ecology, YFV detection in vectors and knowledge of the local community about the transmission and prevention of this arbovirus in La Macarena Meta, Colombia. Entomological surveys (searching for adults and immature stages), were applied in 178 and 103 houses from urban and rural areas respectively, between August and October of 2019. The inspections were conducted in intra, peri and extra-domiciliary environments. Additionally, a survey for community knowledge regarding YFV transmission and prevention was conducted. Immature and adult mosquitoes were taxonomically determined, pooled and preserved in RNAlater® for molecular detection of the virus. A total of 6744 individuals were collected corresponding to 13 genera and 35 species, highlighting the presence of the YF vectors: 1918 *Aedes aegypti* and 61 *Haemagogus janthinomys*. The adult´s infestation of *Ae. aegypti* was 52% in urban and 4% in rural houses and Breteau index was 70.2% and 6% in urban and rural areas respectively. Adults of *H. janthinomys* were found only in rural areas in extra-domiciliary environments and the immature stages were collected from natural breeding places. Currently, the pools of *H. janthinomys* have been processed for YFV detection but all have been negative. Regarding the results of the community knowledge, only 46% of the responders have received information about YFV, 75% knows that YFV is transmitted by a mosquito, 40% recognized the urban vector, 14% recognized the sylvatic vector and only 17% showed the vaccination card. Although YFV has not been detected so far on the mosquitoes from the study area, the high infestation observed by *Ae. aegypti* in the urban area, its presence in the rural area of the municipality, as well as the lack of knowledge about the disease, its prevention and its vectors, are important risk factors for the transmission of YFV and others arbovirus transmitted by *Ae. aegypti*.

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Presencia de Aedes albopictus (Skuse) (Diptera: Culicidae) en una zona urbana hiperendémica al nororiente de Colombia

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Introducción: *Aedes (Ae.) aegypti* y *Ae. albopictus* son los principales vectores en la transmisión del virus Dengue, Zika y Chikungunya y responsables de la incidencia de la gran mayoría de casos de estas arbovirosis, generando un impacto significativo en la salud pública en distintos continentes, así como un alto riesgo para millones de personas en zonas donde circulan los arbovirus. La creciente expansión de *Ae. albopictus* y su registro en áreas urbanas genera preocupación en zonas de alta endemicidad en las cuales *Ae. aegypti* está presente y comparte el escenario ecológico con *Ae. albopictus*. Objetivo: Este trabajo registra la presencia de *Ae. albopictus* en distintos lugares del Área Metropolitana de Bucaramanga – AMB, ampliando el conocimiento de la distribución de estos vectores en zonas urbanas de Colombia. Metodología: A partir de ovitrampas ubicadas en distintas zonas del AMB, se colectó semanalmente durante seis meses muestras de oviposturas, la cuales se llevaron posteriormente al laboratorio para su cría experimental y determinación taxonómica. Igualmente, con los datos iniciales de colecta, se analizaron índice de ovitrampas positivas (OPI), índice densidad de huevos (EDI) e índice de densidad del vector (VDI) para cada localidad. Resultados y Discusión: Se obtuvo un total de 8136 huevos de los cuales se logró establecer 6057 adultos de todas las localidades del AMB. *Ae. albopictus* representa el 35,1% de las muestras, mientras *Ae. aegypti* 64,9%. Se encontró mayoritariamente *Ae. albopictus* al sur del AMB, en áreas con abundante vegetación arbórea. Floridablanca obtuvo mejores resultados en ovitrampas evaluadas con un OPI de 72,1%, EDI 75,3 y un VDI 54,3%, seguida de Bucaramanga OPI de 47,9%, EDI 47,2% y VDI 22,6% y Girón OPI de 31,3%, EDI 38,8 y VDI 12,1%, indicando mayor eficiencia en las ovitrampas dispuestas en Floridablanca. Conclusión: Éste trabajo genera el primer reporte de *Ae. albopictus* en el AMB, teniendo una presencia significativa en zonas de expansión urbanística donde predomina una cobertura vegetal abundante. Además, en algunos sitios los resultados sugieren una competencia interespecífica entre *Ae. albopictus* y *Ae. aegypti*, que puede estar desplazando a este último, tal como se ha reportado en otros estudios.

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Monitoring mitochondrial function of insect cells C6/36 during dengue virus infection

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Aedes aegypti and *Aedes albopictus* mosquitoes are responsible for DENV (DENV) transmission in tropical and subtropical areas worldwide, where an estimated of 3 billion people live with a risk of dengue disease. Human infection with DENV causes a range from sub-clinical or mild to febrile diseases and hemorrhagic fever. Infected mosquitoes do not show detectable signs of disease, even though the virus maintains a lifelong persistent infection. The interactions between some viruses and the host mitochondria are crucial for virus replication and pathogenicity. DENV infection in vertebrate cells modulate mitochondrial function and dynamics to facilitate viral proliferation. Here, we describe the first comprehensive analysis of the mitochondrial functions and morphology during DENV infection in insect cells C6/36 (derived from Aedes albopictus). We showed that DENV infection induces an increase of reactive oxygen species production, enhances mitochondrial transmembrane potential but not induce drastically changes in mitochondrial respiration of mosquito cells. Furthermore, we offer the first evidence that DENV induces translocation of mitofusins to mitochondria.

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Biovaluation of the effectiveness and residuality in the field of pyriproxyfen 0.5% granulated in the control of immature stories (larvas and pupas) of the Aedes aegypti (diptera: culicidae) vector of Dengue virus, Chikungunya and Zika.

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Introduction: Pyriproxyfen is a juvenile hormone analog that affects the metamorphosis of mosquito larvae that develop in water, preventing the emergence of adults. This analogue imitates the juvenile hormone that insects make, in their first (larval) phases, avoiding the change of these larval stages, causing the other quality that it possesses which is also to provoke mortality of the pupae. Objective: Determine the effectiveness and field residuality of the Pyriproxyfen 0.5% G growth regulator in the control of immature stages (larvae and pupae) of *Aedes aegypti*. Determine the percentage of emergency inhibition caused by Pyriproxyfen 0.5% G n) the control of immature stages (larvae and pupae) of *Aedes aegypti*. Materials: 5 Tanks of 200 liters capacity with lid. 5 *Aedes aegypti* mosquito larvae of early stage III or early IV. Methods: This study was carried out following the guidelines of the technical document WHO / CDS / WHOPES / 2001.2, WHO / CDS / WHOPES / GCDPP / 2005.13 and the author's own protocol for evaluation of Pyriproxyfen. Results and Discussion: At 96 days post application the residuality of the Pyriproxyfen 0.5 G growth regulator showed 91.0% larval mortality, 100% pupal mortality and 100% emergency inhibition (PIE). Conclusion: Pyriproxyfen 0.5 G at the field level showed a double control effect: larvicide and pupae, with mortality rates above 90%, at a control period of 96 days after application, likewise the Pyriproxyfen 0.5 G prevented the emergence of adult mosquitoes from larvae exposed to said growth regulator.

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Susceptibility of Aedes aegypti mosquitos from El Paso, Texas to dengue virus

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Dengue is considered the most important viral diseases in humans among the mosquito-borne diseases. Dengue is endemic in tropical and subtropical regions where the vectors *Aedes aegypti*, and *Ae. albopictus*, are distributed. Several dengue outbreaks have been reported in southeast Texas and northern Mexico in the last 30 years. Although *Ae. aegypti* inhabits the entire southern United States (US) border region; there is limited evidence of the susceptibility of this *Ae. aegypti* population to dengue viruses. In this study, we compared the ability of three dengue serotype 2 (DENV-2) genotypes (Southeast Asian, American and Asian/American) to infect low generations of female *Ae. aegypti* (F3) mosquitoes from a US border region (El Paso, Texas). Infectivity rates in *Ae. aegypti* mosquitoes varied for each DENV-2 genotype, ranging from 65% to 88% at 14 days post-exposure. In addition, viral infectious dose and time post-exposure variables were associated with increased infectivity rates. These results demonstrated for the first time that *Ae. aegypti* mosquitoes from this US border community were susceptible to infection with dengue virus. Further vector competence studies involving transmission trials using *Ae. aegypti* mosquitoes for dengue viruses will provide the information needed to assess the risk of DENV transmission in this US border region.

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Intra-specific larval relationship in Aedes aegypti and Aedes albopictus populations under laboratory conditions

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Introduction: Intraspecific density-dependent competition has been considered an important determinant of the dynamics of the larval stages of *Aedes aegypti* and *Aedes albopictus*. The param-

eters of the life cycle that can be altered as a result of the action of intraspecific competition at the level of the larva state are the body weight in a pupa state, the survival, the size of the adults, and the longevity of the females. Taking into account that within the knowledge of the ecology of the vector, the competitive interactions that occur in immature stages of the mosquito can have an impact on the vector competition, the objective of this study was: Determine the effect of larval intraspecific competition caused by high and low density in *Aedes aegypti* and *Aedes albopictus* colonies in the Havana. Methods: The two colonies were evaluated for larval development and survival time, as well as size and survival time in adults. Results: it was found that in high density conditions larval competition significantly shortened development and survival time in the last larval stages. High density during the larval stage also brought about changes in adults from the two colonies, represented by a significantly reduced size and a decrease in survival time. Conclusion: The effect of competition, for density, affects primarily larva to adult survivorship and larval development time. Competitive treatments had no significant effect on the median adult female longevity.

Virology-Pathogenesis-Antivirals

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Antiviral activity of essential oil extracted from Lippia alba against Zika virus

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Introduction: Zika virus (ZIKV) is an emerging flavivirus with an extended geographical distribution and growing importance in public health. Few cases have been reported in Peru; however, after the "El Niño Costero" phenomenon in 2017, the cases of ZIKV increased as a result of seasonal temperature fluctuations. *Lippia alba*, as many other species of the Verbenaceae family, has reportedly showed several medical properties which have promoted its use as sedatives, antidepressant, and pain relievers. Furthermore, current research has proven antiviral activity against the Dengue and Yellow Fever viruses. Objective of the study: To evaluate the antiviral activity of the essential oil from *Lippia alba* against ZIKV using VERO – 76 and C6/36 cell lines Materials and methods: *Lippia alba* samples were collected from the region of Bagua Grande (Amazonas, Peru). The essential oil was extracted by steam drag hydrodistillation and stored at 4°C. The propagation of ZIKV was performed on C6/36 (Salivary glands of *Aedes albopictus*) and VERO–76 (kidney of Cercopithecus aethiops) cell lines. Moreover, the plaque assay was performed using the semi-solid method with a viral quantification of 18 PFU/well, the cytotoxicity test was determined by cell viability stained with naphthol blue-black and the antiviral activity was determined using the plaque reduction inhibitory test (PRIT) in the VERO – 76 cell line. Results: The C6/36 cell line was the best model for the propagation of ZIKV. Moreover, the essential oil of *Lippia alba* elicited cytotoxicity to a concentration $\geq$ 167 µg/ml in VERO-76Finally, the essential oil at a concentration of 13.36 ug/ml reduced approximately 79.4% of ZIKV in 6 days post-infection. Discussion/Conclusion: The essential oil of *Lippia alba* showed antiviral activity in vitro against ZIKV; nevertheless, more research using purified fractionated products from the essential oil and animals models are needed to improve the understanding of their effects and mechanism of action. These results support the use of *Lippia alba* as a possible candidate for the study of antiviral chemical compounds against ZIKV. Ultimately, *Lippia alba* may represent an additional therapeutic option for reducing the viremia caused by ZIKV infection.

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Inflammatory signaling in dengue-infected platelets requires translation and secretion of non-structural protein 1

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Introduction: Emerging evidences identify major contributions of platelets to inflammatory amplification in dengue, but the mechanisms of

infection-driven platelet activation are not completely understood. Dengue virus non-structural protein-1 (DENV NS1) is a viral protein secreted by infected cells with recognized roles in dengue pathogenesis. It has been recently demonstrated that DENV NS1 is able to induce procoagulant events mediated by platelets, but it remains unknown whether NS1 contributes to the inflammatory phenotype of infected platelets. Objective: Here we investigate whether recombinant DENV NS1 induces an inflammatory profile in platelets and if NS1 synthesis and secretion are required for the inflammatory phenotype of DENV-infected platelets. Methods and results: NS1 stimulation induced translocation of α-granules and release of stored factors, namely the chemokines RANTES/CCL5 and PF4/CXCL4, the proinflammatory cytokine MIF and the adhesion molecule P-selectin, which was not inhibited by Polymyxin B. Nevertheless, NS1 did not induce release of newly synthesized IL-1β. Even though both NS1 and DENV were able to induce pro-IL-1β synthesis, only DENV infection signalized for inflammasome activation and IL-1β release by platelets. A more complete thromboinflammatory phenotype was achieved by synergistic activation of NS1 with classical platelet agonists, enhancing α-granules translocation and inducing thromboxane A2 synthesis (thrombin and PAF), or activating inflammasome for IL-1β processing and secretion (ATP). Also, platelet activation by NS1 partially depended on TLR4, but not TLR2/6. Finally, we demonstrate that platelets sustain viral genome translation and replication but do not support the release of viral progeny to the extracellular milieu, characterizing an abortive viral infection. Although DENV infection was not productive, translation of viral genome led to NS1 expression and release by platelets, contributing to the activation of infected platelets through an autocrine loop. Discussion and Conclusion: Altogether, our data demonstrates that platelets are an important source of proinflammatory mediators upon stimulation by endogenous, through an autocrine loop, or exogenous NS1. In conclusion, these data dissect distinct mechanisms for platelet activation in dengue, involving multiple inflammatory signaling from DENV genome translation to engagement of secreted NS1 to platelet TLR4.

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The Role of Dengue Virus Serotype 2 Strain Variation in Differential Neutralization by Dengue Virus Serotype 1 Immune Serum

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A series of dengue virus (DENV) outbreaks in Iquitos, Peru challenges what is currently known about the cross-serotype neutralization abilities of DENV primary immune serum. DENV1 was first introduced into Iquitos in 1990 and elicited DENV1 neutralizing antibodies in approximately 70% of the Iquitos community. Five years later, there was an American genotype DENV2 (AmDENV2) outbreak. Despite a high seroconversion rate during the AmDENV2 outbreak, there were no reported cases of severe dengue disease. Five years after the AmDENV2 outbreak, there was an Asian/American genotype DENV2 (AsAmDENV2) outbreak in Iquitos that caused severe dengue cases. This series of outbreaks demonstrates that in the context of DENV1 followed by DENV2 infection, disease severity depended on the genotype of the DENV2 strain responsible for the secondary infection. DENV1 primary immune serum can neutralize AmDENV2 significantly better than AsAmDENV2. Therefore, we hypothesize that there is a shared structural feature between DENV1 and AmDENV2 that is not present on AsAmDENV2, leading to cross-serotype neutralization of AmDENV2 by DENV1 immune serum. To determine if there is an antigenic structural similarity between DENV1 and AmDENV2, we performed neutralization assays with epitope-mapped monoclonal antibodies (mAbs). DENV1 and DENV2 type-specific mAbs did not uncover any neutralization differences between AmDENV2 and AsAmDENV2. However, a mAb targeting the fusion loop of the E protein revealed differences in the neutralization sensitivity between AmDENV2 and AsAmDENV2.

Preliminary data with DENV1 primary infection serum also demonstrates a similar trend seen with the fusion loop mAb. This suggests that E protein amino acid variations between DENV2 strains may expose the conserved fusion loop region differently in AmDENV2 compared to AsAmDENV2. Comparison of the AmDENV2 and AsAmDENV2 E protein structures indicate that some of the amino acid variations are located at positions that may be important for temperature-dependent viral breathing dynamics. We aim to investigate the effects viral breathing has on cross-serotype neutralization using DENV1 immune serum and AmDENV2 and AsAmDENV2. Understanding viral structural determinants that influence antibody neutralization is important for vaccine development and prediction of outbreak severity.

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Differencial effects of the infection of human platelet precursors MEG-01 cell line with Dengue and Zika virus

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Flavivirus infection is common in tropical countries such as Colombia where Dengue virus (DENV) is considered a public health problem. Dengue symptoms are accompanied by alterations in leukocyte and platelet counts, where thrombocytopenia is frequent during the acute phase. These changes are not commonly found in Zika virus (ZIKV) infection despite its virologic similarities. Peripheral blood is the readily available from patients, but red and white blood cell precursors are localized into the bone marrow and are not typically accessible for research purposes. The goal of this study was to utilize a human platelets precursor cell line to determine the effect of DENV-2 and ZIKV, in vitro. MEG-01 (ATCC CRL-2021), a cell line of megakaryocyte precursors isolated from a chronic myelogenous leukemia patient, were infected with DENV-2 16681 or ZIKV Puerto Rico strain. Relative frequency of infected cells, cell viability and modulation of gene expression were analyzed by flow cytometry, optical microscopy, and quantitative RT-PCR, respectively, at different times of infection. MEG-01 cells were infected by ZIKV and DENV supported by the increased intracellular expression of NS1 and E proteins with a peak at 48 hours post-infection (hpi). It was consistent with intracellular increase of viral RNA. Cell viability measured decreased to 50% at 72 hpi with DENV while ZIKV infection maintained over 90%, similar to Mock treated cells, demonstrating a differential and more pathogenic cytopathic effect to DENV than to ZIKV. CXCL10 and IFN-β gene expression were elevated at 12-24 hpi with DENV treated cells while IDO and IL-1β expression were increased during later period (48-72 hpi). Our preliminary conclusions indicate that both Flaviviruses infect human platelet precursors. DENV but not ZIKV affects the cell viability and generates changes in the gene expression pattern. This finding may suggest these two flavivirus infections affects differentially the thrombopoiesis. Further studies, including those using primary precursors and matures platelets are necessaries to find differential megakaryocyte responses to DENV and ZIKV. This would help to shed light in possible specific markers of disease outcome and help in the clinical differential diagnosis of Dengue vs. Zika disease.

