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Abstract Book

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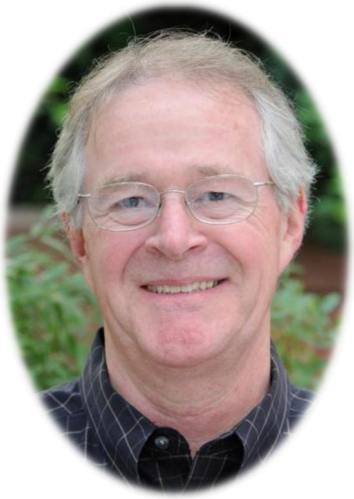

Tom Scott [1]

[1] University of California-Davis, CA, US/Peru

***Abstract*—** In my presentation, I will review highlights of the 24 years (1998-2022) during which I participated in the research program known locally in Iquitos, Peru as, "Proyecto Dengue." We began by testing hypotheses about the relationships among spatial and temporal dimensions of Aedes aegypti populations and dengue virus (DENV) transmission, then quickly expanded into the application of innovative science to enhance disease prevention and to make constructive contributions to the debate over improved public health policy. Our overall goal was to generate the detailed, difficult-to-obtain data that are necessary for assessing current recommendations for disease prevention, for rigorously testing fundamental assumptions in public health policy, and for developing innovative cost and operationally effective strategic concepts and tools for dengue prevention. The program was rooted in a combination of basic and applied science. As our experience in Iquitos grew, we became increasingly interested in how variation in critical components of transmission affect DENV transmission and whether data on those processes can be captured and applied in an operationally amenable way. To that end we (1) characterized and developed theory for the distribution and abundance of Ae. aegypti populations, their interaction with human hosts and DENV infection, and the relationship among entomological measures of risk to patterns of human DENV infection; (2) explored the dynamics of longitudinal DENV transmission patterns; (3) quantified and analyzed how variation in human behavior and human response to infection shape DENV transmission and influence our attempts to control it; (4) developed mathematical and simulation models to systematically study DENV epidemiology and prevention; and (5) carried out 3 clinical trials on novel entomological interventions. This was a highly collaborative program, which benefited from colleagues with diverse expertise. We blended our efforts in ways that created a unique opportunity to contribute to an improved understanding of dengue epidemiology, control, and public health policy.

Oral Presentations

Epidemiology – Phylogenetics

1

Insights From Mathematical Modelling In Understanding The Potential Of Wolbachia To Control Arboviruses

Ribeiro-Dos Santos, Gabriel [1]; Durovni, Betina [2]; Saraceni, Valeria [3]; Souza-Riback, Thais I. [2]; Pinto, Sofia B. [2]; Anders, Katherine L. [2]; Moreira, Luciano A. [2]; **Salje, Henrik** [1]

[1] University of Cambridge, UK; [2] City Health Secretariat, Rio de Janeiro, Brasil; [3] World Mosquito Program

***Abstract*—** The release of Wolbachia infected Aedes mosquitoes is an exciting opportunity to control stubbornly endemic pathogens such as dengue virus as well as epidemic chikungunya and Zika viruses. We have witnessed differing levels of success across release programs, with some places rapidly achieving high introgression and subsequent sharp reduction in dengue incidence, while other locations seeing only intermediate levels of introgression. Understanding the drivers of incomplete introgression and the impact of arbovirus incidence is complex, especially as underlying arbovirus incidence can be highly variable in space and time, and most infections go undetected. I will discuss how we can use mathematical models to help fill this knowledge gap, focusing in particular on the Wolbachia release program in Rio de Janeiro.

2

Butantan Dengue Vaccine Phase 3 Results

Nogueira, Mauricio [1]

[1] Faculdade de Medicina de São José do Rio Preto, Brasil

***Abstract*— Background:** Butantan-DV is a live, attenuated, tetravalent, dengue vaccine produced by Instituto Butantan analogous to TV003 developed by the US National Institutes of Health. We assessed the efficacy and safety of Butantan-DV in participants ages 2-59. **Methods:** Participants were stratified by age (2-6, 7-17, and 18-59 years old) and randomized 2:1 to receive a single dose of Butantan-DV or placebo in an ongoing, phase 3, double-blind trial conducted in 16 sites across Brazil, with projected five years follow-up (NCT02406729). Safety was evaluated as the frequency of participants with solicited (local and systemic) vaccine-related adverse events (AEs). Vaccine efficacy (VE) to prevent symptomatic virologically confirmed dengue (VCD) by RT-PCR after Day 28 postvaccination to any dengue virus (DENV) serotype was determined. Secondary objectives, VE by baseline serostatus, serotype, or against dengue with warning signs/severe dengue, regardless of hospitalization, and a subgroup analysis, VE by age, were also evaluated. **Results:** 16,235 participants were enrolled and received Butantan-DV (10,259) or placebo (5,976); 46.5% of participants were dengue-naïve. Non-serious, solicited systemic vaccine-related AEs were observed in a slightly higher proportion of overall participants receiving Butantan-DV (58.3%) compared to placebo (45.6%) within 21 days postvaccination. The proportion of participants with AEs within each age group was generally comparable to what was observed in the overall population. Enrollment took place between 2016 and 2019 and data through the cut-off (13-JUL-2021; based on the timing when the last participant completed 2 years of follow-up) included 2 to 5 years of follow-up. Through the data cut-off, the overall VE was 67.3% (95% CI: 59.4%-73.9%) and was 64.6% (95% CI: 49.4%-75.5%) in ages 2-6, 70.6% (95% CI: 57.8%-79.8%) in ages 7-17, and 72.8% (95% CI: 57.5%-82.8%) in ages 18-59. Serotype-specific VE was 75.8% (95% CI: 65.8%-83.1%) against DENV1 and 59.7% (95% CI: 46.5%-69.8%) against DENV2. VE against dengue with warning signs/severe dengue was 88.2% (95% CI: 50.8-98.2%). No cases of DENV3 or DENV4 were observed during the follow-up. **Conclusions:** In summary, Butantan-DV was generally well tolerated and efficacious against DENV1 and DENV2 symptomatic VCD, regardless of dengue baseline serostatus or age, and against severe disease through the follow-up period.

3

Chikungunya Transmission During The COVID-19 Pandemic: A Cohort Study In An Urban Informal Settlement

Cruz, Jaqueline S. *[1]; Ticona-Aguilar, Juan P. [2,1]; Anjos, Rosangela O. [1]; Nery, Nivison Jr. [1,2]; Argibay, Hernan [2]; Victoriano, Renato [1]; Xiao, Meng [3,4]; Belitardo, Emilia M. M. A. [1]; Silva, Lara R.*

S.[1]; Aragão-Filho, Ananias S.[1]; De Oliveira, Daiana[2]; Reis, Mitermayer G.[1,5]; Ribeiro, Guilherme S.[1,2]; Costa, Federico[1,2]; Ko, Albert I.[1]

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***Abstract*— Introduction.** Chikungunya virus (CHIKV) have caused seasonal outbreaks in Brasil since the introduction of the ECSA genotype in 2014. These outbreaks appears to be significantly smaller in magnitude and spatial extent compared to transmission of Dengue and Zika viruses. The reasons for the differences in chikungunya's epidemiology, whose agent has similar if not greater vectorial competence to DENV and ZIKV, is not well understood. Furthermore, decreased human movement during the COVID-19 lockdown has been postulated to be the cause of reduced reporting of arboviral disease, yet chikungunya outbreaks have been reported during the pandemic. To address these evidence gaps, we conducted a prospective cohort study, to determine the incidence of CHIKV infection and identify risk factors before and during the pandemic. **Materials and Methods.** We conducted three serosurveys between September 2018 and February 2021 in an urban informal settlement in Salvador, Brasil. We collected sociodemographic data and environmental characteristics and performed ELISAs (Euroimmun) with serum samples to detect anti-CHIKV IgG antibodies. CHIKV infection was defined as seroconversion between the sequential serosurveys. We used generalized linear mixed models that incorporated the household level as a random effect. **Results.** 1,425 residents were followed who had a mean age of 32 years (SD±18), and 61% were female. The cumulative CHIKV incidence was 36% (534/1,425) during the 2.5-year period. The inter-survey period with the highest CHIKV incidence (37%) occurred between Sep-2019 and Feb-2021. Multivariate analysis identified as factors significantly associated with infection: age (OR=1.02 per year,95%CI1.01-1.02) and prior report of a DENV or ZIKV infection (OR=1.57,95%CI1.04-2.36). Reported adherence to social isolation during COVID-19 lockdown was associated with a higher infection risk (OR=1.51,95%CI 1.00-2.26). **Discussion/conclusion.** Our findings indicate that transmission of CHIKV continued six years after the introduction of the virus in Brasil and that outbreaks have not been of sufficient size to impart herd immunity. The higher incidence was observed during the period of COVID-19 lockdown, and increased time in the household was associated with risk for CHIKV infection during lockdown, suggesting that the environment where people reside influences transmission and perhaps the focal nature of chikungunya outbreaks in the setting of urban informal settlements.

4

Prior Zika Or Dengue Virus Infection Increases Risk Of Subsequent Symptomatic Infection By Dengue Virus Serotypes 2, 3, And 4 But Not Serotype 1

Zambrana, Jose V.[1,2]*; Hasund, Chloe M.[3]; Aogo, Rosemary A.[3]; Arguello, Sonia[1]; Narvaez, Cesar[1]; Gonzalez, Karla[1,3]; Collado, Damaris[1]; Miranda, Tatiana[1]; Kuan, Guillermina[1,5]; Gordon, Aubree[2]; Balmaseda, Angel[1,4]; Katzelnick, Leah; Harris, Eva[6]*

[1] Sustainable Sciences Institute, Managua, Nicaragua; [2] Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, USA; [3] Viral Epidemiology and Immunity Unit, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD; [4] Laboratorio Nacional de Virología, Ministerio de Salud, Managua, Nicaragua; [5] Centro de Salud Sócrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua; [6] Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA

***Abstract*—** Infections with dengue virus (DENV) and Zika virus (ZIKV) induce cross-reactive antibodies. We have shown that primary ZIKV infection increases risk of symptomatic and severe disease caused by DENV2 and that DENV immunity results in protection or enhancement depending on pre-existing antibody levels and incoming serotype in our long-standing Nicaraguan pediatric cohort study (2004-present). In 2022, for the first time, all four DENV serotypes caused a major epidemic in Managua. Of 3,386 active cohort participants with longitudinal sampling, 10.6% (n=374) experienced symptomatic DENV infections; most were caused by DENV1 (n=139) and DENV4 (n=133), followed by DENV3 (n=54) and DENV2 (n=9). Prior infection histories were defined using DENV and ZIKV inhibition enzyme-linked immunosorbent assays (iELISAs), the ZIKV NS1 blockade-of-binding (BOB) assay, and laboratory-confirmed cases. Generalized linear and additive models (GLMs and GAMs) adjusted for age, sex, time since the last infection, and individual when appropriate were used to predict

disease risk. During the 2022 epidemic, compared to naïve individuals, a prior primary ZIKV infection significantly increased disease risk of DENV4 (Risk Ratio [RR] = 3.25, 95% Confidence Interval: 1.65-6.41) and DENV3 (RR = 2.90 [1.34-6.27]) but not DENV1 (RR = 1.20 [0.72, 1.99]). Children with a single prior DENV infection and a DENV infection followed by ZIKV infection were also at increased risk of subsequent disease caused by DENV4 (RR = 2.62 [1.48-4.63] and RR = 3.96 [1.93-8.12], respectively). We then performed similar analyses across 19 years of the cohort, finding that flavivirus-immune individuals were at significantly increased risk of dengue disease overall versus naïve individuals, but different effects were seen by serotype. Significantly increased risk was observed for DENV2 (p=1280. Overall, prior ZIKV infection, like prior DENV, increases risk of certain DENV serotypes, underscoring the significance of serotype-specific effects when assessing vaccine safety and efficacy.

5

Oropouche Virus As An Emerging Cause Of Acute Febrile Illness In Colombia

Ciuoderis, Karl A.[1]; Berg, Michael G.[2]; Perez, Lester J.[2]; Hadji, Abbas[2]; Perez-Restrepo, Laura S.[1]; Carvajal-Aristizabal, Leidi[1]; Forberg, Kenn[2]; Yamaguchi, Julie[2]; Cardona, Andres[1]; Weiss, Sonja[2]; Qiu, Xiaoxing[2]; Hernandez-Ortiz, Juan P.[1]; Averhoff, Francisco[2]; Cloherty, Gavin A.[2]; **Osorio, Jorge E.**[3]

[1] Colombia/Wisconsin One Health Consortium (CWOHC), Universidad Nacional de Colombia, Medellín, Colombia; [2] Infectious Diseases Research, Abbott Diagnostics, Abbott Park IL USA; [3] Global Health Institute & Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI

***Abstract*—** Arbovirus infections are frequent causes of acute febrile illness (AFI) in tropical countries. We conducted health facility based AFI surveillance at four sites in Colombia (Cucuta, Cali, Villavicencio, Leticia) during 2019-2022. Demographic, clinical and risk factor data were collected from persons with AFI that consented to participate in the study (n=2,967). Serologic specimens were obtained and tested for multiple pathogens by RT-PCR and rapid test (Antigen/IgM), with 20.7% identified as dengue positive from combined testing. Oropouche virus (OROV) was initially detected in serum by metagenomic next generation sequencing (mNGS) and virus target capture in a patient from Cúcuta. Three additional infections from Leticia were confirmed by conventional PCR, sequenced, and isolated in tissue culture. Phylogenetic analysis determined there have been at least two independent OROV introductions into Colombia. To assess OROV spread, a RT-qPCR dual-target assay was developed which identified 87/791 (10.9%) viremic cases in AFI specimens from Cali (3/53), Cucuta (3/19), Villavicencio (38/566), and Leticia (43/153). In parallel, an automated anti-nucleocapsid antibody assay detected IgM in 27/503 (5.4%) and IgG in 92/568 (16.2%) patients screened, for which 24/68 (35.3%) of PCR positives had antibodies. Dengue was found primarily in children (<18 yr) and linked to several clinical manifestations (weakness, skin rash and petechiae), whereas Oropouche cases were associated with the location, climate phase, and odynophagia symptom. Our results confirm OROV as an emerging pathogen and recommend increased surveillance to determine its burden as a cause of AFI in Colombia.

Modelling – Public Health – Burden of Disease

6

Immune Landscapes Of Dengue Virus

Cummings, Derek [1]

***Abstract*—** In many locations in the world, multiple lineages of dengue virus serotypes co-circulate. The extent to which genetic variation within serotypes affects immunological interactions within and between serotype is not well characterized. Here, we describe efforts to map antigenic variation of dengue viruses using a panel of African green monkey sera and antigenic cartography. We describe temporal dynamics of DENV in Thailand over multiple decades and propose theoretical models that may describe evolutionary forces that lead to antigenic differences between viruses over time. We also describe the relevance of our animal model derived antigenic relationships between viruses and the immune response of humans. Finally, we present evidence that antigenic relationships between viruses in Thailand help to explain, in part, patterns of age and serotype specific risk of medically attended dengue illness in Bangkok, Thailand

7

Global Arbovirus Initiative: Preparing For The Next Pandemic By Tackling Mosquito-Borne Viruses With Epidemic And Pandemic Potential

Rojas, Diana [1]

***Abstract*— The risk of emergence and re-emergence of arboviruses with epidemic and pandemic potential has increased as a global public health threat and will continue to do so in the years to come. The geographic range of arboviruses will also keep extending due to increased human movement, urbanization, climate change with environmental adaptation and uncontrolled expansion of mosquito vector populations. WHO has been working on strategic plans for multipathogen pandemic preparedness and response for health emergencies to strengthen capacities in vulnerable countries from local to national, regional, and global levels. In March 2022 WHO and partners launched the Global Arbovirus Initiative, comprised of six pillars: 1) Monitoring risk and anticipation; 2) Reducing epidemic risk; 3) Strengthening Vector Control; 4) Preventing and preparing for pandemics; 5) Enhancing innovation and new approaches; and 6) Building a coalition of partners. This initiative convenes partners across multiple sectors including health, agriculture, urban administration, and environment, as well as national, academic and private sector partners to forge a collaborative approach that builds on existing disease-specific programs to strengthen national integrated arbovirus disease programs. This will enable optimal use of limited resources to achieve the greatest impact. Epidemic response must be grounded in strengthening of ongoing national efforts to surveil for and respond to endemic transmission and localized outbreaks. Over the first year since the launch of the Initiative, WHO and collaborators have progressed in developing integrated risk maps for Aedes-borne arboviruses, appointed regional arbovirus consultants to drive implementation at regional level, and worked with WHO Regional Offices and Member States to adapt the approach to their specific priorities, frameworks, and needs. The successful implementation of the Initiative hinges on continued and political will, development of a sustainable funding model, and ever greater collaboration by partners to advance the objectives of pandemic preparedness and reduction of disease burden.

8

Projecting The Public Health Impact Of A Novel Dengue Vaccine Using Model-Derived Efficacy Estimates

Alkuzweny, Manar[1]*; España, Guido*[1]*; Perkins, T. Alex*[1]

[1] Department of Biological Sciences and Eck Institute for Global Health, University of Notre Dame

***Abstract*— Background & aims of study.** A safe and effective vaccine that can be universally administered could play an important role in controlling dengue. Takeda's QDENGA, which recently completed phase-III clinical trials and has been authorized for use in several countries, is a potential candidate for such a vaccine. However, trial results have indicated differential protection by outcome, infecting serotype, and serostatus. To understand how this will affect real-world effectiveness and to accurately inform rollout policies and recommendations, it is important to project the potential impact of the vaccine. **Methods & Results.** We first obtained estimates of protection that align with how impact projection models are parameterized by using a multi-level model that is simultaneously fitted to trial-wide, country-level, age-specific, and serostatus-specific clinical trial data on reported cases and hospitalizations. We then incorporated these model-derived efficacy estimates into an agent-based model of dengue dynamics to project the public health impact of administering QDENGA. We found that protection varies by both serotype and serostatus, with protection against disease ranging from 95.9% (95% CI: 86.3, 100.0) among seropositives infected by DENV-2 to -3.8% (95% CI: -78.9, 70.5) among seronegatives infected by DENV-4. Our estimates were less uncertain than trial estimates, as using a multi-level model allowed us to account for shared baseline epidemiological characteristics between arms. This is highlighted by our estimates for protection against hospitalization due to DENV-3 among seronegatives. While the published analysis of trial data estimated an efficacy of -87.9% (95% CI: -573.4, 47.6), indicating enhanced risk of disease, our model estimated a value closer to the null (-6.8%, 95% CI: -71.3, 51.4). Using our agent-based model, we project that QDENGA could avert 38% (95% CI: 36-42) of cases among seropositives and 1% (95% CI: 0.4-17) of cases among seronegatives. **Implications.** Our projections reflect the uneven protection by serostatus afforded by the vaccine. These results suggest that while QDENGA may be an important tool in controlling dengue, the heterogenous protection it offers may hamper its impact. These findings may be important for public health decision-makers to consider as QDENGA is authorized for use worldwide.

9

Developing a dengue virus lineage system to improve genomic surveillance

Hill, Verity[1]*; Vogels, Chantal*[1]*; Chaguza, Chrispin*[1]*; Grubaugh, Nathan*[1,2]

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06510; [2] Department of Biostatistics, Yale School of Public Health, New Haven, CT, USA, 06510

***Abstract*—** Last year, dengue virus caused nearly three million cases in the Americas. As climate change continues and Aedes species increase their geographical range, more populations are at risk of infection, leading to an increase in the disease burden. Interventions, including Wolbachia infection and vaccinations, are likely to be impacted by viral genetic diversity but dengue virus genetic diversity is currently not well described. Dengue virus genomes are categorized as one of four serotypes, and each of those have several genotypes. However, some sequenced dengue viruses do not cluster with the defined genotypes, and they do not capture most transmission dynamics. For example, most of the DENV1 sequences in the Americas fall into the DENV1-V genotype, and so details of how DENV1 spreads between and within countries using only the genotype designations are obscured. Further, any impact of pharmaceutical or biological interventions is not easily monitored, and both would require slower and more complex phylodynamic analyses. Following previous work on SARS-CoV-2 and Rabies virus, we proposed a hierarchical lineage classification system to address this issue. We built on the existing serotype-genotype system to maintain ties with the existing dengue virus research community; and designated all publicly available dengue virus genomes, including new genomes from the Caribbean and Florida. We have also written an accompanying software tool to enable other groups to assign new sequenced viruses for genomic surveillance programs. We then apply this lineage system to explore local and regional dengue virus transmission dynamics without the need for complex phylodynamic analyses.

10

Genomic Surveillance Of CHIKV Circulating In Argentina 2023

Fabbri, Cintia[1]*; Garcia, Jorge*[1]*; Barulli, Cintia*[1]*; Luppo, Victoria*[1]*; Feroci, Mariel*[1]*; Perrone, Sofia*[1]*; Casoni, Doraldina*[1]*; Jackel, Noelia*[1,2]*; Morales, María*[1]

[1] Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui"-ANLIS. Pergamino. Buenos Aires. Argentina; [2] National network of laboratories for the diagnosis of dengue and other arboviruses

***Abstract*—** Chikungunya virus (CHIKV) was detected in Tanzania, Africa, in 1952 and has been recognized in various regions over the years. The first autochthonous detection in the Americas was in December 2013 with the confirmation of the first human cases on the island of Saint Martin, spreading throughout the Caribbean region and later autochthonous transmission was detected in almost all of Latin America. In Argentina, laboratory surveillance of this pathology has been carried out since 2011 using molecular and serological methodologies. In 2016, 434 autochthonous cases were registered in two northern provinces of the country (Salta and Jujuy), identifying the Asian-American genotype in the samples in which genomic partial sequencing could be performed. After 2016, only imported cases were detected in Argentina. From December 2022 (epidemiological week (EW) 31 of 2022 and mid-April 2023 (EW 16), 1,460 autochthonous cases of CHIKV were registered in Argentina reported in 8 jurisdictions: Buenos Aires, CABA, Córdoba, Chaco, Corrientes, Formosa, Salta, and Santa Fe. Library preparation was performed with the Illumina COVID-Seq Assay kit, replacing the SARS-COV2 primers used for cDNA generation with specific primers for complete CHIKV sequencing designed by Quick et al, 2017. The sequencing reaction was performed on a MiSeq (Illumina) sequencing instrument using the MiSeq v2 reagent kit on a 300 cycle program. Genome Detective Virus Tool v2.52 (https://www.genomedetective.com) and CHIKV genotyping was performed with the Chikungunya Virus Typing Tool v3.69 (https://www.genomedetective.com/app/typingtool/chikungunya). Acute serum samples qRT-PCR CHIKV (+) (Ct <28) corresponding to the current outbreak in Argentina with were selected and 23/24 (95.8%) complete genomic sequences were obtained. The phylogenetic analyzes performed on these samples and their comparison with other genomes available in GenBank showed that all of them correspond to the ECSA genotype. This identification marks the introduction of a new genotype in the country. Currently, the ECSA genotype is the predominant one in Paraguay, Brasil and other countries in South América. This results remarks the relevance to continue with genomic surveillance studies to perform analyzes that contribute to a better understanding of the introduction and spread of CHIKV in Argentina during epidemics with an impact on public health.

Vector Biology – Vector-Virus Interactions

11

The Mosquito Virome And The Implications On Vector Competence For Arboviruses

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Abstract— Dengue is the fastest growing vector borne disease worldwide. Increased transmission has been driven by global spread of Aedes aegypti and A. albopictus mosquitoes, that are the major vectors for dengue virus (DENV) and other emerging arthropod borne viruses (arboviruses) such as Zika (ZIKV). Vector abundance has long been used to assess risk of infections, but dengue incidence is not dependent solely on the presence of mosquitoes. Indeed, factors that affect arbovirus transmission by mosquitoes in the wild remain largely unknown. Here we will discuss how the collection of viruses (the virome) naturally circulating in mosquitoes is mostly composed of insect-specific viruses (ISVs) but can directly affect vector competence. Our data also show that the virome is mostly species-specific and that ISVs are commonly associated with both invasive and local species of mosquitoes. This knowledge could help improve risk assessment tools and provide new strategies to control the transmission of arboviruses.

12

Mosquito-Virus Interactions And Zika Virus Emergence

Lambrechts, Louis [1,2]

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Abstract— Zika virus was once considered an innocuous member of mosquito-borne flaviviruses because documented human infections had remained sporadic and benign during several decades following its discovery in Uganda in 1947. The worldwide Zika virus epidemics that have occurred since 2007 and the unexpected link to neuropathologies and birth defects subsequently raised Zika virus to the status of global public health emergency. In the last few years, considerable research effort has been dedicated to retrospectively understand the factors that might have contributed to the spectacular emergence of Zika virus. Relatively less attention has been paid to regions where the virus is present but did not cause large-scale epidemics, such as Africa. In this talk, I will explore the hypotheses that can resolve this paradox and present data that provide a possible explanation.

13

Modifications caused by the NS1 protein of the dengue virus in the intestinal epithelium of the Aedes aegypti mosquito favor virus dissemination

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Abstract— Dengue is a viral infection transmitted by the bite of infected Aedes aegypti and Ae. albopictus female mosquitoes. The dengue virus (DV) RNA encodes three structural (C,prM, and E) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins involved in the virus replicative cycle. NS1 is the sole nonstructural protein actively and continuously secreted by infected cells. In humans, NS1 has been associated with disease pathogenesis through several mechanisms, including disruption of tight junction, thus altering endothelial homeostasis. When the mosquito feeds, NS1 is ingested along with the infectious virus. NS1 is known to promote mosquito infection by decreasing the antiviral response in vectors; however, it is unknown whether NS1 can also alter other mosquito factors that may aid virus dissemination. In this work, we provide evidence that ingested NS1 increases intestinal epithelium permeability and favors the spread of DV infection in Ae. aegypti mosquitoes. Colorimetric assays with blue dye no. 1 showed a significant increase in dye filtering into the mosquito midgut when NS1 was present in the blood meal, at 24h post-treatment. This effect disappeared when NS1 was heat denatured or preincubated with an anti-NS1 antibody ($p<0.01$). This observation suggests that intestinal barrier dysfunction occurs when NS1 is present. Confocal microscopy showed

evident changes in the localization of claudin-1 and β-catenin mosquito-like proteins, which participate in the gut of the tight junction analogs, septate junctions, in NS1-exposed mosquitoes. In addition, conventional H&E histology and transmission electron microscopy showed changes in the villi structure of intestinal cells and brush borders. Finally, qRT-PCR experiments indicated that NS1 helped disseminate the virus to secondary tissues within the mosquito. In mosquito hemolymph and abdomen, we found a higher DV prevalence (more than 40%), at 24 and 48h post-treatment with NS1, compared to mosquitoes that ingested NS1-blocked protein with anti-NS1 antibodies ($p<0.05$). Our results suggest that the NS1 protein facilitates the dissemination of the DV within the mosquito by modifying the mosquito's intestinal epithelium.

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H3 Histone Methylations Patterns In The Midgut Of Aedes Aegypti Mosquitoes Infected With Dengue Virus Serotype-2

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Abstract— Dengue disease is one of the challenges that public health has faced for decades, and the Americas region is one of the most affected. Scientific research is a necessary tool that can contribute to the multidisciplinary approach to EGI-Dengue (Integrated management strategy for dengue prevention and control). The Aedes aegypti mosquito is the main vector for transmission of the virus to humans. Vector genetics is an important intrinsic factor contributing to vector competence. Genes associated with the RNAi pathway such as DICER-2 and Argonaute-2 have been described as effector genes that participate importantly in the antiviral immune response (IR). Also, DICER-2 has been described as a QTL related to the DENV-2 susceptible phenotype, however, its transcriptional regulation is unknown. Epigenetic mechanisms, such as histone modification, could be associated with determining the DENV-2 susceptible phenotype. Differential expression of methyltransferase and acetyltransferase enzymes was observed in strains with resistant and susceptible phenotypes. As well as the enrichment of genes associated with transcription factors of the NOTCH and Jak-STAT pathways was observed in a mapping study of histone epigenetic marks such as H3K27ac, H3K9ac, H3K9me3, and H3K4me. However, in Aedes aegypti the role of histone epigenetic marks has not been extensively studied. Therefore, we proposed a study to determine by a multiplex assay, 21 histone H3 methylation patterns in the midgut of mosquitoes fed with dengue virus and identify the genomic sequences through a ChIP-seq assay associated with the H3K27me3 and H3K4m3 marks. As results, we determined that the Aedes aegypti strains evaluated present phenotype susceptible to DENV-2. Additionally, in silico we identified the presence of histone enzymatic machinery such as Su(var)3-9, EZH, and SET1, in the Aedes aegypti genome. Finally, a purified extract of histones (H3, H2A, H2B, H4) was obtained from the midgut of mosquitoes to measure the methylation pattern.