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Antiviral effect of Metformin in Zika virus infection

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Introduction: The reemergence of different arboviruses around the world has generated a worldwide health alert. Among them, the members of the flavivirus genus such as Dengue (DENV) and Zika (ZIKV) viruses can cause different clinical conditions ranging from fever, to fatal diseases in humans and even death in some cases. The co-circulation of these flaviviruses and the possible immunological complications due to previous infections have made difficult to create an efficient anti-DENV vaccine. Metformin is the most commonly used drug to treat type II diabetes around the world. Recently, retrospective studies

have suggested that this drug may have antiviral properties, which correlates with in vitro studies performed during infection with Dengue. Objective: To analyze the ability of Metformin to inhibit ZIKV infection in vitro. Materials and methods: Different cell lines were infected with ZIKV and treated with metformin. Viral infection was analyzed by flow cytometry, focus forming assay, Western blot and confocal microscopy. Results: Metformin was found to have a broad antiviral spectrum in vitro, as it can inhibit ZIKV, as well as, DENV and YFV. This antiviral effect can be observed in different cell types. Conclusion: Metformin inhibits in vitro infection of ZIKV, DENV and YFV. These results lay the basis for studies of the effectiveness of metformin in the mouse model.

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ZIKV induces cleavage of BRD4 to promote viral replication

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Over the last decade, Zika virus (ZIKV) has emerged as a major public health concern due to its connection with microcephaly and Guillain-Barre syndrome. Recent work identified bromodomain protein 4 (BRD4) as a restriction factor for ZIKV infection. Intriguingly, western blotting with anti-BRD4 antibodies of ZIKV-infected HeLa cells revealed a second reactive band as compared to a single larger band found in non-infected HeLa cells. While BRD4 may be expressed as two functionally distinct isoforms, the antibody used here is isoform specific, thus ruling out the possibility that the smaller band is a result of alternatively spliced BRD4. BRD4 has been shown to relax chromatin through histone acetyltransferase (HAT) activity found within the C-terminal domain (CTD). Following displacement of nucleosomes by BRD4 HAT activity, BRD4 then acts as a scaffold to recruit transcription machinery and increase gene expression. I hypothesize that ZIKV cleaves BRD4 to inhibit chromatin restructuring and subsequent gene expression, thereby promoting ZIKV replication. Further experiments showed that, unlike ZIKV, Dengue and Yellow Fever did not produce the second band suggesting the effect is ZIKV-specific. Additionally, transfection of individual ZIKV proteins or NS2B-NS3 protease did not produce the second band. My project focuses on answering two principal questions. First,by what mechanism does ZIKV induce BRD4 cleavage? Second, how does cleavage-based repression of BRD4 promote ZIKV infection? I will first determine whether BRD4 is cleaved by developing a plasmid expressing a dual-tagged BRD4 to detect and analyze the cleaved fragments to identify the cleavage site via targeted mass spectrometry. Next, using ATAC-seq, a method for characterizing chromatin accessibility, combined with BRD4 targeted ChIP-seq, I will study chromatin remodeling in infected and non-infected HeLa cells and compare this to cells expressing cleavage-resistant BRD4 mutant. I expect to identify BRD4 regulated genes that upon infection are more compact due to ZIKV induced BRD4 cleavage, and therefore are important for productive infection. Characterizing the role of BRD4 in ZIKV infection may elucidate a novel mechanism by which ZIKV promotes replication through epigenetic modification. Clarification of these pathways may lead to novel targets for antiviral therapeutics.

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Identification in silico of natural compounds as possible inhibitors of the Dengue virus NS5 protein

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Introduction: Dengue is one of the viral diseases of greatest medical importance worldwide. Currently, there is no specific treatment to reduce the viral load. Within the dengue virus (DENV) proteins, NS5 has been considered as an attractive therapeutic target, due to it is the most conserved viral protein among flavivirus. Numerous studies have shown the promising antiviral effect of many naturally occurring compounds. Aim of the study: To identify new possible anti-DENV molecules of natural origin using computational tools. Methodology: 1) The 3D structures of NS5 ProSA tools. 2) Virtual screening was performed on the NS5 Methyltransferase and Polymerase domains of each DENV serotype using the DrugDis-

covery @ TACC server. 3) Chemoinformatic filters were implemented including prediction of solubility, compliance with Lipinski rules and prediction of toxicological risks (carcinogenic, hepatotoxic, mutagenic, immunotoxic and cytotoxic effects) using the Swissadme and Protox II tools. Results: A total of 194,090 natural compounds were identified by the initial virtual screening, however the best 1000 compounds for each protein and domain were selected, obtaining a total of 8000 compounds. After applying the different prediction filters, evaluating its performance and binding energies, we finally obtained 9 compounds as possible inhibitors of methyltransferase activity and 10 inhibitors of DENV polymerase activity. According to our results, these 19 compounds of natural origin are potential candidates to be evaluated against the 4 DENV serotypes in in vitro tests. Conclusion: We found compounds similar to other previously reported compounds with alkaloid-indole Hirsutism-type antiviral activity. Therefore, we propose that our compounds could have promising anti-DENV effects.

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Effective Control of Early Zika Virus Replication by Dengue Immunity is Associated to the Length of Time Between the 2 Infections but is not Mediated by antibodies

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Zika virus (ZIKV) infection remains a public health concern with the recent epidemic infecting millions of people in the Americas. This flavivirus 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 studies have claimed that pre-existing immunity to ZIKV may enhance DENV pathogenesis. Prior exposure to a single serotype of DENV predisposes individuals to severe disease upon secondary heterologous DENV infection, but little is known about the contribution of virus-specific and cross-reacting antibodies or the cellular immune response generated by a primary DENV infection on the course of a secondary ZIKV infection in vivo. Here we show that the length of time between DENV/ZIKV infections has a qualitative impact on controlling ZIKV replication. Sixteen rhesus macaques with different immunological history (n=6 and n=4 infected with DENV-2 at two different points of time, n=6 naïve animals) were infected with ZIKV. qRT-PCR for measurement of viremia in serum, plaque reduction neutralization assays, ELISA tests, DENV-2 antibody depletions and cell phenotyping via flow cytometry were performed. qRT-PCR results show that viremia in serum was shorter in macaques that were exposed to DENV twelve months earlier in comparison to naïve animals and the cohort exposed to DENV three months earlier. Although cross-binding antibodies were detected, previous exposure to DENV has little or no impact on ZIKV neutralization. Furthermore, depletion of DENV2-specific antibodies in sera confirmed that these type-specific antibodies generated during a previous exposure do not contribute to ZIKV control. We conclude that there is a degree of cross-protection between primary DENV and subsequent ZIKV infection that is time-dependent. A one year time-lapse between DENV and ZIKV infections could positively modulate the immune response to induce protection, and taken together, our results suggest that early after ZIKV infection the humoral response does not contribute to ZIKV replication control. Other mechanismssuch as the cellular immune response may play a predominant role in mediating this viral control.

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Antiviral activity of beta-caryophyllene sesquiterpene against Zika virus

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Introduction: Beta-caryophyllene is a bicyclic sesquiterpene with a rare 1,1-dimethylcyclobutane ring, commonly found in many essential oils from several plants. Viral infections, such as arboviruses, are a serious public health problem, especially in tropical countries with favorable environmental conditions for the development of vector mosquitoes, such as Brazil. This study aimed to investigate the antiviral activity of beta-caryophyllene against Zika virus (ZIKV). Materials and Methods: All chemicals, including beta-caryophyllene, were obtained from Sigma–Aldrich Co. The sample was diluted in culture medium L15 (Cultilab^{TM}) to a concentration of 2mg/mL by adding specific surfactants (3% DMSO) for the cytotoxicity assay. Vero cells were used to study viral activity, cultured in L15 culture medium, containing tryptose and supplemented with fetal bovine serum. Cells were grown in a greenhouse at a temperature of 37°C, humidified to 5% CO_2 at a cell density of 2x105 cells/mL. Subsequently, Vero cells underwent ZIKV infection. The sample was used at concentrations 1000; 500; 250; 125; 62.5 and 31.25 µg/mL. Antiviral activity assays were used in three distinct tests: pretreatment; simultaneous activity and posttreatment. The cytotoxicity of the sample was evaluated by the MTT colorimetric assay and absorbance reading on a spectrophotometer at 540 nm. All experiments were performed in triplicate and analyzed with GraphPad Prism software 5.0. Results: Cytotoxicity assays are relevant for testing involving viruses as the concentration thresholds that do not cause cell toxicity should be determined and antiviral activity protocols initiated. It was possible to quantify the cytotoxicity on the Vero cell monolayer of cells, whose CC50 was 797.35 μg/mL, indicating low cytotoxicity at the tested concentrations. Beta-caryophyllene exhibited antiviral action at the three stages of the biological test: pretreatment (74.38 μg/mL), simultaneous activity (354.3 μg/mL) and posttreatment (212.7 μg/mL). Discussion/Conclusion: The highest antiviral action was found during the pretreatment test, which allows to verify the ability of the samples to prevent virus adsorption to the cell and/or its cellular penetration. The extracellular action of this sesquiterpene on the viral particle may be associated with the low polarity of these compounds, which may facilitate interaction with the phospholipid bilayer.

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The aryl hydrocarbon receptor inhibition blocks the dengue and Zika flavivirus infections

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that has been classically associated with the clearance of xenobiotics. Recently, numerous studies have indicated that AHR is capable of modulating the immune system. Regarding to viral infections, it was shown that the stimulation of AHR not only reduces the survival of mice infected with influenza A virus, but also enhances the hepatitis C virus assembly and production in vitro. Dengue virus (DENV) belongs to the Flaviviridae family and has four serotypes (DENV-1,2,3,4) that are capable of causing illness in humans after the bite of infected mosquitoes of the genus Aedes. Nowadays, specific treatments do not exist, thus it is crucial the development of new antiviral strategies. Previous results from our group indicates that the blockade of AHR, inhibits the Zika Virus (ZIKV) replication in vitro. In the present study we evaluated the impact of the pharmacological modulation of AHR on the DENV infection in vitro. Agonists and antagonists of AHR were used to treat A549 cell cultures before the infection with DENV1-4. From the supernatants of the cultures, we determined the viral yields. We also performed, real-time RT-PCR and indirect immunofluorescence to quantify the viral genome and protein, respectively. The treatment with 20µM of the AHR antago-

nist CH223191 decreased the viral yield 95±4% and the protein expression in a 84,5±0,5%. The data obtained suggests the AHR signaling pathway is involved in the DENV and ZIKV replication in vitro, whereby it is a potential therapeutic target against flaviviruses.

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MAYV characterization and analysis of antiviral effects of signalling pathway inhibitors

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MAYV belongs to the genus Alphavirus, family Togaviridae that also includes the CHIKV. MAYV is found mainly in Central and South America causing a febrile illness followed by arthralgia that can persist for long periods of time. MAYV fever is considered a neglected disease due the few epidemiologic data and in Brazil is a potential public health hazard with the number of suspicious cases increasing every year. The aim of this work was to characterize our MAYV sample. Accordingly, we started with multiplication curves in VERO cells with different MOIs followed by transmission electron microscopy to the analysis of the multiplication cycle. We observed a fast multiplication cycle, with a short eclipse phase of 4 h.p.i. followed by the exponential phase with the peak of multiplication occurring at 18 h.p.i. with all cell monolayer destroyed at 24 h.p.i. The RNA synthesis started around 2-3 h.p.i. followed by virus production at 4 h.p.i. and particle liberation at 5-6 h.p.i. In the antiviral tests, we observed that the MEK/ERK and JNK inhibitors reduced at more than 90% the viral load at 15 h.p.i. The TEM results corroborated what we saw in the multiplication curves with vesicles with virus (post entry) being observed until 2 h.p.i., spherules and virus budding 4 h.p.i. and cytoplasmic vacuoles 5 h.p.i. We also observed a great variety of forms of cytoplasmic vacuoles surrounded by viral precursors, organized spherules-like structures close to a pseudo viral factory and presence of polymorphic giant forms. We intend to establish a timeline of events during MAYV however we still need more data specially about the earlier stages of infection, for that we intend to analyse the multiplication cycle at a lower MOI by TEM. Besides that, the next steps will be a more robust investigation with the MEK/ERK and JNK inhibitors and the beginning of the animal studies using the C57BL/6 model

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Activation and secretion of NF-Kappa B by platelets exposed to dengue virus

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Introduction:Severe dengue can lead to important vascular complications secondary to haemoconcentration and thrombocytopenia. Physiopathologically, the virus activates platelets by binding to their receptors. Experimental evidence indicates that physiologically the activation of platelets with thrombin involves phosphorylation of the p65 subunit of the NF-Kappa B molecule. However, the role that NF-Kappa B plays in the pathophysiology of dengue infection is unknown and whether this molecule could serve as an early marker of vascular damage, since the activated molecule can be transferred to endothelial cells among others. Objective of the study:To evaluate the activation and secretion of NF-Kappa B in platelets exposed to dengue virus. Materials and methods: Human platelets were washed and the platelet population was characterized by flow cytometry using the CD41 marker (specific platelet marker). -From the supernatants a protein concentration was made with ammonium sulfate followed by a western blot for NF-Kappa B, p-NF-Kappa B and alpha-tubulin. Results: 97.4% of platelets constitutively expressed CD41. Platelet activation corresponds to 44.1% of the population expressing the CD62P marker. The expression of NF-Kappa B in both dengue and untreated platelets was evaluated by western blot. Conclusion: The platelet population is of high purity and expresses the CD41 marker. Platelets constitutively express NF-kappa B and the phosphorylation of NF-Kappa B depends on the stimulus.

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The role of NS2A and NS2B proteins in the activation of NLRP3 inflammasome in an endothelial cell model

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INTRODUCTION: Dengue virus (DENV) is a member of the Flaviviridae family and it causes dengue disease. It has been reported that DENV manipulates processes for its own benefit, such as cell cycle, vesicular traffic, metabolism and functions related to the immune system, like inflammasome. DENV-2 is able of activating the NLRP3 inflammasome, a multiproteic complex that is part of the immune response and participates in the processing and release of proinflammatory cytokines (IL-1β and IL-18). Although the activation of this complex has been reported, the mechanism remains unclear. OBJETIVE: To analyze the proteins of DENV that induces activation of inflammasome in an endothelial cell model. MATERIALS AND METHODS: HMEC-1 (A human microvascular endothelial cell line-1) cells were infected with DENV-2 at 5 MOI at 37°C for 2h. ATP (5mM) stimulation was used as a positive control for NLRP3 inflammasome inducer and cells with heat-inactivated virus as a negative control. On the other hand, HMEC-1 cells were primed with LPS (2μg/ml) followed by transfection with NS2A-GFP and NS2B-GFP proteins. Subsequently, the cells were lysed in RIPA buffer for protein extraction and 30μg of protein was subjected to Western Blot (WB). The expression of the components of the inflammasome was analyzed using complex markers (NLRP3, ASC) and the activation was evaluated by expression of active caspase-1 and secretion of caspase-1-dependent IL-1β through ELISA assay. RESULTS: In this work we address the ability of NS2A and NS2B, two like-viroporins of dengue virus to stimulate the NLRP3 inflammasome pathway. We found that dengue infection increases the expression and ASC oligomerization and further IL-1β secretion after infection, through caspase-1 activation. Thus we observed that NS2A colocalize with NLRP3 and NS2A and NS2B elevated the formation of ASC in a punctate structure that corresponds to inflammasome complex activation as well as being able to induce the oligomerization of ASC and caspase-1 activation. CONCLUSSIONS: Together, our observations provide an insight regarding to the responsible proteins of dengue that induced inflammatory responses and highlight the importance of the NS2A and NS2B in activation of the NLRP3 inflammasome during dengue infection.

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In silico drug repurposing for the identification of potential candidate molecules against arboviral infection

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Arboviral diseases caused by dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) viruses represent a major public health problem worldwide, especially in tropical areas where millions of infections occur every year. Currently, no effective vaccine or selective antiviral therapy exists for most of these arboviral infections. The aim of this study was to identify candidate molecules for the treatment of these diseases among FDA-approved drugs currently available on the market. To do so, we used in silico drug repurposing screening and subsequent in vitro evaluation using cell culture models of DENV and ZIKV infections. Numerous pharmaceutical compounds from antibiotics to chemotherapeutic agents, including ergotamine, antrafenine, natamycin, pranlukast, nilotinib, itraconazole, conivaptan and novobiocin, presented high in silico binding affinity for distinct DENV, ZIKV, and CHIKV proteins, such as the envelope and several non-structural proteins. Several of these compounds were tested in vitro on human monocytic cells (U937-DCSIGN), and pranlukast exhibited the greatest antiviral activity as measured by flow cytometry. Further in vitro assays using pranlukast showed a significant inhibitory effect on DENV and ZIKV infection in human hepatocytes (Huh-7 cells) with potential abrogation of virus entry. Finally, intrinsic fluorescence analyses suggested that pranlukast may interact

to some degree with three DENV proteins: envelope, capsid, and NS1. Due to its promising results and, suitable accessibility on the market compared to other pharmaceuticals, the anti-asthmatic pranlukast is proposed as a potential drug candidate against DENV, ZIKV, and CHIKV infections. Together, these results support the use of drug repurposing screening to find lead compounds that exhibit good affinity scores in silico as therapeutic agents or scaffolds for the development of new drugs against arboviral diseases.

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Minority Gene Expression Profiling: Probing the genetic signatures of Dengue pathogenesis using ribosome profiling

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Minority Gene Expression Profiling (MGEP) refers to a scenario where the expression profiles of specific genes of interest are concentrated in a small cellular pool that is embedded within a larger, non-expressive pool. An example of this is the analysis of disease-related genes within subpopulations of blood or biopsied tissues. These systems are characterized by low signal-to-noise ratios that make it difficult, if not impossible, to uncover the desired signatures of pathogenesis in the absence of lengthy, and often problematic, technical manipulations. We have adapted ribosome profiling (RP) workflows from the Illumina to the Ion Proton platform and used them to analyze signatures of pathogenesis in an MGEP model system consisting of human cells eliciting ¡3% productive dengue virus (DENV) infection. We find that RP is powerful enough to identify relevant responses of differentially expressed genes, even in the presence of significant noise. We discuss how to deal with sources of unwanted variation, and propose ways to further improve this powerful approach to the study of pathogenic signatures within MGEP systems.

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Chikungunya fever triggers platelet activation

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Introduction: Chikungunya fever (CHIKV) is a viral disease transmitted by mosquitoes of the genus Aedes. The infection is usually symptomatic and can cause febrile pictures with pain and severe prolonged and debilitating joint pain, which may persist for years. Although the pathogenesis of CHIKV is not fully understood, the evolution to severe disease seems to be associated with the activation of immune mechanisms and the action of inflammatory mediators. Platelets are recognized as inflammatory cells with fundamental activities in the immune response, maintenance of vascular stability and pathogenicity of various inflammatory diseases. The aim of this project was to analyze platelet activation and the possible role of platelets in the amplification of the inflammatory response. METHODS AND RESULTS: Blood was collected from patients infected with CHIKV during the 2016 epidemic, following previously established exclusion and inclusion criteria described in CEPE 42999214.1.1001.5248. Through flow cytometry we observe that the infection leads to an increase in CD62P expression characteristic marker for platelet activation, interestingly we found that patients who continued to have joint pain one year after infection also had greater platelet activation at the time of blood collection. We also observed, by western blotting, increased in IL-1b cleavage and NALP-3 expression in platelets from CHIKV patients when compared to healthy volunteers, suggesting a role for platelets in inflammatory activation in CHIKV. In addition, we observed increased levels of platelet derived 12- (S) -HETE in the plasma of CHIKV patients, a lipid mediator involved in inflammation and vascular permeability. Conclusion: We observed in this study that the platelets of pa-

tients infected with chikungunya exhibit signs of increased activation and inflammatory mediator release. Collectively, our data suggest an involvement of platelets in the inflammatory amplification and immunological processes triggered by the infection.

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Differential expression analysis and profiling of hepatic miRNA and isomiRNA in dengue hemorrhagic fever

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Dengue virus causes dengue hemorrhagic fever (DHF) and has been associated to fatal cases worldwide. The liver is one of the most important target tissues in severe cases, due to its intense viral replication and metabolic role. microRNAs role during infection is crucial to understand the regulatory mechanisms of DENV infection and can help in diagnostic and anti-viral therapies development. We sequenced the miRNome of six fatal cases and compared to five controls, to characterize the human microRNAs expression profile in the liver tissue during DHF. Eight microRNAs were differentially expressed, including miR-126-5p, a regulatory molecule of endothelial cells, miR-122-5p, a liver specific homeostasis regulator, and miR-146a-5p, an interferon-regulator. Enrichment analysis with predicted target genes of microRNAs revealed regulatory pathways of apoptosis, involving MAPK, RAS, CDK and FAS. Immune response pathways were related to NF-kB, CC and CX families, IL and TLR. This is the first description of the human microRNA and isomicroRNA profile in liver tissues from DHF cases. The results demonstrated the association of miR-126-5p, miR-122-5p and miR-146a-5p with DHF liver pathogenesis, involving endothelial repair and vascular permeability regulation, control of homeostasis and expression of inflammatory cytokines.

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Cholesterol-lowering drugs as host-direct antivirals against dengue, zika and yellow fever viruses

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Introduction: The arboviruses, dengue (DENV), Zika (ZIKV) and yellow fever (YFV) viruses that belong to the Flavivirus genus, are becoming a global health problem. Viruses, as obligate intracellular parasites, can hijack host cell components in order to replicate successfully. One of these components is the cholesterol that ensures an efficient virus production by increasing its levels during flavivirus infection. Therefore, cholesterol has been proposed as a therapeutic target to treat flavivirus infection using FDA-approved cholesterol-lowering drugs. Material and methods: The antiflaviviral effect of ezetimibe and atorvastatin (cholesterol lowering drugs) was analyzed in vitrousing Huh-7 cells infected with DENV 2, ZIKV and YFV, where the CC_{50}, IC_{50} and the SI were determined. In vivo assays were performed using the AG129 mouse model for DENV and ZIKV infection to determine the survival and describe the histopathological changes in the liver of treated and not treated infected mice. The protocol was approved by the Animal Care and Use Committee at CINVESTAV-IPN, Mexico. Conclusion: Our results indicate that ezetimibe and atorvastatin are drugs suitable for inhibiting flavivirus infection. Opening the possibility of initiating clinical trials in humans.

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The effects of a Zika Virus infection on CREB3L1 neuroprotective pathway

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Introduction: Zika Virus (ZIKV) can act as a teratogenic agent causing congenital Zika syndrome. ZIKV can reduce the ability of human neuron progenitor cells and other nerve cells to induce self-repair and cell survival mechanisms. Gene ontology analysis has reported up-regulation of cAMP responsive element binding protein 3-like 1 (CREB3L1) gene during a ZIKV infection, suggesting CREB3L1 neuroprotective pathway is altered in nerve cells infected with ZIKV. Objective: The objective of our study is to assess the expression levels of CREB3L1 neuroprotective pathway during a ZIKV infection. Materials and Methods: SHSY-5Y neuroblastoma cells were differentiated with retinoic acid and infected with ZIKV at a multiplicity of infection (MOI) of 0.1 at different time points. After examining the cultures for cytopathic effects, western blot assay was used to assess protein expression levels and qRT-PCR was performed to evaluate the gene expression of CREB3L1 pathway and ZIKV viral load. Results: Preliminary results indicate that a ZIKV infection at a MOI of 0.1 causes an increase in viral load titers 12, 24 and 48 hours (h) post infection in differentiated SHSY-5Y cells. The RNA and protein levels of CREB3L1 pathway did not show any differences in expression during a ZIKV infection at a time dependent manner. Discussion/Conclusion: Our preliminary studies indicate CREB3L1 genetic and protein expression levels are not altered during a ZIKV infection at different time points. The activity of CREB3L1 pathway and its role in nerve cell survival processes will be evaluated in SHSY-5Y cells infected with ZIKV.

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N69 Glycosylation on Premature Protein is essential for Dengue Virus Replication

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Maturation of Dengue viruses (DENV) alters the structure and infectivity of the virus. The major determinants of DENV maturation rely on the viral premature protein (prM) and its cleavage by the host protease furin. The prM is highly conserved among all four serotypes with 65% sequence identity. In particular, an N-linked glycosylation site, N69, located at the surface exposed Pr portion, is universally present with the exception of two strains out of 5,459 complete genome sequences in the VIPR database. Currently, no known function for the N69 Glycan has been suggested. However, based on the conservation and location of N69, we hypothesized a functional role of the N69 glycan in the viral life cycle, with possible implications on antigenicity. Using a DENV reverse genetic system, we designed four separate loss-of-glycosylation variants, including the sequences of the two above mentioned strains. However, we were unable to generate a replicating recombinant DENV with the loss of N69 glycosylation. Instead, second site revertants with a functional glycosylation motif were rescued after a prolonged period of time. Our data strongly indicate that N69 glycosylation has a critical function in the viral life cycle, possibly during viral assembly.

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Virtual Screenings

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Dengue virus (DENV) is the causative agent of dengue, that affect annually about 400 millions of people around the world and there is no specific treatment. NS5 is the most conserved viral protein and is considered a therapeutic target. We search compounds with properties similar to drugs as possible inhibitors of NS5 of DENV. We construct and validate 3D structure of NS5, using SwissModel, Molprobity and ProSA. We made a virtual screening in 7 regions (GTP and

SAM binding site, KDKE tetrad, Cavity A and B, RNA Tunnel and GDD motif) using DrugDiscovery@TACC server and Zinc database. The compounds were selected by solubility, Lipinski rules and toxicological risks using Swissadme and Protox II. We found 8 compounds with multidomain binding and 80 in single domain, with interaction mainly in KDKE tetrad and GDD motif. The compounds identified can be of interest for the use of them as possible drugs, therefore we suggest the evaluation of these compounds in future research.

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NS2B-NS3 protease from Zika virus alters the nuclear pore complex integrity

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Introduction: Zika virus (ZIKV) is an emerging arbovirus associated with cases of microcephaly in newborn babies and Guillain-Barré Syndrome. The non-structural protein 3 (NS3) has fundamental protease and helicase functions involved in the cleavage of ZIKV polyprotein and during viral replication respectively. Besides, NS3 affects the morphology of the nuclear lamina of the nuclear envelope (NE). Another critical component in the NE is the nuclear pore complex (NPC), the primary regulator of the nucleus-cytoplasmic transport. It has been described that some viral proteases of Poliovirus and Rhinovirus affect the integrity and function of the proteins (nucleoporins, Nups) of the NPC, which results in the affectation of the nucleus-cytoplasm traffic of proteins and mRNAs, reducing cell translation and thus avoiding the antiviral immune response, favouring its replication. Objective: The integrity of Nup62, Nup98, Nup153 and TPR nucleoporins were evaluated during ZIKV infection and transfection NS2B-NS3. Materials and Methods: Huh7 cells were infected with ZIKV or transfected with the active and inactive NS3 protease, analysis of integrity of Nup62, Nup98, Nup153 and TPR were evaluated using confocal microscopy and western blot analysis. Results and conclusion: In summary, our results that during infection with ZIKV the integrity of Nup98, Nup153 and TPR is altered, this effect is also observed during transfection with NS3, demonstrating that NS3 is responsible for the alteration of the NPC. The presence of NS3 in the nucleus of infected cells early after infection support our observation. The disassembly of nuclear structures by NS3 is a new finding that will allow us to understand the virus-host interaction. However, the consequences of the degradation of these nucleoporins are still unknown and require investigation.

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Intrahost diversity of DENV-2 in patients with different clinical outcomes

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Dengue virus (DENV) can cause potentially life-threatening acute diseases in tropical and subtropical countries. DENVs are RNA viruses that within the host exist as genetically diverse populations. Intra-host genetic diversity is thought to facilitate arbovirus adaptation to changing environments and hosts, and it may also be linked to viral pathogenesis. Intending to shed some light on the viral determinants for severe pathogenesis, we analyzed the DENV-2 intrahost genetic diversity in twenty-one patients with different clinical outcomes. Full-length viral genomes were deep sequenced using an amplicon-free approach, on the Illumina NextSeq500 sequencing system. Single nucleotide variants (SNV) and insertions/deletions variants (indels) were determined for each sample. The 21 cases were clinically classified as following: dengue fever without warning signs (n=9), dengue with warning signs (ws) (n=7), and severe dengue (n=5). Mutant swarms of severe cases were characterized by a higher percentage of unique variants (σ=50% vs. 17.5 and 10.6% for cases with or without ws), and a higher frequency for SNVs and indels (Median=13.1 vs. 8.1 vs. and 3.0 for cases with or without ws). Gene variability (percent of varia-

tions per total nucleotide positions) along the viral genome was overall higher for cases without ws (0.51-1.33% vs. 0.19-0.74 and 0.21-1.04% for cases with ws and severe), but presented a similar profile for the three clinical categories, with two exceptions: severe cases exhibited a lower variability in the NS2B gene, while cases with ws in NS4A. However, all variants involved in these two cases were non-synonymous or indels. Finally, 43% (188/439) of all different variants identified in all samples, were consistently found among cases without ws, while, seven non-synonymous variants distributed in the envelope coding gene (1), NS3 (1), NS4A (1) and NS5 (4) were only present amid severe cases and with warning signs. It would be essential to study whether the latter may be involved in severe pathogenesis of DENV-2. The present analysis represents an early effort to correlate DENV-2 genetic diversity to its pathogenic potential and contributes to the understanding of the dynamics of this virus within the human host.

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MBL2 polymorphism influences MBL serum levels and activity during dengue infection in children from a cohort study

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Introduction: In infants, severe dengue often occurs during primary infections. The mannose-binding lectin (MBL) is a component of innate immunity that plays a crucial role in protection during primary infection, in the absence of specific antibodies. Genetic polymorphisms in MBL2 gene have functional effects on serum levels and activity of MBL and may impact susceptibility to dengue virus (DENV) infection. Objective: to assess MBL levels and activity in infants born to DENV-immune mothers and to determine whether or not the variant MBL alleles are associated with symptomatology and susceptibility to dengue infection. Materials and methods: Samples were obtained from a prospective study established at Recife, Northeast Brazil. The study groups included children who presented: (i) symptomatic DENV infection (n=29); (ii) asymptomatic DENV infection (n=17); and (iii) children that did not experienced DENV infection (control group) (n=84) during the first two years of life. All samples were laboratory-confirmed as DENV positive or negative by RT-PCR and/or IgM capture ELISA. MBL levels and activity (binding capacity) were assessed by ELISA. To determine MBL2 polymorphisms in exon 1 (at positions 52, 54 e 57) and the promoter region (at positions -550, -221 and +4), DNA was extracted from clot samples and genotyping assays were performed using the TaqMan system. Results: Serum levels of MBL were significantly higher in control (6041ng/mL) than DENV-infected children (1162ng/mL; pMBL2gene were related to low functional MBL (p=0.0094). There was no difference between genotypes and haplotypes in the DENV-infected group. Interestingly, MBL binding capacity in children with genotypes and haplotypes related with high activity of MBL was lower when compared with high MBL activity in control group (p Discussion/conclusion:Taken together, our results suggest that MBL2 gene polymorphism might influence dengue susceptibility.

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Regulation of the expression of miRNAs by the Zika virus during Central Nervous System development

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Abstract: The CNS is composed of two cell types: neurons and cells of the glia. These cells, derivate from the neuroectoderm, came from a stem cell (SC) with the ability to self-renew and originate more stem cells, which differentiate into neural progenitor cells (NPC). In recent years, it has been shown that gene expression during CNS development can be regulated at post-transcriptional level by small non-coding RNAs, called miRNAs. Recently, it has been an increase in microcephaly cases worldwide caused by infections with the Zika virus. This RNA+ virus infects SC and NPC, causing deregulation in expression of miRNAs, in-

crease apoptosis, reduction in the mitosis of the NPC and inhibition of cell differentiation. On the other hand, during the infection with Zika, the expression of several toll-like receptors, such as TLR 3/4 and 7 are induced and activate the transcription factors IRF3, IRF7, and TNFα, IFN, NF-kB. It is known that miRNAs possess response elements to IRFs, NF-kB and STAT. Objective of the study: Evaluated the expression of miR125a/b, miR7a and miR181a in models of infection whit ZIKV in-vitro and in-vivo. Measure the protein level of pIRF3, pNF-kB or pSTAT3. Materials and methods: The expression of miR125a/b, miR7a and miR181a was determined by RT-qPCR in N1 and N2A cells line, infected whit Zika virus African lineage (MR766). At the same time, a model of intrauterine infection was established in pregnant C57BL/6 mice to evaluate the expression of miRNAs. The levels of TLR3, pIRF3, pNf-kB and pSTAT3 were measured by western blot. Results: The ZIKV infection modulate the expression of miR 125a and 7a in N1 cells line and the induction it dependent of the course of infection. The protein levels of STAT are diminished, and the pNf-kB present a slight increased and the pIRF3 didn't change through the kinetic of infection. Discussion/Conclusion: The ZIKV induce a deregulation in miRNAs expression that have functions in CNS development, however this increase it's not dependent of activation of TLR3 pathway. It's necessary to elucidate the mechanism that induce the incorrect expression of miRNAs, for a therapeutic strategy in the future.

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ZIKV-infected placentae: histopathology, inflammatory profile and ultrastructural analysis in gestations resulting in cases of microcephaly or not

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Introduction: Definitive evidence is lacking for the understanding of histopathological changes in ZIKV infection, the activated immune response in the placenta of the pregnant patient, as well as the implications that the changes in this organ may have and determine a more severe condition in the fetus, such as microcephaly. Objective: In this work, we aimed to investigate the histopathological and ultrastructural changes, and the immunological profile in the placenta of 10 Zika virus (ZIKV)-infected patients during pregnancy, 5 pregnancies resulting in cases of microcephaly and 5 non-microcephaly compared to 5 non-infected control placentae. Methods: Clinical and laboratory examinations of the pregnant women were accomplished. Histopathology by H.E. and Picro Sirius Red stainning, ZIKV immunoassays, and ultrastructural evaluation of the placenta were performed. Results: The evaluation of the abnormally large term placenta revealed severe damage to the maternal decidua and chorionic villi. Maternal portions presented diffuse edema, fibrosis, degeneration, calcification, and focal areas of inflammatory infiltrates. An investigation of the chorionic villi presented an area of extensive calcification, vascular endothelial thickening, and perivascular inflammatory infiltrates. These features were not observed in the controls placentae. Additionally, there was an expressive decrease of collagen (up to 60%) in infected placentas. ZIKV-E and NS1 protein were detected only in samples from the ZIKV infected patients by immunohistochemistry. Increased cellularity (Hofbauer cells and TCD8+ lymphocytes), expression of MMP-2 and MMP-9, as well as local proinflammatory cytokines such as IFN-γ and TNF-α, and other markers such as RANTES and VEGFR2, confirm inflammation. The infection of Hofbauer cells during gestation not only reflects the critical failure of the maternal-fetal protection arrangement, but also highlights a potential pathway for the vertical transmission of ZIKV. Ultrastructural aspects of this sample showed alterrations and intracellular clusters of virus-like particles. Conclusion: The placental changes caused by ZIKV are not pathognomonic, however, we provide evidence that this infection leads to severe placental injury to the maintenance of pregnancy, and these results support the understanding of the Zika disease's immunopathogenesis.

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DENV2 inhibits thrombin-induced oxidative phosphorylation through PI3K/Akt signaling pathway inhibition

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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 platelet dysfunction, in patients with dengue, could be due to direct interaction between platelets and dengue virus. This event induces changes in platelet function and contributes to pathogenesis. To evaluate DENV2 effects on platelet function, morphological changes, platelet activation and aggregation, mitochondrial function and surface markers expression were tested. We found that DENV2 induces conspicuous morphological changes on platelets not induced by other arboviruses; increases CD62P and reduces CD42b expressions on platelet surface; reduces agonist-induced platelet aggregation through increases intracellular NO production; inhibits oxidative phosphorylation through PI3K/Akt signaling pathways inhibition without reduction in DYm or ROS. Surprisingly, rDC-SIGN treatment reverts DENV2 effects on platelet function. The previous results were observed after the purified DENV2 particles were incubated with a platelet suspension for 2 h at 37°C, which suggests that DENV2 particles interaction with platelet receptors has a direct effect over platelet function and may be correlated with the clinical manifestations observed during severe dengue, such as thrombocytopenia and hemorrhages. The overall impact of these changes in signaling pathways related to immune activity remains to be determinate, as does the potential to develop new pharmacological treatments.