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16s rRNA Metagenomic Characterization Of Individual Midgut Microbiota Of Aedes Aegypti Larvae From Hatchery And Two Localities Of Tumbes, Peru

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Abstract— Aedes aegypti is a mosquito vector of dengue, chikungunya, and zika viruses, all of world concern. The characterization of the composition of the microbiota associated with the mosquito is relevant because is involved in several biological aspects of the vector, such as the sensitivity to viral infections, and being able to use these microorganisms as an alternative method of vector control. In this study, the microbiota present in the individual larvae midguts of Aedes aegypti from hatchery, Los Pinos, and Cancas, were characterized by 16s rRNA metagenomics analysis with QIIME2. Additionally, a pool of midguts of newly emerged adult females, 3 pools of midguts, and a poll of ovaries of blood-fed adult females from hatchery were characterized to evaluate the persistence of a possible core microbiota in different stages. At the genera level, midguts of larvae from Los Pinos, followed by Cancas, were more diverse compared with larvae from hatchery, with a mean of 227, 225.6, and 104.7 OTUS, respectively. Individual larvae midguts from Los Pinos, Cancas, and Hatchery shared 69, 67, and 35 OTUS, respectively. All the larvae midguts presented a possible core microbiota composed of the genera Pseudomonas, Mycobacterium, Bacillus, Acinetobacter, Cloacibacterium, and Bifidobacterium.

A possible core microbiota was found on the midguts of larvae and adults female from hatchery, even in the ovary of blood-fed adult females, it was composed of the genera Pseudomonas and Chryseobacterium. Interestedly, the genera Chryseobacterium in newly emerged females had 50.79 % of abundance, and in blood-fed adults females achieve a mean of 14.13%. The genera Pseudomonas had a higher abundance in the ovary of blood-fed females from hatchery with 14.46%. It was concluded how important the study of individual samples could be instead of pooling them, which could overestimate the number of shared microbiota at different stages and organs.

Ecology – Vector Control

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Harnessing heterogeneity to guide innovative Aedes aegypti control interventions in urban areas

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***Abstract*—** Transmission heterogeneity, whereby a disproportionate fraction of pathogen transmission events result from a small number of individuals or geographic locations, is an inherent property of most infectious disease systems. For Aedes-borne viruses, transmission heterogeneity emerges due to multiple drivers, including human mobility and exposure to mosquitoes in places other than their residence, the disproportionate contribution of inapparent infections to virus transmission, and variable Ae. Aegypti spatial distribution, biting and resting behavior. Given such major sources of variability, reactive Ae. aegypti vector control interventions in response to symptomatic cases are predicted to will fail to interrupt virus transmission. In collaboration with the Pan-American Health Organization, we developed an innovative framework that address the complexity of arbovirus transmission in urban areas and help guide the implementation of preventive Ae. aegypti control. I will show results from more than 8 years of research quantifying dengue transmission hotspots in cities of Mexico, Brazil and other countries in Latin America. Harnessing spatial heterogeneity in transmission risk will help deploy novel interventions. To that aim, I will also show results from ongoing studies in Mexico evaluating the epidemiological impact of targeted indoor residual spraying, an approach with potential as a preventive Ae. aegypti control tool. Combined, both studies provide information about emerging approaches with potential to strengthen arbovirus surveillance and Ae. aegypti control in endemic urban centers.

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Wolbachia Method: Results And Perspectives

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***Abstract*—** The World Mosquito Program (WMP) is a non-profit initiative that works to protect the population from diseases transmitted by mosquitoes. An innovation is the method that consists of releasing Aedes aegypti mosquitoes containing the Wolbachia microorganism into the environment, which have a reduced ability to transmit dengue, Zika and chikungunya. Wolbachia is a bacterium present in approximately 60% of insects in the world. WMP researchers managed to introduce this bacteria, which was taken from fruit flies, into Ae. aegypti. When the bacteria is present in the mosquito, these viruses do not develop well, reducing their transmission. The aim of the WMP is to establish populations of Ae. aegypti with Wolbachia. This is possible as the bacteria is transmitted naturally from the female to her descendants, who are already born with Wolbachia, ensuring the self-sustainability of the method. This initiative does not use any type of genetic modification. The Wolbachia Method is safe, natural and self-sustainable and has the potential to achieve a significant impact on public health in endemic areas. Currently, WMP carries out activities in 13 countries. The Wolbachia Method has proven effectiveness. A Randomized Controlled Trial, carried out in Yogyakarta, Indonesia, shows a 77% reduction in the incidence of dengue in areas treated with Wolbachia. In Niterói/Brazil there was a reduction of around 70%, 60% and 40% in the transmission of dengue, chikungunya and Zika, respectively. In complex environments in the city of Rio de Janeiro, a 38% reduction in dengue cases was demonstrated. In 2014, releases began in the pilot areas of Jurujuba, in Niterói, and Tubiacanga, in the city of Rio de Janeiro. Releases stopped in January 2016, since then, monitoring has revealed the establishment of Wolbachia over 90%. In November 2016, there was an expansion in these two cities, covering an area with approximately 1.3 million inhabitants. In 2019, WMP Brazil started acting in three other cities: Campo Grande, Belo Horizonte and Petrolina. The releases in these three municipalities will be completed by the end of 2023. After these initial phases, there are consistent plans for a national roll-out beginning in 2024.

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Rapid detection of dengue virus natural infection in Aedes aegypti from Peru by combined, portable real-time quantitative polymerase chain reaction and next generation sequencing

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Abstract— Aedes aegypti, the yellow fever mosquito which also transmits Dengue virus (DENV), Chikungunya virus (CHKV), Zika virus (ZIKV) and Mayaro virus (MAYV), is one of the most important arbovirus vectors globally. Several different methods have been developed to detect arboviruses in Ae. aegypti. Compared to virus isolation, reverse transcription polymerase chain reaction (RT-PCR) and quantitative real time PCR (RT-qPCR) offer reduced sample processing time and comparable or better sensitivity. We compared two approaches for DENV detection in wild-caught Ae. aegypti, an optimized Flavivirus RT-PCR (FU1/cFD2) combined with Sanger sequencing and a field-deployable RT-qPCR (Biomeme pan-Dengue Virus panel) combined with Nanopore sequencing. A total of 698 Ae. aegypti (65 pools) collected in 2016-2019 from 12 Peruvian military bases and 22 civilian communities located in coastal and jungle regions in Peru were tested. Lab-reared Ae. aegypti experimentally infected with DENV-2 were included as positive controls in addition to standard positive controls. Four mosquito pools were Flavivirus positive by FU1/cFD2 RT-PCR and eight pools were DENV positive (Cq = 8.9 – 23.19) by Biomeme RT-qPCR. All four Flavivirus RT-PCR positive pools corresponded to Biomeme DENV RT-qPCR positive pools with Cq higher than 18 and with a standard amplification curve. Flavivirus RT-PCR amplicons (250 bp) were sequenced by the Sanger method; BLASTn analysis showed 97-99% identity with DENV-3 (accession KJ643590.1). All eight Biomeme DENV RT-qPCR positive pools were sequenced by Nanopore technology using MinION; four pools positive for DENV-3 were detected coinciding with FU1/cFD2 amplicon sequencing. Furthermore, only these four pools were DENV positive when re-tested by Biomeme RT-qPCR. The four DENV-3 mosquito positive pools belong to collections performed in 2017 from military and civilian communities in Piura during a dengue outbreak in this state associated with the coastal "El Niño." Our results indicate that our RT-PCR/amplicon sequencing approach and the combined portable Biomeme RT-qPCR/MinION sequencing approach provide comparable results for DENV natural infection detection in wild-caught Ae. aegypti. Additionally, the Biomeme system is a user-friendly, rapid tool for DENV screening in mosquitos, yet confirmatory testing by sequencing is necessary.

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The Asian Tiger Mosquito invasion story in forest of Central Africa and potential consequences in a One Health perspective

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Abstract— The hypothesis of an invasion of forest environments of Ae. albopictus implies it has the ability to colonize forest ecosystems, including access to potential breeding sites, or feeding on wild reservoirs of arboviruses. The study aimed to investigate the potential invasion of forest environments by Ae. albopictus mosquitoes and their ability to colonize these ecosystems, including accessing breeding sites and feeding on wild arbovirus reservoirs. The study was conducted in the Lopé National Park in Gabon, monitoring Ae. albopictus populations from 2014 to 2018 in both human-influenced and wild areas using various methods such as ovitraps, BG-sentinel traps, and human landing catches. Additionally, a specific survey was carried out for five days, deploying three networks of 30 to 40 ovitraps in three forest sites near human habitations, at distances ranging from 0 to 175 meters from the forest edges towards the inner parts of the forest blocks. Larval surveys were also conducted in natural and artificial water collections within the village and the sylvan region of the park. The findings revealed that Ae. albopictus can persist in sylvatic ecosystems regardless of the degree of human presence, and its establishment in forests does not diminish its tendency to bite humans. The results indicated a continuous colonization process within the

forest, demonstrating the species' capacity to colonize the forest interior. However, a modeling analysis of colonization dynamics showed a progressive decrease in colonization levels with increasing distance from the anthropo-sylvatic forest edges. Furthermore, larval surveys highlighted that Ae. albopictus is more likely to colonize forest groves and galleries in the sylvan areas of the park. These locations serve as circulation hubs for animal reservoirs, suggesting that the mosquitoes may act as bridge vectors, facilitating the transmission of zoonotic viruses between forested regions and human-influenced compartments.

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Exploring Aedes Aegypti Dynamics Through Wolbachia Replacement And Hybrid Suppress-then-replace Strategies For Dengue Control

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***Abstract*— Background.** Wolbachia replacement offers an innovative approach for controlling Aedes aegypti transmitted arboviruses. Field trials have demonstrated a reduction in dengue burden following Wolbachia replacement and maximizing the efficiency of deployment while anticipating potential obstacles to fixation will inform scale-up of Wolbachia. **Methods.** A compartmental entomological model was used to explore how release size, release number and seasonal timing affect replacement speed. Hybrid suppress-then-replace approaches tested whether Wolbachia replacement could be achieved more efficiently if preceded by a mosquito suppression programme such as Wolbachia suppression (male-only release), Sterile Insect Technology (SIT), 1st generation self-limiting technology (1gSLT), environmental management, larvicides, and adulticides. **Results.** Trade-offs are demonstrated such as between the time taken for Wolbachia to reach target coverage and temporary exacerbation in the mosquito population. Suppression, particularly with interventions that induce mosquito sterility, can reduce the number of Wolbachia mosquitoes that need to be released to achieve fixation by up to 80% and achieve fixation faster. We predict that the optimal time to begin replacement-only or hybrid programmes is just before the seasonal lowest point in mosquito abundance. **Conclusions.** These results show that a hybrid intervention approach and timing of deployment significantly affect the rate at which Wolbachia reaches fixation. These conditions, in addition to potential trade-offs, can be managed when planning Wolbachia replacement to improve deployment efficiency.

Virology

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What Makes An Effective Live-Attenuated Vaccine? New Insights Into Molecular Mechanisms Driving The Innate Cellular Response To Yellow Fever Virus 17D (YFV-17D)

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***Abstract*—** The live-attenuated yellow fever virus strain 17D (YFV-17D) is considered one of the safest and most effective vaccines ever developed, conferring lifelong immunity with a single dose. Although the vaccine has been used for over 80 years, the basis for its immunogenicity remains poorly understood. In vaccinated humans, transcriptional profiling of PBMCs suggests that innate immune gene signatures correlate with protection, while integrated stress response gene signatures predict the magnitude of CD8 T cell responses. To determine how YFV-17D infection induces these responses, we examined dynamics of IFN expression and virus replication compared to the parental strain, YFV-Asibi, and an additional hepatotropic flavivirus, dengue virus (DENV2). Indeed, YFV-17D induced significantly higher type-I interferon (IFN) expression than YFV-Asibi or DENV2. IFN expression required the signaling

adaptor MAVS but not STING, suggesting a central role for the mitochondria in driving these IFN dynamics. Biochemical analysis of mitochondrial function by Seahorse analysis and LC-MS for metabolites revealed that YFV-17D uniquely upregulated mitochondrial respiration, and induced mitochondrial uncoupling associated with depletion of intermediates from the glycolytic, pentose-5-phosphate and tricarboxylic acid cycle pathways. Importantly, pharmacological inhibition of specific mitochondrial stress pathways eliminated IFN expression without affecting virus replication in tissue culture, and significantly reduced innate immune gene signatures of infected primary human dendritic cells. Thus, mitochondrial dysfunction in response to YFV-17D infection is a key driver of innate immunity, which has implications for further design of live-attenuated flavivirus vaccines.

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Molecular Studies On The 3UTR Of The Chikungunya Virus Genome

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Abstract— Chikungunya virus (CHIKV) is an important human pathogen transmitted by mosquitoes that has caused recent epidemics in the Indian Ocean and the Americas. Re-emerging lineages have fixed mutations in the coding sequence and large variations in the 3'UTR. As a result, these lineages feature 3'UTRs carrying different copy numbers of conserved sequence blocks referred to as direct repeats (DRs). To investigate the dynamics of the 3'UTR, we performed in vitro evolution experiments of viral populations. We found that genomic recombination occurs with high frequency in the 3'UTR of the viral genome, promoted by specific sequence and structural RNA elements. As a result, a spectrum of viable and non-viable variants with different number of DR copies are generated. Importantly, the CHIKV 3'UTR is under opposite selective pressures in mammalian and mosquito hosts. Whereas DRs are dispensable in mammalian cells, they enhance viral replication in mosquito cells. Consequently, recombinant viral variants lacking DR copies are widely generated and positively selected in mammalian cells, but are cleared when the virus replicates in mosquitoes. To study the relevance of RNA recombination in vivo, we experimentally infected reference laboratory mosquito strains. We observed that a deletion mutant that exhibits impaired growth rates in mosquito cells in vitro has also impaired transmission in vivo. Moreover, the 3'UTR of this deletion mutant undergoes RNA recombination in mosquitoes giving raise to new variants that acquired DR copies. Functional studies revealed that these variants have a clear advantage to replicate in mosquito cells over the parental mutant virus, underlining the relevance of RNA recombination for host adaptation. Based on these results, we have proposed a model to explain the function of the 3'UTR during host switching, in which genomic recombination acts together with natural selection to shape the composition of the viral population, enabling CHIKV to efficiently cross species barriers.

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Identification Of An Essential RNA Element Located Just Downstream Of The Stop Codon In The Dengue Virus Genome

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Abstract— The DENV genome consists of a single open reading frame flanked by highly structured non-coding 3' and 5' UTRs crucial for viral replication. The 3'UTR contains two Stem Loops (SLI and SLII), two dumbbells (DBI and DBII), and a 3' Terminal Stem Loop. SL and DB structures halt the activity of the host XRN1 exoribonuclease, leading to the accumulation of subgenomic flavivirus RNAs (sfRNAs) associated with immune evasion and pathogenesis. These structures are referred to as xrRNA. just downstream of the translation stop codon, preceding SLI, there is an unstructured sequence called the hypervariable region (HVR), which varies in sequence and length across DENV serotypes and genotypes. Deleting the HVR in a DENV2 infectious clone abolishes viral infectivity. To understand its role in viral replication, we designed a battery of mutants that gradually reduced its length, modified its

sequence, or introduced stable RNA structures at different positions. We found that viral replication requires at least 20 nucleotides between the stop codon and the first xrRNA1; otherwise, the viral RNAs are non-infectious. We hypothesize that the compact xrRNA structure following the stop codon interferes with viral translation termination or alters the proper folding of the viral 3'UTR. To investigate the mechanism of this observation, we search for revertant viruses after transfection of a viral RNA with a complete HVR deletion. We obtained a pseudo-revertant virus that recovered full replication, carrying a single mutation within xrRNA1 structure. Analysis of RNA folding revealed that this mutation destabilizes the xrRNA1 structure, likely interfering with its ability to halt the XRN1 activity. To confirm this idea, we examine the revertant virus property to generate sfRNAs in infected cell extracts using Northern Blot analysis. We found that the mutation was sufficient to impair sfRNA1 accumulation in infected cells, resulting in the generation of shorter sfRNA species. Together, our data confirm that the compact xrRNA1 structure following the stop codon at the viral 3'UTR is responsible of abolishing DENV replication. These findings provide new information about the complex interplay between translation termination, sfRNA formation, and viral RNA folding, which is essential for viral infectivity.

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Constraining Arboviruses To Their Arthropod Vectors

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Abstract— The compositional properties of viral genomes reveal their origin and evolution. Arboviruses (arthropod-borne viruses) replicate in insects that transmit them to vertebrates, their second host. Thus, arboviruses are exposed to the compositional biases of both hosts from distinctly different phyla. Compositional traits are genomic signatures across a genome and result in different arrays of oligonucleotides. To reduce the high dimensionality of the data and extract meaningful patterns, we applied principal component analysis (PCA) and multidimensional scaling (MDS) approaches to more than 8000 reference viral genomes. We found that arboviruses are dinucleotide underrepresented in CpG and UpA, whereas insect-specific viruses (ISVs) are only underrepresented in UpA. Using Mayaro virus (MAYV) as a model, we have rationally altered this dinucleotide frequency balance towards insect-specific viruses (ISVs) by computational design and synthetic biology. Our results show that recoded MAYVs grow as wild-type viruses in insect cells, but are significantly attenuated in mammalian cells and in the mouse model. Importantly, the attenuated phenotype of recoded MAYV can be reversed by targeting the zinc finger antiviral protein (ZAP). Collectively, we propose here insights into the influence of both arthropod and vertebrate immune systems in shaping the genetic composition of arboviruses.

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Mayaro Virus Pathogenesis And Immunity In Rhesus Macaques

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Abstract— Mayaro virus (MAYV) is a mosquito-transmitted alphavirus circulating in Central and South America that causes debilitating and persistent arthritogenic disease. While MAYV was previously reported to infect non-human primates, extensive characterization of MAYV pathogenesis is currently lacking. In this study we characterized MAYV infection and immunity in rhesus macaques. To inform the selection of a viral strain for NHP experiments, we evaluated five MAYV strains in mice and showed that MAYV strain Buenos Aires 505411 induced robust tissue dissemination and disease. Rhesus macaques were subcutaneously infected with 10^5 plaque-forming units of this strain into the arms. Peak viremia occurred at 2 days post-infection (dpi) with evidence of protracted viremia in one animal and rebound viremia at 10 dpi in two other animals. The NHPs were taken to necropsy at 10 dpi to assess viral dissemination, which included the arm and leg muscles and joints, lymphoid tissues, major organs, male reproductive tissues, as well as peripheral and central nervous system tissues. Histological examination demonstrated that MAYV infection was associated with appendicular joint and muscle inflammation as well as presence of perivascular

inflammation in a wide variety of tissues. One of the animals developed a maculopapular rash and two NHP had viral RNA detected in upper torso skin samples, which was associated with the presence of perivascular and perifollicular lymphocytic aggregation. The analysis of longitudinal peripheral blood samples indicated a robust innate and adaptive immune activation, including the presence of anti-MAYV neutralizing antibodies with activity against related Una virus and Chikungunya virus. Inflammatory cytokines and monocyte activation also peaked with viremia. The rhesus macaque model of MAYV infection recapitulates many of the aspects of human infection and is poised to facilitate the evaluation of novel therapies and vaccines targeting this re-emerging virus.

Virology – Pathogenesis

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Genetic Screens Expose ER Protein Biogenesis As Achilles Heel For Dengue Virus Replication

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Abstract— Dengue virus infection causes a range of symptoms in infected individuals, from mild febrile illness to severe disease associated with life-threatening plasma leakage and shock. A systematic understanding of host factors influencing viral infection is critical to elucidate virus-host interactions and could lead to antiviral strategies targeting host proteins. We have used genome-scale loss-of-function screens and identified cellular factors essential for dengue virus replication. We found a striking dependence on ER-associated membrane complexes uncovering ER-biology as Achilles heel for virus infection. I will present recent findings that provide remarkable insights into the molecular mechanism by which dengue virus has hijacked these cellular pathways.

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Searching For New Functions And Protein-Interactions Of The Dengue Virus NS1 Protein

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Abstract— Dengue is the most important viral disease transmitted to humans by mosquitoes. Aedes aegypti and Aedes albopictus are the two species of mosquito mainly responsible for the urban cycle of dengue. The dengue virus RNA genome encodes for 10 proteins: 3 structural (C, prM and E) and 7 non-structural proteins (NS1-NS5). The NS1 protein is a multi-functional, conserved glycoprotein that is present only in vector-borne flaviviruses. Intracellular NS1 participates in viral replication and virion morphogenesis. In addition, NS1 is secreted as a hexamer and soluble NS1 have been implicated in dengue pathogenesis through several different mechanisms. Much less is known about NS1 functions in the mosquito cells than in the vertebrate cells; a gap that needs to be closed toward better dengue control measurements. Evidence gathered by us and others, suggest that relevant differences in NS1 biology exist between dengue virus infected mammalian and mosquito cells. For example, while in vertebrate cells NS1 is secreted following a classical secretory route, in mosquito cells NS1 seems to be secreted by an unconventional secretory route that bypasses the Golgi complex, in association with caveolin-1 and several chaperones. To better understand NS1 functions in the mosquito, we used a proximity biotinylation system combined with mass spectrometry, which enabled the identification of over 800 potential proteins as part of NS1 interactome in mosquito cells. Groups of proteins associated with the ribosome, the endoplasmic reticulum and the Golgi were identified as expected. However, other protein pathways associated with the nucleus, and not previously reported, such as epigenetic regulation and RNA silencing, were also found. The presence of a significant fraction of the NS1 intracellular pool in the cell nucleus was verified by fractionation and confocal assays. Thus, we are currently focused on studying the participation of kinesins motor proteins in the traffic of NS1 from the ER to the extracellular space in mosquito cells, the importance of the presence of NS1 in the cell nucleus for virus replication, and new functions for NS1 in the mosquito. Results that add to the understanding of NS1 as a multifunctional protein will be presented and discussed.

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Accumulation Of Zika Virus Noncoding Subgenomic Flavivirus RNAs Downregulates The Translation Of Antiviral Genes In Human Host

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Abstract— The Flavivirus genus is a diverse group of viruses that includes a wide range of arthropod-borne pathogens, posing significant threats to human and animal health. Flaviviruses are single-stranded RNA viruses with a positive-sense genome, and are primarily transmitted through the bites of infected mosquitoes and ticks. This group encompasses several well-known and medically important viruses, such as dengue virus, Zika virus (ZIKV) and yellow fever virus. Noncoding subgenomic flavivirus RNAs (sfRNAs) are intriguing and vital components found among the Flaviviridae genus. They result from partial degradation of the viral genome and act as regulatory elements, playing a crucial role in modulating viral pathogenicity and host immune responses. Understanding the mechanisms and functions of sfRNA will provide valuable information into the complex interactions between Flaviviruses and their hosts, opening up avenues for the development of novel antiviral strategies. To dissect the functions and mechanisms of sfRNAs during ZIKV infection, we constructed infectious clones from local Argentinean isolates and developed recombinant viruses that are unable to generate sfRNAs (ZIKV sfRNAnull). We performed comparative studies using infections with wild type (WT) and sfRNAnull ZIKVs in human cells, and we confirmed that accumulation of sfRNAs was necessary to counteract the innate antiviral response. While the WT infection resulted in cytopathogenic effect after 48 hours post infection (hpi), the sfRNAnull virus efficiently infected human cells, but the infection was cleared at 72 hpi. Using ribosome profiling and RNA sequencing in infected human cells with both viruses, we observed a significant decrease in translation efficiency during infections with the WT virus. Specifically, the generation and accumulation of sfRNAs resulted in a significant reduction in the translation efficiency of Interferon Stimulated Genes (ISGs), which play a critical role in the host's antiviral defense mechanism. In contrast, ZIKV sfRNAnull did not exhibit this particular phenotype. These findings unveil a novel mechanism through which sfRNAs restrict the host's antiviral response by suppressing the translation of antiviral genes. Our data support the idea that the accumulation of sfRNAs creates a more favorable environment for viral infection and propagation.

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Genetic Ancestry-Associated Differences In Dengue Virus Infection In Human Skin

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Abstract— Host genetic ancestry has previously been implicated as a risk factor for severe dengue, however the lack of translatable models hinders studying this relationship. We used an ex vivo model of dengue virus (DENV) infection in human skin collected from healthy adults undergoing elective cosmetic surgeries to assess the potential effects of ancestry. We used a panel of 128 ancestry informative markers for determining European ancestry (EA) and African Ancestry (AA) on each donor (n=38). Skin explants were inoculated with DENV-2 (strain 16681) and analyzed at 24 hours post-infection (hpi) by confocal microscopy using antibodies for macrophages (CD163), DENV non-structural protein 3 (NS3), and inflammatory (IL-1β) or antiviral (IFN-α) markers. DENV infection in the epidermis and dermis was strongly correlated with EA. EA donors had a three-fold increase in recruitment of CD163+ macrophages to the site of infection and a two-fold increase in infection of those cells. When measuring cytokines, AA donors show a robust anti-viral response to DENV infection with significantly higher IFN-α production, while EA donors have a pro-inflammatory cytokine response with significantly higher IL-1β production. To investigate further, we obtained single nucleotide polymorphism (SNPs) data for genes known to be associated with ancestry and/or severe disease from all donors. 25 SNPs were associated with higher levels of NS3+ infection in the dermal layer. Four of these SNPs were in the retinoid x receptor-α (RXR-α) gene, encoding a ligand-dependent transcription factor ubiquitously expressed in the skin. Since previous studies show that RXR-α can inhibit IFN-α production and that SNPs in RXRA gene are associated with protection against severe disease in individuals of AA, we measured protein expression of RXR-α. Our preliminary results show that at baseline EA and AA donors have similar levels of RXR-α, but at 24hpi AA donors have a drastic decrease in expression. This downregulation could be related to the optimal IFN-α response and limited IL-1β seen in AA donors. These results provide evidence for the first time of ancestry-associated differences in the innate response to DENV infection in human skin and provide insight into potential targets for therapeutic drug development against severe disease.

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Co-translational proline hydroxylation is essential for flavivirus biogenesis

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***Abstract*—** Viral pathogens are an ongoing threat to public health worldwide. Dissecting the viral dependence on host biosynthetic pathways could lead to effective therapies[1]. Here we integrate polysome proteomics analyses with functional genomics and pharmacologic interventions to define how entero- and flaviviruses redirect the polysomes of infected cells to synthesize viral proteins and disable host protein production. We find infection with either Polio, Dengue or Zika virus drastically remodels polysome composition along similar principles, without major changes to core ribosome stoichiometry. These viruses use different strategies to evict from polysomes a common set of translation initiation and RNA surveillance factors, while recruiting host machineries that adapt polysomes for viral biogenesis. The factors recruited to viral polysomes provide effective targets for antiviral interventions. Surprisingly, we find both Dengue and Zika promote recruitment of the entire collagen prolyl-hydroxylation machinery to polysomes, to mediate co-translational modification of conserved prolines in the viral polyprotein. Genetic or pharmacologic inhibition of prolyl-hydroxylation blocks viral polyprotein folding and induces viral nascent chain degradation. This intervention abrogates viral polysome remodeling and blocks virus production. Our findings define the modular nature of polysome specialization at the virus-host interface and establish a powerful strategy to identify targets for selective antiviral interventions.

Pathogenesis – Antiviral

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The Architecture Of The Mature Flavivirus Particle Impacts The Neutralization Breadth Of Cross-Reactive Antibodies Against Dengue Virus

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***Abstract*—** In this presentation I will revisit structural studies that led to the characterization of human monoclonal antibodies (Mabs) potently neutralizing dengue virus serotypes 1 to 4 as well as Zika virus, and discuss the reasons for such an extraordinary cross-reactivity. I will further present more recent results on antibodies targeting domain III of the envelope protein and certain features of the epitope landscape of this domain. We found that domain III displays a surface patch that is recognized by Mabs cross-neutralizing Zika virus and dengue serotype 1, while an adjacent patch is recognized by Mabs neutralizing Zika virus and dengue serotypes 2-4, but not serotype 1. I will conclude by discussing the implication of such surface patches bound by cross-reactive antibodies with the potential interaction of domain III with a cell surface receptor common to all four dengue virus serotypes.

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Multidisciplinary Research On Neglected Arboviruses At The Brasilian Synchrotron Source

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***Abstract*—** Using the emerging mosquito-borne arbovirus Mayaro virus (MAYV) as an example, I will show how multiple technologies can be employed for the study of neglected infectious diseases, aimed at a more complete understanding of viral biology and proposition of much needed countermeasures against disease. Among the research projects performed by our team and collaborators, I will mention the identification and characterization of structural features of MAYV proteins and the viral particle, the insights leading to the search of virus-targeted antiviral compounds and how can we validate protective effects in vivo, including the use of a novel X-ray tomography technique at Sirius.

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Induction Of A Pro-Inflammatory Profile In Human Polarized Macrophages Following Chikungunya Virus Infection

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Abstract— Chikungunya virus (CHIKV) is a mosquito-borne pathogen that causes nearly 1.1 million human infections annually. Many patients recover within a few weeks of infection, but up to 50% develop chronic symptoms such as joint pain and swelling. Although the possible role of macrophages in promoting a local inflammatory response has been described, the pathogenesis of this condition is not well understood. It is known that, depending on the environment surrounding these cells, their phenotype and response to a specific stimulus can lead to an inflammatory or anti-inflammatory immune response, defining two distinct cell subsets known as M1 and M2 macrophages, respectively. In this context, there is no evidence on the ability of CHIKV to infect polarized macrophages or its role in inducing/modulating macrophage polarization. To describe the response of macrophages during CHIKV infection, we performed cellular and functional characterization of infected cells by flow cytometry, plaque assays and gene expression. To understand the functional effect of CHIKV infection on polarized macrophages, we quantified cytokines released into the supernatant by flow cytometry. We also confirmed macrophage infection by flow cytometry and indirect immunofluorescence. Our results showed for the first time that CHIKV is able to infect human polarized macrophages in different magnitudes, with M2 and M0 macrophages being more susceptible to infection than M1 macrophages. In addition, gene expression analysis and cytokine quantification in the supernatant showed that infected polarized macrophages upregulate the expression of pro-inflammatory proteins regardless of their polarization pattern. These results suggest a direct relationship between CHIKV infection of macrophages and the maintenance of a pro-inflammatory cellular microenvironment that is not polarization dependent.