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Effect of cellular lipids on Dengue and Chikungunya replication levels

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Introduction: It has been discovered that arboviruses have developed a unique ability to form cytosolic membranes to build a new factory RNA replication There is evidence that host factors work a crucial role in how they work. It has been reported that metabolic pathways of cholesterol and lipids involved and are modulated during arbovirus infection, it has been determined that specifically lipids capable of destabilizing or causing curvatures in membranes, as well as lipids that affect their permeability, are abundant in arbovirus infected cells. Objective: Evaluate the effect of lipids on Dengue and Chikungunya replication levels in vitro. Methods: To evaluate the cytotoxicity of lipids at different concentrations, a cell viability test was performed, subsequently the internalization of lipids in Huh-7 cells was confirmed using the oil red technique, finally, the effect of lipids was evaluated in dengue by Plaque Formation Units (PFU) assay and RT-qPCR. Results: The spread of Dengue and Chikungunya in C6/36 cells, was confirmed by PCR. Non-toxic concentrations of 0.25 mM and 0.75 mM were selected for palmitic acid and stearic acid, as well as concentrations of 0.15 and 0.3 mM for cholesterol, internalization of the different lipids in the cells was evaluated, confirming the maintenance of 100% of these inside the cell for up to 72h. Subsequently, the effect of palmitic acid on chikungunya replication was evaluated by observing an increase in replication 19 and 51 times at 24 and 48 h respectively with the concentration of 0.75mM. As for dengue, a greater cytopathic effect is observed in infected cultures by PFU assays after 8 days of incubation as the concentration of palmitic acid, stearic acid and cholesterol in the medium increases. Conclusion: The increase in concentration of lipids in culture of cells infected by chikungunya and dengue causes a significant increase in the levels of replication.

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Agaricus brasiliensis sulfated polysaccharide inhibits Dengue virus infection and dengue virus nonstructural protein 1-mediated pathogenesis

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Dengue virus (DENV) severe infections are characterized by increased vascular permeability and hemorrhagic manifestations. Despite its substantial morbidity and mortality, no therapeutic agents exist for treatment of dengue, and the currently available vaccine does not confer full protection. Thus, development of therapeutic and/or preventive drugs is urgently needed. Nonstructural protein 1 (NS1) plays important roles in host immune evasion and viral pathogenesis by directly triggering endothelial barrier dysfunction and inducing inflammatory responses, contributing to vascular leak in vivo. Here, we evaluated the in vitro and in vivo efficacy of the (1-6,1-3)-β-D-glucan isolated from *Agaricus brasiliensis* fruiting bodies (FR) and its sulfated derivative (FR-S) against DENV infection and DENV NS1-mediated pathogenesis. FR-S, but not FR, significantly inhibited DENV2 (strain 172-06) replication in human monocytic U937-DC-SIGN cells (EC50=30.46$\mu\mu$g/mL) when added simultaneously with viral infection. No inhibitory effect was observed when FR or FR-S were added 1 hour post-infection, implicating viral entry inhibition as the main antiviral mechanism of action of FR-S. In an in vitro model of endothelial permeability, FR (0.25ug/mL) significantly inhibited Trans-Endothelial Electrical Resistance (TEER) reduction (>50%) induced by DENV2 NS1 treatment of human pulmonary microvascular endothelial cells (HPMECs), while FR-S displayed 100% efficacy at 0.12ug/mL. Confocal microscopy assays revealed 63.9% and 72.8% inhibition of DENV NS1 binding to HPMECs by treatment with 0.25ug/mL of FR and FR-S, respectively. Further, FR-S significantly reduced (41.8%, p=0.048) hyperpermeability in mouse skin induced by DENV2 NS1 injected intradermally into wild-type mice (C57BL/6). In summary, these results demonstrate FR-S in vitro efficacy against DENV infection, as well as against NS1-induced endothelial disruption in vitro and permeability in vivo. These findings stimulate further exploration of FR-S and other glycan candidates for dengue treatment alone or as combination therapies with compounds with different mechanisms of action.

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Analysis of the interaction between Dengue virus 2 NS1 protein and human CD14 protein in monocytes

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NS1 protein is essential for Dengue virus (DENV) and can be secreted into the serum of infected patients. NS1 protein can activate monocytes and macrophages, by a mechanism not yet fully elucidated. Our group has identified that the NS1 protein is capable of interacting with hepatocytes' proteins, among them, CD14 protein, which is a membrane receptor found mainly in monocytes. In these cells, CD14 and the TLR4 membrane receptor are responsible for recognizing bacterial products, such as LPS. In this context, it is interesting to evaluate the role of NS1 protein in modulating signaling by CD14, as it may reveal a new target for dengue treatment. To confirm the interaction in vitro, an enzyme-linked immunosorbent assay (ELISA) and co-immunoprecipitation (Co-IP) were realized using the purified CD14 and NS1 proteins. Fluorescence optical microscopy assays with primary monocytes infected with the DENV2 virus or treated with purified NS1 were used to verify the colocalization of NS1 and CD14 in primary monocytes. To verify the interaction by molecular docking, using the three-dimensional models of the NS1 and CD14 proteins obtained in the Protein Data Bank. An immunophenotyping analysis of monocytes treated with NS1 and LPS-RS (TLR4-signaling antagonist) was performed for 48h, seeking to verify the cell activation by flow cytometry. Further, the supernatants of these monocytes were analyzed by capture ELISA for

cytokine analysis. The interaction between NS1 of DENV2 and CD14 was confirmed by all interaction assays. Molecular docking also suggests that NS1 and LPS share the same site of interaction with CD14. Monocytes treated with NS1 expressed more CD14. There was also a 50% increase of the HLA-DR molecule, responsible for the presentation of antigen and LPS-RS rescued the phenotype caused by NS1. NS1 also induced secretion of IL-8 and IL-6 cytokines. As perspectives, we aim to perform other approaches to confirm the contribution of these receptors.

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Investigation of the interaction between human apoliprotein A1 and Dengue virus NS1 protein

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

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A novel nonhuman primate model for Kyasanur forest disease virus and Alkhurma hemorrhagic disease virus

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Kyasanur forest disease virus (KFDV) and closely related Alkhurma hemorrhagic disease virus (AHFV) are two emerging flaviviruses in India and Saudi Arabia that cause severe hemorrhagic disease in humans. No disease model exists for KFDV or AHFV that accurately mimics the disease progression in humans because mice and nonhuman primates infected with KFDV experience lethal neurological illness rather than hemorrhagic signs. Our group recently showed that an interferon stimulated gene called tripartite motif protein 5 (TRIM5a) has potent antiviral protection against select tick-borne flaviviruses such as KFDV. The TRIM5 gene is modified in pigtailed macaques and the pigtail TRIM5 does not restrict the tick-borne flaviviruses. Therefore, we hypothesized that the pigtailed macaques would be more susceptible to KFDV. Upon infection with KFDV, the macaques developed an acute febrile illness characterized by epistaxis, slow and careful movements, and dehydration. At peak illness, some animals were unbalanced and using their cage for

support or reluctant to move. All animals had a severe drop in platelets, monocytes, and neutrophils. Four of six animals met the euthanasia criteria within eight days of infection. All animals had infectious virus detectable in the gastrointestinal tract, and virus was recovered from most lungs, and lymph nodes, but rarely detected in the CNS. Furthermore, two of six animals had infectious virus recoverable in the rectal swabs, demonstrating that the animals were capable of shedding virus. Analysis of cytokines and chemokines in the blood showed elevated levels of IFNg, IL-6, MCP-1, and IL-1RA. Pigtailed macaques infected with AHFV had almost identical symptoms to that of KFDV and a similar disease progression. Two of four animals met the euthanasia criteria, and these animals also had virus detectable in the gastrointestinal tissues, lungs, and lymph nodes. This work characterizes for the first time a nonhuman primate model for KFDV and AHFV that closely follows clinical disease observed in human cases. We plan to use the pigtailed macaque model for testing vaccines and antivirals as well as modeling the innate and adaptive immune response to KFDV and AHFV.

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Immunity to DENV4 results in better control of Zika replication and higher magnitude of neutralization compared to DENV3 in macaques

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Zika and Dengue are antigenically similar viruses from the Flaviviridae family. Since 2015 Zika cocirculate in areas endemic for Dengue (DENV) in the Americas. The association of Zika with neurological damage and congenital defects raise questions about the role of previous immunity to Dengue and other flavivirus in secondary infections. Since DENV occurs in 4 serotypes and severe pathogenesis is associated with heterologous infections, it has been proposed that pre-existing immunity to DENV could affect Zika pathogenesis due to cross-reactive non-neutralizing immunity. However, it also has been proposed that previous DENV immunity could play a protective role against Zika virus (ZIKV). This emphasizes the need for understanding the role of cross-reactive immunity in flavivirus pathogenesis, specially analyzing all DENV serotypes. Here we study the effect of long-term immunity (3 years after Dengue infection) in ZIKV infection using a Rhesus macaque model previously exposed to either DENV serotype 3 (DENV3) or DENV4 compared to a naïve group. For this work we quantified viral RNA loads in serum and urine, liver enzymes and other clinical parameters. We also performed antibody neutralizations assays to evaluate the role of humoral immunity and cytokine analysis to evaluate any modulation of immune response in these cohorts. We found that animals with a previous exposition to DENV4 had a shorter viremia period when compared to DENV3 immune and the control group. DENV4 immune animals also had higher magnitude of neutralizing antibodies to ZIKV when compared to DENV3 immune cohort. Since ZIKV viremia on DENV4 immune cohorts start to decline before we detected ZIKV specific neutralizing antibodies, it can be suggested that at early stages after infection cross neutralizing antibodies from previous dengue infection play a limited role in ZIKV neutralization. This data demonstrates that long term immunity to DENV3 or DENV4 is not associated with enhancement, however immunity to DENV4 may have a better protection role during secondary infections to ZIKV.

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Zika virus infection in pregnant Cynomolgus macaques

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Zika virus (ZIKV) infection during pregnancy is associated with increased risk of congenital abnormalities that affect fetal growth and central nervous system development. Rates and severity of disease vary widely, and the factors that influence outcome of ZIKV infection remain unclear. In this study, we infected cynomolgus macaques with ZIKV in the first trimester of pregnancy by the intravaginal route to model sexual transmission of ZIKV and to investigate features of maternal infection and its effect on fetal development. Following inoculation with ZIKV French Polynesian or Puerto Rico (PRVABC59) strains, 5 out of 6 dams seroconverted to produce neutralizing ZIKV-specific antibodies. Sporadic virus shedding in bodily fluids was observed. However, viremia was transient, a finding that is in contrast to reports of prolonged viremia in ZIKV-infected pregnant rhesus macaques compared to non-pregnant ZIKV-infected animals. Despite the presence of virus in the blood, RNA sequencing analysis of whole blood collected at various timepoints after inoculation revealed a notable lack of transcriptional changes compared to mock-inoculated controls. These results suggest that cynomolgus macaques are relatively resistant to ZIKV following intravaginal infection. However, viral antigen and/or genomic RNA was detected in trophoblasts of the placenta when assessed near-term of pregnancy, indicating viral persistence in placental tissue months after the initial infection. Furthermore, placental damage characterized by necrosis, neutrophil infiltration and deciduitis was also observed in 3 out of 5 seroconverted dams. These pathological changes were limited to the maternal side of the placenta juxtaposed to normal, healthy tissue on the fetal side. Despite persistent viral antigen and placental pathology, no viral antigen or pathology was detected in the fetus. Thus, placental pathology in the cynomolgus macaque model is insufficient to cause overt defects in fetal growth and development. These data suggest that sexual transmission of ZIKV can result in placental pathology in primates. However, our results also suggest that compared to rhesus macaque models, the cynomolgus macaque is relatively resistant to placental transfer of virus to the developing fetus.

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Nuclear location of non-structural protein 3 (NS3) during DENV2 infection in Huh7 cells

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Introduction: Dengue virus (DENV) from genus Flavivirus, is a single-stranded RNA virus of positive polarity that encodes a polyprotein which is translated into three structural proteins and seven non-structural proteins (NS). Although viral replicative cycle takes place in the cytoplasm, the nucleus may play an important role during replication as some flaviviral proteins such as the C and NS5 proteins are translocated to the nucleus. The NS3 protein of the japanese encephalitis virus (JEV) was also observed in the nuclei of infected cells by transmission electron microscopy; moreover, our group reported the nuclear localization of NS3 during DENV infection in C6/36 cells; however, the nuclear localization of DENV NS3 protein in mammalian cells has not been completely studied. Aim: To evaluate the nuclear location of NS3 protein during DENV2 infection in the mamalian Huh7 cells. Materials and methods: An in silico analysis for the prediction of nuclear localization (NLS) and export sequences (NES) was performed in cNLS Mapper and WREGEX software. The prediction of the tertiary structure of the protein was designed in Raptor X software. The subcellular location of the viral proteins E and NS3 in DENV-infected Huh-7 cells was analyzed by confocal microcopy and by western blotting of nuclear and citoplasmic protein fractions from infected Huh-7 cells using anti-NS3 and anti-E antibodies. Results: The in silico analysis of NS3 revealed the presence of a NLS and a NES motifs in the helicase domain of the protein. Both sequences are conserved in different members of the Flavivirus genus. By confocal microscopy the nuclear localization of the NS3 protein of DENV2 early after infection was determined. This result was confirmed by cell fractionation and Western Blot analysis. Conclusion: Early after DENV2 infection in Huh7 cells, the NS3 protein is translocated to the nucleus and later, the protein returns to the cytoplasm. The importance of the nuclear-

cytoplasmic shuttling of NS3 protein during viral infection is currently being studied by our group.

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Antiviral effect of Vitamin D and the microbial peptide LL-37 against ZIKV infection in macrophages

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Introduction: The infections caused by Zika Virus (ZIKV) are a major trait for world public health. Although, most of infections are asymptomatic or mild diseases with good prognostic, complications related to ZIKV infection such as Guillain-Barré syndrome and microcephaly of ZKIV-infected mother's newborn children are major concerns. Vitamin D (VitD) has emerged as an alternative therapy for Flaviviruses infections such as dengue virus, due to its antiviral and immunomodulatory effects. Aim: The objective of this study was to evaluate the antiviral and immunomodulatory effect of VitD and the microbial peptide LL-37 against ZIKV infection. Materials and Methods: For this, we obtained macrophages from primary monocytes differentiated in the presence (D3-MDMs) or absence (MDMs) of VitD and infected with them with ZIKV. Furthermore, since VitD is known for increasing the expression of the antimicrobial peptide LL-37, we evaluated the antiviral effect of this peptide against ZIKV infection by treating MDMs with exogenous LL-37. Results and discussion/conclusion: We found a significant decrease in the percentage of infected D3-MDMs measured by flow cytometry, when they were differentiated with 1nM of VitD. Also, ZIKV viral load in supernatant significantly decreased using this concentration of VitD. The antiviral effect of VitD was accompanied by a decrease in the production of the inflammatory cytokines, such as IL-6 and TNF-α, quantified by ELISA in supernatants. In addition, We found a dose-dependent antiviral effect of LL-37 against ZIKV infection of MDMs measured by flow cytometry, which was also accompanied by a decrease in the production of IL-6 and TNF-α. In conclusion, we found that VitD decreases the infection of ZIKV and the production of inflammatory cytokines by D3-MDMs, similar to that observed with exogenous treatment with cathelicidin LL-37. Further studies are needed to evaluate a possible relationship between VitD-induced LL-37 and its effect in ZIKV replication.

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Regulation of innate immune response by Vitamin D in dengue virus 2 infected macrophages

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Introduction: The symptomatic infections caused by the dengue virus (DENV) are a major problem of public world health worldwide. Several studies suggest a relationship between a dysregulated inflammatory response that involves proinflammatory cytokine production and cell activation, in the immunopathogenesis of DENV. Besides traditional support therapies, alternative therapies that can modulate the inflammatory response and restrict DENV replication could be a promising method to mitigate disease pathogenesis. Bioactive Vitamin D (VitD) has been showed to reduce DENV replication and the inflammatory response, however the effect of this hormone on the innate immune responses and cell activation during DENV infection has not been studied in detail. Aim of the study: The aim of this study was to determine if Vitamin D is able to regulate the innate immune response in macrophages infected with dengue virus 2. Materials and methods: Therefore, in this study, monocytes from healthy individuals were differentiated to macrophages in the presence (D3-MDMs) or absence of VitD (MDMs), and infected with DENV-2 for 2, 8 and 24 hours. Then, pattern recognition receptors expression, cytokines production, and Interferon-Stimulated Genes were evaluated. Results/Discussion: We found that D3-MDMs showed partial resistance to DENV-2 infection and produced lower levels of IL-6, TNF-α and IL-10. qPCR and flow cytometry analysis showed that D3-MDMs expressed lower levels of RIG-I, TLR3, TLR7 and TLR9, as well as, higher levels of SOCS-1 in response to DENV-2 infection. In addition, we found that DENV-2 infected D3-

MDMs produced lower levels of ROS compared to MDMs, which was associated with the down-regulation of TLR9 expression. Finally, although VitD treatment neither modulates the expression of IFN-α nor IFN-β, higher expression levels of PKR and OAS mRNA were found in D3-MDMs. Taken together, our results suggest that VitD can modulate the inflammatory and antiviral response of macrophages in response to DENV-2 infection, which might involve various pathways: i) down-regulation of TLR3, TLR7, and TLR9, ii) decrease of ROS production and iii) upregulation of SOCS-1 and interferon-stimulated genes (ISGs) such as PKR and OAS.

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A Host-Targeted Antiviral ferruginol analog affects viral protein translation as well as actin remodeling during in vitro Dengue virus infection

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Introduction: Dengue virus (DENV) infection is the most important arbovirosis around the word. However, no antivirals drugs have been approved for its treatment. As a new antiviral research approach, the named Host-Targeted Antivirals (HTAs) appears as a promising strategy, mainly by its high barrier to resistance and low probability to select drug-resistant viral strains. Objective. In this study, we attempted to identify the potential mechanism of action of the 18-(Phthalimide-2-yl) ferruginol (also named compound 8), a semi-synthetic ferruginol analog, previously reported as a potent agent against Dengue virus type 2. Methods and Results: Using plaque-forming units assays, molecular docking, fluorescence microscopy and image analysis, we found that compound 8 couples with high affinity to RhoA GTPase (-9.8 kcal/mol) and to non-structural protein 5 (-9.6 kcal/mol). Also, this molecule reduces dramatically the stress fibers and induces cellular morphological changes, when it was added to cell cultures prior to or after infection, but has no effect on microtubules. Further, compound 8 decreases the fluorescence intensity of viral envelope protein, NS3 protein and double-stranded RNA (dsRNA), with drastic changes in NS3 and dsRNA distribution pattern along with virus yield reduction, especially when was added after 6 and 12 h.p.i. Western blot and RT-qPCR assays reveal that this molecule affects early viral protein translation when a DENV-2 replicon stable cell line and recombinant RLuc-tagged DENV-2 were used. Treatment before and in the early stages of infection does not affect viral attachment and/or entry. Additionally, flow cytometry and wound-healing experiments hint that cellular effects prompted for this compound do not relate to early apoptotic events and it could be reversible. ADME prediction suggests good drug-likeness and the potential oral bioavailability of this small molecule. Conclusion: Overall, our findings strongly suggest that 18-(Phthalimide-2-yl)-ferruginol has an HTA-related mechanism of action, possibly disrupting the polyprotein translation of DENV-2 via alteration of actin remodeling and other related cellular processes that probably are required for the replication complex formation. Further experiments are necessary to confirm or refute this hypothesis.

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Tumor necrosis factor-alpha is involved in the genesis and maintenance of hypernociception in a model of chikungunya virus infection

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Chikungunya fever is a viral disease that has as etiological agent Chikungunya virus (CHIKV), mainly transmitted by the mosquitoes *Aedes aegypti* and *Ae. albopictus*. CHIKV was first identified in Brazil in 2014, where it has been associated with an increased number of Chikungunya cases throughout the country. Disease is characterized by fever, headache, erythematous and

maculopapular rashes, back pain, myalgia, and symmetrical biphasic arthralgia, which most often develop into chronic. Currently, there is no specific antiviral treatment or vaccine available for the treatment of chikungunya fever. The therapy in use supports the symptoms through the use of analgesics and antipyretics, hydration and rest. Here, a model of CHIKV infection was established by injection of 1x106 PFU of CHIKV subcutaneously into four-week-old C57/BL6 mice. Clinical, inflammatory, virological and morphological analysis were performed at different time points post infection. Inoculation of CHIKV to WT mice induced paw edema and hypernociception from days 1 to 21 of infection which was associated with increased levels of TNFα, IL-6, CCL2, CCL4, CCL5, CXCL-2 and CXCL-9 mediators in the paw and an elevated TNFα and CXCL-1 in the dorsal root ganglion. Viable virus was recovered from paw, popliteal lymph node, quadriceps and spleen up to day3 of infection. Paw histopathological analysis revealed an intense inflammation with loss of tissue architecture at day 7. Next, the role of tumor necrosis factor-a (TNF-a) molecule was investigated. WT or TNFαR1-/- were used. Alternatively, TNF-a was pharmacologically inhibited by etanercept. Results revealed that both TNFαR1-/- and WT mice treated with etanercept showed reduced paw edema and abrogation of hypernociception. Therapeutical etanercept administration (from day 3 or 7) rescued hypernociception induced by CHIKV. TNFαR1-/- mice presented higher viral loads in different tissues (paw, ankle, popliteal lymph node, knee, spleen and plasma), which was associated with an inefficient assembly of the host's inflammatory response to infection. Thus, these data suggest that TNF-a may contribute to the genesis and maintenance of hypernociception induced by CHIKV possible through control of virus replication. These findings are promising and suggest the use of TNFα as a potential therapy against CHIKV infection.

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Mayaro virus infection induces osteoclast and osteoblast activation triggering bone loss : Role of CCL2 - CCR2 axis on disease pathogenesis.

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Mayaro virus (MAYV) is an emergent arbovirus responsible for sporadic outbreaks of acute febrile illness in countries of South America, including Brazil, where it is considered endemic. Mayaro Fever (MF) is a self-limited disease, which can range from mild to moderately severe. As with other alphavirus infections, rheumatic disease drived by MAYV is characterized by disabling pain, arthritis and myositis, which may be acute and chronic. Our aim was to establish a murine model of MAYV infection in immunocompetent mice and to study the role of CCL2-CCR2 axis on bone loss induction. Four-week-old mice (wild type, C57/BL6 and CCR2-/-) were infected with 1x106 PFU of MAYV in the right footpad. Clinical signs (paw edema/hypernociception), viral loads (plaque assay) and immunoinflammatory parameters (MPO, NAG, flow cytometry, cytokine and chemokines) were assessed. Bone loss was measured by Micro-CT and immunohistochemistry analysis (TRAP staining). The potential of the MAYV to infect osteoclasts and osteoblasts was evaluated in primary cultures in vitro. A macrophage murine lineage (RAW264.7) was used for the osteoclast culture, which was differentiated into osteoclasts with RANKL. Osteoblast culture was obtained by isolation of bone marrow cells, which were placed in osteogenic medium for differentiation into osteoblast. Results demonstrate that MAYV induces paw edema at initial days after infection, prolonged hypernociception up to 28 days post-infection and massive viral dissemination to several tissues (paw, popliteal lymph node, knee, spleen, liver, jaw and plasma). Cytometry analysis revealed predominant migration of CCR2+ cells into muscle tissue and increased expression of CCL2 by ELISA was observed in plasma, maxilla, spleen and muscle. Micro-CT analyzes of tibia and maxilla from MAYV-infected WT mice revealed increased bone loss from 14 to 21dpi that was massively reduced in CCR2-/- mice. Data from osteoblast and osteoclast cultures have demonstrated that MAYV is able to replicate in both cell types and induced a strong inflammatory response in later timepoints. Treatment of these cells with a CCR2 inhibitor prevented excessive inflammation induced by MAYV infection. Overall, these data show that the C57BL/6 mice are a promising model for studying MAYV-induced arthritogenic disease specially the mechanisms involved with bone loss

architecture.

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New expression of type 3 InsP3 receptor in hepatocytes is a protective mechanism in human Yellow Fever infection

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Yellow fever (YF) is a viral hemorrhagic fever that typically involves the liver. Brazil recently experienced its largest recorded YF outbreak, and the disease was fatal in more than a third of affected individuals, mostly because of acute liver failure. Affected individuals generally are treated only supportively, but during the recent Brazilian outbreak, selected patients were treated with liver transplant. We took advantage of this clinical experience to better characterize the clinical and pathological features of YF-induced liver failure and to examine the mechanism of hepatocellular injury in YF, in order to identify targets that would be amenable to therapeutic intervention to prevent progression to liver failure and death. Patients with YF liver failure rapidly developed massive transaminase elevations, along with jaundice, coagulopathy, thrombocytopenia, and usually hepatic encephalopathy and acute kidney injury as well, along with pathological findings that included microvesicular steatosis and lytic necrosis. Hepatocytes began to express the type 3 isoform of the inositol trisphosphate receptor (ITPR3), an intracellular calcium (Ca2+) channel that is not normally expressed in hepatocytes. Experiments in an animal model (A129 mice), isolated hepatocytes, and liver-derived cell lines infected by both 17DD vaccine or an virulent wild-type strains showed that this new expression of ITPR3 increases nuclear Ca2+ signaling, promotes hepatocyte proliferation, and reduces the steatosis and cell death induced by the YF virus. Yellow fever often induces liver failure characterized by massive hepatocellular damage plus steatosis. New expression of ITPR3 also occurs in YF-infected hepatocytes, which is an endogenous protective mechanism that may suggest new approaches to treat affected individuals before they progress to liver failure, thereby decreasing the mortality of this disease in a way that does not rely on the costly and limited resource of liver transplantation.

Late Breaker Abstracts

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An experience of health education in cooperation with public schools – Dengue on focus

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Informing people may not necessarily lead to change of behavior.A code of behavior created and negotiated with and by students inside schools may develop a sense of community and well being for all, generating engagement to programs such as dengue control.Here, we generated a code of conduct inside schools and used the scientific method to generate ideas and didactic resources based on students' experience to ameliorate community communication and behavior towards dengue. This study was conducted from 2010 to 2015 with students from seven public schools (elementary and secondary) in Belo Horizonte, Brazil. Level of knowledge of students about dengue (the mosquito, cycle, transmission) was evaluated using questionnaire before and after activities. Students were invited to create a code of behavior for their activity at schools and actively produced didactic material under the supervision of students and researchers. Material consisted of videos, photography exposition, maps of dengue incidence in their region and families and interviews. The initial questionnaire showed that most students were well informed about dengue disease and transmission. However, knowledge was not associated with a preventive posture. Boys had lower level of informa-

tion, delivering home tasks to someone else(where most mosquitos concentrate). Girls had better scores in the questionnaires, knew who had gotten dengue in the family and which care was taken. Such behavior expresses a traditional family arrangement(home care and caring)that can compromise health campaigns.This experience understands that a sense of community needs to be built from inside it. Educative materials should be able to create self-identification among people involved, representing their voices, local values and specificities. The community itself can stablish their understanding of well being and forms of control themselves. And, any social program that may eventually change people's behavior will need to be continuously financed and really understand each community's voice. This may improve community engagement and eventually lead to change in behavior.

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Potential Displacement of Dengue Virus Serotype 2 by Dengue Virus Serotype 1 in Iquitos, Peru

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Introduction: Dengue virus (DENV) poses a significant health threat to United States operational forces worldwide; it is listed third in the US Department of Defense 2019 global ranked list of infectious disease threats. There are four serotypes that circulate worldwide: DEN1-4. DEN1 was first detected in the America's in 1977 in Jamaica. In Peru, the first documented DEN1 outbreak was reported in 1990 in the Amazonian city of Iquitos, and DEN1 continued to be detected up until 2014. Objective: Report the reintroduction of DEN1 into Iquitos. Materials and methods: The U.S. Naval Medical Research Unit No. 6 has been working in Peru since 1980 and conducts an IRB-approved clinic-based surveillance throughout Peru, including Iquitos. Subjects with acute fever or history of fever of $\leq$5 days of duration and $\geq$5 years old are invited to participate. After an informed consent is obtained, a blood sample is taken. Samples are processed to detect dengue and other arboviruses by RT-PCR. Results: From 2000 to 2019, a total of 2,500 dengue-cases were detected in Iquitos: DEN1 (43/2,500), DEN2 (2019/2,500), DEN3 (281/2,500), DEN4 (154/2,500), DEN2+DEN3(1/2,500), DEN2+DEN4 (1/2,500) and 1 unsubtyped DENV. The distribution of DEN1 detection by year is2/43(2000), 1/43(2001), 2/43(2005), 1/43(2010), 14/43(2011), 1/43(2013), 3/43(2014)and 19/43(2019). In 2019, DEN1 cases were again detected and the number of positive cases increased during October 3 to December 5. Most participants positive forDEN1 reside in the peri-urban area of Iquitos.DEN1 and DEN2 are currently co-circulating in Iquitos. Discussion/conclusion: The reintroduction of DEN1 in Iquitos after 5 years and its rapid spread could displace DEN2 in Iquitos. Understanding the dynamics of DENV transmission and circulation is important to evaluate the role of natural immunity and determine the regional epidemiology of disease for product development of therapeutics. Continued surveillance in Iquitos and additional analysis of our data will help determine the origin of DEN1 and assess its virulence to support Force Health Protection.

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Dengue, chikungunya and zika virus coinfection: results of the national surveillance during the zika epidemic in Colombia

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Abstract: Our objective was to determine the frequency of zika (ZIKV), chikungunya (CHIKV) and dengue (DENV) virus coinfection and describe the mortality cases that occurred during the epidemiologic surveillance of the ZIKV epidemic in Colombia. We analysed all cases of suspected ZIKV infection that were reported to the National Institute of Health (October 2015 – December 2016). DENV, CHIKV and ZIKV RNA were detected in serum or tissue samples using polymerase chain reaction assay. Medical records of the fatal cases were reviewed. We identied that 23871 samples were processed. The frequency of viral agents was 439 (1.84%) for DENV,

257 (1.07%) for CHIKV and 10118 (42.38%) for ZIKV. Thirty-four (0.14%) cases of coinfection were identied. The CHIKV–ZIKV coinfection was present in 28 cases (82.3%), DENV–CHIKV in three (8.8%) and DENV–ZIKV in three (8.8%). Seven (20.6%) coinfection cases were fatal (two DENV–CHIKV cases and veCHIKV–ZIKV cases). Two cases were foetal deaths and the others were related to neurological syndrome and sepsis. In conclusion, the frequency of arbovirus coinfection during epidemic of ZIKV was low, and CHIKV–ZIKV coinfection was the most common. Mortality was high among coinfection patients. The role of each virus in the mortality cases of coinfection warrants further studies.

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Diseño In silico de pequeños RNAs de interferencia (siRNA) contra el Virus del Dengue

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Introducción: El virus del dengue es un flavivirus envuelto, con un genoma de RNA de polaridad positiva el cual seune a ribosomas y traduce una poliproteína indispensable para su replicación. Los siRNA son pequeñas secuencias de RNA que regulan la expresión genética. Sintéticamente se han utilizado para el silenciamiento de genes virales, sin embargo, el diseño de siRNA implica parámetros necesarios para una inhibición de genomas virales efectivo lo cual es de importancia para el desarrollo de diferentes procesos deinvestigación. Objetivo: Diseñar pequeños RNAs de interferencia (siRNA) contra el virus del Dengue (DENV) mediante herramientas computacionales. Metodología: Se identificaron genomas completos de los 4 serotipos del DENV mediante la base de datos ViPR, se realizaron alineamientos mediante Clustal Omega y se generaron secuencias consenso mediante Jalview, se analizaron secuencias conservadas y se diseñaron siRNA teniendo en cuenta las reglas U.R.A mediante siDirect2 y BLOCK-iT™RNAi Designer de Thermo Fisher. Una vez obtenidas las secuencias se analizó: lugar de reconocimiento en el genoma del virus, y especificidad de cada siRNA mediante megaBLAST, análisis estructural y termodinámico mediante RNAstructure, se Calculó la capacidad calorífica y concentración dependientes de la temperatura de fusión mediante Dinameltwebserver y finalmente se validaron mediante siRNAPred server y se realizó una interacción RNA-proteína mediante ClusPro. Resultados: el análisis de secuencias preliminar mediante alineamientos múltiples y eliminación de secuencias redundantes con identidad del 95% permitió obtener finalmente 6 secuencias de DENV1, 13 de DENV2, 4 de DENV3, 7de DENV 4, con estas se realizaron secuencias consenso para cada serotipo. Finalmente se obtuvo un total de 84 siRNA específicos para Dengue, de estos 24 cumplieron con todos los parámetros necesarios para un silenciamiento efectivo tales como; reglas U.R.A, termodinámicos, la validación y eficacia basada en una máquina de vectores arrojó números cercanos a uno.Se logró diseñar 24 secuencias de siRNA mediante parámetros computacionales que permiten establecer especificidad y eficiencia de silenciamiento de la expresión del genoma del virus del Dengue que permiten ser considerados como candidatos a ser evaluados *In vitro*.

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Persistence of infectious Zika virus in breast milk after maternal Zika infection in pregnancy

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Introduction: Despite of the progress made in Zika virus (ZIKV) pathogenesis since 2015-16epidemic in the Americas, gaps remain about the impact of maternal infection during pregnancy on post-partum risk of exposure for the infant. Characterizing ZIKV persistence in mother's bodily compartments and across populations is crucial to understanding risk factors for mother to child transmission and establish proper transmission prevention measures. Objective: To characterize ZIKV persistence and shedding in maternal bodily compartments with that may facilitate peri- or post-partum neonatal exposure. Materials and Methods: We collected placenta, amniotic fluid, serum, urine and breast milk samples from8 women in Vitoria, Brazil, with confirmed ZIKV infection during pregnancy at delivery. A breast milk follow up sample was collected approximately 3 months after delivery.Infants saliva and urine samples were collected at birth. RT-

PCR was performed in all samples and the positive ones were inoculated in Vero cell cultures in order to assess for the presence of infectious viral particles.Results: ZIKV RNA was only detectable in breast milk, in 3 out of 8 individuals. Of these 3, two presented ZIKV infection in second trimester and one in third trimester of gestation. One had infective ZIKV in breast milk at 114 days (3,8months) after the onset of symptoms. None of the 8 infants had ZIKV detectable in urine or saliva. Infants were followed up to 3 months and none presented signs or symptoms of acute or congenital ZIKV infection. Follow up breast milk samples were negative. Conclusion: Following ZIKV infections that occurred during pregnancy, breast milk was the only ZIKV positive specimen in the delivery time point. Some women exposed to ZIKV in pregnancy can shed infective virus in breast milk, which may be potentially transmitted to the infant. However, we found no clinical evidence of infection of the infants after the breastfeeding ZIKV exposure. We do not observe any evidence that contrasts to the current WHO recommendations for breastfeeding after maternal ZIKV exposure, but we raise the plausibility of testing this specimen in addition for diagnostic, specifically for those mothers with exposure in second trimester of gestation or later.

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Changes in Monocytes Subsets During CHIKV Infection

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Cells and molecules that signal innate immune responses are primarily responsible for the initial defense of organisms against invading agents. Among the leukocytes responsible for innate immunity are monocytes -a population suffering from bacterial and viral infections. Monocytes are subclassified into 3 groups (classical, intermediate and non-classical) that appear to respond heterogeneously to different pathogens. It is through PRRs -recognizing PAMPs- that occurs with cellular activity and transcription of factors that culminate in the production of cytokines and chemokines. Among PRRs, the TLR superfamily can be highlighted, where TLR7 and more recently TLR2 and TLR4 play an important role in the course of viral infections. They lead to the production of IFNs in addition to the other proinflammatory molecules responsible for lysis of infected cells, recruitment of other cell subtypes, and viral release. CHIKV is an arbovirus transmitted by *Ae. aegypti* that has arrived in Brazil in recent years, causing myalgia, fever, skin manifestations and disabling joint pain that may extend for months or years. The mechanisms for the occurrence of such intense symptoms and establishment of chronic conditions are still unclear. Also, little is known about the cellular immune status of patients who are co-infected with CHIKV virus and other arboviruses circulating in Brazil (ZIKV and DENV). Thus, the aim of this study was to evaluate the anti-virus immune response of monocyte subpopulations and their activation pattern in CHIKV infected patients, at different stages of the disease, and in patients co-infected with viruses. After analyzing the cells by flow cytometry from naturally CHIKV-infected and co-infected patients we observed that there is an increase in the frequency of intermediate monocytes in CHIKV mono and co-infected patients compared to controls. We also demonstrated an increase in CD163 and HLA-DR expression in this same subpopulation in all patient groups. Regarding the analysis of TLR2 and TLR4 expression, these TLRs are increased in classical monocytes (TLR2) and intermediates inpatients with CHIKV co-infection. However, a subpopulation of non-classical monocytes showed a decrease in TLR2 in the chronic phase, and was the same also seen for TLR7.