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Spondweni Virus Infection In Pregnant Rhesus Macaques Causes Prolonged Viremia And No Apparent Fetal Harm

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Abstract— The 2015—2016 Zika virus (ZIKV) epidemic demonstrated the potential for flaviviruses to rapidly emerge, cause severe disease, and have unexpected impacts, such as harming pregnancies. In 2016, Spondweni virus (SPONV), the closest known relative of ZIKV, was detected in a pool of mosquitoes in Haiti, suggesting it also has the potential to emerge in the Western Hemisphere. Our previous study using a mouse model of infection during pregnancy found that SPONV can cause fetal demise and placental pathology similar to ZIKV. In our current study, we infected four pregnant rhesus macaques with SPONV (SA-Ar94 strain) during the first trimester (gestational day 30) and compared their viral loads and fetal development with four pregnant macaques infected with ZIKV (Dakar-Ar41524 strain; gestational day 45). We collected blood and urine samples throughout gestation to assess viral RNA (vRNA) levels and conducted regular ultrasounds to track fetal growth. The results showed that SPONV infection in pregnant rhesus macaques led to prolonged viremia (>21 days) in two of four individuals (23 and 31 dpi). Similarly, two of four ZIKV-infected macaques had prolonged viremia (28 and 77 dpi). We performed Cesarean deliveries (approximately gestational day 155) to examine maternal, fetal, and maternal-fetal tissues for viral infection using quantitative RT-PCR. Although SPONV vRNA was not detected in any tissues, low levels of vRNA below the limit of detection were found in the placenta and maternal spleen. None of the fetuses from SPONV-infected mothers showed any apparent adverse outcomes, such as growth restrictions, demise, premature birth, or gross physical abnormalities, similar to our ZIKV group. Placental and fetal tissue histology is ongoing. This study represents the first investigation of SPONV infection in pregnant rhesus macaques, demonstrating its potential to persist in plasma during pregnancy. Overt signs of fetal harm, as observed in previous mouse studies, were not detected in this study. Still, pathological complications from viral exposure in utero may be delayed or difficult to detect. While SPONV may not currently pose a significant global health threat, studying its effects during pregnancy helps us understand virus-host interactions and prepare for future outbreaks of emerging flaviviruses.

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Monoclonal Antibody against Zika Virus E protein EDI/II domain

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***Abstract*— Introduction and objective.** Zika virus (ZIKV) infection represents an emerging infectious disease that poses an increasing threat to human health, especially after the ZIKV outbreak in Brasil in 2015. This virus was associated with a significant increase in microcephaly in babies born to infected mothers. Unfortunately, there is still a lack of highly effective antiviral drugs or vaccines against ZIKV. The viral envelope protein (E) consists of three domains (EDI, EDII and EDIII) and is the main target of protective neutralizing antibodies. A comparison among ZIKV and the four DENV serotype (DENV1-DENV4) E proteins shows 55% identity. This high identity leads to cross-recognition of the different proteins by the immune system, which has implications for both protection and pathogenesis. Therefore, the main objective of this study was to produce and characterize human monoclonal antibodies (mAbs) using an approach consisting in the cloning and expression of DNA sequences that encode the variable regions of B cell receptors specific for the E protein obtained from a donor previously infected with ZIKV. **Methods and Results.** We obtained one mAb, A9Z, that recognized a conformational epitope in domains I and II of the ZIKV envelope protein. We showed that this mAb cross-reacts with the E protein of DENV1, 2 and 3 serotypes, in addition to neutralizing these viruses in vitro. We assessed the therapeutic relevance of A9Z using a lethal ZIKV challenge model in immunocompromised mice. Here, we showed that A9Z mAb confers better protection in ZIKV-infected mice compared to unrelated mAb or untreated mice. **Conclusion.** The administration of A9Z mA one day before and one day after virus challenge showed its protective potential as a drug to be administered both prophylactically and therapeutically. In summary, the present study suggests that A9Z mAb may be employed against ZIKV infection.

Clinical

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Dengue severity by serotype in 19 years of pediatric studies in Nicaragua

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***Abstract*—** The four dengue virus serotypes (DENV1-4) cause a range of clinical manifestations, from mild to severe and potentially fatal disease. Understanding dengue severity by serotype is critical, particularly in the context of the introduction of new vaccines that have differential efficacy by serotype. We studied the clinical spectrum of all 4 DENV serotypes over 19 years of two ongoing cohort studies in Managua: the Pediatric Dengue Cohort Study (since 2004; PDCS) and the Pediatric Dengue Hospital-based study (since 2005; PDHS). Study participants 6 months to 17 years of age were followed during their hospital stay or as ambulatory patients. Cases were confirmed by molecular, serological, and virological methods. Cases were classified according to the World Health Organization 1997 and 2009 guidelines. We enrolled a total of 14071 participants of which 2954 were positive for DENV (21%) laboratory-confirmed as DENV-positive. Of 2512 cases with serotype result by RT-PCR, 560 corresponded to DENV1, 1032 to DENV2, 745 to DENV3 and 175 to DENV4. Secondary (2°) cases were more prevalent in DENV2 and DENV4 infections, with 824 (76.3%) and 140 (83.3%), respectively, while primary infections occurred in 282 (52.1%) DENV1 and 369 (51.5%) DENV3 cases. Secondary infections presented a higher proportion of SD in all DENV serotypes, except for DENV-3, which presented a similar proportion of severe cases in primary and secondary infections (50.7% and 49.3%, respectively). When assessing the clinical manifestations by serotype, DENV3 was more related to pleural effusion (OR 2.66), poor capillary filling (OR 2.1), compensated shock (OR 2.24) and hypertensive shock (OR 2.84) which would explain why Children who present DENV3 have a 2.9 times greater risk of

presenting severity according to the traditional classification WHO 1997 and 2.5 times greater risk of severity according to the WHO 2009 classification, in addition to having a 2.56 times greater risk of entering intensive care unit. Overall, we found that clinical manifestations of dengue differ by serotype and immune response, with DENV2 and DENV3 being most associated with severity. Our findings indicate that the clinical spectrum of all serotypes should be considered in the development of a safe and effective vaccine.

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Development Of Maternal Neutralizing Antibodies During Pregnancy Post Zika Virus Infection Is Associated With Lower Risk Of Microcephaly And Structural Brain Abnormalities In Exposed Infants

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***Abstract*— Background**: Zika virus (ZIKV) infection produces robust humoral immune responses in adults. The role of maternal neutralizing antibodies (nAb) to ZIKV during pregnancy and potential associations with adverse infant outcomes following antenatal ZIKV exposure has not been elucidated. Methods: During the Zika epidemic of 2015-2017 in Rio de Janeiro, Brazil we established a cohort of pregnant women with ZIKV PCR-confirmed infection and followed participants over time. Maternal sera was collected during symptomatic infection (rash in the preceding 5 days) and periodically as of 14 days post-acute infection. ZIKV-infection was diagnosed by RT-PCR of blood and/ or urine at the time of clinical presentation. ZIKV plaque reduction neutralization assays with 90% neutralization (PRNT90) were performed, using a PRNT90 cut-off of 1:240. The magnitude of maternal Nab titers following infection during pregnancy was evaluated, with associations between maternal nAb activity and infant birth outcomes explored using multivariate linear regression. **Results**: PRNT90 assays were performed in serial serum specimens for 137 women with ZIKV-infection during pregnancy. Mean maternal age was 30.5 years (SD:6.51) with mean gestational age of infection 18.9 weeks (SD:8.6); 29% of women were infected in the first, 50% in the second and 21% in the third trimester of pregnancy. The mean ZIKV nAb titer > 2 weeks post-infection was 64,258 (SD:213288); 9% of PRNT90 titers were < 500, 12% between 500-1000 and remaining > 1000. 15% of evaluable infants had adverse findings: microcephaly (MC), structural brain abnormalities on imaging, hearing and/ or eye abnormalities. Protective factors associated with MC included infection later in gestation: aHR: 0.06, p=0.036 and presence of maternal Nab titers: aHR 0.17, p=0.014. Absence of structural brain abnormalities was associated with later maternal infection in pregnancy: aHR: 0.16, p = 0.017 and maternal nAb titers: aHR: 0.34, p=0.012. After adjusting for trimester of infection, the higher the maternal Nab titer, the lower the risk for MC and the lower the risk for structural brain abnormalities. **Conclusions**: Development of high maternal nAb titers during pregnancy following ZIKV infection was found to be protective against adverse infant outcomes such as microcephaly or structural brain abnormalities.

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Clinical Characterization And Severity Of Patients Diagnosed With Dengue In Different Age Groups During The 2019 Dengue Epidemic In Brasil

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***Abstract*—** Dengue is the main arbovirus in terms of morbidity-mortality. Clinical presentation ranges from mild, asymptomatic to severe disease and can differs between age groups, leading to a difficulty of diagnostic delaying an appropriate clinical treatment. Thus, this analysis evaluated the clinical characteristics in dengue-confirmed patients in different age groups, during the 2019 Brasilian epidemics. For that, data from 1365 patients were analyzed retrospectively, based on medical records and serum samples in 4 groups: child - 1 to 12 y.o.;

adolescent - 13 to 17 y.o.; adult - 18 to 64 y.o.; elderly - 65 y.o or older. The patient's samples were confirmed for DENV by NS1, RT-PCR and serological test (anti-dengue IgM detection). According to the WHO clinical classification the severe forms of the disease (DWS/SD) where more frequently in elderly (45,34%; p< 0.001), consequently with more hospitalization (58,02%; p<0.001). In the child group Exanthema was predominant (43,97%; p<0.001), while myalgia, arthralgia, headache and ocular pain were less frequently (41,84%; 5,67%; 48,94%; 24,11% respectively with p<0.001). In the elderly group, fever was less frequently (78,26% p 0.018) and leukopenia more often (45,63% p<0.001). Considering the range of the child age group, the difficulty of expression in younger ages and the subjectivity of symptoms such as myalgia, arthralgia, headache and ocular pain, this group was sub divided in 2 (1 to 5 y.o and 6 to 13 y.o). Myalgia, headache and ocular pain were more frequently in the 6-13 y.o. group (47,75% p 0.006; 57,66% p<0.001; 27,93% p 0.042). In DWS/SD vascular disease with fluid accumulation, respiratory distress and shock prevailed in the child group (35% p<0.001; 11,76% p<0.001; 11,76% p 0.008). Adolescent presented more abdominal pain (82,35% p<0.001) and bleeding (35,295 p 0.027). And, Elderly group presented more signs of hematologic involvement with predominance of thrombocytopenia (82,95% p 0.044) and hemoconcentration (46,88% p 0.015). Considering the results, to understand the particularities of the clinical characterization and severe manifestations of Dengue in different age groups is essential to a better comprehension of the disease, diagnosis and treatment to mitigate the evolution to severe forms of the disease in all ages.

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Dengue Virus Serologic Prevalence And Prospective Cohort In Adult Population From Metropolitan Area Of Medellín, Colombia 2018-2022

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***Abstract*— 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, information which would facilitate evaluating the implementation of antivirals for prevention and treatment for Dengue or how to introduce vaccines. **Objective.** Determine the seroprevalence and the incidence of primary Dengue virus (DENV) infection (symptomatic and asymptomatic) among adult population from metropolitan area of Medellin. **Materials and methods.** A prospective, community-based, phase 0 study was conducted in 3 independent cohorts of adult participants between Feb2018 and Dec2022. To determine the incidence of DENV infection a serological survey was performed through Panbio® indirect anti-DENV IgG, with semiannual sample collections. In addition, an active recruitment of febrile patients was performed. **Results and Discussion.** Over the three cohorts, 4885 volunteers were contacted, of which 38% showed antibodies against the DENV. 3008 seronegative volunteers were enrolled in the study. The average age of the evaluated volunteers was estimated at 22.5 (SD=7.74), 1076 (35.8%) were men and 1932 (64.2%) were women. Preliminary results indicated a seroconversion rate of <5% and none of these seroconverted volunteers showed symptoms. **Conclusion.** The results of this study provide epidemiological evidence of asymptomatic dengue cases, their immunological behavior, and knowledge of circulating serotypes. This facilitates the design of future studies for testing new interventions against dengue.

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Characteristics of dengue index cases enrolled to prospective phase 0 study of dengue infection in index cases and household contacts in Nha Trang – Vietnam

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***Abstract*— Introduction:** Dengue is among the top 10 global health threats, with half the world population considered at risk. Antivirals could have a role in controlling outbreaks or decreasing transmission in endemic areas in addition to other measures such as vaccines and vector control. We are developing a first-in-class pan-serotypic dengue antiviral small molecule (JNJ-1802). **Objective.** To validate the design of a phase 2, community-based, dengue index case (IC)/household contact (HHC) efficacy trial, by evaluating study operational aspects and virological parameters. **Results.** We conducted a prospective, community-based, phase 0 study with an IC/HHC design, between November 2021 and March 2023, starting with the identification of symptomatic dengue cases (classified as ICs) and their asymptomatic HHCs from Nha Trang, Vietnam. We enrolled 130 dengue ICs aged ≥ 1 year and 301 HHCs aged $\geq 18 - \leq 65$ years. We obtained information on the likelihood of reaching ICs (within 72 hours of symptoms onset) and HHCs (within 48 hours of IC screening). We also established the feasibility of following up HHCs through household or health facility visits twice a week for blood sample collections. We were able to evaluate the incidence of dengue virus (DENV) infections in HHCs through RNA detection. In addition, in an ongoing phase 2a, randomized, double-blind, placebo-controlled trial (NCT05048875) in adults, we are evaluating the antiviral activity, safety, and pharmacokinetics of different oral JNJ-1802 dose regimens using an attenuated DENV-3 human challenge prophylactic model. Initial results for one of the dose regimens showed a statistically significant difference in JNJ-1802 antiviral activity versus placebo, measured as the reduction in the area under the DENV-3 RNA viral load concentration-time curve from baseline to 28 days post-inoculation. No safety concerns were identified. **Conclusion.** We found that a community-based IC/HHC study design may be feasible in dengue-endemic areas. We also showed that JNJ-1802 displayed antiviral activity against an attenuated DENV-3 in a human challenge model. Based on these learnings, we will conduct a phase 2 community-based trial to further assess the efficacy of JNJ-1802 against naturally circulating dengue serotypes.

Arbovirus Research In Peru

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Situación Del Dengue En El Perú

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***Abstract*—** El dengue es una enfermedad ocasionada por el virus del dengue y transmitida por la picadura del mosquito Aedes aegypti. El dengue genera epidemias explosivas principalmente relacionadas con el ingreso de un nuevo serotipo, pérdida de la inmunidad en la población, variabilidad climática ("Fenómeno El Niño"), así como la persistencia del dengue se relaciona con la abundancia del vector, los determinantes sociales y las desigualdades. Actualmente el país atraviesa por una epidemia de dengue sin precedentes en su historia que se ha extendido a 20 regiones y que se ha atizado por la presencia de "El Niño" costero y el Ciclón "Yaku".

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Diagnosis and Surveillance of Dengue Serotypes and Genotypes in Peru, 1990-2023

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***Abstract*—** Dengue entered Peru in 1990, being the DEN1 serotype with which the epidemic began in the northern Amazon and spread to other Amazonian areas and the northern coast. The National Institute of Health (INS) is the head of the national network of public health laboratories, for which it carries out specialized diagnosis and carries out technological transfers, conducts the production and coordinates the distribution of diagnostic kits, manages the evaluation program of quality, directs laboratory-based surveillance and research development with the purpose of strengthening dengue prevention and control measures. In this sense, since the entry of dengue into the country, diagnostic tests have been implemented for the determination of IgM and IgG and later for the determination of NS1 using ELISA. Then, the dengue serotyping test was implemented by real-time RT-PCR and genotypic characterization by genomic sequencing, under the CDC/PAHO VIGENDA Protocol. Currently, in Peru 21 of 24 regions (57 laboratories) have the capacity to

diagnose and characterize dengue, improving the opportunity for care.

Various research groups such as the INS continue to make efforts to complement and understand more and more the behavior of dengue and the relationship with the vector and the host. In this sense, it is important to continue strengthening the diagnosis and surveillance of dengue since it allows us to know the circulation and distribution of dengue serotypes and genotypes in a timely manner, being a key tool for adequate decision making in Peru and in the countries in the region.

SEROTYPE	YEAR OF ENTRY TO PERU	CIRCULATING GENOTYPES (UNTIL 2019)	CIRCULATING GENOTYPES (2023)
DEN1	1990	Genotype III Genotype V	**Genotype V**
DEN2	2001/2010 2019	America/Asia America **Genotype II Cosmpolitan**	 **Genotype II cosmopotitan #**
DEN3	2001	India Genotype III	
DEN4	2001	Indonesia Genotype II	

Currently prevalent genotype

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Comparison Of In Silico Predicted Linear B-Cell Epitopes For Dengue Virus With Epitopes Discovered In A Proteome-Wide Peptide Microarray Analysis

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***Abstract*—** Identification of seromarkers with higher diagnostic accuracy for dengue virus (DENV) has become crucial given the growing co-circulation of clinically indistinguishable arthropod-borne viruses. Nowadays, serodiagnosis is complicated as serologic tests suffer from false positive results caused by cross-reactivity amongst members of the Flavivirus family. This underlines the need for a finer delineation of the smallest antigenic regions in the DENV protein that are recognized by type-specific (TS-) antibodies and/or B-cell receptors, and are known as B-cell epitopes (BCEs). Although most BCEs are conformational, linear BCEs have been exploited for the development of immunodiagnostics, peptide vaccines and therapeutic antibodies and therefore are the focus of this study. Previously in silico and functional methods have been successfully used for the identification of BCEs for common viral pathogens with remarkable examples such as human immunodeficiency virus, influenza virus and SARS-CoV-2. However, the comparison of in-silico predicted epitopes with in-vitro results is needed to further assess the usefulness and accuracy of the BCE predicting tools. In a previous study, we designed a custom high-density 15-mer peptide microarray covering the entire proteome of dengue, Zika and yellow fever viruses to profile the IgM and IgG antibody response in DENV-infected individuals. Highly reactive peptides across the DENV proteome were identified as seromarker candidates, and twenty DENV-specific 15-mer peptides were further evaluated on their utility to diagnose dengue patients. In this study, we attempt to assess the utility of linear BCE prediction tools by verifying whether the experimentally validated peptides previously identified are also picked up by the computer-based models. For this purpose, the sequence database that served to generate the peptide microarray will be used for the prediction of BCEs using popular machine learning algorithms including ABCpred, BepiPred and BepiBlast. The matched epitopes will be mapped on available DENV protein structures using Pymol. Additionally, we will explore to what extent changes in the amino acid (AA)-composition of the identified BCEs impact antibody recognition, providing new insights into AA-variety at the epitope level and into the potential of computational models to predict this.

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Knowledge, Attitudes, And Practices Regarding Dengue And Malaria In Remote Communities Of The Peruvian Amazon

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***Abstract*—** In the Peruvian Amazon, dengue is causing an increasing number of outbreaks in remote communities with little access to health information and care. Here, we report the results of nearly 600 knowledge, attitudes, and practices (KAP) surveys regarding dengue and malaria. The surveys were conducted with community members across 20 sites along the Ucayali, Puinahua, Marañon and Amazon Rivers. The sites represent a spectrum of urbanization,

healthcare access, and connectivity (both physical and virtual). Utilizing these surveys, we sought to identify possible gaps in knowledge regarding dengue and malaria, as well as the impact of knowledge on personal protective behaviors. Our results help to inform targeted outreach messaging for health centers in rural areas that are newly at-risk for dengue transmission.

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Targeted Indoor Residual Spray (TIRS) for Aedes aegypti with novel chemical compounds

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Abstract— Current efforts to reduce the burden of Aedes-borne viruses (ABVs), principally dengue, as well as more recently emerged Zika, and Chikungunya viruses are proving futile. A relatively new vector control alternative for Aedes aegypti is targeted indoor residual spray (TIRS) in which residual insecticide is applied to exposed interior surfaces where Aedes aegypti are known to rest. More specifically, residual insecticide is applied on wall surfaces < 1.5 m in height and under furniture. The TIRS application time per household is relatively fast (approximately 10 min) compared to traditional IRS (> 1hr). This study aimed to assess the effectiveness of TIRS for the control of Ae. aegypti in the city of Iquitos, in the Peruvian Amazon. We evaluated the efficacy and duration of two residual formulations, organophospate pirimifos-methyl (Actellic® 300 CS) and pyrrole Chlorfenapyr (Certero® 240 SC) on four types of wall surface (wood, brick, concrete and plywood) using WHO cone bioassays, and used the most effective product, pirimifos-methyl, in a cluster randomized control trial (CRCT). Thirty clusters with 50 houses were selected for the study, 15 clusters were randomly selected for TIRS with pirimifos-methyl and 15 served as untreated control. Adult mosquito surveys using prokopack aspiration were carried out pre-treatment and at ~15 d and 30-45d post-TIRS application in all households within the cluster (includes untreated houses in TIRS clusters). Mortality across all wall surfaces in WHO cone tests was 93% (1 d) and 76% (30 d) for pirimifos-methyl, and 6% (1 d) and 2% (30 d) for Chlorfenapyr. The CRCT trial showed an average adult Ae. aegypti reduction per household of 93% (74% - 99% range) in TIRS treated clusters compared to 12% in control clusters. Adult Ae. aegypti per household decreased by 97% in treated houses. Furthermore, space spray interventions carried out at the same time due to a dengue outbreak in Iquitos showed no measurable reduction in Ae. aegypti populations. Our results suggest that TIRS is a viable and more effective Ae. aegypti control strategy than currently used methods when applied at recommended dosage and flow rate.

Immunology

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Molecular Mechanisms Underlying Flavivirus-Dendritic Cell Interactions

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Abstract— Dendritic cells (DCs) are among the first cellular hosts of DENV and ZIKV and are thus critical players in both viral pathogenesis and immunity; however, the mechanisms by which DENV and ZIKV interact with human DCs are poorly understood. Previous studies have examined unseparated mixed populations of DENV- (or ZIKV)-infected and uninfected (bystander) DCs together, limiting the sensitivity and specificity for identifying viral-regulated processes. Consequently, these studies have yielded conflicting data regarding the impact of ZIKV and DENV infection on DCs. In the present study, we have overcome these limitations by performing transcriptional and functional analyses of purified populations of infected vs uninfected human monocyte-derived (moDCs) from multiple donors. Specifically, we performed RNA-seq on infected and uninfected bystander moDCs exposed to DENV or ZIKV and demonstrated that infection with ZIKV, but not DENV, downregulates transcripts involved in inflammatory signaling and T cell antigen presentation. Accordingly, ZIKV-infected moDCs exhibit a severely impaired ability to undergo maturation, produce proinflammatory cytokines, and stimulate allogeneic T cell responses. This is in striking contrast to DENV-infected moDCs, which exhibit upregulated expression of genes associated with cytokine and inflammatory

signaling. Our results provide a foundation for investigating how ZIKV and DENV induce opposing effects in moDCs and thus modulate their immune functions. This information will be crucial to the development of DENV-ZIKV vaccines and therapeutics.

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Multi-tissue transcriptomic study in severe dengue disease: In silico analysis of potential drugs for its management

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***Abstract*—** Dengue is a major global health concern given the increase on cases and fatalities, and virus and vector dispersions. Here we analyzed transcriptomic data in the key tissues of liver, spleen and blood profiles. Gene expression evaluation was done by Next Generation Sequencing, using Ion AmpliSeq Transcriptome Human Gene Expression Kit (10.8 million reads per sample) in liver, and spleen of dengue fatal cases and blood tissues of dengue severe cases. It was found upregulation of immune components pathways, like phosphatidylinositol 3-kinase binding, inflammasome and MHC complex; and metabolic components pathways, like insulin-like growth factor binding and cholesterol-binding proteins. It was observed downregulation of STAT protein phosphorylation pathways, RIG I like and type 1 IFN receptor binding, and liver metabolism. Despite transcriptomic differences due to tissue specialization, the common mechanisms of action "Adrenergic receptor antagonist", "ATPase inhibitor", "NFkB pathway inhibitor" and "Serotonin receptor antagonist" were identified as druggable to oppose the effects of severe dengue infection in the studied tissues. These are good candidates for future functional evaluation and clinical trials. Transcriptomics, proteomics and pathogen-host interactomics data is being explored for in silico informed-selection of drugs, prior to its functional evaluation. The effectiveness of this kind of strategy has been put into test in the current COVID-19 pandemic, and it has been paying off, leading to a few drugs being rapidly repurposed as treatment against SARS-CoV-2 infection. Several neglected tropical diseases, for which treatment remains unavailable, would benefit from informed in silico investigations of drugs, as we tried to perform in this work for dengue fever disease.

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Dengue virus NS5 modulates pro-inflammatory cytokine production in a serotype—specific manner

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***Abstract*—** Dengue pathogenesis and disease severity are associated with increased production of pro-inflammatory cytokines. This cytokine production varies among the four DENV serotypes (DENV1 to 4) and poses a challenge for live DENV vaccine design. In this study, we identified a viral mechanism that limits nuclear factor-κB (NF-κB) activation and cytokine secretion during DENV infection. Using proteomics, we detected that NS5 binds and degrades the host protein ERC1 to antagonize NF-κB activation and limit pro-inflammatory cytokine secretion. We found that ERC1 degradation involves unique properties of the methyltransferase domain of NS5 that are not conserved among the four DENV serotypes. Based on this observation, we mapped the amino acid residues in NS5 responsible for ERC1 degradation. We generated a recombinant DENV2 that exchanges serotype properties with DENV4 by a single amino acid substitution. Infection with this virus led to higher levels of cytokine expression and secretion, resembling that reported for DENV4. This work uncovers a novel function of the viral protein NS5 to antagonize immune signaling and limit cytokine production in a dengue virus serotype-specific manner. Importantly, these findings provide relevant information for improving tetravalent live attenuated vaccines.

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Antibody Correlates Of Severe Disease In Secondary Dengue Virus Infection After A Primary Zika Virus Infection: A Possible Role For IgA

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Abstract— Sequential infections of dengue virus serotypes 1-4 (DENV1-4) and Zika (ZIKV) can lead to protection or severe disease. We observed in our long-standing pediatric cohort in Nicaragua that prior ZIKV infection increases risk of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) during secondary DENV2 infection, potentially due to antibody-dependent enhancement. However, immune correlates of disease severity are not completely understood. Here, we analyzed antibody characteristics in samples collected after primary ZIKV infection that were associated with DHF/DSS versus dengue fever (DF) in a subsequent secondary DENV2 infection in 24 children (n=12/group) from our Nicaraguan cohort study. We characterized anti-DENV and anti-ZIKV antibodies (IgG1-4, IgA, IgM) in pre-secondary infection samples using a multiplex Luminex assay against recombinant E protein (E), E domain III, and nonstructural protein 1 (NS1) of DENV1-4 and ZIKV, and analyzed the Fc effector functions antibody-dependent complement deposition (ADCD) and antibody-dependent cellular phagocytosis as well. After modelling a dose-response curve, the effective dilution at which mean fluorescence intensity was reduced by 50% (ED50) was significantly associated with DHF/DSS for IgA but not for other isotypes. A bivariate logistic binomial regression showed that a 0.1Log10 increase in the ED50 of anti-DENV2 NS1 IgA and anti-ZIKV NS1 IgA, among others, increased odds of DHF/DSS by 3.07 (95% Confidence Interval [95%CI] 1.62-9.77) and 2.02 (95%CI, 1.31-4.34), respectively. A LASSO multivariate regression selected ED50 of anti-DENV2 NS1 IgA as the most relevant feature associated with DHF/DSS. Finally, a Bayesian network analysis revealed that ED50 of anti-DENV2 NS1 IgA and anti-DENV4 NS1 IgA increased the probability of DHF/DSS. We are currently analyzing the amount of IgA1/IgA2 and dimeric vs. monomeric IgA, the avidity of IgA against DENV/ZIKV antigens, and markers of neutrophil function in pre-secondary infection and acute-phase samples from these individuals. We hypothesize that IgA may be involved in a pathogenic pathway associated with DHF/DSS that deserves further study.