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Assessing Zika, Dengue and Chikungunya in Peruvian and Brazilian blood donors

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Introduction: Zika virus (ZIKV), is an arthropod-borne virus (arbovirus) belonging to the family Flaviviridae that caused widespread outbreaks in Latin America during 2015-2016 and documented virus introductions in the US. Furthermore, there was concern about exposure of US military stationed throughout the US Southern Command. The high rate of asymptomatic ZIKV infection raises strong concerns blood transfusion transmission due infected blood donors. Objective: To assess the prevalence of arbovirus infection in adult blood donors at four blood banks in Peru and one in Brazil areas that experienced ZIKV and other arbovirus epidemics. Materials and methods:Our study was conducted between November 2017 and December 2018, shortly after large ZIKV outbreaks in Peru (Hospital EsSalud III, Hospital Regional, Hospital Santa Gema and Centro Hemodador Regional) and Centro Estadual de Hemoterapia e Hematologia (HEMOES) in Brazil). After providing written informed consent, donors provided blood samples arbovirus screening. The samples were tested with the Trioplex real-time RT-PCR assay, designed by the Centers for Disease Control and Prevention that detects ZIKV, dengue virus(DENV) and Chikungunya virus (CHIKV). Plasma samples from Brazil were screened for IgG against ZIKV, DENV and CHIKV by ELISA. Results: All 2,783 blood samples (1,203 from Peru, 1,580 from Brazil) were negative for ZIKV, DENV and CHIKV by RT-PCR. IgG ELISA of the Brazilian samples (n= 1,580) showed previous exposure to all three arbovirus infections. The majority of the subjects (55.9%) had IgG antibodies to one or more of the three infections, whereas 44.1% had no evidence of prior exposure. Most had antibodies to both DENV and ZIKV (47.1%), followed by single infections with DENV (7.6%), ZIKV (1.08%)or CHIKV (0.07%). Additionally, one person had antibodies to all 3 viruses and another to DENV and CHIKV. Discussion/Conclusion: Based on our results, there is minimal risk of arboviral (ZIKV, DENV and CHIKV) contamination in the blood supply of the examined blood banks, although at the time of sample collection there were no concurrent large epidemics of ZIKV, DENV or CHIKV. The serological analysis suggests that donors in Espirito Santo have moderate levels of exposure to Flaviviruses (ZIKV and DENV) and low levels of exposure to CHIKV.

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Evaluación de desempeño de una técnica de RT-PCR en tiempo real para la detección de RNA específico de virus dengue

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Los virus transmitidos por mosquitos son amenazas sanitarias cada vez más importantes y se propagan rápidamente a nivel mundial. Actualmente el dengue es un problema de salud creciente en las áreas subtropicales y templadas de Argentina. La disponibilidad de insumos comerciales puede significar un incremento en el acceso al diagnostico para la población por su mayor facilidad de manejo en laboratorios clínicos al compararlo con una metodología "in house". El presente trabajo tuvo por objetivo evaluar la sensibilidad y especificidad analítica así como la capacidad diagnóstica del kit RealStar®DENGUE RT-PCR kit 2.0 (Altona Diagnostics) para DENV (sin determinar serotipo) comparándolo con la técnica multiplex de referencia para detección y serotipado de DENV(CDC DEN1-4 Real time RT-PCR). Se determinó el título de cepasde referenciade DENV-1, 2, 3 y 4 al mismo tiempo que se tomó una alícuota parala extracción del genoma viral. A partir del ARN se realizaron diluciones seriadas 1/10 (10-1a 10-7) para evaluar la sensibilidad analítica. Para estudiarla especificidad analítica, se procesaron ARN virales de diferentes Flavivirus de circulación en Argentina (YFV, ZIKV, SLEV, WNV). Para la evaluar la capacidad diagnóstica fueron procesadas muestras de 25 pacientes con resultados positivos para distintos serotipos y carga viral detectados recientemente en el país(14)y negativos (11).La Sensibilidad para los distintos serotipos medida en UFP/ml fue: DENV-1=1.1, DENV-2=3.5, DENV-3=0.6 y DENV-4=0.9. El estudio de la especificidad analítica no mostro reacciones cruzadas con ninguno de los patógenos estudiados. El 96% de las muestras clínicas analizadas fue identificado correctamente. La sensibilidad para DENVes ligeramente inferior respecto del protocolo "in house", pero similar a otros reactivos disponibles en el mercado y evaluados previamente en el INEVH. En todas las muestras procesadas se detectó el control interno (parámetro de calidad y validación de la metodología). El reac-

tivo resulta muy sencillo en su manipulación así como en la posibilidad de detección en una única reacción del target y del control interno. Los resultados de la evaluación del kitRealStar®DENGUE evidencian su aptitud para la aplicación al diagnóstico de dengue en la fase aguda de la infección.

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Evaluación externa internacional para diagnóstico molecular de DENGUE por rRT-PCR, Red Nacional de Laboratorios para Diagnóstico de dengue y otros Arbovirus de Argentina

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En Argentina, el algoritmo de diagnóstico etiológico de dengue que aplica la Red Nacional de Laboratorios para diagnóstico de dengue y otros Arbovirus indica que en muestras de pacientes con 6 o menos días de evolución desde el inicio de los síntomas se realicela detección de antígeno NS1 y/o detección de genoma viral, dependiendo de las capacidades y estructura laboratorial. Actualmente, la red cuenta con 24/65 laboratorios provinciales con capacidad para realizardetección molecular por RT-PCR en tiempo real (rRT-PCR). Desde 2014 se utiliza el protocolo desarrollado por CDC-Dengue Branch-Puerto Rico: kit CDC DEN1-4 Real time RT-PCR.En 2015 se realizó una proficiencia Intra-Red en colaboración con CDC-Dengue Branchen la que participaron 17 Laboratorios provinciales obteniéndose valores de Sp=100% y Se=83-100%.En 2019-2020 se organizó una segunda ronda de evaluación externa, siguiendo la misma estrategia colaborativa INEVH-CDC-Dengue Branch. El objetivo de éste trabajo fue continuar el monitoreo de la metodologíade diagnóstico molecular para dengue implementada en los Laboratorios de la Red Nacional de Laboratorios de Argentina. El panel consistió en 14 muestras liofilizadas: 12 muestras positivas, 3 de cada serotipo con viremias alta, intermedia y bajay dos muestras negativas. Dicho panel fueprovistos por CDC-Dengue Branch. El mismo fue verificado en INEVH previo a la distribución y los valores obtenidos, junto a los datos originales de CDC fueron tomados como valores de referencia. Se distribuyeron un total de 19 paneles y hasta fin de enero de 2020 respondieron 13 laboratorios (70%). El análisis de los resultados permitió determinar que la especificidad fue de 100% para todos los laboratorios evaluados. Todos mostraron una correcta identificación de los cuatro serotipos con los siguientes valores de sensibilidad (Se) e Índice Kappa (K): Se= 100%, K=1 (7/13), Se=92%, K= 0.76 (3/13); Se=83%, K=0.6(3/13). Los valores másbajos de Sese observaron enlas muestras con menor viremia. La evaluación externa permitió generar ciclos de mejora con los laboratorios de modo de optimizar la sensibilidad de la detección molecular de los serotiposde dengue circulantes en el país y garantizar la confiabilidad de los resultados.

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Ginkgolic acid inhibits Alphavirus and Flavivirus replication

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The Alphavirus (Togaviridae) and Flavivirus (Flaviviridae) genera are arthropod-borne viruses (arboviruses) with a widespread distribution in tropical and subtropical regions. The alphaviruses Chikungunya (CHIKV), Mayaro (MAYV)and Una (UNAV); and the Flavivirus Zika (ZIKV) are emerging and re-emerging pathogens that have caused outbreaks affecting populations in Latin America. Despite the large impact of these arboviruses on the health systems inthe region, there are not any approved vaccines or drug treatments to combat these infections. Natural products are a rich source of molecules with diverse biological activities. Ginkgolic acid is a natural compound isolated from the seed coats or leaves of Ginkgo Biloba, a plant that is widely used in traditional Chinese medicine. Previous studies have been demonstrated that Ginkgolic acid has antitumoral, antibacterial, antiparasitic and anti-HIV activity. Furthermore, this compound is able to inhibit the SUMOylation, a post-translational protein modification that regulates key processes in the cell. Nevertheless, Ginkgolic acid's ability to

disturb arboviruses replication, it remains unexplored.The aim of this study was to evaluate the antiviral activity of Ginkgolic acid against the arboviruses CHIKV, MAYV, UNAV and ZIKV.The cytotoxicity of Ginkgolic acid was tested in Vero and HeLa cells using the MTT method. Viral progeny production in cell supernatants treated or not treated with Ginkgolic acid was quantified by plaque assay.The presence of viral antigens in the cell lines was evaluated by immunofluorescence experiments. Viral proteins expression in Ginkgolic acid-treated and DMSO-treated cells was determined by western blot.Cell viability was not affected by Ginkgolicacid after 24 h of treatment. Infection kinetic experiments in Vero and HeLa cells pre-treated with Ginkgolic acid showed a reduction in viral progeny yield for all viruses tested, and this effect was dose-dependent. Immunofluorescence assays revealed that in the Ginkgolic acid-treated cells there was a decrease in the number of viral antigen-positive cells. Additionally, western blot experiments demonstrated that Ginkgolic acid treatment suppressed the expression of viral proteins. Altogether,these results suggest Ginkgolic acid has antiviral activity against Alphavirus and Flavivirus.

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Circulation of phylogenetically diverse Dengue virus serotype 1 strains in Peru

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Introduction: Dengue virus (DENV) is the most widely circulating mosquito-borne viral disease in the world and is recognized as an important threat to operational readiness to the US military.Continued surveillance and bioinformatics analysis of circulating dengue genotypes in conjunction with clinical data can help assess the risk the virus poses to regional operations. The etiology of severe dengue cases is not completely understood, but secondary infection and/or variation in virus virulence have been associated with the outcome of infection. The Pan American Health Organization calculated that the number of dengue cases reported in 2019 is the largest recorded in the history of the Americas. All four dengue serotypes have circulated in South America and in Peru,and DENV-2 has been predominantly circulating from 2010 until 2019 although sporadic DENV-1 and DENV-3 have been reported during that time. In 2019, DENV-1 was identified in Piura and Tumbes near the border with Ecuador, Puerto Maldonado near the border with Brazil and in Iquitos, the largest Peruvian Amazon city. Objectives: Perform bioinformatics analysis to genetically characterize DENV-1 strains detected in Peru and identify potential routes of entry. Methods: Bioinformatics analysis was performed on sequences of 90 DENV-1 obtained from human sera from Peru and neighboring countries collected between 2005 and 2019 through an IRB-approved study. A maximum-likelihood phylogeny of the E gene was inferred under a TN93+I+G nucleotide substitution model. Results: Preliminary analysis determined that all strains currently belong to genotype V but they were grouped in distinct, statistically supported clades. Our data suggests Peru experienced at least six introductions of DEN-1: four introductions via a northern route from Ecuador/Colombia, and two introductions via eastern routes from Brazil/Bolivia/Paraguay/Argentina. Regarding intra-Peru phylodynamics, the results suggest that diffusion of DENV-1 within Peru occurred mainly between coastal and north-eastern regions. Discussion and conclusions: DENV-1 has likely been introduced multiple times into Peru from various countries. There is evidence of sustained circulation of DENV-1 in Peru in 2019 and additional data is required to assess the clinical symptoms associated with these DENV-1 to determine the risk of severe disease.

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Microneutralization Assay for the Measurement of Neutralizing Antibodies to Zika Virus

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Introduction: Zika virus (ZIKV) is a flavivirus transmitted by Aedes mosquitoes that represents a threat to worldwide public health and to deployed military service members. Objective: This study investigates the presence of antibodies against ZIKV as its effective detection is critical to support Force Health Protection. Materials and Methods: Here we aim to present the development of a simple and high reproducible microneutralization test (MNT) for detecting ZIKV neutralizing antibodies because of its simplicity

and rapidness than to the plaque reduction neutralization test (PRNT). This assay was validated by selecting 51-paired samples (acute and convalescent) from Colombia, Honduras, Venezuela and Peru. Acute samples were confirmed by real-time RT-PCR to all three ZIKV, Dengue and Chikungunya viruses. Convalescent samples were tested by MNT and PRNT and were adapted to a 96-well plate with serial 2-fold dilutions of serum with a pre-determined dose TCDI50 ZIKV. After an 1-hour incubating period, we added [2x105cells/ml] susceptible Vero 76 cells to the mixture and incubated it for four days, followed by the staining of this monolayer. A50% cytopathic effect (CPE) in the last dilution expresses a neutralizing titer for cut off. Results: They were confirmed by the gold standard, PRNT assay. Analytical sensitivity and specificity of the MNT was 96.7% (95% CI: 82.8 to 99.9%) and 100.0% (95% CI 83.9% to 100.0%), respectively. The MNT reliability was estimated through the kappa value, resulting in k=0.96 (95% CI: 0.88 to 1.00). Discussion & Conclusions: Our findings suggest that the MNT for ZIKV is reliable in areas with previous dengue exposure. Moreover, MNT demonstrated a successful performance than to the PRNT, including lower costs and using a more efficient format.

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Diseño y evaluación de péptidos inhibidores de la interacción proteína-proteína (iPPI) dirigidos contra el dominio III de la Envolturab (ED3) del Virus Dengue 2 (DENV2)

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Introducción: El dengue es una enfermedad endémica que afecta a 50 millones de personas al año; causada por el virus del dengue (DENV); la infección por este virus esta mediada por la interacción entre la proteína E del virus y receptores celulares. Este estudio tiene por objetivo diseñar *In-silico* y evaluar *In vitro* iPPI, dirigidos contra el ED3 del DENV2, y evaluar su interacción en la invasión viral. Metodología: Se diseñaron In-silico modelos de interacción entre el ED3 del DENV2 y los receptores celulares CD44, CD206 y CD209 empleando el servidor Cluspro2.0 (`https://cluspro.bu.edu/login.php`),con estos modelos se buscaron iPPI empleando Rosetta Online-Server (`http://rosie.rosettacommons.org/peptiderive`). Se determinó hemolisis (eritrocitoshumamos) y citotoxicidad (BHK,Huh-7). Péptido control positivo DN59, Hrobowski, et al 2005. Análisis estadísticos: Se realizaron 3 ensayos independientes por triplicado. Estadística descriptiva para citotoxicidad y hemolisis. ANOVA de un factor para determinar diferencias de UFP (Unidades Formadoras de Placa) entre el grupo tratado, control positivo y negativo; y comparación múltiple de Dunnett. Aspectos bioéticos: Res.8430/1993-Res.0314/2018-Colombia. Acta de aprobación comité de bioética de investigaciones Nº2101-06-2018, Facultad Ciencias de la Salud/Universidad del Quindío. Resultados: Los modelos obtenidos empleando ClusPro (`https://cluspro.bu.edu/help.php`),fueron subidos a Rosetta Online (`https://rosie.graylab.jhu.edu/peptiderive`) para obtener los iPPI (denominados PD1-PD4); estos fueron sintetizados Peptide2.0,[4mg] desalados-99% de pureza. Ninguno de los péptidos genero hemolisis, ni toxicidad en Huh-7 ni BHK, a partir de 100µM. Los ensayos en BHK mostraron que PD1[50µM] disminuye la invasión viral(MOI:0,1) de forma signicativa, en comparación con el control de infección; 95% de confianza, p-valor¡0,05. Discusión: Interacción iPPI (CD-1ACD-4) con ED3 DENV: Se identifico que los IPPI, In silico reconocen la región de la ED3 de todos los serotipos de DENV; debido a que se unen a una región conservada en la envoltura viral, no solamente en los DENV sino en todos los flavivirus. Conclusión: Las herramientas de bioinformática empleadas en este estudio permitieron identicar un péptido candidato que hasta el momento ha demostrado tener la capacidad de disminuir las UFP/mL. Esta estrategia de búsqueda acelera la identicación de moléculas con potencial de drogas antivirales.

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Current status of insecticide resistance in populations of Aedes aegypti in Ecuador

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Frequent and inappropriate use of insecticides is one of the factors influencing the development of *Aedes aegypti* resistant populations worldwide. In Ecuador, insecticide resistance has been a ne-

glected topic and the loss of susceptibility to the insecticides deltamethrin and temephos was reported for certain areas of the country. The aim of this research was to describe the recent state of resistance of *Ae. aegypti* to the main insecticides used for vector control in different provinces of Ecuador. This is a descriptive study conducted in populations of *Ae. aegypti* collected in 65 locations in Ecuador from 2018 to 2019. The larvae were bred in an insectarium and evaluated using bioassays with diagnostic doses for malathion and deltamethrin and serialized doses for temephos. Lethal concentrations of insecticide were obtained using samples collected in the field and compared with the reference strain ROCK MRA-734. As a result of this study, 38 locations showed resistance to deltamethrin, 16 locations to malathion, and 25 locations to temephos. The results obtained in this research present the current state of insecticide resistance in Ecuador providing important information that might be used by healthcare decision-makers to improve vector control in Ecuador. Rotation of insecticides and alternative biological vector control strategies should be considered to address the insecticide resistance and prevent selective pressure observed in *Ae. aegypti* populations in Ecuador.