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Depletion of CD8+ T Cells Reveals CD4+ T Cell Contribution in Controlling Zika Viremia in Dengue-Immune Macaques

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Abstract— The introduction of Zika virus (ZIKV) in tropical/sub-tropical regions that are endemic to other close-related flaviviruses such as Dengue virus (DENV) has raised concerns, mainly because of their cross-reactivity in both humoral and cellular responses. The correlates of protection generated by a primary DENV infection on the course of a secondary ZIKV infection, and vice-versa, are poorly understood. This study aims to better characterize the contribution of T and B cells during a secondary heterologous flavivirus infection between DENV and ZIKV in non-human primates (NHP). To test this, we performed CD4+ depletions on Rhesus macaques, followed by a primary DENV-2 infection. After one year, CD4+ or CD8+ depletions were performed, followed by a secondary ZIKV infection (N=6: CD4-/DENV2/CD4-/ZIKV; N=6: CD4+/DENV2/CD4-/ZIKV; N=6: CD4-/DENV2/CD4+/ZIKV; N=6: CD4+/DENV2/CD8-/ZIKV; and N=5: CD4+/DENV2/CD4+/ZIKV as a control group). As expected, after the primary infection CD4-depleted animals have a longer DENV viremia compared to the control group. Additionally, CD4+ T cell depletion resulted in a delay in the expansion of DENV-2 neutralization titers during primary infection in comparison with the control group. We identified two remarkable results during the secondary ZIKV infection. First, CD8-depleted animals have less viremia compared to any other group including the control group. These results reinforce the main role

for CD4+ T cells controlling viremia as CD8+ T cell depletion resulted in a compensatory increase of CD4+ T cell frequency as shown in the immunophenotyping profile. Second, we found that the absence of CD4+ T cells during the primary DENV infection resulted in a higher neutralization magnitude against ZIKV. This result suggests that CD4+ T cells may interfere in delivering an efficient neutralization during ZIKV infection where type-specific antibodies generated during a previous DENV exposure do not contribute to secondary infection control. In addition, we show the impact of different CD4+ T cell depletion conditions in flavivirus epitope recognition by Luminex Multiantigens Platform. Our work sheds new light on the dynamic of the neutralization response and the multifactorial nature of the cellular immune response to flavivirus during subsequent infections and provides new insights into developing efficient DENV and ZIKV vaccines.

Immunology – Vaccines

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Immunity To Dengue Virus

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***Abstract*—** (not included)

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What We Have Learned About Protective Immunity From Dengue Controlled Human Infection And Live Attenuated Vaccine Studies

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***Abstract*—** Dengue vaccines must induce protection against each of the 4 dengue virus (DENV) serotypes as each serotype can cause the full spectrum of disease. An ineffective dengue vaccine may not only fail to protect against disease, it may actually predispose vaccinated individuals to more severe disease upon natural DENV infection. We have conducted more than 30 randomized, placebo-controlled clinical trials of monovalent and multivalent candidate dengue vaccines developed by the National Institutes of Health. We have also developed 2 dengue controlled human infection models (CHIMs) to assess vaccine efficacy and the protective immune response. These studies have afforded us the opportunity to evaluate the infectivity, replication kinetics, serologic and cellular immune responses of these monovalent and multivalent vaccine candidates. We will describe the clinical, virologic, and immunologic characteristics of three live attenuated tetravalent dengue vaccines that have either been licensed or are nearing licensure and apply the lessons learned from these studies to better understand the efficacy results reported for these vaccines. The extent to which each of these vaccines is able to induce homotypic immunity and how to best assess this will be discussed.

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Efficacy and Safety of Takeda's Tetravalent Dengue Vaccine Candidate (TAK-003) After 4.5 Years of Follow-Up

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***Abstract*—** **Background.** An ongoing long-term efficacy trial in eight dengue-endemic countries is evaluating a recombinant tetravalent dengue vaccine based on a DENV-2 backbone (TAK-003), with exploratory data after 4.5 years of follow-up presented here. **Methods.** From September 2016 to March 2017, healthy 4–16-years-old children (n=20,099) were randomized 2:1 to receive two doses of TAK-003 or placebo three months apart and were under active febrile illness surveillance to detect symptomatic dengue (both outpatient and hospitalized) using a serotype-specific RT-PCR. Serious adverse events (SAEs) were collected throughout the trial. **Results.** 20,071 children received ≥1 dose of TAK-003 or placebo; 27.6% (5547/20,063) were seronegative at baseline. 18,260 (91.0%) completed up to 4.5 years post vaccination follow-up and 27,684 febrile illnesses were reported. These led to detection of 1007 RT-PCR confirmed dengue cases, 188 of which required hospitalization.

The cumulative vaccine efficacy (VE) from first dose until 4.5 years after the second dose was 61.2% (95% CI 56.0-65.8) against virologically-confirmed dengue (VCD) and 84.1% (95% CI 77.8-88.6) against hospitalized VCD. VE in baseline seronegative participants against VCD was 53.5% (95% CI 41.6-62.9) and 79.3% (95% CI 63.5-88.2) against hospitalized VCD. In baseline seropositive participants, VE was 64.2% (95% CI 58.4-69.2) against VCD and 85.9% (95% CI 78.7-90.7) against hospitalized VCD. Efficacy was maintained beyond 3 years after vaccination regardless of baseline serostatus with sustained robust protection against hospitalized VCD. Rates of SAEs were similar between the vaccine and placebo groups and no important safety risks were identified. **Conclusion.** Two doses of TAK-003 three months apart were well tolerated and protected against symptomatic dengue through 4.5 years after vaccination in both dengue-naïve and pre-exposed children in dengue endemic countries. Efficacy was higher and sustained against dengue leading to hospitalization.

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Tumor Necrosis Factor (TNF) Contributes To Joint Inflammation, Hypernociception And Disease Severity In Chikungunya Virus Infection: Implications For Therapeutic Interventions

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Abstract— Arthralgia, characterized by joint pain, is a prominent symptom of chikungunya virus (CHIKV) infection and can significantly impair individuals' quality of life. The underlying mechanisms associated with the pathogenesis of CHIKV-induced arthralgia are not fully understood. In this study, we present a novel mouse model of CHIKV infection in immunocompetent mice and investigate the role of tumor necrosis factor (TNF) in the development of the disease. We also assess the potential therapeutic effects of etanercept, a TNF inhibitor, in CHIKV-infected mice. C57BL/6 wild type (WT) or deficient in TNF receptor 1 (TNFR1-/-) mice were inoculated with 1 ×106 PFU of CHIKV in the paw. Additionally, WT mice were treated with etanercept to inhibit TNF. We evaluated hypernociception, inflammatory responses, and virological parameters in the infected mice. Inoculation of CHIKV into WT mice resulted in persistent hypernociception, indicative of enhanced pain sensitivity. We observed significant viral replication in target organs and local production of inflammatory mediators during the early stages of infection. CHIKV infection also induced specific humoral IgM and IgG responses. In TNFR1-/- mice, the hypernociception threshold was decreased, suggesting increased pain sensitivity, which was associated with a milder local inflammatory response in the paw but delayed viral clearance. Treatment with etanercept, either locally or systemically, alleviated CHIKV-induced hypernociception.This study provides the first evidence of hypernociception, a clinical correlate of arthralgia, in immunocompetent mice infected with CHIKV. We demonstrate the dual role of TNF in contributing to viral clearance while driving tissue damage and hypernociception. Inhibition of TNF with etanercept may offer therapeutic benefits in CHIKV-infected individuals by reducing pain sensitivity. However, the role of TNF in viral clearance suggests that monitoring viral levels in CHIKV-infected patients is essential, and TNF inhibitors should ideally be used in combination with antiviral drugs.

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Development Of A Powassan Virus Like Particle Vaccine Adjuvanted With Novel Toll-like Receptor Agonists

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Abstract— Powassan virus (POWV) is an emerging tick-borne virus of the Flaviviridae family. Although

the number of people infected per year is relatively low, the range of the vector (Ixodes scapularis) that transmits POWV is expanding within North America, and the number of infections is rising in recent years. Infection in humans can be quite severe and lead to serious neuroinvasive disease with up to a 20% fatality rate. There are currently no vaccines or treatments available for POWV infection. Research in our lab is focused on development of flavivirus-targeting virus like particle (VLP) vaccines, composed of the preMembrane-Envelope (prME) region of the genome. Previous work has demonstrated that a VLP vaccination strategy is highly effective at preventing Zika virus (ZIKV) infection in murine and nonhuman primate (NHP) models. We have adapted this strategy to target POWV and are characterizing the immune responses and efficacy of VLPs when paired with several novel adjuvants. The University of Montana component of our team has patented a novel lipidated TLR7/8 agonist, UM-1007, and a novel TLR4 agonist, INI-2002. UM-1007 is a nucleolipid derivative that can effectively skew the response towards Th1-type whereas INI-2002 is a TLR4 agonist that demonstrated ability to enhance Th1/Th2 responses while also increasing antibody titers in primed mice. We have demonstrated that POWV VLPs when combined with UM-1007 or INI-2002 induce significantly higher levels of POWV-binding antibodies than VLP alone or VLP adjuvanted with alum. Furthermore, UM-1007 adjuvanted VLP vaccination results in the elicitation of a strong neutralizing antibody response, reduced viral load in the brain, and complete protection against POWV challenge in a murine model. We have also identified multiple lipid nanoparticle formulations of UM-1007 that synergistically enhance the antibody response to the vaccine. Current studies are focused on exploring the mechanisms by which specific adjuvants program the immune response toward an optimal anti-flavivirus response.

Diagnostics – Severity Markers – Prognostics

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Multiplex Sample-Sparing Antibody Assay Platform For Arbovirus Surveillance And Diagnosis

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1. ***Abstract*— Background:** Arboviruses such as Chikungunya, dengue, and Zika (CHIKV, DENV, and ZIKV) co-circulate and cause similar clinical symptoms. Pre-existing immunity to one of the four serotypes of DENV or ZIKV is a risk factor for progression to severe disease during secondary DENV infection. Due to the extensive antibody cross-reactivity, current serological tests for arbovirus have limited effectiveness for surveillance, pre-vaccine screening, and predicting the risk of severe illness. **Methods:** We developed sample-sparing, multiplexed, microsphere-based assays utilizing the antigenically distinct regions of the domain III (DIII) of the envelope protein from a panel of medically relevant alpha and flaviviruses. We developed algorithms to detect and differentiate acute infections using acute-convalescent paired sera from DENV, ZIKV, and CHIKV-endemic countries. We also defined the number of past infections using a single serum sample collected at the acute or late-convalescent phase. **Results:** Compared to RT-PCR and paired serology using whole viruses, the multiplex assay demonstrated exceptional sensitivity and specificity for detecting acute DENV or ZIKV infections. The envelope DIII-based multiplex assay also accurately detected past DENV, ZIKV, and CHIKV infections in patients who had previously tested positive by the live-virus neutralization tests. **Conclusions:** The multiplexed EDIII bead assay is robust, requires a small sample volume, and does not require handling virus to accurately detect acute infection and determine the history of prior infection with DENV serotypes 1-4, ZIKV, or CHIKV. This assay emerges as a powerful serological test for supporting seroprevalence and serosurveillance efforts, improving early diagnosis by assessing risk based on previous infection history, and screening for vaccine eligibility.

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Evaluation of Commercial Anti-Dengue Virus IgG Test Performance to Facilitate Dengue Immunization

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***Abstract*—** The Advisory Committee on Immunization Practices (ACIP) recommends screening children aged 9-16 years living in dengue-endemic areas of the United States with anti-dengue virus (DENV) IgG tests to confirm prior DENV infection before vaccination with Dengvaxia®. The minimum recommended test performance is ≥ 75% sensitivity and ≥ 98% specificity. We evaluated the performance of seven commercial anti-DENV IgG tests using serum panels from early convalescent specimens paired with acute specimens characterized by DENV and Zika virus (ZIKV) RT-PCR. The five best performing tests along with two additional tests specifically designed for pre-vaccination screening were evaluated with a panel of 44 specimens collected from healthy 9-16-year-old children in Puerto Rico. Four of these tests had promising discriminatory capacity for past DENV infections and were further evaluated using 400 specimens from the same population. Specimens from this population were classified as DENV exposed, ZIKV exposed, DENV and ZIKV exposed or unexposed using DENV and ZIKV virus Focus Reduction Neutralization Tests in combination with an in-house DENV IgG ELISA. No single test met the recommended performance to identify children eligible for Dengvaxia®; but the Euroimmun anti-DENV NS1 Type 1-4 ELISA combined with the CTK OnSite Dengue IgG rapid test R0065C with a visual test read yielded 80.3% sensitivity and 100% specificity. To meet the recommended performance standards, these two manufacturers modified their tests to stand on their own. Using the automated ALTA rapid test reader, the modified CTK OnSite Dengue IgG rapid test R0065C yielded 76.2% sensitivity and 98.1% specificity. The preliminary performance for the modified Euroimmun anti-DENV NS1 Type 1-4 ELISA was 76.6% sensitivity and 99.1% specificity. The modified tests that were developed by the manufacturers of the Euroimmun anti-DENV NS1 Type 1-4 ELISA and the CTK OnSite Dengue IgG rapid test R0065C met the recommended performance standards for identifying children eligible for vaccination and help to ensure that only children with prior DENV infection are vaccinated.

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Development of chimeric Zika virus proteins for serological diagnosis of low cross-reactivity

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***Abstract*—** Arboviruses constitute a group of viruses transmitted primarily by mosquitoes. They represent a major public health problem worldwide. The emergence of Zika in Brazil (2015) drew attention because despite being an acute and self-limiting disease, part of the individuals had severe neurological manifestations, congenital complications, and Guillain-Barré syndrome. The great antigenic similarity between flaviviruses makes diagnosis difficult. Thus, there is a need to develop new diagnostic tools. This work aimed to generate chimeric proteins capable of serologically differentiating ZIKV or DENV infections. The recombinant proteins (ZIKV-1, ZIKV-2, ZIKV-3) were designed and their respective genes were subcloned into a pET21a expression vector. The recombinant proteins were expressed and purified and antigenicity was validated. Purified proteins were tested as solid-phase antigens in standard ELISA protocols for the detection of anti-ZIKV IgG antibodies. The results obtained after standardization are promising, and the tests elaborated with the recombinant antigens showing high sensitivity and specificity, in addition to low cross-reactivity with the interferent with other viruses. The ZIKV-1 protein showed 91% sensitivity and 97% specificity, the ZIKV-2 protein showed 95% sensitivity and 96% specificity, and the ZIKV-3 protein showed 66% sensitivity and 84% specificity.

Regarding the DENV interferent, there was 10% cross-reactivity for ZIKV-1 and 19% for ZIKV-3, ZIKV-2 did not show cross-reactivity. The assays were also carried out in another laboratory and presented similar results, demonstrating reproducibility and robustness. The results found for ZIKV-1 and ZIKV-2 show great potential for developing a specific diagnostic test for detecting IgG antibodies to ZIKV using the produced chimeric proteins.

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The Serum Metabolome Reveals Insights Into Dengue Virus Infection And Disease Severity

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***Abstract*—** The four dengue virus serotypes (DENV1-4) place 3.9 billion people at risk of infection each year, rendering them the most prevalent arboviruses worldwide. DENV1-4 are the etiologic agents of dengue fever, which is a self-limiting disease, but some cases progress to dengue hemorrhagic fever and dengue shock syndrome with vascular leakage leading to shock and potentially death. Severe manifestations present within 4-7 days of fever onset, and reliable biomarkers that predict this progression are urgently needed. Here, a dynamic metabolic response to DENV infection and disease was measured in human serum, presenting a viable option for dengue disease state classification, biomarker detection and insights into the biology of dengue disease severity. Serum samples from 535 Nicaraguan children (1-14 years old) were collected during days 1-7 of illness. Clinical metadata included disease classification, sex, virus serotype, and prior DENV infection history. The serum metabolome was measured using liquid chromatography-mass spectrometry-based untargeted metabolomics that revealed 3,807 dysregulated molecular features across disease states. To unlock the underlying metabolism and biology within this dataset, pathway analysis was performed via the mummichog algorithm to glean information on dysregulated metabolic pathways. Forty-five metabolites were identified that are involved in the metabolic pathways of tryptophan, omega-6 and omega-3 fatty acids, fatty acid biosynthesis, eicosanoids, prostaglandins, purines, small peptides, amino acids, carnitines, glycerophospholipids and bile acids. Dysregulation of identified and tentatively identified serum metabolites were used to make inferences about the biology and progression of dengue disease. The dysregulated serum metabolites were used to classify patient disease outcome and identify novel, and recapitulate known, metabolic shifts related to dengue disease state.

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When Computer Science Met Virology: Methodologies For Quantitative Antigen Testing Of The Four Serotypes Of Dengue Virus

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***Abstract*—** One of the significant set backs for the use of antigen tests is their relatively low sensitivity, specially when compared to molecular techniques like qRT-PCR. In part, the lack of accurate sensitivity calculations methodologies of an antigen test is responsible for this bias view of antigen tests. Whereas molecular techniques require RNA integrity for its detection and amplification, lack of low temperature preservation of the nucleic acid and the need for far distant diagnostics adds value to using antigen detection, high sensitive methods in place for timely diagnosis of dengue. We propose the use of antigen test to detect NS1 protein of dengue serotypes 1, 2, 3 and 4 individually and to innovative the analysis based on image analysis and computer vision techniques. We present quantitative laboratory experimentation that predicts real-world performance by applying Bayesian probability to calculate the Percent of Positive Agreement function. The proposed appraisal methodology would be a useful tool for antigen test evaluations; it simplifies the process to assess the sensitivity of the test during clinical studies. The approach would allow researchers to standardize, democratize, and speed-up the process of validation, comparative analysis, and performance assessment of antigen tests for dengue diagnosis. The methodology would contribute towards more effective public health response strategies for test validation and the establishment of uniform analysis.

We describe these quantitative methods for dengue NS1 and unfold the predictive relational models using pixel intensity of the antigen test as a quantitative

outcome to derive corresponding qRT-PCR cycles, ng/ml of NS1 protein or plaque forming units (pfu/ml) of virus converting qualitative to a quantitative outcome.

Human Behavior & Community Engagement

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Participatory Research In Nicaragua: Data And Community Mobilization In The Control Of Aedes Aegypti

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***Abstract*— Dengue, Chikungunya, and Zika viruses pose significant challenges for public health disease management. Approaches to control disease transmission have traditionally focused on the mosquito vector using insecticide and non-insecticide methods. Traditional efforts have fallen short to control arbovirus transmission, and while novel technologies such as have shown promising results for suppression and replacement of Aedes populations, they are still being evaluated in different geographic settings. To test an alternative strategy, we implemented a sustainable community-focused Participatory Action Research methodology for Aedes aegypti control based on the Care Group and DengueChat programs in Managua, Nicaragua. We incorporated key principles from the Camino Verde - Socialization of Evidence for Participatory Action (SEPA) approach, and Communication for Development (C4D) strategies. Between 2017 and 2020, we conducted house-to-house visits to promote best Aedes control practices and water container management. These visits were carried out by community "brigadista" (volunteer) and a household participant. All containers that accumulated water were thoroughly inspected and their status recorded. The brigadista, rather than removing the positive container themselves, used well-structured lesson plans and flipcharts to encourage desirable behaviors and promote residents agency. The Care Groups coordinated "socialization" meetings with neighbors to build a sense of community and share achievements and neighborhood progress. These meetings were done in a way to prevent the stigmatization of specific households. Concurrently, the Ministry of Health carried out routine control measures in the area. Our results underscore the significance of involving communities and local leadership in the management and control activities of Aedes aegypti within neighborhoods. After the implementation of our control strategy, we observed a reduction of 81% for pupae/household, 64% pupae/container, and 81% pupae/person. With a sustained reduction of productive containers in the area. The successful combination of Care Groups, Community Based Participatory Research principles, key messages and proven communication strategies and the implementation of mobile technologies were successful in reducing Aedes mosquitoes in Managua.**

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Characterization Of Human Mobility To Improve Dengue Prevention Strategies

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***Abstract*— Background:** In contrast to the trend expected based on existing prediction models, dengue incidence was historically low in most endemic countries during the COVID-19 pandemic mobility restrictions of 2020-2021. This could indicate that current transmission models do not correctly take into account the human mobility and space where people reside during day-time transmission moments. Therefor we revisited a recent study that evaluated the association of arbovirus cases with visits to popular/crowded spots. **Methods:** We conducted a retrospective case-control study in Santiago de Cuba and Cienfuegos province between 2018-2019 characterizing 'regular mobility patterns'. For each case, two controls matched on residence neighbourhood, were selected. Cases were selected among the confirmed dengue/Zika cases from the routine surveillance list (IgM ELISA confirmed for dengue, PCR confirmed for Zika). We used a mobility survey, including geolocalization of residences, to characterize regular mobility paths of cases and controls. **Results:** The final sample size was 134/61 cases and 286/144 controls for Santiago/Cienfuegos respectively. The majority of participants are working outside their home, not being different between cases and controls (p= 0.33 for Cienfuegos, and 0.93 for Santiago). Occupation, gender and age were not significantly associated with being a case. In both locations, being a case was associated with regularly visiting during day-time specific 'popular/crowded spots', such as a street frequented for pendular movement (p=0.016) and a school-complex (p=0.032) in Santiago or an 'open square where people are connecting with wi-fi'(p=0.036) and a primary school**

(p=0.028) in Cienfuegos. **Conclusions:** People spend more time outside their homes than being in their homes, so the focus of epidemiological attention for epidemic control should not only be centered on individuals' homes. Places where many people are gathering during day-time and the movement between such spaces and the residences could be an essential driver of spatio-temporal heterogeneity of DENV transmission dynamics.

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Estrategias Educativas y Comunicativas Para El Control Del Dengue En Dos Poblaciones Con Alta Carga De La Enfermedad: Un Enfoque En Educación En Salud

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***Abstract*— Antecedentes.** El dengue representa una problemática de salud en la subregión del Urabá, Colombia; los casos notificados en el año 2018 representaron el 37% de la morbilidad del departamento de Antioquia y el 42% de la mortalidad por esta causa. A pesar de las diversas estrategias comunitarias implementadas, la apropiación ha sido limitada por parte de las comunidades afectadas, lo que se ha reflejado en la poca participación para la reducción de casos de dengue; conscientes de esta realidad y reconociendo la importancia del trabajo intersectorial y comunitario de las poblaciones, se co-diseñaron e implementaron estrategias educativas y comunicativas desde un enfoque de educación en salud en dos localidades de los municipios de Apartadó y Turbo, que presentan alta carga de la enfermedad. **Métodos.** se realizó una investigación cualitativa de corte participativo, apoyado en el enfoque de educación en salud, que se asume como un proceso que combina la formación y la comunicación, en la perspectiva de adoptar medidas destinadas a mejorar la salud desde el desarrollo de habilidades personales y sociales que beneficien a las comunidades, en especial, aquellas sumergidas en situaciones de riesgo por factores relacionados con la salud pública. Se implementaron técnicas interactivas con grupos poblacionales que facilitaron el co-diseño y la apropiación de estrategias educativas y comunicativas para el control del dengue. **Resultados.** los productos estuvieron relacionadas con el desarrollo de competencias en conocimiento general del dengue, particularidades de la cultura ambiental, y actividades de comprensión, transformación y participación de los pobladores e instituciones del territorio, para el control del mosquito vector y su prevención, de allí las diversas estrategias construidas: cartilla, videos, audios, jingles, podcast, entre otros. **Conclusiones.** la dinámica de interacción social e institucional posibilitaron procesos de co-construcción desde las iniciativas de los participantes poniendo en evidencia sus saberes y prácticas, que enriquecieron la experiencia del cuidado de la salud mediada por la Apropiación Social del Conocimiento.

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Efectividad De La Estrategia Municipal Para El Control Integral Del Dengue En Xochitepec, Morelos, México

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***Abstract*—** La transmisión del dengue es determinada por una compleja y diversa interacción de factores biológicos, sociales, económicos, demográficos y ambientales. Objetivo: Evaluar la efectividad de una estrategia de control integral del dengue durante tres periodos administrativos (2013- 2015, 2016-2018, y 2019-2021) en el municipio de Xochitepec, Morelos.

Materiales y Métodos: Investigación Acción Participativa con análisis epidemiológico (casos confirmados), entomológico (patios controlados) y actividades de control integral del vector. Aplicación de peces para el control biológico del vector, saneamiento físico y químico, promoción de la salud, educación ambiental, coordinación intersectorial y municipal (sala situacional). Resultados: Participación y formación de 3100 promotores comunitarios. Eliminación de 298, 371 y 281 toneladas de cacharros en cada trienio de gobierno, respectivamente. Incremento en cada periodo de patios sin riesgo entomológico de 76% a 97.6%, de 93% a 97.9% y de 94 a 98%, así como disminución de presencia de larvas de 25.49% a 7.58%, de 14.6% a 3.1% y de 13% a 2%, respectivamente. Los casos confirmados de dengue no grave en cada periodo fueron 111, 9 y 82 y de dengue grave 75, 1 y 4 respectivamente en cada trienio, sin reporte de defunciones por dengue. Reducción sostenida del uso de insecticida en 50% y de 25% de larvicida, con reducción progresiva del control biológico. Conclusiones: Se demuestra la efectividad de alternativas de control integral con participación social y comunitaria bajo la coordinación municipal en el control efectivo del dengue y enfermedades transmitidas por Aedes aegypti, a pesar de los cambios de administración municipal.

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Dengvaxia® vaccine acceptability and identification of reasons for vaccine hesitancy among parents from a community cohort in Ponce, Puerto Rico

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Abstract— Dengue vaccines will be a key component for any dengue elimination strategy. Dengvaxia®, the first dengue vaccine recommended in the US and its territories, is available for individuals aged 9–16 years (y) residing in Puerto Rico (PR) and other dengue endemic areas and with laboratory confirmation of a previous infection. Dengvaxia® has been available in PR since September 2022. However, as of May 2023, only 31 individuals have been vaccinated, highlighting the need to understand the factors associated with parental intention to vaccinate their children. We interviewed parents of children <18y enrolled in Communities Organized to Prevent Arboviruses, a cohort study in southern Puerto Rico, to identify potential barriers to Dengvaxia® vaccination. Interviewers read parents a short informational script about Dengvaxia® and then asked about their intention to vaccinate their children. We used descriptive analyses and adjusted risk ratios (RR) to identify factors associated with Dengvaxia® vaccine intention. During December 2021–April 2023, we interviewed 1,103 parents. The median age was 40y (interquartile range 34–45), and 68% were female. Overall, 92% were unaware that Dengvaxia® was available. However, 67% intended to vaccinate their children, with no significant differences before and after Dengvaxia® availability (p=0.99). Parents with an annual income of ≥$30,000 were less likely to vaccinate their children compared to those earning <$10,000 (RR 0.87 [95%CI 0.78,0.96]). After adjusting for income, parents who completed high school (RR 0.80 [95%CI 0.72,0.89]) or an associate or higher degree (RR 0.75 [95%CI 0.67,0.84]) were less likely to vaccinate their children compared to those that did not complete high school. In contrast, parents who considered their children at high risk for dengue in the following 12 months were more likely to intend to vaccinate their children (RR 1.17 [95%CI 1.02,1.34]) compared to those who did not. Among 360 parents who were unsure or would not vaccinate their children, 36% reported concerns about vaccine safety and 28% needed more information on how the vaccine works. In PR, territory-wide vaccine messaging about dengue vaccines, including focused interventions for parents with higher incomes and education levels, could decrease vaccine hesitancy and increase dengue vaccine coverage.


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Highlighted Posters

Diagnostics — Prognostics — Clinical

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Assessment of a phase 2 community-based study design to inform on the clinical evaluation of JNJ-1802, a first-in-class dengue antiviral under development

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***Abstract*— Introduction**: Dengue is among the top 10 global health threats, with half the world population considered at risk. Antivirals could have a role in controlling outbreaks or decreasing transmission in endemic areas in addition to other measures such as vaccines and vector control. We are developing a first-in-class pan-serotypic dengue antiviral small molecule (JNJ-1802). **Objective.** To validate the design of a phase 2, community-based, dengue index case (IC)/household contact (HHC) efficacy trial, by evaluating study operational aspects and virological parameters. **Results.** We conducted a prospective, community-based, phase 0 study with an IC/HHC design, between November 2021 and March 2023, starting with the identification of symptomatic dengue cases (classified as ICs) and their asymptomatic HHCs from Nha Trang, Vietnam. We enrolled 130 dengue ICs aged ≥ 1 year and 301 HHCs aged ≥ 18— ≤ 65 years. We obtained information on the likelihood of reaching ICs (within 72 hours of symptoms onset) and HHCs (within 48 hours of IC screening). We also established the feasibility of following up HHCs through household or health facility visits twice a week for blood sample collections. We were able to evaluate the incidence of dengue virus (DENV) infections in HHCs through RNA detection. In addition, in an ongoing phase 2a, randomized, double-blind, placebo-controlled trial (NCT05048875) in adults, we are evaluating the antiviral activity, safety, and pharmacokinetics of different oral JNJ-1802 dose regimens using an attenuated DENV-3 human challenge prophylactic model. Initial results for one of the dose regimens showed a statistically significant difference in JNJ-1802 antiviral activity versus placebo, measured as the reduction in the area under the DENV-3 RNA viral load concentration-time curve from baseline to 28 days post-inoculation. No safety concerns were identified. **Conclusion.** We found that a community-based IC/HHC study design may be feasible in dengue-endemic areas. We also showed that JNJ-1802 displayed antiviral activity against an attenuated DENV-3 in a human challenge model. Based on these learnings, we will conduct a phase 2 community-based trial to further assess the efficacy of JNJ-1802 against naturally circulating dengue serotypes.

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Efficiency of automated viral RNA purification for pediatric studies of dengue and zika in hyperendemic areas

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***Abstract*—** The isolation of nucleic acids is a critical and limiting step for molecular diagnosis, which prompted the arrival in Colombia of automated purification instruments during the SARS-CoV-2 pandemic. The local application of this technology in

the study of tropical diseases, such as dengue and zika, is beginning to be tested. We evaluated the efficiency of the automated extraction of viral RNA for studies of pediatric dengue and zika. Clinical samples of children with dengue that were well characterized through RNA isolation by silica columns and serotype-specific nested RT-PCR, in addition to 40 pediatric plasma samples spiked with ZIKV (strain PRVA BC59) and 209 from negative pre-epidemic patients, were analyzed. RNA from patients was extracted by two automated standard and high-throughput protocols on the automated KingFisherTM Flex instrument. The isolated RNA was evaluated for concentration and purity by spectrophotometry, for structural and functional integrity by electrophoresis and expression of the RNase P gene, and usefulness in serotype-specific DENV detection by conventional and real time RT-PCR. For the evaluation of ZIKV RNA, the commercial TaqMan Triplex assay was used, along with a well-tested in-house RT-qPCR assay. The concentration of RNA and the number of integral bands were higher with the high-throughput protocol. However, the number of specimens serotyped for DENV by RT-qPCR was 20% higher with the standard protocol. The cycle thresholds of the Taqman Triplex commercial kit and the in-house assay for the detection of plasma ZIKV RNA isolated with the standard protocol showed a strong association ($r2 = 0.87$, $P < 0.0001$) and a sensitivity and specificity of 96% and 100%, respectively, when all 249 samples were analyzed. Our findings support the application of automated instruments to studies of cocirculating flaviviruses that have represented a public health problem in recent decades in Colombia. They boast advantages such as efficiency, precision, time savings, and lower risk of cross-contamination.

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Circulating Extracellular Vesicles Increase Endothelial Permeability In Dengue Through Inflammatory Cytokines And Eicosanoids Signaling

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***Abstract*— Introduction.** Dengue is the most prevalent human arbovirus disease worldwide, causing diseases from mild to severe dengue syndromes. Even though the mechanisms underlying severe dengue are not completely understood, overwhelmed inflammation plays major roles in dengue pathogenesis. Extracellular vesicles (EVs) are small vesicles shed from cellular membranes with recognized roles in cellular communication in immunity and inflammation. **Objective.** We aimed to investigate circulating EVs in dengue patients and whether their cellular sources and inflammatory content associate with disease severity and endothelial cell (EC) function ex vivo. **Methodology and results.** We quantified EVs in plasma from 65 dengue patients included according to IRB and identified EV cellular sources through flow cytometry. We observed increased levels of circulating EVs in dengue compared to healthy controls. Dengue-infected patients presented lower percentages of platelet-EVs alongside an enrichment in RBC-, lymphocyte-, monocyte- and EC-EVs. Despite lower frequencies, platelet-EVs' numbers were higher in dengue patients compared to control. Importantly, higher levels of platelet- and monocyte-EVs correlated to signs of increased vascular permeability, RBC- and EC-EVs associated with severe dengue, and EVs from CD8+ and NK cells associated with mild dengue. To identify inflammatory mediators carried by EVs, we quantified mediators in whole plasma and paired EV-depleted plasma. The levels of the cytokines IL-1β, VEGF and PDGF, but not TNF-α and IFN-γ, were completely blunted by EV-depletion in plasma from dengue patients. Moreover, 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 EVs. Similarly, the platelet-derived eicosanoid 12(S)-HETE, but not TXA2, also circulate mainly in EVs. We confirmed through Western blot the presence of 12-lypoxygenase, caspase-1 and pro- and cleaved-IL-1β in microparticles from dengue-infected patients, with higher IL-1β processing in severe dengue. Through mechanistic experiments with endothelial cell cultures, we showed that 12(S)-HETE reduces VE-cadherin staining and generates intercellular gaps in EC monolayers, increasing permeability in vitro. Importantly, EC stimulation with plasma from dengue patients reduced VE-cadherin expression and increased endothelial permeability depending on EVs, IL-1R and the 12(S)-HETE receptor BLT2. **Conclusion.**

We show that EVs levels, source and cargo associate with disease severity and contribute to endothelial permeability in dengue.

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Longitudinal Analysis Of Antibody Trajectories Against Dengue And Zika Viruses In A Cohort Living In The Yucatan Peninsula

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***Abstract*—** Dengue (DENV) and Zika (ZIKV) viruses are Aedes-borne flaviviruses (ABFs) that circulate in tropical regions and pose a significant threat to global public health. The presence of four serotypes of DENV and the potential for co-transmission of the related ZIKV makes cross-reactive and cross-neutralizing antibodies an important factor for protection from disease. Little is known about how repeated ABV exposure over many years can affect antibody selection in humans. We performed a longitudinal study to analyze how antibody heavy and light chain usage, levels of somatic hypermutation, and antibody-mediated neutralization changed over time and how each was affected by previous ABV exposure. We collected peripheral B cells and serum from children participating in a field trial in the DENV-endemic city of Mérida, Yucatán, Mexico. Baseline samples were obtained from ~500 participants in 2020, 2021, and 2022 and follow-up samples were collected after laboratory-confirmed infection with an ABV. We observed significant increases in antibody binding, tested by virus capture ELISA, and virus neutralization by focus reduction in serum samples provided by subjects that seroconverted during the study. We chose subjects who either seroconverted during the study period or had high ABV neutralization titers to perform next generation single cell sequencing on B cells. Virus-specific B cells were detected and isolated by flow cytometry with a more than 10-fold increase in virus-specific cells captured in recently infected individuals compared to those infected before the study period. VDJ libraries were constructed for each participant from non-specific, activated peripheral B cells as well as virus-specific B cells. Phylogenetic and statistical analyses showed a diverse antibody response both among and between participants with a significant increase in clonality in pools taken within a month of infection. Our results describe the change in the immunological landscape of a population under ABF pressure over time.

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The Type I And Type III Interferon Responses Protect Against Tick-Borne Flaviviruses Through IFI6

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***Abstract*—** Tick-borne flaviviruses (TBFVs) are a growing health concern of global dimension. Various representatives of this viral family have been described to cause fatal neurological disease in humans with increasing case numbers throughout the last decades. The innate immune response, especially in the form of interferon-dependent signaling pathways, is an essential part of the human defense system to counteract infection with TBFVs. Previous studies disclosed that even though sharing a similar signaling cascade after binding to their respective receptor complexes, interferons belonging to the type I and III families differ in the cluster of genes they stimulate. However, which genes the different interferon families activate and how these are modulating infection with TBFVs remains poorly characterized. With our work, we show that both interferon families are crucial for protection against infection with various tick-borne flaviviruses in vitro and in vivo, likely relying on the same antiviral factors. Monitoring protection against tick-borne encephalitis virus (TBEV), the prototypical TBFV, in human cell lines representing two tissues relevant for TBEV pathogenesis, the intestine and the brain, established how different tissues depend on different interferons for effective protection against viral infection. We could further establish a murine in vivo model with

knockouts for either interferon type I or III receptors highlighting the importance of both interferon families for effective protection against TBFVs. To assess the importance of single interferon-dependent effectors for modulating TBFV infection, we screened a gRNA library targeting around 2000 different interferon-stimulated genes against interferon-mediated protection against TBEV infection in human cells. This CRISPR-based loss-of-function screening approach identified IFI6 as a central player for interferon type I- and III-driven responses against TBFVs. Using a variety of different methods, we could validate its antiviral function on multiple levels (viral RNA, protein and production of infectious virions), against several TBFVs and in cell lines derived from different human tissues. We are currently working on pinning down the mechanistic details of this candidate. With this work, we aim to open new perspectives for targeting weakness points in the life cycle of TBFVs that afterwards might pave the way for the development of new antiviral therapeutics.

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Use Of A Stabilized Conformational Dengue Virus Serotype 2 Envelope Antigen To Isolate Memory-Derived Neutralizing Monoclonal Antibodies From A Convalescent Patient

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Abstract— Dengue is a mosquito-borne disease caused by four serotypes of dengue virus (DENV), each of which can elicit serotype-specific or cross-reactive antibodies that target the envelope (E) structural glycoprotein. Understanding how DENV-specific B cell responses drive antibody-mediated protection is important to vaccine design. To directly study this, we used a recently developed recombinant E protein stabilized dimer (recED) derived from DENV serotype 2 to probe DENV-specific memory B cells from a patient who recovered from dengue. Complementary approaches including direct sorting of recED-binding memory B cells followed by antibody gene sequencing or culture and characterization of secreted antibody from recED-sorted cells yielded 25 novel lineages of DENV-reactive monoclonal antibodies (mAbs), at least three of which exhibited neutralizing activity against multiple serotypes including DENV2. Unlike other DENV cross-reactive mAbs, some of which show neutralizing activity against Zika virus, the characterized recED-binding mAbs recognized, but did not neutralize Zika virus. These results indicate that recED can be used to tag DENV2-reactive surface immunoglobulin-positive memory B cells and isolate DENV-neutralizing antibodies from patients. These data extend in vivo studies showing that immunization of animals with recED generates DENV2-neutralizing responses. We hypothesize that this antigen can be used to define the landscape of DENV-specific B cell responses in defined vaccination and viral challenge settings.

Vector Biology — Ecology — Control

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A Cluster Randomized Controlled Trial Assessing The Efficacy Of Preventive Targeted Indoor Residual Spraying To Reduce Aedes-Borne Viral Illnesses In Merida, Mexico

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Abstract— The control of Aedes aegypti, the major vector of dengue, Zika, and chikungunya, is in critical need of improvement. An approach, termed Targeted Indoor Residual Spraying (TIRS), consists of the selective application of residual insecticides on Ae. aegypti indoor resting sites, such as exposed lower sections of walls (< 1.5 m), under furniture, and on dark surfaces. Here, we will present the design of the TIRS trial, a two-arm, parallel, unblinded, cluster

randomized controlled trial quantifying the efficacy of TIRS in reducing the burden of laboratory-confirmed ABV clinical disease (primary endpoint) or infection (secondary epidemiological endpoint), and over the density and arbovirus infection rates of Ae. aegypti (secondary entomological endpoints). Entomological preliminary results after the first year applying pirimiphos-methyl (Actellic 300CS, Syngenta) in ~8,000 houses was quantified by measuring indoor adult Ae. aegypti density quantified with Prokopack aspirators in a sub-sample of 1,500 houses. At baseline (pre-TIRS application), Ae. aegypti indices (total adults, females, and blood-fed females) were not different between treatment and control arms. The Overall effect (accumulated across the 6 months post- TIRS) led to a 77.4% reduction in positivity compared to the control arm. Similarly, for adult density, the overall percent reduction was 62%. Furthermore, we observed a reduction in efficacy as we reach the maximum residual lifespan of the insecticide at 6 months. TIRS has also shown high levels of satisfaction and uptake in the community. Our findings confirm the high and sustained entomological efficacy of TIRS and the value of pirimiphos-methyl for the residual control of indoor Ae. aegypti. Epidemiological data collection is ongoing and will be analyzed at the end of the trial (2025). TIRS is a promising approach, driving a paradigm shift in Ae. aegypti control because it can be deployed preventively (before the transmission season) rather than reactively (in response to symptomatic cases).

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Association of water availability and Aedes aegypti pupae and adults in an urban/rural mosaic in Nicaragua

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Abstract— The increase in dengue virus (DENV) epidemics and the latitudinal expansion of Aedes mosquitoes globally has made it critical to understand the ecological and social factors that modulate its population abundance locally. We used multilevel models to evaluate the different life stage abundance of Ae. aegypti collected from October to December of 2022 (rainy season) in 500 households in 21 urban and peri-urban neighborhoods in District 3 of Managua, Nicaragua, as part of an arbovirus surveillance study (A2CARES). A total of 1,194 pupae and 201 (indoor: 129, outdoor: 72) adult female mosquitoes were collected. We used a GLMM and GAMM approach to estimate both pupae and adult female indoor and outdoor abundance. The number of pupae found in a home was directly associated with the number of containers present (exp= 1.41, SE= 0.09, p< 0.001) and outdoor female specimen abundance (exp= 2.73, SE= 0.47, p< 0.03). We observed that outdoor female abundance increased with the frequency of water service interruptions per day (exp= 1.07, SE= 0.02, p< 0.01), total number of pupae found in the household (exp= 1.027, SE= 0.009, p< 0.01) and neighborhood of collection (exp= 0.501, SE= 0.027, p=0.01). The GAMM smooth for water interruptions was also significant, increasing with the number of female mosquitoes outdoors. Indoor female abundance was only associated with the number of pupae found in a home (exp= 1.021, SE= 0.008, p< 0.01). We are currently untangling the fine-scale spatial patterns for mosquito abundance, human density, access to water and services, and most productive containers to evaluate how these variables impact mosquito ecology using a urbanicity mosaic perspective within our study site. Our results suggest that for our geographic setting, pupae can be an adequate proxy for female abundance. More importantly, the inclusion of stakeholders involved in household water container management and municipal services are critical for future intervention projects in the region. Fine-tuning hotspot analysis of mosquito abundance to identify key factors that modulate their population is critical to improve vector control activities in limited-resource settings.


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Accidente cerebrovascular isquémico durante la infección por dengue: reporte de dos casos. Medellín, Colombia.

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***Abstract*— Introducción.** La arbovirosis más importante en Colombia es la causada por el virus del dengue (DENV). Según la Organización Mundial de la Salud, la enfermedad por DENV se clasifica en dengue sin o con signos de alarma y dengue grave. Esta última categoría incluye el daño a órganos. Uno de estos órganos es el sistema nervioso central y aunque en la actualidad se reconoce la asociación de varias complicaciones neurológicas con la infección por DENV, el accidente cerebrovascular (ACV) isquémico es considerada una complicación rara. A continuación, se presentan dos casos de pacientes con ACV isquémico asociado a la infección por dengue. **Reporte de casos..** La primera paciente fue una mujer de 51 años que presentó monoparesia, parálisis facial, disartria, Babinski y síncope al 7º día de iniciado un cuadro febril. Ella tenía antecedentes de aterotrombosis y accidente cerebrovascular. La resonancia magnética nuclear (RMN) mostró un infarto agudo en el territorio de la arteria cerebral media derecha. Dengue fue confirmado por la presencia de anticuerpos NS1 e IgM en suero. El segundo paciente fue un hombre de 50 años que consultó por cefalea, alteración del estado de conciencia y mutismo tras un episodio febril una semana antes. Además, presentó afasia nominal, signo de Parineaud's. El puntaje de la escala de coma de Glasgow fue 9/15. El paciente tenía antecedente de accidente cerebrovascular, resección de glioblastoma multifocal, epilepsia, hipotiroidismo y diabetes. La RMN mostró un evento isquémico subagudo de la arteria cerebral media derecha. El diagnóstico de dengue se confirmó por la presencia de anticuerpos NS1 e IgM en suero y RT-PCR en suero y líquido cefalorraquídeo (LCR). DENV-1 se observó en ambos fluidos. Ambos pacientes presentaron trombocitopenia y leucopenia y el segundo caso, también presentó hemoconcentración y alteración de pruebas hepáticas. **Conclusión.** En países tropicales y subtropicales, dengue podría ser una importante causa de ACV isquémico en pacientes con comorbilidades, incluido el ACV. Es importante que el médico interrogue a los pacientes que consulten por déficit neurológicos focales y alteración del estado de conciencia, sobre la presencia de fiebre para descartar infección por DENV.

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Clinical And Laboratory Behavior In The Different Phases Of Dengue In A Reference Pediatric Population In Cartagena De Indias During An Epidemic

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***Abstract*—** El virus del dengue (DENV) es una arbovirosis de importante impacto a nivel mundial y que hace parte de las enfermedades tropicales desatendidas es la infección por el virus del dengue. La presencia de la enfermedad por el virus del dengue se encuentra ampliamente distribuida en países tropicales y subtropicales y en lugares como Colombia. Dentro del espectro de manifestaciones clínicas del dengue se encuentra el dengue sin signos de alarma el dengue con signos de alarma y el dengue grave para la cual se dispone de signos clínicos y de laboratorio, así como factores pronósticos para evaluar y evitar algunas complicaciones de la

enfermedad. Dentro de la población a riesgo de padecer dengue se encuentra la población infantil. Por lo anterior el objetivo de este trabajo es establecer y estimar el comportamiento de las distintas variables clínicas y de laboratorio alrededor de las distintas fases del dengue en población pediátrica de una institución de salud de referencia en población pediátrica en la ciudad de Cartagena de Indias durante una situación de epidemia. Estudio retrospectivo de cohortes observacional se diseñó un instrumento para el seguimiento de pacientes desde el ingreso en la urgencia al alta o muerte en un hospital pediátrico para un total de aproximada. En total se obtuvieron más de 4000 historias para el análisis y validación del proyecto encontrando patrones de manifestaciones clínicas muy particulares y diferentes al modelo actual.

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Clinical and laboratory markers of the concurrent bacterial infection in children with dengue

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***Abstract*— Background.** Bacterial coinfection may occur in a fraction of individuals with dengue and can result in longer hospital stays, higher mortality, and increased costs in health services. However, the rate of this comorbidity in children with dengue is partially known, as well as the risk factors and potential biomarkers useful to identify this entity. **Methods.** We conducted a retrospective multicenter study in a dengue hyperendemic region of Colombia, enrolling 1,597 children from two pediatric cohorts. We included children with confirmed dengue, ranging from mild to severe disease, and evaluated the rate of bacterial coinfection, their clinical characteristics, and diagnostic predictors. We also assessed the diagnostic performance of the proinflammatory circulating interleukin (IL)-6 for detecting bacterial coinfections in children with dengue. **Results.** The rate of bacterial coinfection in children with dengue with warning signs in cohorts 1 and 2 was 2.4.% and 7.3%, respectively, and this rate reached 30.7% and 38.2% in children with severe disease in both cohorts. The presence of a higher total leukocyte count and C-reactive protein levels, as well as high IL-6 plasma levels at hospital admission, in children <48 months of age were indicative of bacterial coinfection in dengue. Moreover, bacterial coinfection conferred a higher risk of developing organ dysfunction in children with severe dengue, the requirement of a longer hospital stay, and a 2.3-fold increase in direct health-related costs. **Conclusions.** An important proportion of children with dengue, particularly those with severe disease, course with bacterial coinfection and have a higher risk of morbidity. Physicians should perform microbiological analyses to identify children with risk factors for concurrent dengue and bacterial infection, to rationally and timely provide antimicrobial therapy.

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Clinical Impact Of DENV And SARS-CoV-2 Co-Infection In Hospitalized Patients

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***Abstract*—** Since its emergence in 2019, coronavirus disease (COVID-19) has spread worldwide consuming public health resources. However, the world still needs to deal with the burden of other infectious

diseases that continue to thrive. Countries in the Tropics, including Brasil, are affected by cyclic dengue epidemics on an annual basis, with some regions being hotspots for other flavivirus transmissions. To date, little is known about the impact of co-infections between DENV and SARS-CoV-2. Our study was performed on 400 serum samples collected from laboratory-confirmed COVID-19 patients between February to June 2021, months historically related to DENV outbreaks. The samples were tested by serology and molecular assays for the presence of DENV. While no DENV PCR positive was observed, 78% of the samples were DENV IgG positive, 6% DENV IgM positive, and 0.25% DENV NS1 positive by ELISA. DENV IgM and IgG antibodies were isolated by chromatography from co-positive samples, and 62.5% of the samples were positive for neutralizing antibodies (FRNT80) for DENV IgM, suggesting a recent infection. We also observed increased levels of IL-1β in SARS-CoV-2/DENV co-infected patients. Intriguingly, diabetes was the only relevant comorbidity (p=0.046), though at a lower frequency than expected. A high rate of hospitalization (94.9%) and mortality (50%) were found, with a significant increase in invasive mechanical ventilatory support (86.96%) in co-infected cases, suggesting a severe impact on patients' clinical outcomes. When analyzing previous exposure to DENV, secondary dengue patients (IgG/IgM ratio of >1.10) co-infected with SARS-CoV-2 more frequently presented dyspnea and respiratory distress. However, DENV primary infected patients had fever and cough in a higher proportion than patients with secondary dengue (87.50% vs. 33.33%, p=0.027 for fever), presented longer hospital and ICU stays (4 and 20.29 days, respectively) and a higher mortality rate (60%) compared to patients with secondary dengue, where the majority were discharged (62,50%). Our data demonstrate that differentiation between both diseases is a great concern for tropical countries and should be further explored to improve patient management and the effectiveness of their surveillance.

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Clinical Presentation Of Acute Arboviral Infections During 2022 In The Tirs Project Cohort

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Abstract— Aedes borne diseases (ABD), such as dengue, chikungunya and Zika, are caused by viral infections transmitted to humans through bites of infected mosquitoes. The increase in their incidence and geographical distribution is a major public health problem in the Region of the Americas. The COVID-19 pandemic has led to a more complex epidemiological situation because of the possibility of misdiagnosis due to similar clinical presentations. The aim of this study was to describe the clinical characteristics of the ABD cases from the last active surveillance season (July-December 2022) in the TIRS trial cohort of children aged 2-15 in Merida, Yucatán, Mexico. This cohort includes ~4600 children followed prospectively for signs of ABD in houses receiving and not receiving the spraying of the insecticide pirimiphos methyl. Through household visits, phone calls, phone messages and a toll-free line, 437 reports of potential ABD symptoms were observed. Of these, 201 (46%) were considered as suspected ABD cases. The age range was 3.5-17.7 years old, with a mean of 10.4±3.8 years, with slightly more males (51.3%, n=102). A total of 182 participants provided a blood sample. From these, 87 cases were detected (47.8%) by PCR or IgM results. Dengue represented 49% of the cases (n=43), followed by Zika 43% (n=37) and different coinfections 8% (n=7: DENV-ZIKV, n=4; ZIKV-CHIKV, n=2; and DENV-CHIKV, n=1). Patients with confirmed ABD were slightly older than the negatives (5.1-17.7 years, 11.6±3.5 vs 3.5-16.9, 9.4±3.9) but there were no significant differences between age nor sex. The passive surveillance system of Merida reported 269 DENV cases, and no ZIKV or CHIKV infections. The clinical presentation of the studied cases was diverse, with fever, myalgia and headache as the most common signs/symptoms reported. Gastrointestinal and respiratory

signs/symptoms were also observed. No ABD severe cases were observed. Despite the ongoing COVID-19 pandemic, only 6% (n=11/182) reported a personal contact with an ongoing suspected case of COVID-19. Of these, 4/11 (36.4%) tested positive for any ABD. This results, reinforce the importance of strengthened surveillance to detect silent ZIKV transmission and describe the variable array of symptoms involved in DENV ambulatory infections during COVID-19 pandemic.

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Comparación De Hallazgos Clínicos Y De Laboratorio Entre Niños Y Adultos Con Infección Por Virus Dengue En Los Municipios De Turbo Y Apartadó, Colombia Estudio Prospectivo, 2020-2022

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***Abstract*— Antecedentes.** El perfil epidemiológico del dengue ha cambiado y actualmente se caracteriza por un aumento en el número de casos en niños; también se reconocen diferencias clínicas y de laboratorio entre adultos y niños. **Materiales y métodos.** Se incluyeron 192 pacientes con dengue confirmado por laboratorio en los municipios de Turbo y Apartadó. Los casos fueron agrupados así: primera Infancia (0-5 años), Infancia (6 - 11 años), Adolescencia (12 - 18 años) y adultos (mayores de 18). **Resultados.** de los 192 pacientes, 56,3% correspondía a hombres, siendo 22,9% población de primera Infancia, 34,9% de infancia, adolescencia 26,0% y adultos 16,1% para un total de 80,7% de pacientes menores de 18 años. El 3,1% fueron casos de dengue grave; dengue con signos de alarma, 58,3 % y dengue sin signos de alarma 38,5%. El 89,1% cursó con infección secundaria, esta presentación fue significativamente menor en el grupo de 0 a 5 años p= 0,004, sin embargo, este grupo tuvo mayor frecuencia de dengue grave. La mediana (RIC) de los días para recuperación de los síntomas fue menor en niños que en adultos [10 días (8- 13) Vs 15 días (8,5- 18)] p=0,006. Los síntomas respiratorios (36,1% Vs 32,4%), hepatomegalia (10,3% Vs 8,1%), bradicardia (18,1% Vs 13,5%) y derrame pleural (6,5% Vs 2,7%) fueron más frecuentes en niños, con diferencias no significativas. Las hemorragias fueron más frecuentes en adultos (64,9% Vs 57,4%), a excepción de epistaxis y melenas que fueron más frecuentes en niños. La frecuencia de brote fue inversamente proporcional al grupo de edad: 70,5% primera infancia, 47,5% infantes, 56,0% adolescentes y 32,3% adultos, p<0,001. Lo mismo ocurrió con prurito: 56,8%, 55,2%, 46,0% y 29% p=0,065, y edema en cara: 22,7%, 20,9%, 16,0% y 0,0 p=0,04. Los hallazgos de laboratorio fueron semejantes en los grupos. **Conclusiones.** se evidenció mayor afluencia de niños con dengue a los servicios de salud, también fueron los de más rápida recuperación y los de más alta frecuencia de manifestaciones en piel y edema en cara, lo cual puede ayudar a la sospecha del diagnóstico clínico. En esta población no se evidenció diferencias en hallazgos de laboratorio.

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Dengue Asociado A Enfermedad Neurológica: Frecuencia Y Diferencias Clínicas Y De Laboratorio Entre Pacientes Con Enfermedad Neurológica Asociada A Dengue Y Solo Con Enfermedad Neurológica

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***Abstract*— Antecedentes.** Aunque está demostrado el compromiso del sistema nervioso por el virus del dengue (DENV), este tema aún requiere mayor investigación. **Materiales y métodos**. Se realizó un estudio prospectivo en el que se captaron 39 pacientes con enfermedad neurológica, a los cuales se les indagó por antecedente de fiebre 21 días antes del diagnóstico neurológico. A todos se les descartó dengue mediante pruebas de laboratorio y se les hizo registro de hallazgos clínicos y de laboratorio. Los pacientes con diagnóstico confirmado de dengue fueron clasificados como (EN+D) y en quienes se descartó dengue fueron clasificados como (EN-D). **Resultados**. La frecuencia de dengue en los casos de enfermedad neurológica (EN+D) fue 17,95%. El diagnóstico se confirmó en todos los pacientes por presencia de anticuerpos IgM en sangre y en cuatro de éstos también por antígeno NS1. En dos casos se detectó RNA viral por RT-PCR en sangre y líquido cefalorraquídeo (LCR), identificando los serotipos DENV-1 y DENV-3 en ambas muestras. Los diagnósticos neurológicos de los pacientes con EN+D fueron: encefalitis (6 casos), accidente cerebro vascular (ACV) (1 caso) y ACV y encefalitis (1 caso). Los casos de ACV se confirmaron por Resonancia magnética. La mediana de la edad en años en los casos de EN+D fue mayor que en los de EN-D, (50,0 vs. 28,5, p=0.058). En los casos con EN+D fue más frecuente la bradicardia (71,4% vs. 6,3%, p=0,001). En cuanto a los parámetros de laboratorio, se identificó mayor frecuencia de hemoconcentración (42,9 vs. 3,1%, p=0,014), leucopenia (71,4% vs. 0,0%, p<0,001), trombocitopenia (71,4% vs. 3,1%, p<0,001), niveles elevados de aspartato aminotransferas (100,0% vs. 41,7%, p=0,037) y niveles elevados de proteína C reactiva (50% vs. 7,7%, p=0,034), en pacientes con EN+D comparado con los casos de EN-D. En el LCR los leucocitos estuvieron elevados de forma más frecuente en el grupo de EN-D respecto al otro grupo (60,9% vs. 0,0% p=0,041). **Conclusiones.** Se observó una frecuencia elevada de dengue en pacientes con enfermedad neurológica, La bradicardia y algunos hallazgos de laboratorio tanto en sangre como LCR podrían orientar la sospecha de este compromiso del SNC asociado a dengue.

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Effect of previous Zika virus exposure on acute dengue infection

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***Abstract*—** In recent years the arbovirus infections have become a significant health concern worldwide. The simultaneous circulation of flaviviruses in tropical regions has led to the hypothesis that immunity generated by a previous flavivirus infection could promote severe disease outcomes in subsequent infections by heterologous flavivirus. Considering the current epidemiological context of arboviruses in the country, especially in São José do Rio Preto, São Paulo, this study analyzed the influence of antibodies produced in response to Zika infection on the clinical course of a subsequent infection with dengue serotype 2 in patient samples collected during the epidemics in 2019 and 2022 in the municipality. We enrolled 1,043 laboratory-confirmed dengue patients through the Polymerase Chain Reaction (PCR) method and investigated their prior infection to Zika or dengue using an enzyme-linked immunosorbent assays (ELISA). Furthermore, we assessed the

cytokine expression profile in the patients' samples and conducted statistical analysis on our data. Severe forms of dengue disease were more frequent in patients with previous Zika infection, but not in those previously exposed to dengue. Our observations suggest that previous ZIKV infection increased the risk of severe forms of dengue and hospitalizations, similar to what has been observed in secondary DENV infections. In contrast, our study suggests that the observed severity of dengue clinical outcomes don't seem to be influenced by ADE (increased viral load and anti-inflammatory cytokine levels), as prior infection to dengue was associated with a lower risk for development of severe forms of dengue disease. Conversely, patients with a previous Zika infection had neither higher viral titer nor cytokine profiles similar to ADE. For this reason, the mechanism involved in the clinical disease exacerbation by prior ZIKV immunity seem to differ from the classic ADE mechanism observed in secondary dengue infections. Lastly, as a subsequent course of action, we will endeavor to validate these data using in vivo murine infection models, in order to gain insights into this correlation and ascertain the potential cellular and molecular mechanisms associated with this phenomenon.