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Climate and dengue epidemics in Madre de Dios, Peru

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Introduction: Dengue is currently regarded globally as the most important mosquito-borne viral disease. In Peru, dengue fever annually causes epidemics in the coast and Amazon region, therefore understanding the Spatio-temporal dynamics of the dengue and its relationship with weather will improve preemptiveness measures. Objectives: To assess the relationship between dengue epidemics and climate as well as the synchronicity of dengue epidemics waves across districts of Madre de Dios (MDD). Methods: We did a coherence wavelet analysis to assess the relationship between dengue epidemic and climate and to assess the synchrony of dengue cases between districts. We used satellite precipitation data from the Global Precipitation Measurement (GPM), and air temperature data from the Global Land Data Assimilation System (GLDAS). Results: Further, we obtained the weekly number of dengue cases at the district level during the period 2009 -2019 from the Health Ministry's epidemiological surveillance. Results: We found strong coherence (correlation) between climate and dengue cases for the period 2009-2016 period at an annual level. Moreover, the peak in precipitation and the minimum temperature tends to occur before dengue cases peak; however, the maximum temperature and the thermal range were asynchronous. The coherence analysis of dengue epidemic curves (2019) of the 11 districts of MDD studied showed that there is synchrony among them. So, the dengue epidemic travels from east to west districts. Furthermore, the synchrony is stronger between the districts with more cases (Tambopata, Las Piedras, Inambari), and Tambopata is the one leading the dengue epidemic. We did not observe this behavior before 2019. Conclusion: Our study showed that there is a relationship between some climate variables and dengue epidemic. Moreover, dengue epidemic travel waves move from east to west, being the Tambopata district, the one that leads the epidemic.

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Epidemiological and Molecular Surveillance of Dengue Virus in Cartagena, Colombia, 2019

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Introduction: Dengue fever is a disease caused by dengue virus (DENV). There are four serotypes that circulate worldwide(DENV-1to -4),and its main route of transmission is through the bites of infected *Aedes aegypti*. Symptoms vary from fever and headache, to more severe cases with

vomiting, abdominal pain and bleeding. In Colombia, dengue fever is endemic and poses a risk to both public health and deployed US military service members. Objective: Evaluate the presence of DENV in subjects with acute febrile illness in Cartagena. Materials and methods: From January to December 2019, we enrolled 64 subjects in four urban health facilities. The inclusion criteria was defined as:fever with $\leq$ 5 days of duration of illness, $\geq$ 5 years old and with no apparent reason of fever. From those who accepted to participate, we collect epidemiological/clinical data,and a whole blood sampleto isolate sera. RNA from sera was tested by RT-PCR for DENV, Zika and Chikungunya virus. Results: The average age of participants was 22 years old(range from 5-71) and distribution by sex was comparable (30 females, 34 male). We detected 11/64 (17%) DENV-positive samples;7/11 for DENV-1, 2/11 for DENV-2 and 2/11 for DENV-3. The number of DENV-positives was higher in males than females by a 10:1 ratio. There was also an increase in the number of cases in the months of higher rainfall(September to December).Among DENV-positive participants, the most frequent symptom was general malaise, followed by headache, chills and anorexia; five of them presented warning signs according to the WHO classification(vomiting, abdominal pain and mucosal bleeding). Discussion/conclusion: Multiple serotypes continue to co-circulate in Cartagena, highlighting the continued risk of exposure and potential for severe disease associated with secondary infections. In our study, we detected 3 serotypes (DENV-1, -2 and -3), while the Colombian MoH only reported two serotypes in the region (DENV-1 and-3). The detection of DENV-2 highlights the importance of our surveillance network to provide comprehensive pathogen detection data to support force health protection.

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Potential impact of macroclimatic variability on the epidemiology of Dengue in Bolívar, Colombia, 2015-2017

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Introduction. During the last decades, evidence has been collected on the association between the El Niño and La Niña climatic phenomena with the incidence of dengue in many regions of Latin America and the world, however, knowledge about such associations in regions of Colombia is scarce. For this reason, more studies of this type are required that may be useful in developing appropriate and timely strategies for the control of dengue in Colombia.Objective. In this study we evaluated possible associations between macroclimatic variations and dengue cases in the department of Bolivar, Colombia, in the period 2015-2017.Materials and methods. The epidemiological data were obtained from the National Public Health Surveillance System of Colombia (SIVIGILA) and macroclimatic data were based in the Oceanic Niño Index(ONI), according to National Oceanographic and Atmospheric Administration (NOAA, USA) classification.The months were categorized as El Niño, Neutral and La Niña to establish differences in the dengue incidence according those periods.Linear regression models were performed for climatic and epidemiological variables using SPSS v.19.0. Statistical significance was defined as ($p<0.05$).Results. During the study period, a total of 1.606confirmed cases of dengue were reported in Bolívar. The mean number of dengue cases per month was 46.33 $\pm$ 53.19 (range, 3-224). During El Niño, cases were significantly higher(media 86.06)than in the Neutral (14.91) and La Niña (14.11) periods(ANOVA F=14.002; p¡0.01).Linear regression models showed a significant association between the incidence of dengue and the ONI index, with higher values of ONI (above 0, El Niño periods) higher incidence of dengue was observed(r2= 0.554; p¡ 0.05). Discussion. The results of this preliminary study show associations between macro climatic phenomena and dengue epidemiology in Bolivar, Colombia, over a 3-year period. Similar facts have been observed in studies conducted in other regions of Colombia and Latin America. An additional study that covers a longer time period and includes microclimatic variables is needed to support these findings.

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Development and validation of an in house ELISA to determinate antibodies against Chikungunya virus

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Chikungunya virus (CHIKV) is an alphavirus which is mainly transmitted by infected Aedes species. The rapid spread of CHIKV in several countries in the Americas during 2015 was unprecedented and mainly associated with mild symptoms including joint pain. However, severe symptoms and disabling CHIKV cases were also reported. CHIKV has become a health threat to both military and civilian populations. The serologic diagnosis of CHIKV is a major challenge in regions with other non-CHIKV alphavirus etiologies such as Mayaro (MAYV) and Venezuelan Equine Encephalitis virus (VEEV) due to cross-reactivity of antibodies to homologous antigens among the viral family. Hence, the development of highly specific serological diagnostic techniques to differentiate infections caused by closely related viruses is needed. Here we describe the development, validation and performance of a novel IgM antibody capture ELISA(MAC–ELISA) against CHIKV. Briefly, viral antigen was prepared using a CHIKV isolated in Vero-76 cells from a Colombian human case. The MAC-ELISA validation was conducted using serum samples collected from febrile patients enrolled in Colombia, Venezuela and Peru during 2015 to 2016 as part of an ongoing febrile surveillance study in Latin America. Serum samples were laboratory confirmed as positive or negative by seroconversion and qPCR in their corresponding acute paired samples. A total of 64 CHIKV-positive convalescent serum samples and 52 CHIKV-negative convalescent serum samples were analyzed the novel MAC-ELISA assay. Additionally, cross-reactivity was evaluated in 18 and 12 convalescent positive-samples for MAYV and VEEV, respectively. The MAC-ELISA's performance was compared to the gold standard, the microneutralization assay. The analytical sensitivity and specicity, as well as predictive values of the novel MAC–ELISA was 100% (95% CI 93.2%-100.0%). We did not observe cross-reaction to MAYV nor VEEV. Our findings suggest that our new MAC-ELISA can be used as a highly specic assay for detection of CHIKV infection in regions with circulation of non-CHIKV alphavirus.

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The Global Vector Hub – a new platform to revolutionise vector control worldwide

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Introduction:More than half the world's population is at risk of vector-borne diseases, yet no single, all-encompassing resource for researchers and health workers involved in vector-borne disease control exists. Until now: the Global Vector Hub (GVH) is an exciting new online platform soon to be launched at the London School of Hygiene & Tropical Medicine. The objectives of the GVH are to assist in capacity building for vector control globally, establish a community of practice for vector control interventions, and enable stakeholders to make evidence-based decisions on vector control interventions. The main audiences of the GVH are public health officials, vector control agents and vector researchers, but it is open to all.Methods: The GVH consists of a community-led, online, open-access web platform to provide comprehensive information on vector control and vector biology. The three main components of the Global Vector Hub are: 1.Data: We are providing a comprehensive database of geo-tagged entomological data (vector abundance data, insecticide resistance monitoring, and pathogens vectored) and epidemiological data that will offer a clear overview of the vector situation in any country or region. Registered users can also share their data, for example from vector research projects or surveillance programs. 2.Resources: To assist in vector control capacity building, we are continuously creating a large repository of vector control resources, such as guidelines from WHO/PAHO and the CDC, toolkits, manuals, and online tutorials. We are also heavily involved in an upcoming Massive Open Online Course (MOOC) on disease vectors and their control. All material from this MOOC will also be integrated into our resource section. 3.Networks: We are building a searchable registry and worldwide network of vector researchers and vector controllers that will allow registered users to identify others working on similar vectors, diseases or in the same coun-

try/region, thus fostering the exchange of information and reducing wasteful duplication of efforts. Results and Conclusions: The first stage of the GVH focuses on South and Central America, and on Aedes mosquitos as vectors of arboviral diseases. Upcoming stages will expand into other regions, and also include malaria vectors, subsequently followed by other disease vectors groups.

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Isolation and identification of an echarate-like virus from chanchamayo, peru

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Introduction: Infectious diseases pose an important threat to operational readiness to the US military, and continued surveillance is critical to identify novel or emerging pathogens. Detection ofphleboviruses has increased in recent years and they can cause non-specific symptoms in humans that isoften misdiagnosed as dengue fever, malaria, or an influenza infection. Objective: Identify biological pathogens associated with febrile disease. Methods: NAMRU-6 conducts anIRB-approved passive surveillance study throughout Peru. Subjects$\geq\geq$5 years of age and reporting a fever for$\leq\leq$5 days are invited to participate and a blood sample is collected followed by serum separation. Sera is tested by RT-PCR for a panel of arboviruses;negative samples are passaged on Vero 76 cells followed by indirect immunofluorescence with antisera against Echarate, Murutucu, Catu, Itaqui, Apeu, Marituba, Bunyanwuera, and Itayavirus and four distinct pools of polyclonal antisera:1, yellow fever and dengue virus serotype 3;2, Venezuelan equine encephalitis, Eastern equine encephalitis, and Mayaro virus;3, encephalomyocardiovirus, Allpahuayo and Tacaribe;and 4, Guaroa, caraparu, Maguariand Oropouche.Sequencing was performed using high-throughput sequencing methods to generate unbiased amplifications of viral nucleic acids.The raw sequence data was processed using custom scripts. All contigs generated were identified by blast search. Maximum likelihood phylogenies for all segments was inferred under Jones-Taylor-Thornton (JTT) model with Gamma distribution with invariant sites (G+I).Statistical support was assessed using 1000 bootstrap replicates. Results: A 20 year-old male from Chanchamayo, Peru reported to a health clinic with 2-days of fever, malaise, chills, myalgias, arthralgias and headache. The serum sample from the subject was negative by RT-PCR and therefor submitted for virus isolation. A positive signal was observed with Echarate virus antisera. Phylogenetic trees of the L, M and S segments identified the isolate as a phlebovirus and found that the gene segments group within the Candiru complex genetic clade. Discussion and Conclusions: While there are well described arboviruses such as dengue virus, the risk exists of novel viruses emerging and becoming widespread. Sustained biosurveillance is necessary for the early identification of infectious diseases in order to develop effective mitigation strategies.

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Prevalence of Chikungunya Antibodies in a population from a prospective cohort study

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The Chikungunya virus (CHIKV) is the pathogenic agent responsible for the Chikungunya fever, which symptomatically varies from a non-apparent infection to rashand severe arthralgia. In recent years, the circulation of CHIKV in Brazil increased until its infection was detected in all states, mostly due to the high prevalence of the main vector *Aedes aegypti* mosquitoes (followed by *Ae. albopictus*) in urban and peri-urban areas. The aim of this work was to verify the circulation of CHIKV through theprevalence of antibodies in participants of a prospective cohort conducted in São José do Rio Preto (SP), Brazil, an endemic area for flavivirus circulation. For this, sera samples and a sociodemographic survey were collected in four different timepoints Oct2015-Mar2016 (FB), Oct2016-Mar2017 (A01), Oct2017-Mar2018 (A02) andOct2018-Mar2019 (A03) and the seroconversion of the subjects were analyzed. We

tested 341 paired samples to IgM and IgG anti-CHIKV by ELISA. The results showed that 32 (9.4%) participants were IgM positive or borderline during the four years of the follow-up. Considering the IgG results, we detected 04 (1.2%) FB participants positive or borderline, 05 (1.5%) in A01, 09 (2.6%) in A02 and 09 (2.6%) A03. The incidence in the period was 14.2 cases/1,000 habitants. The majority of the positivity occurred among participants from 21 to 60 years old and the females were the most affected (57.5%). All samples tested by ELISA assay were also analyzed by PRNT 80 and only one sample showed seroconversion with a title 1:40. Our results suggest the silent circulation of CHIKV in a flavivirus endemic area, mainly dengue and zika viruses. Epidemiological surveillance programs should be concerned in detecting evidences of the circulation of new viruses to prevent it to spread to different locations between naïve population avoiding major epidemics and becoming a public health problem.

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Development of a long-term dengue cohort in La Union, Piura – Peru

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Introduction: Dengue virus (DENV)is rapidly expanding around the world, threatening operational readiness of US military personnel deployed to endemic areas. To date there is no licensed therapeutic and only one licensed vaccine available with limited efficacy. As therapeutics and novel vaccines are being developed, there is a need for well described populations with defined rates of disease for product testing. Objective: To report preliminary DENV seroprevalence in a cohort study in Piura, northern coast of Peru. Methods: Enrollment began in July 2019through an IRB-approved protocol. 2316 households were randomly selected and subjects $\geq$5 years of age were invited to participate. Baseline sera samples were collected at enrollment and follow up sero-surveys will be conducted every year.Active surveillance is performed twice per week via phone calls looking for potential DENV cases.The inclusion criteria is fever for $\leq$5 days without an identified cause. Blood samples from cases are obtained within the first 5 days and convalescent samplesfrom 10–15 days. Baseline samples are tested by an ELISA IgG detection test for DENV using serotype 1-4 antigens. Positive results are corroborated by a microneutralization test against all fourDENV serotypes. Acute samples are tested by RT-PCR detecting DENV serotypes 1-4. Selected positive samples will be analyzed by full-genome sequencing.Results To date,1,020 participants have been enrolled in the study. Samples from 235 participants (21/235 under the age of 18) have been processed by ELISA for IgG against DENV and 83/235(35%) were positive. 9/83(10%) were positive only to one serotype (1/9 to DENV-2 and 8/9samples to DENV-3); and 72/83 samples (86%) reacted to multiple serotypes. 71/84 (84%) samples demonstrated reactivity to DENV-3. Discussion and Conclusions: Our preliminary data suggests that seroprevalence levels in La Union are lower than those reported inIquitos which is approximately 80% for the general population,and ranges from 67.1% to 89.9% for age-adjusted groups.Once data is collected in the following year, we will be able to determine incidence and have a well-characterized cohort to conduct product development.

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Natural Variation within Genotypes Regulate Escape from Highly Potent DENV3 EDII Type-Specific Human Antibodies

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Dengue virus (DENV) is a positive-stranded RNA flavivirus with four distinct serotypes (1-4). Each of these serotypes is further clustered into two or more genotypes that are genetically distinct. Individuals who acquire a primary DENV infection normally develop type-specic (TS) antibodies, which typically infers life-long immunity to the infecting serotype. Those who develop a secondary DENV infection generally develop long-lasting, cross-reactive (CR) antibodies that are protective against all four serotypes. However, with a secondary infection, a small portion of the population may develop more severe symptoms resulting in dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which can progress to death. A primary target for anti-DENV antibodies is the envelope protein (E-protein), which is comprised of a series of rafts—each encompassing three homodimers that contain two anti-parallel

protomers. These protomers are made up of three domains each (EDI, EDII and EDIII). We have identified DENV3 TS EDII human monoclonal antibodies (hmAbs) from a cohort in Nicaragua. Interestingly, all of the subjects in the cohort produced strongly potent neutralizing antibodies to EDII. Natural genotypic variation in the EDII regions of different DENV3 genotypes confer susceptibility or resistance to neutralization by two of the EDII hmAbs (DENV-115and-419). We know these hmAbs bind to EDII and that they can neutralize genotypes I, II and III but not genotype IV of DENV3. The goal of the study was to map individual variation responsible for the neutralization potencies of these EDII DENV3 hmAbs. Using reverse genetics, we implanted clusters of specic EDII residues from the susceptible genotype (GIII) into the resistant genotype backbone (GIV) to determine which residues are most functionally able to promote neutralization in a resistant genotype backbone. Our data reveal novel residues and genotype strains that regulate the neutralization with highly potent DENV3 EDII human antibodies. These data are important because high numbers of DENV3 break through infections have been noted in at least two different leading tetravalent vaccine formulations used in human populations.

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Mitogen-activated protein kinases p38 and JNK regulate Mayaro virus replication

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Mayaro virus (MAYV) is an Alphavirus belonging to the Togaviridae family. MAYV infection is associated with symptoms such as fever, myalgia, headache,rash and polyarthralgia. This arbovirus is transmitted through the bites of sylvatic mosquitoes, mainly members of the Haemagogus genus. Recently, a MAYV infection detected in a child in Haiti alerted health authorities about the potential spread of this virus to new territories. There are no approved vaccines or antivirals to combat this infection,but new cases indicate an urgent need to identify possible treatments. Mitogen-activated protein kinases (MAKP)are signal transduction pathways that regulate fundamental cell events, including proliferation, differentiation,cytokine production and apoptosis. Classical MAPK are extracellular signal-regulated kinases (ERK1/2), p38 andc-Jun N-terminal kinase(JNK). These kinases are activated in response to a variety of extracellular stimuli, and the signal is propagated through the phosphorylation of diverse substrates.Among these substrates are transcription factors, which modulates specific gene expression programs that allow cells to adapt to different physiological conditions. Viruses are able to exploit MAPKs'activity to favor their replication. However, whether MAYV is capable of manipulating these pathways to efficiently replicate has not yet been investigated. The aim of this study was to assess the effect of MAPK inhibition on the MAYV replication.Viral titers in supernatants from cells treated or not treated with MAPK inhibitors U0126 (ERK1/2), SB203580 (p38) and SP600125 (JNK) were measured by plaque assay. The cytotoxicity of MAPK inhibitors was assessed in human dermal fibroblasts (HDFs)and HeLa cells using the MTT method. Viral proteins expression in MAPK inhibitor-treated and control cells was evaluated by western blot.The inhibition of p38 and JNK in HDFs and HeLa cells reduced MAYV progeny production, whereas ERK1/2 inhibition had almost no effect. At the tested concentration of MAPK inhibitors, we did not observe any cell toxicity. On the other hand, we found a strong suppression of MAYV proteins expression in the cells treated with p38 and JNK inhibitors when compared to control cells.These results indicate that the MAPK p38 and JNK are required for efficient replication of MAYV.