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Evidence Of Dengue Viral Markers Among Blood Donors In Two Selected Blood Centres In Kenya: A Prospective Serological Study

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***Abstract*— Background Information**: For patients who receive donated blood, blood transfusion as a life-saving procedure offers both advantages and disadvantages in terms of their health. The transmission of a bloodborne pathogen is one of the threats. In addition to being spread by mosquitoes, the dengue virus can also spread through blood transfusions and organ transplants, making it a disease of concern in Kenya's coastal counties. In recent years, dengue outbreaks have been common in these areas. **Objective**: This study aimed to determine the seroprevalence of the dengue virus among blood donors in Kenya. **Methods**: Between December 2023 and June 2024, we conducted a prospective cross-sectional design study in Mombasa and Nairobi counties. During data collection, a research questionnaire and serological testing were used as study methods. **Results.** A total of 203 blood donors consented to participate in the study. The prevalence of dengue virus was n = 11/203 (6%) for anti-dengue IgM, n=30/203 (15%) for IgG, and n=2/203 (1%) for NS1 antigen. Mombasa County had the highest percentage of donors with detectable IgM markers at 11% compared to Nairobi County at 2%. Only 2% of the Mombasa County blood donors had the NS1 antigen detected, while none of them was in Nairobi County. The relationship between the study site and IgM positivity was statistically significant, with chi-square values of 6.377 (p=0.0019). Furthermore, IgM / IgG / NS1 showed a comparable association with donors with a history of fever in the last six months, with a chi-square value of 13.001 (p=0.001), 20.782 (p =0.000), and 7.299 (p=0.046) correspondingly. Participants who indicated that they had lived with a subject to dengue fever had a substantial correlation with IgM positivity, with chi-square values of 12.3 (p=0.007). **Conclusions, recommendations, and implications**. According to the research, Mombasa County blood donors are more likely to have exposure to dengue virus. In the coastal counties of Mombasa, Kilifi, Kwale, Lamu, and Taita Taveta, immediate action is needed to assess whether blood donors need to be tested for the dengue virus. This would be a wise preventative approach to safeguard blood recipients in these areas, particularly during dengue outbreaks.

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Implementation Of A Protocol For The Detection Of Dengue, And Chikungunya Viruses In Biological Samples By RT-LAMP

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***Abstract*—** Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) arboviruses are arthropod-borne viruses that share a common vector, the Aedes aegypti mosquito. In the acute stage, they cause similar and non-specific symptoms, but the outcome and clinical management of these diseases are different, so early and accurate identification of the virus is necessary. Although these tests are sensitive and specific, they have disadvantages in terms of the time required for detection and the high cost of consumables and equipment. This study evaluated the performance of an RT-LAMP assay for the detection of dengue, chikungunya in biological samples, as LAMP is a method that is easy to implement, has a faster turnaround time and is less expensive than molecular methods. A set of primers was designed for each DENV serotype and for two CHIKV genes, and the analytical sensitivity of each assay was determined, as well as the clinical sensitivity and specificity in biological samples. The RT-LAMP assays, using RNA purified from infected C6/36 supernatants and serially diluted in 10-fold decrease, showed equivalent

performance to the RT-qPCR assays. Additionally, the assays were validated using RNA from patients with febrile illness and demonstrated a sensitivity and specificity greater than 85%, allowing them to be used as diagnostic tests for arboviruses in patients with suspected infection.

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InBios' Dengue Immunoassays: Current Performance And Future Objectives

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Abstract— Dengue virus (DENV) is the most widely transmitted mosquito-borne disease, infecting almost 400 million people annually. This flavivirus is composed of four dengue serotypes, DENV-1, -2, -3 and -4, and previous exposure to a given serotype will not necessarily provide protective immunity to future infections. While a variety of diagnostic tests have been developed over the past three decades to detect DENV infection, including enzyme linked immunoassays (ELISA), rapid tests, and polymerase chain reaction (PCR) assays, the accurate diagnosis of acute and past dengue infections remains challenging, particularly in resource-limited areas where other circulating flaviviruses may cause confounding interpretations. Furthermore, new dengue vaccines have been approved for use only with individuals who have been previously infected with the virus, further increasing the need for the specific detection of past dengue infection. Here, we evaluate the past and current performance of the dengue immunoassays available from InBios International, Inc., including the FDA cleared DENV Detect IgM Capture ELISA (510(k) #K100534) and DENV Detect NS1 ELISA (510(k) #K181473). Additionally, the preliminary performance of a new dengue IgG anti-NS1 indirect ELISA and corresponding rapid test is evaluated, and we discuss the cross-reactivity observed with Zika infections and compare the results with more traditional E-protein targets. Other rapid tests for dengue include the Dengue NS1 Detect Rapid Test and preliminary performance data will be shown. We discuss future objectives and provide preliminary data evaluating the performance of a new-gen multiplexed flavivirus immunoassay that can be easily adapted into the clinical and POC setting. This multiplexed assay simultaneously queries the antibody response to several relevant targets including dengue NS1 from DENV-1, -2, -3, -4 and Zika NS1; additional envelope and control proteins are included in this array-based format to provide significantly greater data for properly analyzing and categorizing a test specimen.

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Long-Term Symptoms Associated With Chikungunya Virus Infection: A Prospective Community-based Cohort Study

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Abstract— Chikungunya virus (CHIKV) infection is associated with chronic sequelae. However, existing longitudinal studies have focused on patients and lacked appropriate control groups. In this community-based prospective cohort study, we identified CHIKV infection among participants who experienced an outbreak caused by the ESCA genotype. We followed a cohort of residents aged ≥5 years from a slum community in Salvador, Brasil. Five biannual serosurveys were conducted between 2019 and 2023. CHIKV infection was determined by IgG seroconversion between the 1st (September–November 2019) and 2nd (November 2020–February 2021) surveys. Structured questionnaires collected data on self-reported symptoms, sociodemographic characteristics, the Chalder Fatigue Scale (CFS), and the Short Form 12 (SF-12) quality of life scores. Among 1,532 enrolled participants, 793 (52%) participated in the five follow-up surveys. In the interval between the 1st and 2nd surveys [7.4(SD±0.9) months], 26.4% (209/793) of participants had serologic evidence of CHIKV infection. When comparing the 209 participants with CHIKV infection to the 584 participants without CHIKV infection, we found significantly increased reporting of joint pain (37% vs 10%; p<0.01), body pain (33% vs 13%; p<0.01), and fever (33% vs 19%; p=0.01). Furthermore, CHIKV infected individuals had significantly lower quality of mental health scores (mean 47 vs 54; p=0.01), albeit no significant differences found for physical health scores. During the 3rd survey conducted 15.1(SD±0.6) months after the outbreak peak, previously infected individuals has significantly greater risk of reporting body pain (7% vs

2%; p<0.01) than previously uninfected individual but did have significant differences in additional symptoms or the SF-12 scores. During the 4th and 5th surveys conducted 24.0(SD±1.0) and 32.1(SD±1.2) months, respectively, after the outbreak peak, there were no significant differences in reported symptoms or SF-12 scores between previously infected and uninfected groups. The findings, although limited by recall bias, suggest a lower symptomatic attack rate for CHIKV infection (rate difference, 27%) compared to previous beliefs. Furthermore, except for body aches, we did not observe a significantly increased risk of symptoms or lower quality of life 15.1 months after acute infection. These findings highlight the importance of re-evaluating evidence from patient cohort studies on chronic sequelae attributed to CHIKV infection.

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Misuse Of Transfusion In Recent Dengue Epidemic In A Public Hospital In Lima, Peru 2023 Preliminary Report

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***Abstract*— Introduction.** Dengue incidence has increased in endemic areas of the tropics and is expanding into non-endemic areas such as Lima, Peru. During the first half of 2023, Lima is having a major dengue outbreak, and for health personnel there, it represents their first experience treating the disease. Thrombocytopenia is a common symptom associated with dengue, in contrast to hemorrhage which is rare. Edgardo Rebagliati Martins Hospital (HNERM), EsSalud, a national reference center, treated suspected dengue cases with moderate to severe thrombocytopenia, some of whom received transfusions. Herein, we compare the clinical outcomes in dengue patients with thrombocytopenia receiving transfusion with those who did not. **Methods.** We reviewed the medical records of pediatric and adult dengue patients who received emergency care or were hospitalized from January to April 2023. Of 55 suspected cases, 29 cases were confirmed by Elisa IgM by the Peruvian National Institute of Health. **Results.** The average age was 37 years [6-38), of which six are pediatric [6-13 years], and 28% (17/29) female. About half, 51% (15/29) were infected in Lima, whereas the remainder had a travel history to a dengue endemic city outside of Lima. Of these, 28 presented with warning signs. The mean platelet count was 90,000/uL [21,000-186,000]. Twenty % (6/29) presented mild hemorrhage. Transfusion (6 with platelets only) was prescribed for 24% (7/29). For those receiving platelets the average platelet count was 51,000/uL [21,000-52,000], only one had moderate bleeding but it was not active, and 4 presented ascites. None were classified as severe dengue. **Conclusion.** Although platelet transfusions are only appropriate with severe and active hemorrhage (thrombocytopenia <20,000/uL), in our setting the transfusions were given without meeting this criteria. This suggests the need for rapid clinical management training for physicians in health facilities in Lima.

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Plasma Metabolomics For The Identification Of Potential Diagnostic Biomarkers Of Dengue Infection In An Acute Febrile Illness Cohort In Colombia

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***Abstract*—** Clinical diagnosis of dengue virus infection (DENV) presents significant challenges due to the considerable overlap of symptoms with other causes of acute febrile illness (AFI) in tropical regions. In resource-constrained areas, it is essential to develop

novel, affordable, scalable, and ideally point-of-care diagnostic strategies to accurately identify the causative pathogen. To achieve this goal, it is crucial to identify differentially expressed biomarkers among endemic pathogens causing AFI. In this study, we aimed to identify potential biomarkers of DENV through metabolomic analysis of individuals with confirmed infections in an AFI cohort in La Virginia, Risaralda, Colombia. The ongoing cohort consists of 415 individuals recruited between 2019 and 2023 from the Coffee Triangle region of Colombia. A subset of 28 individuals (DENV=8, Fever of other origin=20) matched for age, height, and weight were subjected to reverse-phase liquid chromatography coupled with time-of-flight mass spectrometry analysis. Clinical groups were compared using principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA). Differentially expressed metabolites were identified based on a p-value < 0.05 and a Jack-knife confidence interval > 1. Subsequently, a LASSO regression was performed to select the most relevant metabolites, followed by a ROC analysis using the top 5 selected features. We identified a total of 35 differentially expressed metabolites belonging to various chemical families, such as carboxylic acids, fatty acids, glycerophospholipids, sphingolipids, steroids, indoles, among others. The LASSO regression identified Urobilin (AUC 0.963, Sensitivity 100% Specificity 80%), Biliverdin (AUC 0.894, Sensitivity 100% Specificity 60%), Bilirubin (AUC 0.738, Sensitivity 100% Specificity 65%), LPC 18:2 (AUC 0.744, Sensitivity 100% Specificity 20%), and CAR 12:0;O (AUC 0.719, Sensitivity 100% Specificity 60%) as the most relevant metabolites and potential biomarkers. We are currently performing analysis on additional samples to include an increased number of dengue cases, malaria and Covid, as well as additional etiologies of fever in the tropics. These findings suggest that the identified circulating metabolites in plasma during acute infection may serve as biomarkers for etiological diagnosis of dengue fever in the tropics.

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Predicting Clinical Outcomes Of Dengue Fever: An Observational And Analytical Cohort Study Using Machine Learning Techniques

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Abstract— Dengue is a highly prevalent vector-borne viral disease with diverse clinical presentations, ranging from asymptomatic to severe, potentially fatal forms. The pathogenesis remains unclear, there is no specific treatment, and it is uncertain when an individual will develop severe illness. This study aimed to predict dengue clinical presentation in the population of Urabá Antioqueño, Colombia. A prospective observational analytic cohort study was conducted on patients diagnosed with dengue. Patients underwent a complete clinical evaluation, disease diagnosis and seroclassification, co-infection evaluation, and genetic characterization. The dependent variable was the presentation of severe disease associated with clinical symptoms, infection conditions, paraclinical factors, and sociodemographic conditions. A total of 137 dengue patients were included. Most patients presented symptoms such as fever, myalgia, arthralgia, asthenia, fatigue, and chills. Most infections were secondary, and co-infections were identified in the minority. The severe disease occurred in 4.4% of the patients, while 95.6% had uncomplicated dengue. An exploratory analysis was performed using Python. To address the class imbalance, oversampling of severe dengue cases was conducted using SMOTE from sci-kit-learn. Cross-validation was used to select the best classification model. The Random Forest model was selected, and hyperparameters were adjusted using GridSearchCV. Although the model demonstrated high performance with an AUC of 0.92, the high likelihood of overfitting due to oversampling of the minority class calls for further patient inclusion, particularly those diagnosed with severe disease. We have obtained additional data from more patients with severe dengue and are completing the genetic analysis of polymorphisms in the TNF-α gene. This is aimed at addressing the model's overfitting issue. Although these results are preliminary, they are expected to be finalized by the time of the conference presentation. These findings are valuable in advancing our understanding of severe disease development.

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Predictors Of Severity In Acute Dengue Infection During The 2019 Epidemic In Brasil

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***Abstract*—** Dengue is the most important arbovirus in terms of morbidity and mortality worldwide. The pathogenesis of severe forms is quite complex and involves a series of viral and host interactions. The occurrence of second episode of dengue is classically known as a risk factor for hospitalization and severity, while other conditions have been starting to stand out as additional risk factors. This study aimed to investigate the influence of comorbidities as potential host risk factors for the occurrence of dengue with warning signal (DWS) and severe dengue (SD), by including 769 patients confirmed for dengue by RT-PCR positive and/or NS1 detection during the 2019 epidemic in a hyperendemic region for the virus. Among the patients enrolled, 86.2% of patients presented as dengue without warning signal (DwWS), 12.8% as DWS and 0.9% as SD, according with the WHO Clinical Classification (2009). Besides, 82.7% had history of previous infection by DENV and 2.59% by Zika. Univariate binary logistic regression analysis showed that individuals older than 60 years (OR = 2.19; 95% CI 1.37- 3.52; p = 0.001), whose with diabetes mellitus (OR = 2.35; 95% CI 1.31 – 4.20; p= 0.004), with high blood pressure (OR = 1.8; 95% CI 1.10 – 2.95; p = 0.018), and with peptic disease (OR = 8.52; 95% CI 1.88 – 38.63; p = 0.005) had higher risk for severe forms (DWS + SD). When analysis was based on history of dengue, diabetes mellitus was risk factor in uni- and multivariate analysis in naïve patients, but not when the previous dengue was identified. Once the mechanism by which only a few individuals progress to severe forms of dengue is still unclear, including in not-secondary dengue, the identification of predictors of disease severity, as presented here, is essential to guide the appropriate clinical management already in the early stages of the disease, contributing to favorable outcomes.

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Production Of Dengue Virus' Recombinant Proteins From Serotypes 1 To 4 In Eukaryotic Cells For The Development Of Diagnostic Tests

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***Abstract*—** There are two main types of diagnostic tests for Dengue: serological tests, which detect the presence of antibodies against the virus or viral antigens, and molecular tests, which detect the presence of the virus's genetic material. The first ones require the availability of antigens and/or specific antibodies, usually obtained as recombinant products. With the emergence of new diseases, such as COVID-19, and the co-circulation of CHIKV (Chikungunya Virus) and ZIKV (Zika Virus), the differential diagnosis of these diseases is essential, mainly because early detection, diagnostic accuracy and access to proper medical care reduces morbidity and mortality. The production of accurate serological Dengue tests present several limitations: recombinant proteins of this virus are difficult to express in eukaryotic cells whereas recombinant proteins produced in prokaryotic systems have shown little specificity and/or sensitivity. These proteins have distinct characteristics depending on the type of cell in which they are produced and in cells of eukaryotic organisms, they have the advantage of the presence of specific post-translational modifications, which can interfere with the antigen-antibody interaction and even in the immune responses generated from immunization. In this project, recombinant Dengue virus E proteins from serotypes 1 to 4 were produced using two different eukaryotic platforms. The lentiviral vector-infected eukaryotic cell platform, and the pcDNA3.1 vector transfection, both aiming to the generation of cell clones stably expressing the antigens. The E protein sequence was designed using 80% of the protein E gene, removing the transmembrane portion and adding the TPA (tissue plasminogen activator) signal peptide for secretion. A Kozak consensus sequence was also added for mRNA optimization. Proteins from the 4 serotypes were produced in EXPI293 cells and purified by affinity chromatography, with titers of approximately 3µg of protein per mL of culture. Production is being optimized using cell sorting to isolate clones and labeling with specific antibodies for population enrichment by flow cytometry. These antigens will be used in diagnostic tests and evaluated in terms of sensitivity and specificity for their ability to be recognized by specific antibodies.

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Risk factors associated with severe dengue during the 2019 epidemic: A cross-sectional study from southern Colombia

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Abstract— In 2019, there was the highest number of registered cases of dengue in the Americas with 127,000 events in Colombia. Of all patients with severe dengue (SD) in the country during that year, 20% came exclusively from the hyperendemic department of Huila, one of the 32 departments dividing Colombia, demonstrating the devastating local impact of dengue virus (DENV) infection, and supporting the need to identify characteristics associated with this outcome. Here, we analyzed 5,494 cases of dengue and 251 of SD from the department of Huila, southern Colombia, with confirmed infection reported to the national epidemiological surveillance system in 2019. Epidemiological, clinical, and virological variables were included in the univariate, bivariate, and multivariate statistical models. The warning signs evaluated were significantly more frequent in the SD group, compared to the group with dengue, supporting an adequate classification. The most frequent form of SD was massive vascular leakage, followed by organ failure, and bleeding with 76%, 13%, and 3%, respectively. The 8% of SD cases had more than two conditions defining SD. 572 cases were serotyped by conventional and real-time RT-PCR and DENV-2 infections were associated with SD despite the higher circulation of DENV-1 during the same period in Colombia. Multiple logistic regression models showed that age less than 5 years (OR: 6, CI: 2.5-14.1), age 5 -15 years (OR: 3.4, CI: 1.5 - 7.7), diarrhea (OR: 2.7, CI: 1.7-4.8), increased hematocrit (OR: 2.4, CI: 1.3-4.5), abdominal pain (OR: 4.4, CI: 2.7-7.2), hepatomegaly (OR: 4.9, CI: 3.1-7.7) and signs of fluid accumulation (OR: 11, CI: 6.1-23.3), were the factors associated with the outcome of severity due to DENV infection. These results provide information for the design of medical approach strategies for patients at risk of severe DENV infection, especially in children.

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Sensitivity of anti-Dengue IgM serological tests fluctuate in hyperendemic arbovirus areas

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Abstract— Brasil is one of the nations with the highest dengue (DENV) fever burden. Due to Brasil´s continental dimensions, rapid serologic tests (RDTs) targeting IgM pose as a valuable diagnostic tool considering cost-benefit and are largely employed in the public health system for early DENV diagnosis. Nevertheless, IgM-based diagnostic might display limitations in dengue-endemic countries considering its sensitivity in secondary DENV-infection. To explore the utility of DENV-specific IgM tests as a marker of early dengue infection in a hyperendemic flavivirus zone, the present study assessed the analytical performance (sensitivity and specificity) of RDTs in serum samples collected in São José do Rio Preto (SJRP) during the 2019 DENV-2 outbreak. This retrospective study was conducted with two distinct sampling panels. Panel A: to determine the population of SJRP´s DENV-immune status, 819 sera with DENV-infection confirmed on RT-PCR and/or NS1 antigen were used for primary/secondary infection classification (IgG/IgM ratio of 1.2). Commercial ELISAs were performed for anti-DENV IgG and IgM detection. Panel B: 1388 cases presenting anti-DENV IgM antibodies screened using RDT and with DENV-infection confirmed (or not) by RT-PCR and/or NS1 antigen assays were extracted from the national public database SINAN. Out of the 819 sera, 42.8% (351 patients) had secondary DENV-infection, corroborating the hyperendemicity of flavivirus in SJRP. Elderly >61 years exhibited a higher positivity rate of secondary DENV-infection (15%), reinforcing the historical circulation of DENV in the region. Overall, IgM RDTs showed a sensitivity of 71% (CI95% 67.2-74.6) and a specificity of 67.5% (CI95% 63.7-71.0), with a positive predictive value of 67% (CI95% 64.1-69.5), demonstrating the limitations of IgM RDTs in DENV diagnosis in a secondary infected population. Most strikingly, IgM sensitivity decreased as patients became older with 86.4% (CI95% 71.9-95.6) in children ≤ 10 years and reaching 56% (CI95% 46.5-65.2) in elderly >61 years. On the other hand, specificity increased with aging from 60% (CI95% 47.6-71.5; ≤ 10 years) to 78.2% (CI95% 68.9-85.8; >61 years). In conclusion, IgM-based RDTs are not suitable for early DENV-infection diagnosis in dengue-endemic areas possessing a high rate of secondary DENV-infected population, and viral-driven direct tests (i.e., RT-PCR, NS1) should be considered as an alternative.

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Soluble Triggering Receptor Expressed On Myeloid Cells (sTREM-1) In Patients With Dengue, Paraguay

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Abstract— Dengue is an acute arboviral disease of great global impact in terms of morbidity, mortality, and economic costs. Most cases present as dengue with or without alarm signs (DWS+ or DWS-), but some may progress to potentially fatal severe dengue (SD). sTREM-1 is the soluble form of TREM-1, a transmembrane protein expressed in innate immune cells. It is released by proteolysis and can be detected in biological fluids such as serum and plasma. Recent studies indicate its usefulness as a severity marker for various diseases, although there is still little data on its usefulness for severe dengue. The aim of this study was to analyze sTREM-1 levels in patients with dengue classified according to severity (WHO, 2009), and to evaluate its behavior in relation to demographic and clinical variables. A cross-sectional study was performed, and 124 serum and plasma samples were analyzed from patients who attended healthcare centers in Central Department, Paraguay between 2018 and 2020 (44 DWS-, 56 DWS+ and 24 SD). 57% (71/124) were female and the median age was 34-year-old (21-56). DENV-4 was the predominant serotype. Higher concentrations of STREM-1 were found in SD cases (range 0-1329pg/mL) in comparison to DWS-and DWS+ (range 105-6030 pg/mL; $p<0.0001$) and an OR of 1.4 for every 100 units increase of sTREM-1. Higher concentrations were detected until five days after the symptom's onset. Correlation with lipopolysaccharide binding protein (LBP) and chymase was analyzed, finding a moderate correlation with sTREM-1 in both cases (Rho=0.401 and 0.505, respectively). This data shows sTREM-1 potential as a marker severity, but its performance as prognostic tests warrants further evaluation.

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The NS1-antibody response facilitates the identification of children with dengue and zika during different clinical and epidemiological outbreaks of flaviviruses

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Abstract— The infections by Dengue virus (DENV) and Zika virus (ZIKV) have some similar symptoms and a high cross-reactive antibody response. The objective of this study was to evaluate the virological and NS1-specific antibody responses to DENV and ZIKV in children with suspected dengue in different epidemiological settings in a hyperendemic area of southern Colombia. Methods: The viral RNA, circulating NS1, and IgM/IgG NS1-specific for DENV and ZIKV were evaluated by RT-qPCR and ELISA in children suspected of dengue enrolled in a hospital setting during the ZIKV epidemic (n=105), and a primary healthcare setting during a DENV epidemic (n=196). For detection of IgM DENV and ZIKV-specific, a new indirect ELISA NS1-based was performed using 351 clinical samples previously characterized. Results: DENV RNA or NS1 antigen was detected in 66% of all children and in none the ZIKV was detected. ELISA NS1-based for DENV and ZIKV IgM showed a sensitivity/specificity of 92/80% and 98/75%, respectively. Of 102 children without detectable viremia or antigenemia, 56.8%, 18.6%, and 24.5% were IgM-DENV+, IgM-ZIKV+, and IgM-DENV+ZIKV+, respectively. The IgM-NS1 ZIKV/DENV ratio improved the identification of the infecting flavivirus in the IgM-DENV+ZIKV+ children, with a high predominance of DENV infections in the two pediatric settings. Conclusion: Overall, 89% of the evaluated children had an identifiable flavivirus infection, with 82% caused by DENV and 7% by ZIKV, demonstrating active viral cocirculation in the pediatric population of Colombia. The IgM-NS1 detection improved the identification of flavivirus pediatric infections, suggesting it is a helpful tool for medical approaches in tropical regions with high viral cocirculation and different clinical sceneries.

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Validation of an NS1 and IgM Rapid Test In The Early Diagnosis of Dengue In a Primary Healthcare Centre in Bucaramanga, Colombia During The Years 2018-2020

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Abstract— Dengue has a broad spectrum of manifestations that difficult the clinical confirmation in regions where the acute syndrome febrile is multi-aetiology. The laboratory diagnostic requires specific and sensitive tests with the good advantage of cost, time, and easier manipulation. The rapid tests to detect NS1, play an important role in the early diagnosis of dengue however the implementation in primary health care is not the rule and the routinary IgM detection may not be useful during the acute phase. This study aimed to validate the use of the NS1 and IgM rapid test in primary healthcare facilities in the early detection of dengue cases during an outbreak in Colombia. From 2018 to 2020, a system was established in the healthcare of public networks in Bucaramanga, Colombia. After the blood counts and using the remaining sample, a commercial rapid test was run for antigen NS1 and IgM. One RT-PCR was performed for the detection of Zika, chikungunya, and dengue viruses. The quick test and the RT-PCR were performed independently and in a specific order: first, the rapid test and second the RT-PCR were done blinding. We estimated sensitivity, specificity, and positive and negative predictive values (SE, SP, PPV and NPV, respectively) for NS1 and IgM against PCR stratifying by disease's duration, considering a range of prevalence between 10-50%. We evaluated 566 patients with complete clinical and diagnostic data (32% were PCR positive). Overall, SE and SP were 81% and 72% for NS1 and 38% and 62% for IgM, respectively. SE increased with the disease's duration from 33.3-54.0% for IgM but remained stable around 80.0% for NS1, whereas SP was similar for both tests and showed a decreasing trend at larger disease duration. PPV and NPV ranged from 52.9-74.2% and 73.6-100.0% for IgM, and from 58.7-95.9% and 98.7-100.0% for NS1 at the disease's onset, respectively. Testing for NS1 is not only feasible in the context of primary care but also a highly accurate approach to diagnose tools for dengue in endemic areas where the acute syndrome febrile is multi-aetiology, allowing an early and suitable diagnosis to reduce complications due to the disease.

Immunology – Vaccines

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A Gold Nanorod-Nanostructured Subunit Vaccine Against Dengue Generates Robust Immune Responses And Protects Against Disease In Mice

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Abstract— Despite the emergence of SARS-CoV-2, dengue remains one of the most important infectious diseases in tropical regions of the globe, and significant outbreaks of the disease continue to erupt during the COVID-19 pandemic. The development of an effective vaccine has been considered a priority for years, and in 2016 a chimeric vector vaccine was

finally licensed for use in many countries. Nonetheless, the efficacy of this vaccine has fallen short of expectations, compromising its large-scale utilization. Meanwhile, other vaccines have been licensed or are close to be; nonetheless, novel vaccine development strategies are still needed in order to maintain an array of feasible options. In this regard, nanotechnology is a field of interdisciplinary research involving chemistry, engineering, biology, and medicine, and potential applications include the development of methods of detection, diagnosis, and treatment for an array of different diseases. Gold Nanorods (GNRs) are of particular interest. We have designed and tested an immunogen against dengue virus (DENV) based on GNRs covalently functionalized with a recombinant DENV-3 envelope protein (GNRpE). The correct assembly of the GNRpE immunogen was confirmed by UV-visible spectroscopy, transmission electron microscopy, and atomic force microscopy. The targeted protein uptake in murine macrophages was more than 2-fold higher when associated with the GNR than administered isolated in the cell media. Upon mice immunization with the experimental immunogen, high levels of anti-DENV neutralizing antibodies and DENV-specific cell-mediated responses were detected. The pattern of cytokines elicited in the group immunized with GNRpE immunogen suggests a tendency towards Th1 and Th17 biased responses. Also, vaccination of immunocompetent mice with GNRpE conferred protection against signs of disease following DENV-3 challenge, and the immunogen prevented all hematological alterations and the vascular leakage elicited by the viral infection. Protection was associated with high DENV-specific cytokine responses and accumulation of effector memory CD8+ T cells in the spleen of immunized mice. Such results are significant as an effective dengue vaccine has remained elusive despite the many efforts and the use of different vaccine strategies and approaches.

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A Humanized Mouse Model For Dengue Virus Infection And Vaccination

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***Abstract*—** 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.

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A Randomized Phase III Trial Of The Efficacy And Safety Of TAK-003 After 45 Years Of Follow-Up: Results Of Children | Adolescents From Brasil

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***Abstract*—** **Background**: Dengue virus (DENV) is a major public concern in Brasil; therefore, an effective vaccine against dengue is needed to reduce the disease burden. An ongoing, long-term phase III trial has evaluated the efficacy and safety of TAK-003, a live-attenuated tetravalent vaccine, in children/adolescents across eight dengue-endemic countries. We present the efficacy and safety results of 4.5 years of follow-up, including subgroup analyses of participants from Brasil. **Methods**: DEN-301 (TIDES) is a multicenter, double-blind, placebo-controlled trial of TAK-003 among 20,099 healthy children/adolescents aged 4–16 years at enrolment across Asia and Latin America. Participants were randomized 2:1 to receive two doses of TAK-003/placebo, subcutaneously, three months apart. Safety was evaluated, and participants were under active febrile illness surveillance. A serotype-specific RT-PCR was used to identify virologically confirmed dengue (VCD). **Results**: Overall, 20,071 participants received ≥ 1 dose of TAK-003/placebo; 91%

completed the 54-month post-vaccination follow-up; 27.7% were seronegative at baseline. In total, 27,684 febrile illnesses were reported. Of these, 5.8% placebo and 2.5% TAK-003 participants had VCD. The vaccine efficacy (VE) to 4.5 years of follow-up was 61.2% (95% CI 56.0—65.8) against VCD (baseline seropositive: 64.2% [95% CI 58.4—69.2]; baseline seronegative 53.5% [95% CI 41.6—62.9]) and 84.1% (95% CI 77.8—88.6) against hospitalization (baseline seropositive: 85.9 [95% CI 78.7—90.7]; baseline seronegative 79.3 [95% CI 63.5—88.2]). There were slightly more serious adverse events in the placebo group, and no important long-term safety risks were identified. In Brasil, 1773 participants received ≥ 1 dose of TAK-003/placebo. In this subgroup, 29.3% were seronegative at baseline, and 1614 febrile illnesses were reported. Of these, 4.2% placebo and 0.9% TAK-003 participants had VCD. VE to 4.5 years of follow-up was 82.2% (95% CI 61.8—91.8) against VCD (baseline seropositive: 86.5% [95% CI 63.7—95.0]; baseline seronegative: 69.6% [95% CI —7.9 to 91.5]). Two participants with VCD were hospitalized in the placebo group versus zero in the TAK-003 group. **Conclusions**: Irrespective of baseline serostatus, TAK-003 was well tolerated and protected against symptomatic dengue and hospitalization over 4.5 years in children/adolescents in the overall study population and the subgroup from Brasil.

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An Open-Label, Phase 2 Study Evaluating Cell-Mediated Inmune Response And Safety Of a Tetravalent Dengue Vaccine In Children And Adolescents Aged 4-16 Years

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Abstract— **Introduction.** TAK-003, a tetravalent dengue vaccine, demonstrated efficacy and safety against symptomatic and hospitalized dengue in a long-term phase 3 study. As a robust cell-mediated immune (CMI) response is important for immunity, this open-label phase 2 study evaluated T-cell responses to TAK-003 in healthy 4- to 16-year-old participants in dengue-endemic regions (NCT02948829). **Methods.** 200 participants were enrolled to receive TAK-003 at Days 1 and 90. Dengue serostatus was tested at baseline (seropositivity: reciprocal neutralizing antibody [NAb; MNT50] titer ≥ 10 for ≥ 1 serotype). The primary objective was CMI response rate at Day 120 using T cell interferon-gamma (IFN-γ) enzyme-linked immunospot assay [ELISPOT]. Peptide pools for non-structural (NS) proteins NS1, NS3, and NS5 matching DENV-1, -2, -3, and -4 were used for stimulation. Secondary objectives included further evaluation of CMI, NAb responses, and safety. Participants were followed up to 3 years post-second vaccination. Here we report results for CMI responses up to Day 270 (6 months after administration of the second TAK-003 dose) and overall safety data. **Results.** IFN-γ T-cell response rate against any peptide pool at Day 120 was 76.0% in seropositive participants and 83.1% in seronegative participants and remained stable through Day 270. IFN-γ T-cell response rates at Day 120 to peptide pools matching DENV-1, -2, -3, and -4 were 58.0%, 75.0%, 60.0% and 50.0% in seropositive participants and 59.7%, 83.1%, 47.4% and 39.0% in seronegative participants, respectively, and remained elevated through Day 270. Multifunctional (secretion of ≥ 2 cytokines among IFN-γ, interleukin-2 and tumor necrosis factor-α) CD4+ and CD8+ T-cell responses were observed, independent of baseline serostatus. NAb titers and seropositivity rates remained high against all four DENV serotypes through Year 3. TAK-003 was well-tolerated with no important safety risks identified. **Conclusions.** TAK-003 elicited multifunctional, cross-reactive T-cell responses against all four DENV serotypes, irrespective of participant baseline serostatus, in 4- to 16-year-old participants living in dengue-endemic regions

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Antibody Correlates Of Protection Against Severe Dengue Disease

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***Abstract*—** Four dengue virus serotypes (DENV1-4) are associated with an estimated 50 million cases of dengue annually worldwide, presenting as mild (dengue fever, DF) or severe (dengue hemorrhagic fever/dengue shock syndrome, DHF/DSS). Primary DENV infections generate circulating cross-reactive antibodies capable of mediating protection from or risk for developing severe dengue upon a subsequent secondary heterotypic infection. However, characteristics of antibodies associated with protection from developing future severe disease remain elusive. We performed systems serology in plasma/serum samples collected before secondary heterotypic DENV infections (primarily DENV2 and DENV3) resulting in DF (n=33) or DHF/DSS (n=33) cases from our longstanding pediatric cohort study in Nicaragua. We measured binding antibodies by Luminex using beads conjugated to recombinant envelope (E), E domain III, and nonstructural protein 1 (NS1) of DENV1-4 and the related Zika virus (ZIKV). For Fc effector function assays (antibody-dependent complement deposition [ADCD] and antibody-dependent cellular phagocytosis [ADCP]), we used beads conjugated to DENV2, DENV3, and ZIKV antigens. Higher levels of total IgG, IgG2, IgG3, IgG4, and both ADCD and ADCP were associated with protection against DHF/DSS, while neutralizing antibodies to mature DENV2 and DENV3 virions, as measured by focus reduction neutralization test on Vero cells, were not significantly different. Although the samples were derived from ZIKV-naïve individuals, the association of ADCD activity with protection was stronger when assays were conducted with ZIKV antigens. We validated these findings with a complement-mediated virolysis assay using DENV2, DENV3, ZIKV, West Nile virus, or yellow fever virus virions. We found that virolysis of ZIKV virions mediated by samples from DENV-exposed ZIKV-naïve individuals was most strongly associated with protection, suggesting that 1) anti-DENV antibodies that cross-react with ZIKV, 2) antibodies that target virion-specific epitopes, and 3) antibody Fc effector functions mediating complement deposition and virolysis are correlated with protection from severe dengue disease. In sum, we identified protective Fc biophysical features and effector functions of DENV-ZIKV cross-reactive antibodies that may support the design and evaluation of dengue vaccines.

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Antigenic Diversity and Association with Outcome of Dengue Infection Following Tetravalent Dengue Vaccination

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***Abstract*—** **Introduction**: Dengue disease is caused by four related, but distinct, virus serotypes. Takeda's live attenuated tetravalent dengue vaccine (TAK-003) is comprised of structural proteins from each serotype in an attenuated dengue virus type 2 (DENV-2) genomic backbone. Data from the pivotal, Phase III clinical trial (DEN-301) showed that TAK-003 is safe and efficacious regardless of baseline dengue serostatus. However, vaccine efficacy varied by serotype and exploratory analysis suggests lack of efficacy against DENV-3 in baseline seronegative recipients. The virus envelope (E) protein, the main target of the neutralizing antibody (nAb) response, is known to accumulate intra-serotype genetic diversity over time. Antigenic diversity could potentially impact vaccine coverage against circulating dengue strains. In the present study, we asked if virologically confirmed dengue (VCD) and disease outcome were linked to antigenic diversity between vaccine and contemporaneous DENV-3 strains. **Methods**: Vaccine DENV-3 E protein sequence was aligned with viral E protein sequences from confirmed cases of DENV-3 in Asia during the DEN-301 study, and sequences of circulating DENV-3 strains from collaborating laboratories. Phylogenetic trees were generated to assess genetic diversity across viral strains. Regression analysis was performed to assess possible links between genetic diversity of DENV-3, viral load in VCD cases, and outcome of DENV exposure when adjusting for age, gender, and treatment group. **Results**: Most incidences of DENV-3 infection throughout the entire DEN-301 clinical trial occurred in the Philippines, followed by Sri Lanka, Thailand, and Colombia. DENV-3 strains causing VCD, clustered with circulating strains of DENV-3 during the trial. Additionally, neither phylogenetic distance between virus E sequences, nor single or cumulative amino acid changes in the E sequences from DENV-3 infections, were associated with viral load post-infection, or hospitalization due to dengue during the trial. **Conclusions**: Phylogenetic analysis reveals evolution of DENV-3 E protein sequences from time of isolation of the vaccine strain in 1964, to contemporaneously circulating DENV-3 strains. However, regression

analysis revealed no association between amino acid variation of virus E protein and the outcome of DENV-3 exposure in the phase 3 efficacy trial.

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Diversity, quality, magnitude, and functionality of the humoral response to a live-attenuated tetravalent dengue vaccine in pediatric participants from a phase 3 efficacy trial

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***Abstract*— Background.** Most dengue cases occur in children, and severe dengue drives the global economic burden of the disease. Immune responses targeting viral structural and nonstructural (NS) proteins have a role in protection against severe dengue. Takeda's live-attenuated tetravalent dengue vaccine, TAK-003, is comprised of structural proteins from each serotype in an attenuated dengue virus (DENV) serotype 2 (DENV-2) genomic backbone. TAK-003 was designed to elicit a dengue-specific immune response against the entire DENV backbone. **Methods.** Samples from children and adolescents aged 4—16 years participating in a phase 3 efficacy trial (DEN-301, NCT02747927) of 2 doses of TAK-003 were assessed for magnitude and functionality of antibody (Ab) responses to structural and NS DENV proteins. Measurements included concentration and avidity of vaccine-elicited binding Abs; breadth and specificity of neutralizing Abs (nAbs); magnitude of complement-fixing Abs (CFAs); and Abs against the viral toxin NS1. **Results.** TAK-003 elicited robust, tetravalent humoral responses against DENV in both baseline seronegative and seropositive vaccine recipients, including high avidity anti-DENV binding Abs, cross-reactive and serotype-specific nAbs with coverage across genetically diverse DENVs, CFAs, and anti-NS1 Abs. These Ab responses peaked at 30 days post-second vaccination and remained above baseline levels through the end of 1 year post-second dose. The data also showed high correlation and concordance with the nAb responses observed in DEN-301 against all 4 DENV serotypes. **Conclusion.** This is the first comprehensive characterization of the magnitude, quality, and functionality of the humoral immune response to a dengue vaccine in a pediatric population. The diversity of TAK-003-driven Ab responses observed in this analysis may contribute to the robust efficacy against hospitalized dengue cases reported in children and adolescents during the pivotal efficacy trial.

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Complement-Fixing Antibody Response Following Vaccination With a Live-Attenuated Dengue Vaccine in A Phase III Clinical Study with Seronegative Adult Participants From Non-Endemic Area

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***Abstract*— Introduction.** The magnitude of complement deposition mediated by complement-fixing antibodies (CFA) following natural dengue virus (DENV) exposure has been associated with protection against symptomatic disease. However, the role of CFA responses in dengue vaccine-mediated efficacy remains unknown. The DEN-301 trial (NCT02747927) demonstrated TAK-003, a live-attenuated tetravalent dengue vaccine, to be well-tolerated and efficacious against virologically confirmed disease and hospitalization caused by any of the four DENV serotypes (DENV1-4) in participants 4-16 years old from eight different endemic countries. TAK-003 has also been shown to elicit a multi-pronged immunity against DENV, including humoral and cellular responses. Furthermore, we have previously reported that TAK-003 elicited CFA in response to an earlier formulation of each vaccine component during Phase II studies in young individuals. This study aimed to investigate if the final formulation of TAK-003-induced CFA responses against DENV1-4 and gain further insight into the associated antibody isotype/subclass responses. **Methods.** DEN-304 (NCT03423173) was a randomized, double-blind, placebo-controlled, phase III trial of TAK-003 in adults from non-endemic areas across the United States. Participants (18-60 years) received 2 doses of the final formulation of TAK-003 subcutaneously at months 0 and 3. For exploratory analyses, serum samples from 48 study participants were collected at months 0, 9, and 15 and analyzed for anti-DENV CFA, IgG1, IgM, and IgA responses using Luminex-based multiplex assays. Neutralizing antibody responses (nAb) were quantified using a DENV microneutralization assay. **Results.** TAK-003 stimulated comparable CFA responses against DENV1-4 in seronegative adults. Further characterization of the antibody isotype repertoire revealed that IgG1, but not IgA or IgM, likely accounted for the vaccine-stimulated complement fixation effector function. Additionally, nAb responses (primary endpoints across several other TAK-003

clinical studies) showed high correlations with CFA and IgG1 across DENV1-4 and moderate correlations with IgA and IgM. **Conclusions.** These results suggest that CFA, IgG1, and to a lesser extent, IgA or IgM play a role in TAK-003-mediated virus neutralization and highlights the need for further expanding vaccine immunogenicity characterization. Assessment of CFA against DENV1-4 and associated antibody isotype/subclass responses will support a more holistic understanding of vaccine-driven protection mechanisms across population demographics and dengue endemicities.

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Deciphering The Role of CD4+ T Cells in Modulating B Cell Repertoire Diversity and Functionality After a Heterologous ZIKV Challenge in Subjects with Prior DENV Immunity

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Abstract— The intrinsic mechanisms and interactions between CD4+ T cells and B cells in priming a humoral response promoting cross-protection in secondary heterologous flavivirus infections remain significantly understudied. The use of single-cell RNA sequencing allows us to assess the B cell receptor repertoire and establish which activation pathway Dengue (DENV) cross-reactive memory B cells take during a secondary infection with Zika (ZIKV), be it T cell-dependent or T cell-independent activation. Such pathways can be characterized by changes in somatic hypermutation rates between the primary and secondary infection. We used single-cell RNA sequencing to characterize the B cell repertoire, machine learning for epitope prediction, Immunophenotyping of B cell subsets, and neutralization tests to assess the role of CD4+ T cells in modulating the humoral response towards a ZIKV infection in DENV2 immune nonhuman primates. Preliminary results using bulk B cell repertoire sequencing on PBMC samples collected during a tertiary DENV4 infection showed that CD4+ T cells can modulate B cell repertoire diversity, as CD4+ T cell-depleted animals showed distinct usage of antibody Variable gene segments, compared to undepleted controls. We show that CD4+ T cells have a significant role in modulating neutralizing capacity during a primary DENV2 infection, seen as a delay in DENV2 neutralizing antibody titers at 15 days post-infection. Furthermore, CD4+ T cells have a significant role in priming a humoral response during a secondary ZIKV infection as animals depleted of CD4+ T cells before the secondary ZIKV challenge, showed a delay in neutralizing antibody titers at 15 days post-infection, compared to undepleted controls. Also, our results suggest that CD4+ T cell depletions before the primary infection with DENV2 did not impact neutralizing capacity toward the secondary ZIKV infection. Our model can provide novel data on what specific B cell populations express cross-reactive or type-specific neutralizing antibodies that can be targeted for clonal expansion, significantly contributing to the development of new DENV/ ZIKV vaccine platforms, limiting setbacks such as Antibody-Dependent Enhancement.

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(RETRACTED)

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Development Of A Nucleic Acid Vaccine For Dengue Virus Serotypes 2 And 3

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Abstract— Dengue is the main arbovirus affecting the human population. As pinpoint differential diagnosis and efficient vaccines are still important issues, the disease remains considered as one of the neglected diseases with greatest impact on Tropical Countries' health systems. The development of a prophylactic vaccine. Nucleic acid vaccines have become excellent options allowing for fast development and adaptability. Therefore, our aim is the development and evaluation of the immunogenicity a nucleic acid vaccine encoding

the envelope (E80) and non-structural protein 1 (NS1) for dengue serotypes 2 and 3. We independently prepared DNA and mRNA molecules coated with lipid nanoparticles and immunized C57BL/6 mice in a two-dose schedule with an interval of 21 days. Blood was drawn between doses for evaluation of the humoral response, and euthanasia 30 days after the boost dose, when spleens were harvested to evaluate the cellular immune responses. Specific humoral responses were observed in primed animals immunized with NS1 D2 and D3, and there was a significant increase in IgG titers after the boosting dose. Such responses were similar for both DNA and LNP mRNA vaccines. As for IFN-gamma production in immunized animals, an increase in titer was generally detected after stimulation. As for immunizations with E80 D2 and D3, was observed specific humoral responses after the booster dose with DNA, including the generation of neutralizing antibodies. These results are encouraging and support the further development of a genetic vaccine to Dengue, including immunogens against the other Dengue virus' serotypes.

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Different Species Of Non-Coding RNA Generated In DENV2 And DENV4 Infections

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Abstract— The molecular mechanisms underlying DENV infection and the host immune response are crucial for developing effective vaccines, diagnostic tools and therapeutic interventions. During viral replication, small non-coding viral RNAs called sfRNAs (small flavivirus RNAs) are accumulated by partial degradation of the viral genome. This process is regulated by complex RNA structures present at the viral 3'UTR that halt the XRN1 exoribonuclease activity and leads to accumulation of sfRNAs in infected cells, which have been associated to pathogenicity and evasion of innate immune responses. Intrigued by the differences found in the 3'UTR of different DENV serotypes and the fact that the NIH vaccine attenuation strategy includes a deletion in this region (TV003), we evaluated the properties of sfRNAs produced during DENV2 and DENV4 infection. For this, we developed genetic tools to manipulate the viral genomes, including the construction of reporter viruses for both serotypes. Using Northern Blot analysis employing specific 3'UTR probes labeled with digoxigenin, we detected different species of sfRNA. In mosquito cells, DENV2 accumulated mainly three species of sfRNAs (sfRNA1, sfRNA3 and sfRNA4, by stalling XRN1 at SLI and both DBs, respectively) while DENV4 generates only two (sfRNA1 and sfRNA3, by stalling XRN1 at the unique SL and DB2). sfRNA1 from DENV4 is shorter due to the lack of one SL. Interestingly, in human cells these viruses produced mainly sfRNA1 (of 420 and 375 nucleotides long, for DENV2 and DENV4 respectively). In addition, we evaluated the impact of the attenuating deletion of the vaccine candidate Delta30 on sfRNA formation for both serotypes. We observed in both viruses the main accumulation of sfRNA1 in human cells. In mosquito infected cells with the vaccine candidate sfRNA2 and sfRNA3 disappeared. Despite that the Delta30 deletion is far from the SL elements, the overall level of sfRNA was reduced in human cells, and in the case of DENV4, sfRNA levels were almost undetectable. Evaluation of the innate antiviral response in infections with DENVs that produce different species of sfRNAs will help to understand unique properties of DENV serotypes and vaccine candidates.

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Characterization Of CD4+ T Contribution During Secondary ZIKV Or DENV2 Exposure In Dengue-Immune Non-Human Primates

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Abstract— Dengue (DENV) and Zika (ZIKV) viruses are vector-borne flavivirus infections transmitted through Aedes and Culex species mosquito bites. Both infections are estimated to impact 100-400

million people worldwide each year. Severe pathogenesis and symptoms are more likely to occur in individuals primarily exposed to a heterologous dengue infection due to natural infection and/or vaccination. On the other hand, activated pTfh cells have been correlated to induced elevated frequencies of antibody-secreting cells (ASC) and high neutralizing antibody responses during DENV infection, Influenza, and Yellow Fever Vaccination (YFV) in humans. There are many aspects still to be investigated related to the cellular immune response of CD4+ T cells and their subsets of peripheral follicular T helpers' cells (pTfh). Particularly, on how these subsets modulate the generation of long-term neutralizing antibody responses in the context of heterologous secondary flavivirus infections. In this study, we compare the contribution of pTfh cells controlling specific cellular activation and neutralizing cross-reactive antibody response after an initial and a second heterologous flavivirus infection [E.g. DENV4→DENV2 (Group 1); DENV2→ZIKV (Group 2); ZIKV→DENV2 (Group 3). Characterization of the frequency and phenotypic profile of CD4+ cells and pTfh subsets in the blood of rhesus macaques, allow the identification of CD4+ T expressing CXCR5, CXCR3, PD-1, and ICOS. Single-cell RNA sequencing and transcriptomics profile of CD4+ T cells, demonstrate how different orders of flavivirus infections modulates humoral immune recall responses to a secondary heterologous viral infection. Quantification of high neutralizing antibody responses also correlates with the frequency of CD4+ T cells and pTfh subsets. Interestingly, we found that total CD4+ T cell depletion during a primary DENV2 infection has a limited impact on the outcome of the humoral immune response to ZIKV infection. Here, we demonstrate the critical role of these CD4+ subsets in priming an early effective humoral response during sequential flavivirus infections. Our study provides a foundation for the development of biomarkers of strong humoral immune responses creating better validation platforms for new vaccine candidates.

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Durable Type-Specific Cytolytic CD4+ T Cell Immunity After Denvax (TAK001) In Flavivirus-naives

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Abstract— Immunity after secondary dengue infection correlates with protection from severe dengue (Olkowski, 2013), however factors mediating protection are incompletely understood. Work by others (Weiskopf, 2015) demonstrated immunity after secondary dengue is associated with a cytolytic CD4+ T cell phenotype not seen after primary infection. We determined whether dengue-specific CD4+ memory T cells were present 8+ years after secondary vs. primary infection in American veterans, and in flavi-naïve recipients of DENVax/TAK001, an earlier formulation of TAK003 tetravalent dengue vaccine. PBMCs from veterans who had primary or secondary dengue infections (determined from history and serologic responses to the 4 dengue serotypes) and vaccinees collected 8 or more years after exposure were restimulated with live dengue 1-4 for 7 days, then fixed and stained for CD3, CD4, CD8, CD45RA, CCR7, IFNγ, granzyme B, perforin, and CD107a. We found that both veterans who had secondary dengue infection and DENVax/TAK001 vaccinees had type-specific re-expandable cytolytic (CD107a+ granzyme B+ Perforin+) CD4+ T effector memory (CCR7-CD45RA-) cells to the infecting or vaccinating serotypes but veterans who had only one dengue infection did not. Vaccination also induced cytolytic (CD107a+granzyme B+ Perforin+ IFNγ+) memory CD4+ and CD8+ TEMRA cells while infection rarely did. While the NIH DLAV dengue vaccine also induces type-specific cytolytic CD4+ T cells (Graham, 2020), we are the first to show these cells persist for 8+ years after vaccination of flavi-naives or secondary infection. In phase 3 trials, TAK003 has shown 79% protection from severe dengue in previously dengue seronegative people, and no increased risk of severe disease in 4.5 years of followup (Biswal, 2022). How TAK003 mediates protection from severe dengue in dengue seronegatives is not fully understood, as the antibody and T cell responses focus on dengue 2 but protection to dengue 1 is also seen and there is no enhanced disease to dengue 3. Our data supports the hypothesis that durable cytolytic CD4+ T cells are induced by secondary but not primary infection and live attenuated dengue vaccination, and may play a role in protection from severe dengue in both people recovered from secondary infections and after live vaccination of seronegatives.

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Evaluation Of Systemic Immune Response After Yellow Fever Infection: An Observational Study On Patients From The 2017-2018 Sylvatic Outbreak In Brasil

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Abstract— Between 2016-2018 major yellow fever (YF) outbreaks occurred in Brasil, in which 2,155 cases and 745 deaths were reported, most of these in Minas Gerais (MG) state. Patients from these outbreaks represented a unique opportunity to assess the immune response triggered by wild-type (WT) strains of the yellow fever virus (YFV) in humans. This study aimed to evaluate the immune response of patients from 2017-2018 outbreak in MG with different disease outcomes, using plaque reduction neutralization test (PRNT) protocols with WT and vaccine 17DD YFV strains, to better understand the response elicited against both. For this, WT YFV strain (Hu-BR2018) isolated from a patient from the same outbreak and vaccine 17DD YFV strain were used in PRNT protocols to assess the immune response of naturally infected YF patients, in comparison with healthy vaccinees. Levels of circulating soluble mediators (CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL4, CCL5, IFN-α, TNF-α, IL-1b, IL-6, IFN-γ, IL-1ra, IL-4, IL-5, IL-10, IL-17A, G-CSF, CM-CSF, and M-CSF) were also measured and determined for patients from the YF outbreak, and for control groups containing healthy individuals seronegative for YF, and healthy vaccinees. Results showed that both YF patients and vaccinees presented different levels of anti-YFV neutralizing antibodies (nAb) against WT and 17DD strains, and overall, the level of neutralization against different strains of YFV varied homotypically and heterotypically. Results based on geometric mean titers (GMT) of naturally infected individuals and vaccinees suggested that the humoral immune response after a natural infection of YFV can reach higher levels than that induced by vaccination (GMT of YF patients against WT YFV compared to GMT of vaccinees; $p<0.0001$). Similar patterns were observed for soluble mediators. This study provided evidence that the humoral and systemic immune response triggered by 17DD vaccine and WT strains of YFV is different, which could be explained by the presence of genetic/antigenic differences between these strains and by differences between vaccination process itself and the natural infection. These data suggest a need to update current means of assessing the immune response in naturally infected YF individuals, as well as immunological surveillance methods in areas with intense viral circulation.

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Fc Gamma Receptor IIb Plays An Essential Role In Controlling Chikungunya Virus-induced Arthralgia

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Abstract— Chikungunya virus (CHIKV) is an arbovirus that causes acute febrile illness and severe chronic arthritis in over 60 countries. The pathogenesis of alphaviral infections involves a complex interaction between viral and host factors, and understanding these factors is crucial for comprehending the disease and developing targeted treatments. Within the host, Fc gamma receptors (FcyRs), which recognize the Fc portion of immunoglobulins G (IgG), play a pivotal and dual role in the inflammatory process. This study aimed to evaluate the involvement of FcyRIIb and FcyRIII receptors in the pathogenesis of CHIKV infection. Four-week-old wild-type (C57Bl/6), FcyRIIb-/-, and FcyRIII-/- mice were intraplantarly infected with an inoculum containing 1x106 PFU of CHIKV. Mice were assessed for hypernociception, viral titers in target organs, neutrophil and macrophage infiltration, cytokine profile production, and tissue damage. CHIKV infection in mice was associated with hypernociception lasting approximately 21 days, recovery of viral titers from

several organs at early time points of infection, and increased neutrophil and macrophage infiltration. Tissue damage in hindpaws and joints correlated with elevated levels of cytokine detection. FcyRIIb-/- mice exhibited exacerbated and prolonged joint hypernociception persisting until the 28th day after infection. In contrast, hypernociception in FcyRIII-/- mice was similar in intensity and duration to wild-type (WT) mice throughout the evaluated period. Viral titers in hindpaws and plasma were higher in FcyRIIb-/- mice compared to WT animals, while they were lower in FcyRIII-/- mice. Analysis of neutrophil infiltration on the 1st and 10th day post-infection showed increased myeloperoxidase (MPO) enzyme levels in FcyRIIb-/- animals, accompanied by increased levels of IL-1ß, CXCL1, and IL-6. Evaluation of macrophage infiltration in FcyRIIb-/- mice revealed increased N-acetylglucosaminidase (NAG) enzyme levels on the 10th and 14th day, whereas WT littermates peaked on the 7th day. Tissue lesions peaked on the 7th day post-infection in both groups, although they were more severe in FcyRIIb-/- animals. Overall, results demonstrate that the absence of the FcyRIIb receptor plays a crucial role in the persistence of hypernociception and the exacerbation of disease induced by CHIKV. The molecular mechanisms involved in this process remain unknown and are under investigation.

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Inhibition Of Pi3kγ Attenuates Neuroinflammation And Neuronal Cell Death Induced By Zika Virus: Implications For Novel Treatment Strategies

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***Abstract*—** Zika virus (ZIKV) is an arbovirus belonging to the Flaviviridae family and Flavivirus genus. ZIKV infections have emerged as a global public health emergency, associated with neurological complications including microcephaly. ZIKV displays neurotropism and triggers neuroinflammation and neuronal cell death. Phosphatidylinositol 3-kinase γ (PI3Kγ), an enzyme involved in cellular functions such as cell growth, proliferation, and survival, is constitutively expressed in various cell types, including leukocytes and neuronal cells. This study aimed to investigate the role of the PI3Kγ pathway during Zika virus infection. In vivo experiments were conducted using interferon α/β receptor knockout mice (A129) infected with ZIKV (4x103 PFU) via intravenous injection. A PI3Kγ inhibitor (AS605240) was administered as a therapeutic strategy, one hour before infection and at 2 days post-infection (dpi), subcutaneously once daily (30 mg/kg), until the peak of disease (5 dpi). Mice were euthanized, and their brains were collected for assessment of viral titers, levels of inflammatory mediators, histopathology, immunohistochemistry, and flow cytometry. The experimental protocol was approved by the Committee on Animal Ethics of the Federal University of Minas Gerais (CEUA/UFMG), under permit protocol no. 72/2020.Concurrently, undifferentiated primary neurons were isolated from wild-type (C57BL/6) and catalytic subunit knockout (PI3Kγkd/kd) mice. Additionally, human neuroblastoma cells (SH-SY5Y) were infected with ZIKV (MOI 1) and treated with AS605240 (0.5-50 μM). Cell viability (MTT/LDH) and ZIKV titers were assessed at 48 dpi. Pharmacological inhibition of PI3Kγ with AS605240 in infected A129 mice attenuated ZIKV-induced intraocular pressure, reduced viral replication in the brain, mitigated brain histopathological damage, and inhibited astrocyte (S100β) and microglia (Iba-1) activation. Furthermore, PI3Kγ blockade resulted in reduced TNF expression in activated microglia, along with increased production of brain-derived neurotrophic factor (BDNF), an important mediator of neuronal function in the brain. Similarly, inhibition of PI3Kγ in undifferentiated neurons and human neuroblastoma cells significantly reduced ZIKV-induced neuronal death and viral titers at 48 hours post-infection. These findings suggest that activation of the PI3Kγ pathway promotes Zika virus replication and contributes to neuroinflammation. Blocking the PI3Kγ pathway using drugs during ZIKV infection could potentially benefit the host as a therapeutic strategy.