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Primate antibodies neutralize a sylvatic strain but not the 17DD vaccine strain. What are the implications of this finding?

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Yellow fever (YF) is a zoonotic disease whose sylvatic transmission cycle of YF virus involves primarily monkeys and mosquitos, and humans are

accidentally infected. The most severe YF presentation is characterized by hepatic failure and hemorrhagic manifestations. IgG antibodies are detected during YF convalescence period, as well as on serological surveys. Plaque Reduction Neutralizing Test (PRNT) is the gold standard technique to detect specific antibodies against flaviviruses. Although this technique is very sensitive and specific, a few laboratories are capable of performing it due to availability of many different protocols and the labor intensity associated to it. The 17D/17DD vaccine strains are used in laboratories worldwide, mainly, because of its safety to workers. However, since the 50's, many authors have shown differences between the sylvatic virus and the vaccine strain, what could implicate in false-negative results, depending on the strain used on PRNT. Taking this into consideration, our aim was to investigate YFV infection in a monkey population captured in an area of occurrence of a positive human case. PRNT was performed on monkey samples (serum or plasma) using both, 17DD and JabSPM02 strains, the latter, a sylvatic strain isolated from a monkey in 2016. Out of 39 samples collected (38 marmosets and 01 capuchin monkey), none was positive by PRNT50 (cut off 1:10) when 17DD strain was used and 87,18% (34/39) were positive when PRNT was performed with JabSPM02 virus. Of those, 28,2% (11/39) yielded titers 40. PRNT was also done on the sample of the confirmed human case, yielding a titer of 1:80, only to the sylvatic strain. Based on these results, we were concerned if antibodies resulting from 17DD immunization would neutralize the sylvatic virus. However, antibodies present on sera from 17DD immunized people neutralized both viruses. This study confirmed the distinction of sylvatic and vaccine strains and demonstrated that choosing a wrong YFV strain interfere with serologic results on monkey sera, leading to a wrong conclusion that monkeys are not being infected by YFV and thus, avoiding the detection of an ongoing and past YF outbreak.

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Detection of Non-Epidemic Arboviruses Using HighlyConserved Primers

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INTRODUCTION: Arthropod-borne viruses (arboviruses) are a constant threat to Public Health, due to endemic, emerging and reemerging viruses and mosquito and tick vectors. Extensive available genetic and epidemiological data are suitable for computational biology studies that can model infection dynamics. Current molecular detection methods commonly rely on single or minimal number of pathogens and sensitivity is still an issue in sequencing methods. AIM: To design and standardize an RT-qPCR multiparallel detection assay for specific and sensitive detection of most pathogenic Alphavirus, Flavivirus and Orthobunyavirus known to date. For this study, epidemic arboviruses such as dengue, chikungunya, Zika and West Nile viruses were excluded as we already developed the standardized assay using the same approach. METHODS: Pathogenic viruses were identified and respective whole-genome alignments were built from those available in GenBank and ViPR databases. Regions with the lowest nucleotide diversity (Pi) and suitable G+C content were selected for primer design under stringent conditions in PrimerBLAST. Finally, those primers with the highest database coverage and the least number of degenerated bases were selected for assays. Primer concentration and dilutions of multipositive control plasmids were tested for optimized results in multiparallel integrated nanofluid circuits for the BioMark (Fluidigm) platform. RESULTS: Mosquito and tick-associated virus clusters were identified and at least two primer pairs were designed for each cluster. Thirty pairs of primers were included. Most of the primers are free of degenerated bases and can be used to discriminate between various pathogens given characteristic high resolution melting curves. CONCLUSION: We designed a panel for the detection of mosquito and tick-associated Alphavirus, Flavivirus and Orthobunyavirus genera members with human pathogenic potential, composed by 30 primer pairs presented here, complementary to a 13 primer-pair panel previously published for epidemic viruses. This new panel allows the RT-qPCR identification of multiple pathogens by the generated melting curve. Further work will be conducted to determine limit of detection, analytical sensitivity, precision, accuracy and analytical specificity for each new included assay. Computational biology approaches towards clusters of viral species provide improved preparedness against emerging,

reemerging or even recombinant viruses.

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Using Dengue prediction by tweets for vigilance in Brazil

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Dengue is a fast-growing mosquito-borne viral disease. Mentioning a disease in social networks is correlated with disease incidence and can capture health-seeking behavior at earlier stages of disease progression. Tweets were shown to be strongly associated with dengue cases, and a useful tool for estimating and forecasting dengue cases. The model for dengue cases estimation based on twitter data was applied for dengue surveillance in an endemic city in Brazil, to better understand the potential to support decision making and assist traditional surveillance. During 2019, dengue cases were estimated by the model every 2 weeks and indicated the peak of dengue incidence and amount of cases to occur in weeks 16 to 19, reaching around 14,000 weekly notified cases. The actual number of cases in the peak week of the season was 14,789 cases at week 19. Regarding future prediction of cases in the future, we observed good correlation between tweets and cases until 5 weeks in the future. The tweets model could explain the variance in dengue cases at approximately 91% in the nowcast or the actual week and 85% in the future week 4 or a month ahead. As expected, relative error of the estimation was bigger in the beginning and end of the epidemic curves, with a mean relative error raging from 30 to 60%. The official notification of Dengue had a delay of approximately 5-8 week; ie. the actual number of a given weeks is only known 5-8 weeks later. Therefore, dengue cases estimated by the tweets Appeared to be a valuable information for the decision making in dengue disease prevention and control in a health municipal secretary. The peak epidemic and worst scenario of the disease was possible to predict and estimated in a real case situation, also the model could help understand and evaluate in a fast pace the impact of the prevention and control actions in the population.

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Homoharringtonine Inhibited Dengue and Zika Viral Replication in Cell Lines and Prolonged Survival of Mice Infected with a lethal Dose of Dengue Virus

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Dengue and Zika virus (DENV and ZIKV) are single-stranded RNA viruses transmitted by the mosquito vector *Aedes aegypti* and are widely endemic in the tropical and subtropical regions of the world. Clinical symptoms caused by DENV include dengue hemorrhagic fever and shock syndrome which can be lethal if not treated promptly. Clinical symptoms caused by ZIKV includes microcephaly and other congenital diseases. Currently, there is no licensed drug for treatment of DENV and ZIKV infection. Harringtonine is a known inhibitor of the eukaryotic large ribosomal subunit that has been previously shown to possess antiviral activity against Chikungunya virus and Sindbis virus. Using DC-SIGN Raji cells as target cells for DENV and ZIKV infection, we found that homoharringtonine inhibited replication of all 4 serotypes of DENV and as well as ZIKV. The EC50 for serotype 1-4 of DENV were all below 0.1 μM. When tested in vivo in AG129 IFN a/b/gR-deficient mice infected with a lethal dose of DENV-2, the drug significantly prolonged survival of AG129 mice in a dose dependent manner without causing any drug-related clinical symptoms or weight loss. The data suggested that homoharringtonine is an effective antiviral small molecule compound and a potential host-based drug lead against arboviral infections. Further characterization of homoharringtonine on the timing of the treatment and on the mechanisms of its antiviral activity is underway.

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A cocrystal structure of dengue capsid protein in complex of inhibitor

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We report a high-resolution cocrystal structure (1.5Å) of DENV-2 capsid protein in complex with an inhibitor that potently suppresses DENV-2, but not other DENV serotypes. The inhibitor induces a "kissing" interaction between two capsid dimers. The inhibitor-bound capsid tetramers are assembled inside virions, resulting in defective uncoating of nucleocapsid when infecting new cells. Resistant DENV-2 emerges through one mutation that abolishes hydrogen bonds in capsid structure, leading to a loss of compound binding. Structure-based analysis has defined the aminoacids responsible for inhibitor's inefficacy against other DENV serotypes.The results have uncovered a new antiviral mechanism through inhibitor-induced tetramerization of viral capsid and provided essential structural and functional knowledge for rational design of pan-serotype DENV capsid inhibitors.

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IgG cross-reactivity against mayaro and chikungunya viruses in the Peruvian Amazon

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Chikungunya virus (CHIKV) and Mayaro virus (MAYV) are alphavirus that co-circulate in South America with others arthropod borne viruses, especially with Dengue virus (DENV). In areas with high DENV endemicity, human infections with these alphaviruses is frequently misdiagnosed due to similar clinical symptomatology (fever and arthralgia). The molecular assay (PCR) is highly specic and sensitive during the acute phase of the disease. However, these viruses only remain for two to six days from the beginning of the infection, therefore, serological assays are required to confirm the diagnostic. The high genomic similarity among alphaviruses renders problematic serologic diagnosis due to cross-reactive antibodies. The goal in this study was to evaluate the IgG antibody responses against CHIKV and MAYV in 75 dengue PCR (+) acute samples (AS). IgG/IgM ELISA kits from Euroimmun were used to measure the antibody responses. Samples were collected in Iquitos and Yurimaguas, both cities located in the Loreto region at the Peruvian Amazon. AS were collected between 7 days after the onset of symptoms (AOS) and the convalescent samples (CS) until 203 AOS. All samples were negative to CHIKV by PCR. None patient showed IgM antibodies against CHIKV, while IgG antibodies against CHIKV and MAYV were detected in 13/75 and 20/75 AS, respectively. Twelve of these samples were IgG positive for both viruses, with higher titres against MAYV. We evaluated neutralizing antibodies against CHIKV in ten samples, all with negative results, including two CHIKV ELISA(+). Neither patient showed a IgG seroconversion against CHIKV and MAYV in the evaluated CS. The high correspondence in the samples positive to both CHIKV and MAYV, suggest serologic cross-reactivity. However, for surveillance purposes, further analysis such as neutralization tests and/or more specific diagnostic tools are needed to rule out this question.

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Caracterización de los mecanismos de resistencia metabólica de Aedes aegypti en los municipios de Villavicencio, Ibagué y Yumbo

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Introducción: El estudio de la resistencia metabólica a través de pruebas bioquímicas se ha trabajado por décadas en la región de las Américas identificando a las esterasas, oxidasas de función múltiple (MFO) y glutatión-s-tranferasas(GST) como las familias enzimáticas que al alterar su actividad facilitan la resistencia a insecticidas. En consecuencia, los sinergistas actúan como inhibidores de enzimas, facilitando que el insecticida alcance su sitio blanco, y de esta manera, supere la resistencia. Objetivo: Caracterizar las enzimas de detoxificación involucradas en la resistencia metabólica de *Aedes aegypti* al insecticida

Deltametrina a través de la evaluación con sinergistas y pruebas bioquímicas en las poblaciones de Villavicencio, Ibagué y Yumbo. Metodología: Se evaluó el efecto de la exposición previa a los sinergistas PBO y DEF sobre la expresión de la resistencia al insecticida Deltametrina en las hembras de las poblaciones naturales de *Aedes aegypti* Villavicencio, Ibagué y Yumbode 1 a 4 días de emergidas y sin alimentación sanguínea, posteriormente, se realizaron pruebas bioquímicas de las mismas poblaciones evaluando la actividad 7 enzimas detoxificantes. Resultados. La evaluación de la eficacia del sinergista PBO en la mejora del efecto tóxico al insecticida Deltametrina, no evidenció un aumento significativo en la mortalidad de las poblaciones naturales, cuyos porcentajes no superaron el 50%. Asimismo, DEF presenta un aumento significativo en la mortalidad pero sin lograr una restauración completa de la susceptibilidad. Por otra parte, las pruebas bioquímicas exhibieron una actividad alterada y altamente alterada en las OFM en todas las poblaciones a excepción de Villavicencio – comuna 4, mientras las esterasas mostraron una actividad alterada variable en todas las poblaciones. Conclusiones: El efecto sinérgico de PBO y DEF no logra la restauración completa de la susceptibilidad en las poblaciones silvestres evaluadas, denotando que otros mecanismos, además de la sobreproducción de enzimas están involucrados en la alta resistencia al insecticida Deltametrina.

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Endoglin (Eng) and Syndecan-1 (SDC1) levels during the course of Dengue Infection

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Introduction: Dengue is a vector-borne viral infection caused by the dengue virus with potentially fatal complications. Clinically, dengue infection is categorized into febrile, defervescence & convalescent phases. Defervescence phase is considered as a critical phase because some of the febrile dengue cases either wane or develops into severe dengue cases. Thus, the identification of host factors during defervescence phase may be used as effective predictive markers. Studies have reported that plasma leakage is one of the hallmark symptoms of dengue infection. Several factors produced by endothelial cells have been associated with infectious diseases. Endoglin & Syndecan-1 are the transmembrane proteins highly expressed in endothelial cells and involved in the maintenance of vascular integrity. Several studies have demonstrated the role of Eng & SDC1 in the infectious disease pathogenesis related to the vasculature. Objective of the Study: To assess the dynamics of mRNA expression and protein level of two endothelial markers (Eng& SDC1)in Dengue patients. Materials & Methods: The study involved Dengue patients (n=25), Other Febrile Illness (n=10) and healthy controls (n=10). Samples were collected at 1st day (time of admission), 7th day (defervescence phase), and 14thDay (convalescence phase). The Dengue patients were categorized according to WHO classification consists of Dengue without Warning Sign (DWOW), Dengue with Warning Sign (DWW) and Severe Dengue (SD).The mRNA expression level & the serum concentration of two endothelial markers were evaluated using qRT-PCR and ELISA kit. Statistical analyses were performed using SPSS software, significance was measured atp$\leq$0.05. Results: The two endothelial markers Eng & SDC1 were significantly (p$\leq$0.05) increased during the defervescence phase in SD & DWW groups both at the expression level & protein level. Similarly, these endothelial markers were significantly increased in SD cases during day 7when compared to OFI & HC. Notably, we found a significant positive correlation between the serum concentrations of Eng & SDC1 during the defervescence phase of dengue cases. This reveals that these molecules are specifically associated with disease severity. Conclusion: Since defervescence is the critical phase wherein severity develops, differential expression of Eng & SDC1 observed in the current study shows that they are potentially involved in the dengue pathogenesis, however further study is needed to decipher the exact role of Eng & SDC1 in the disease virulence.

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Valoración de la temperatura, humedad y precipitación con la ocurrencia de casos de dengue en Guayaquil y sus alrededores

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Introducción: Las variaciones estacionales favorecen la diseminación de brotes en la transmisión del dengue, producto del aumento en la densidad poblacional del mosquito vector, *Aedes aegypti*. El objetivo de este trabajo fue asociar las variables temperatura, humedad y precipitación con la ocurrencia de casos de dengue en la ciudad de Guayaquil y alrededores, durante el año 2017. Metodología: Se efectuó una investigación descriptiva, de campo, no experimental y de corte transversal, desde junio adiciembre de2018. Para la recolección de la información se consultó la Gaceta Epidemiológica SIVE-ALERTA (MSP-Ecuador), donde se obtuvo el número de casos confirmados mensualmente, y se revisó el reporte diario del Boletín Meteorológico de Guayaquil y sus alrededores (INAMHI); posteriormente, se calculó la media mensual de las variables en estudio y se asociaron,utilizando el Coeficiente de Correlación de Pearson. Resultados: El mayor número de casos confirmados fue (n=642) y el menor registro de temperatura (24.4°C)en el mes de abril. La temperatura máxima (33.8°C) al igual que la humedad relativa del ambiente (78-87%) incrementaron en el mes de marzo. Los valores de precipitación, aumentaron entre marzo y abril (63%). Se encontró una asociación estadísticamente significativa (0,70 -0,84). Discusión: La temperatura mínima constante, pero gradual, estaría relacionaría al incremento dela transmisión del virus en la ciudad de Guayaquil y alrededores, motivadoa la humedad relativa que impera en épocas invernales. La humedad relativa incide enla eclosión de huevos y al asociarse con la temperatura, facilita la transmisión vectorial. Al ocurrir mayores precipitaciones, coexisten un mayor número de criaderos, y se prolonga la transmisión. En áreas con mayor precipitación, se esperan densidades vectoriales más estables, siguiendo un patrón endémico y en áreas de menor precipitación,la densidad vectorial fluctúa más, representando un patrón epidémico con brotes esporádicos en algunas épocas del año. Conclusión: Los casos confirmados de dengue incrementaron a finales del primer trimestre de año; asi mismo, los casos disminuyeron en la segunda mitad del año, en especial en el último trimestre. La temperatura mínima constante fue el predictor ambiental más importante encontrado, hallazgo que coincide con estudios relacionados.

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Investigation of Saint Louis Encephalitis and West Nile Viruses in equines from the state of Rio de Janeiro

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Epidemics and epizootics caused by arboviroses are reportes wordwide. The Saint Louis encephalitis virus (SLEV) and West Nile virus (WNV) are arboviruses medical and veterinary magnitude in the americas. The WNV, since 2009, had a new expression in Brazil when it's identification in a man in Piauí as a causative agent of neurological disorder and horses with neurological symptoms in Espírito Santo and Ceará, in 2018 and 2019. Previously only the circulation of WNV in the country was evidenced in serological surveys. in asymptomatic horses, in the Pantanal of Mato Grosso do Sul, in the state of Mato Grosso (MT), in the northeast and in the south of RJ. SLEV was evidenced in viral meningitis in 2006 in São Paulo and in an equine brain with encephalitis in Minas Gerais. Although the state of Rio de Janeiro is a large herd of horses, there are only few studies on the circulation of these viruses. In this study, through a serological epitope-blocking enzyme-linked immunosorbent assay (blocking ELISA) we performed an investigation for the circulation of WNV and SLEV in healthy horses of different regions of RJ. From a total of 435 serum samples submitted to the blocking ELISA, 38 (8.7%) were monotypic reactions to SLEV and 89 (20.5%) presented monotypic reactions to WNV. The highest number and frequency of flavivirus positive animals were in the North (65.8%) and Northwest Fluminense (67.10%) mesoregions. Detection of positive samples in the epitope-blocking ELISA suggests that RJ horses have been exposed to WNV and SLEV.

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