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Modulation Of Complement Regulatory Molecules In Infected And Bystander Cells During Dengue Virus Infection

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***Abstract*—** Dengue virus (DENV) is a flavivirus with four known circulating serotypes (DENV 1-4). Infection with DENV can result in a wide spectrum of disease: primary infections tend to produce milder disease while secondary infections can be associated with more severe disease, though the mechanisms behind this phenomenon are not well defined. The complement cascade seems to play an important role during DENV infection, as cleaved complement factors such as the anaphylatoxins C3a and C5a have a potent effect on the permeability of the capillary vasculature. Recent studies suggest that complement dysregulation and overactivation may play a role in the development of severe dengue disease, especially dysregulation of the alternative pathway (AP). In this study, we aimed to investigate the effect of infection on the expression of complement regulatory molecules, particularly decay accelerating factor (DAF/CD55) on both infected and bystander cells. HepG2 hepatocellular carcinoma cells were infected with DENV-2 16681 (MOI = 1) for 48- and 72-hours. Cells were then stained with anti-CD55 and anti-DENV antibodies and analyzed by flow cytometry to determine the expression of CD55. Infection with DENV-2 resulted in 13-18% of total cells expressing DENV E protein. During DENV-2 infection, a significant increase in the expression of complement regulatory molecule CD55 was observed at both 48- and 72-hours post infection (hpi) in infected (DENV E protein positive) cells, while expression levels in mock-infected and bystander cells remained similar. We then moved these studies to a novel human skin explant model to further investigate modulation of the complement cascade by DENV. Preliminary data from primary epidermal cell suspensions infected with DENV-2 shows the same differential expression of CD55 on infected cells. Our results suggest that DENV-infected cells can augment expression of complement regulatory molecules to help prevent complement-mediated cell damage or death. Going forward, we will continue to use the human skin explant model to investigate the mechanism by which DENV modulates expression of complement regulatory molecules on infected cells while inhibiting their expression on bystander cells.

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NS1 Specific Antibodies Elicited By The Yellow Fever Vaccine 17D

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***Abstract*—** Mosquito transmitted yellow fever virus (YFV), is a flavivirus endemic in regions of Africa and South America, and the WHO estimates that over 400 million people are at risk of YFV infection. YFV is highly pathogenic, causing multi-organ failure with up to 50% patient mortality. There are no YFV-specific treatments, but the live attenuated YFV vaccine 17D is highly protective against disease. Flavivirus genomes encode 3 structural proteins, including envelope (E) glycoprotein - the target of neutralizing vaccine elicited antibodies, and 7 non-structural proteins, including the non-structural protein 1 (NS1). NS1 plays multiple roles in the flavivirus lifecycle: it can form a dimer that is required for viral replication that can also be displayed on the surface of infected cells and is also secreted (sNS1) as a hexamer. sNS1 has been shown to increase vascular endothelial permeability, which may contribute to both disease and virus dissemination. Several studies provide evidence that anti-NS1 antibodies following flavivirus infection provide protection against more severe forms of disease, but the presence and functions of anti-NS1 antibodies from 17D vaccination is unknown. I hypothesize that, as a live attenuated virus, 17D vaccination produces durable, potentially protective anti-NS1 antibodies. To begin to test this hypothesis, I developed and optimized a single-dilution YFV NS1-specific ELISA for YFV NS1 specific antibodies in human vaccinee sera. Following optimization, I characterized anti-NS1 antibodies in a cohort of 155 17D vaccinees vaccinated.

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Potency And Breadth Of 17D-Elicited Neutralizing Antibodies Against Wild-Type Yellow Fever Viruses In A Non-Endemic Cohort With Diverse Flavivirus Infection Histories

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***Abstract*—** The yellow fever (YF) live-attenuated vaccine 17D, licensed in 1938, is considered one of the most successful vaccines to date. Vaccination campaigns have achieved significant control of yellow fever virus (YFV), protecting populations from severe febrile illness and death. Despite great success, elimination of YF is unlikely due to non-human primate reservoirs of YFV, transmitted by Aedes and Haemagogus spp. mosquito vectors. In 2016, the historical regime of prime vaccination followed by a boost every 10-years was amended to a single dose, by both the CDC and the WHO. However, data from our lab and others have demonstrated that 90% neutralization (NT90) serum titers are <1:10 in up to 20% of single-dose-vaccinees. Furthermore, serum NT90 titers are typically established using the attenuated 17D vaccine strain, thus, the correlation between 17D-based titers and neutralization against clinically relevant wild-type (WT) YFVs remains largely unknown. Using a panel of 17D-immune sera from a non-endemic human cohort <10-years post-vaccination (n = 50), we investigated breadth and potency of serum NAbs using focus reduction neutralization assays using a panel of WT YFVs representing the seven YFV genotypes found globally, including contemporary isolates of clinical significance. We found significantly reduced potency of NAbs against WT viruses compared to 17D. Potency against strains belonging to the South American I genotype, responsible for the 2016-2019 YF epidemic in Brasil, was particularly reduced, but to a lesser degree in vaccinees with history of other flavivirus infection, suggesting a degree of cross-protection. To further understand the variables that may play a role in directing antibody responses <10-years post-vaccination, we employed multiple variable regression evaluating the effects of time since vaccination, age at vaccination, and flavivirus infection history. Importantly, for subject serum samples with neutralization titers against 17D at or just above the neutralization threshold of detection, we define the limits of neutralization against WT YFVs, offering a more authentic and stringent indicator of protection. These data suggest an increased proportion of vaccinees at elevated risk of vaccine breakthrough, which may better inform future vaccination recommendations.

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Relation Of Cocirculating Dengue Virus, NS1-specific Antibody Response And Clinical Outcome In Colombian Children

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***Abstract*—** Southern Colombia has been severely affected by the dengue disease, and the highest number of cases and severe infections have been regularly reported from this region through the last decade. Here, we studied the serotype cocirculation of dengue virus 1-4 (DENV), the virus-specific antibody response, and clinical aspects of the 2,200 children from the Department of Huila (region of southern Colombia) enrolled from 2011 to 2020 ranging from mild to severe dengue. From 2,086 RT-qPCR performed an infecting DENV serotype was identified in 920 cases (44.1%). DENV-1 (47.1%) and DENV-2 (35.1%) were the serotypes more frequently detected, followed by DENV-3 (16.3%) and DENV-4 (0.6%) during this time. DENV-1 was the major circulating virus from 2012 to 2013 and 2018 to 2020, whereas DENV-2 and DENV-3 were the dominant serotypes detected from 2014 to 2018. Infections by DENV-2 were significantly more frequent in children with severe dengue (SD) than children with dengue without warning signs (DNS) and dengue with warning signs (DWS), supporting a relation of this serotype with the clinical severity. To evaluate the virus-specific antibody response, we validated a DENV NS1-based indirect ELISA, which showed a specificity/sensitivity for IgM and IgG of 90/85% and 94/90, respectively. The analysis of the virus-specific antibody response showed that most of the patients clinically classified as DNS suffered primary infection, while children with DWS and SD had subsequent infections. Seroconversion was identified in >70% of primary and secondary infections when DENV-NS1 IgG was evaluated in acute/convalescent paired samples. The magnitude of the antibody response was related to clinical severity and the relative amount of circulating DENV IgM and IgG-NS1 were significantly higher in children with SD than in DWS and DNS. Furthermore, the NS1-antibody response correlated with tissue injury markers such as liver enzymes. These results

demonstrated the active cocirculation of DENV 1-4 serotypes in the last decade in southern Colombia and supported the relationship between the magnitude of anti-NS1 humoral immune response and clinical outcome in pediatric dengue offering new tools to medical personnel in hyperendemic areas.

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Structural Studies Of Flavivirus – Antibody Interactions

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Abstract— We have been studying flavivirus – antibody complexes using cryo-electron microscopy. Extensive analyses of the assembly and maturation of the virus particles has previously been described although the dynamic properties of these viruses, especially of dengue virus, remain to be completely described. A comprehensive analysis of the structural landscape of the humoral immune response against flaviviruses is crucial for understanding the role of antibodies in controlling virus infection. The structures of several new flavivirus – antibody complexes will be shown and interpreted. In addition, antibodies against the dengue virus prM protein, can have protective properties in a mouse model of dengue virus. Antibodies against flaviviruses have been shown to have multiple diverse interactions and have revealed multiple mechanisms for antibody-induced virus neutralization. The presentation will compare multiple flaviviruses, including dengue, and their antibody complexes to demonstrate mechanisms of action with some common and some unique binding modes. This fundamental knowledge of antibody-mediated neutralization may be useful in the design of immunogens for future vaccines.

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TLR2-mediated infection in classical monocytes drives inflammatory response to dengue virus

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Abstract— Systemic inflammation, which leads to the disruption of microvascular endothelium integrity, is a crucial factor in the development of a severe dengue virus (DENV) infection. In our previous research, we discovered that Toll-like receptor 2 (TLR2) on monocytes as a key sensor of DENV infection in peripheral blood mononuclear cells. We found that sustained high expression of TLR2 on classical monocytes in acutely infected patients is associated with disease progression. In this study, we investigated the mechanism behind TLR2 axis activation during DENV infection in purified blood monocytes by antagonizing TLR2, its coreceptors and other intracellular pattern-recognition receptors known to sense DENV. Our data identified TLR2, CD14, CD36 and TLR3 as the primary contributors to the immune responses induced by DENV-2 infection. TLR2 engagement facilitated viral entry and, in conjunction with CD14 and CD36, enabled efficient virus replication in classical monocytes, but not in intermediate and nonclassical monocytes. Blocking TLR2-mediated infection prevented TLR3 activation and the release of NF-κB-dependent inflammatory and antiviral responses, indicating the sensing of viral replication intermediate following TLR2-mediated entry. Furthermore, inhibiting TLR2 axis-mediated responses in monocytes through immunological or pharmacological means prevented the loss of endothelial cells integrity. Overall, our study provides novel and significant insights into the connection between TLR2 and the function of classical monocytes in DENV pathogenesis.

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Unraveling The Crucial Role Of The CCR2|CCL2 Axis In Mayaro Virus-Induced Bone Pathology

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***Abstract*—** Mayaro virus (MAYV) is an emerging arbovirus belonging to the Togaviridae family and Alphavirus genus. MAYV infection leads to an acute febrile illness accompanied by persistent polyarthralgia and myalgia. Understanding the mechanisms involved in the development of alphavirus-induced arthritis is of utmost importance for the development of targeted and effective therapeutic interventions. In this study, we investigated the role of the CCR2/CCL2 axis in the pathogenesis of MAYV infection. To accomplish this, we infected hind paws of WT C57BL6j mice and CCR2-/- mice with MAYV and evaluated the progression of the disease. MAYV infection induced an acute inflammatory disease in WT mice, characterized by an upregulation of inflammatory mediators including IL-6, TNF, CCL2, and CCL5. Elevated levels of CCL2, both locally and systemically, resulted in significant recruitment of CCR2+ macrophages and orchestrated a cellular response mediated by these cells. In contrast, CCR2-/- mice exhibited increased levels of CXCL-1, accompanied by a shift from macrophage infiltration to neutrophil recruitment. Furthermore, the absence of the CCR2 receptor provided protection against MAYV-induced bone loss. Silencing the expression of the chemokine CCL2 in vivo using siRNA led to improved clinical outcomes, including reduced hypernociception in animals, accompanied by a significant decrease in systemic CCL2 production and expression of ccl2, ccl5, and Tnf in target tissues such as the paw. Cell culture data further supported the mechanism underlying MAYV-induced bone pathology, demonstrating that infection promoted a pro-osteoclastogenic microenvironment mediated by IL-6, TNF, and CCL2. Migration of osteoclast precursors was found to be dependent on the CCR2/CCL2 axis, and MAYV-induced bone resorption was mediated by osteoclast activation rather than impaired osteoblast function. These findings significantly contribute to our understanding of the pathophysiology of MAYV infection and hold promise for the development of future targeted therapies.

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A field analysis of the site selection behavior for oviposition of Female Aquatic Mosquitoes (Diptera: Culicidae)

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***Abstract*—** Vector control targets the mosquito's capacity to spread the infection in each stage of the mosquito's life cycle. Common strategies used in the field, such as insecticides and traps affect directly the adult mosquito. Other techniques like ovitraps and larval source management focus on the oviposition stage of female aquatic mosquitoes. However, the oviposition site selection behavior needs a better understanding to develop effective national vector-control programs. The mosquitoes (Diptera: Culicidae) Aedes, Anopheles, and Culex can transmit diseases that are a public health burden in the health system of Peru. Temperature increase, droughts, and urbanization affect mosquito infection spread and life cycle, leading to urban outbreaks. We need to understand whether the optical sensitivity or chemical cues are relevant for the mosquitoes to lay their eggs on specific water bodies and how their interactions within the mosquito species may affect the oviposition preference. For this study, we sampled 115 breeding sites across Lima, Peru, between 2020 and 2021. We used an in vivo and in vitro quantitative analysis of the physical-chemical parameters of water samples from breeding sites. We used a camera with a lens with multiple wavelength polarized filters in the field to recreate the mosquito vision. We run a statistical analysis for the water physical-chemical parameters data and two types of polarized light patterns. Our findings align with recent studies that suggest the spread of mosquitoes in new areas and the survival of larvae in polluted breeding sites. According to the results, some parameters, such as total dissolved solids, pH, alkalinity, phosphate, and nitrate, significantly impacted larval abundance. While there was not enough evidence to imply the positive role of the polarization effect on oviposition site selection, larval density was significant in habits with a circularly polarized light pattern. Likewise, pH correlated positively with the light pattern captured by the circularly polarized lens filter. These findings suggest that these mosquitoes could have circularly polarized sensitivity and constrained polarotaxis that could vary

within the species, which could be a critical factor in the mosquito egg-laying stage and control strategies.

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Análisis Comparativo De La Morfometría Alar De Mosquitos (diptera:culicidae) Entre Las 2 Vertientes De Los Andes Del Perú, En La Gradiente Altitudinal

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***Abstract*—** En Perú, la información disponible sobre características morfológicas de vectores es limitada y no hay estudios de variaciones fenotípicas según distribuciones geográficas. La morfometría geométrica es una técnica que permite comparar puntos de referencia o landmarks (LM) en estructuras fijas entre organismos; sin embargo, un gran problema en estudios tradicionales taxonómicos es la preservación total del individuo y el entrenamiento para reconocer estructuras, por lo que usar las alas es una ventaja al ser una estructura solamente bidimensional, tener puntos fáciles de distinguir al usar las intersecciones naturales de las venaciones y ser estructuras resistentes. El primer objetivo de este estudio fue evaluar la utilidad de la morfometría alar para distinguir entre especies de Culícidos. El segundo objetivo fue visibilizar las variables fenotípicas intraespecíficas dependiendo de los lugares, ecosistemas y altitudes. En este estudio 3 géneros de culícidos se han evaluado. Anopheles sp, Aedes sp y Culex sp. Todos conocidos por ser importantes transmisores de enfermedades tropicales como el dengue, la fiebre amarilla y la malaria. Se han evaluado 4 departamentos: Piura, Lima, Cuzco y Madre de Dios. Los cuales presentan diferentes ecosistemas (desierto costero, zonas urbanas, bosque nublado y amazónica tropical), que van por ambas vertientes de los Andes y logrando una gradiente de elevación (0 – 3000 msnm). Se analizó el ala derecha de los individuos, basándose en 19 puntos de referencia. Se utilizó un análisis de componentes principales (PCA) para explicar la varianza total. En general, se observó una diferenciación entre forma y tamaño dependiendo de las especies, lo que permite afirmar el uso de esta técnica para identificación taxonómica. Formándose un clúster por especie identificada. Pero, las mismas características evaluadas dentro de la especie dependiendo de variables geográficas no presentan una variación fuertemente respaldada. Como confirmación se realizó un análisis UPGMA tomando las mismas variables, lo que permitió visualizar la separación entre especies, pero no entre localidades. Estos resultados se podrían comparar en eficacia con identificaciones moleculares o servir para la creación de algoritmos de aprendizaje automático que permitan la identificación automática de especies a futuro.

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Country-wide scope of pyrethroid resistance in Aedes aegypti in Peru and implications for resistance management

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***Abstract*—** Aedes aegypti reinvaded Peru in 1984, with initial detection in Amazonian states near Brasil and recent establishment in southern states near Chile. More than 240,000 cases of dengue have been reported in 2017-2022. Collaborative vector surveillance work with local Ministry of Health and Military units allowed collection of 2,555 Ae. aegypti from 60 locations in coastal and jungle states from 2017 to 2023. Specimens were tested for knockdown resistance (kdr) mutations V1016I and F1534C, and a sub-set for phenotypic resistance to pyrethroids. Mosquitoes from northern coastal and four northern jungle states were fixed or nearly fixed (≥ 95%) for 1534CC; 1534FF mosquitoes (29-100%) were found in southern and one northern jungle states. Moderate to high levels of 1016II were found in northern coastal states (20-86%); low to high levels of 1016II were found in northern jungle states (0-89%); and low to moderate levels of 1016II were found in southern states (0-24%). Eight genotypes were observed (IICC, IIFC, VICC, VIFC, VIFF, VVCC, VVFC, VVFF) with resistant genotypes (VICC, IICC) indicative of higher resistance (20X-60X), recorded in northern states, and less resistant genotypes (VVCC, VVFC, VVFF) indicative of lower resistance (4X) in southern

states. A strong positive correlation between the IICC genotype percentage and both toxicologic performance (LD50) and resistance assessed by CDC bottle assays was found in northern jungle mosquitos. Our results showed that kdr mutations are broadly distributed in Peruvian Ae. aegypti, yet with considerable variation even within close geographic proximity. A higher proportion of more resistant genotypes were found in northern states, which have been subject to many years of control efforts than in southern regions, more recently colonized locations or locations with little vector control activity. This may explain decreased effectiveness of pyrethroid insecticides for Ae. aegypti control in northern Peru. Furthermore, a strong correlation between the kdr genotype and phenotypic resistance to pyrethroids validated the use of these genetic markers as a rapid insecticide resistance screening tool. This study provides insights into pyrethroid resistance mechanisms and may guide mosquito control operations in Peru.

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Detection Of Dengue And Chikungunya Viruses In Mosquitoes From Paraguay

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Abstract— Arboviruses are viruses transmitted by arthropods, predominantly mosquitoes. In Paraguay, dengue (DENV), Zika (ZIKV), yellow fever, and chikungunya (CHIKV) viruses have been reported. In terms of cases recorded, DENV and CHIKV are those with the greatest impact on public health in our country. Moreover, studies that look for uncommon arboviruses or perform arboviral detection in mosquitoes from Paraguay are scarce. Therefore, the aim of this work was to search for arboviruses of medical importance in mosquitoes collected in Paraguay. A total of 3847 mosquitoes from the Capital and Central, Cordillera, Caaguazú, Alto Paraná, Itapúa and Boquerón Departments were collected (2018-2023). Mosquitoes were classified according to species, date and location of collection, and were macerated into 439 mosquito pools containing 1 to 35 mosquitoes per pool. 25 species of mosquitoes were identified; the most frequent was Culex quinquefasciatus (2008 mosquitoes, 52%), followed by Aedes (Ochlerotatus) scapularis (605 mosquitoes, 15.7%) and Culex coronator complex (545 mosquitoes, 14.2%). Aedes aegypti was less frequent (130 mosquitoes, 3.4%). To date, 298 selected pools (3001 mosquitoes) of different species (prioritizing Culex species) were qRT-PCR analyzed for West Nile, Saint Louis encephalitis and Venezuelan equine encephalitis viruses. None of these viruses were detected. A qRT-PCR multiplex was performed to all 67 pools of Aedes species (134 mosquitoes) for DENV, ZIKV and CHIKV detection. We found 3 positive mosquito pools for CHIKV (11 mosquitoes) and 3 for DENV (12 mosquitoes). Successful amplicon primer tilling nanopore sequencing was performed on these positive samples, obtaining nucleotide coverage of 94.2%, 90%, 89% for CHIKV and 91.9%, 87.2%, 93.8% for DENV. Consensus sequences were assigned by the Genome Detective DENV/CHIKV Typing tools as: 1 DENV-4 Genotype II, collected in 2020 from Cordillera Department, during the largest outbreak of DENV-4; 2 DENV-2 Genotype II – Cosmopolitan, collected in 2023 from Capital and Boquerón Department and 3 CHIKV East-Central-South-African (ECSA) lineage, all collected in 2023 from Capital and Central Department, during the first country-wide outbreak. To the best of our knowledge this is the first report of DENV and CHIKV detection and near-full genome sequences obtained from naturally infected Aedes aegypti mosquitoes in Paraguay.

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Distribución espacial e influencia climática en áreas infestadas de Aedes aegypti (Diptera: Culicidae) en la transmisión de dengue en Antioquia, Colombia

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Abstract— **Antecedentes.** El Dengue es un problema grave de salud pública en la región de Urabá, Antioquia, Colombia. El aumento del número de casos se relaciona con la población de vectores, por lo cual dos áreas de transmisión activa del virus del dengue en Urabá, Antioquia, Colombia fueron vigiladas mediante indicadores entomológicos tradicionales y densidad del vector. El objetivo fue evaluar la distribución espacial de Ae. aegypti y valorar la influencia de las condiciones climáticas en áreas vulnerables de los municipios de Apartadó y Turbo, Antioquia, en 2021-2022. **Métodos**. Estudio transversal con aplicación de ocho encuestas entomológicas trimestrales, según muestreo aleatorio, en 1.518 viviendas de tres barrios de los municipios de Apartadó y Turbo entre 2021 y 2022. Las viviendas fueron inspeccionadas para observar la presencia de formas inmaduras del vector en los depósitos de agua y se capturaron los mosquitos adultos mediante una aspiradora prokopack; luego se calcularon los índices de vivienda, depósitos, Breteau y los índices de densidad de mosquitos Ae. aegypti por vivienda y por habitante. Cada vivienda fue georreferenciada para realizar los análisis espaciales mediante la densidad de Kernel y se estimó la correlación con las variables climáticas de humedad relativa, temperatura y precipitación. **Resultados**. Se recolectaron 20.050 especímenes, 5.408 de los cuales eran Ae. aegypti, siendo 2.564 hembras y 2.844 machos. Los índices globales de vivienda, depósitos y Breteau fueron 48,9% (IC95%: 39,2-58,6 ± 24,2), 29,5% (IC 95%: 25,1-34,0 ± 11,1) y 70,2% (IC 95%: 53,6-86,9 ± 41,6), respectivamente. La densidad media de hembras adultas de Ae. aegypti fue de 1,47 (IC95%: 0,94-2,0 ± 1,32) hembras/vivienda y 0,51(IC95%: 0,39-0,63 ± 0,29) hembras/habitante. Se presentó correlación significativa de las variables climáticas en los barrios de Turbo, Antioquia, con las densidades de hembras de Ae. aegypti por habitante en las viviendas inspeccionadas. **Conclusiones.** Los índices entomológicos y la densidad de hembras en las viviendas, junto con el análisis de factores climáticos, pueden ser buenas estrategias para el monitoreo de áreas vulnerables a la transmisión del virus del dengue en regiones endémicas de Antioquia, Colombia.

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Effect of Bisphenol a (BPA) Exposure on Development, Immune Response, and Vector Competence to Dengue Virus in Aedes Aegypti Mosquitoes

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Abstract— The effects of BPA in other organisms have been associated with pathologies of degenerative and infectious diseases, however the repercussions and effects that these derivatives have on vector borne diseases have not yet been described. Because BPA is considered an compound endocrine disruptor, it is capable of modulating the development process in organisms that are constantly exposed to it. The early life stages (larvae and pupae) of the Aedes aegypti mosquitoes may be exposed to BPA, thereby causing changes in development, fecundity and sex ratio in this mosquito. Preliminary data obtained by our group have allowed us to evaluate the concentration of BPA in aquatic niches where we have found populations of Ae. aegypti, which showed that in these sites, the larvae and pupae of these insects are in contact with BPA. Therefore, we also propose to evaluate the biological parameters: 1) Development time between stage; 2) Percentage of pupation; 3) of emergence; 4) of hatching; 5) Survival; 6) Oviposition; 7) Sex ratio and 8) Weight, in a reference mosquito (Rockefeller strain) and in a field mosquito (Cuernavaca strain). The purpose of observing if the effects of exposure to BPA are the reflection of populations conditioned or adapted to continuous exposure to BPA, or if BPA per se is a causal agent and responsible for changes in the biological parameters. We evaluated the effects of BPA on the antiviral immune response of the Ae. aegypti post-exposition and infected with dengue virus (DV). For that, we analysed the expression of transcripts of the main molecules of the antiviral immune response: 1) Toll (Relish 1, Cactus, Defensin, Cecropin); 2) Jak-STAT (Dome, Hop, Stat) and 3) siRNA signaling pathway (Ago2, Dcr2, R2D2), at 0, 3 and 7 days post-feeding and post-infection. The last but most important will be to evaluate if the effect of BPA has repercussions on the susceptibility of these vector to DV infection. For this, the viral load will be obtained in different specific tissues of the mosquito (intestine, carcass and salivary glands) by qPCR, obtaining the number of viral copies per strain to be evaluated.

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Effectiveness of CYTROL 10.8 ULV (Technical Cypermethrin 10.8% P/V and Piperonyl Butoxide 4.5% P/V) In Aedes(S) aegypti (Diptera: Culicidae), Control in Cárdenas, Matanzas Cuba. 2023

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***Abstract*— Introduction.** Matanzas usually carries out intensive control of Aedes aegypti using pyrethroids as adulticides, however, the Cárdenas municipality presents health areas with high House and Breatau Indices (CI) (IB). **Objetive.** Cytrol 10, 8 ULV is a novel formulation for the control of mosquitoes with adulticide treatment, which is being studied for the first time in Cuba. Objective.Evaluate the efficacy of CYTROL 10.8 ULV (Technical Cypermethrin 10.8% P/V and Piperonyl Butoxide 4.5% P/V) is a mixture of two molecules, a potentiated pyrethroid with a synergistic molecule in this case piperonyl butoxide, it provides to the formulation a great effectiveness when applied in intra-home thermonebulization. The treatment was carried out in homes of the popular council of Varadero in the Ramón Martínez health area in Cárdenas municipality. **Materials and Methods.** The state of susceptibility to cypermethrin was evaluated using the methodology of the impregnated bottles. Subsequently, a block with 130 homes was selected, which in one week underwent three treatments according to what was established for intra-domiciliary spatial treatments and a control. The efficacy was determined by the house and Breatau indices and the results from weekly surveys of ovitraps and capture of adult mosquitoes by BG Sentinel traps **Results**. Four months after treatment, the infestation indices remained at zero in the treated block compared to the control. **Conclusion.** The intra-home thermonebulization treatment with this formulation produced a positive impact on the selected block, constituting a good option for reducing Ae. aegypti infestation indices.

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Entomological risk stratification as an essential tool for the surveillance of Aedes aegypti (Diptera:Culicidae) in the current Cuban context

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***Abstract*— Introduction.** Historically, but also today, the operating costs and investment expenses for the maintenance of Aedes aegypti control programs are high, due to the use of - often insecticide-based - control methods with high coverage. To lower costs, surveillance and control of this species could be diversified and guided by an entomo-epidemiological risk stratification. **Objective.** To validate with scientific evidence the entomological risk stratification together with a modified sample size as essential tool for the surveillance of Ae. aegypti. **Materials and methods.** The study was carried out in blocks identified with high entomological risk belonging to four municipalities in Havana province, Cuba. In each municipality, the surveillance team visited during one week 33% of the total number of houses in the selected blocks from March 5 till April 2, 2019, which was later compared and integrated with the values obtained by the 100% sampling of the houses made by the program. Information was obtained about Ae. aegypti pupal productivity. **Results and discussion.** A total of 6 647 containers were inspected from 1599 houses in the four study areas of which only 500 (7.5 %) were positive for Ae. aegypti immature stages. Aedes infestations levels were high with a general Breteau Index of 31.3 a Container Index of 7.5 and a House Index of 18.3. All municipalities showed mainly presence inside the houses. It was shown that Ae. aegypti continues to breed in the same breeding sites, namely water storage and non-destructible artificial containers inside the houses, despite the control exercised for more than 42 years. This was evidenced in the 33% targeted sample, as good as in the 100% coverage surveillance data. These findings provide evidence for addressing the loss of credibility of the routine indicators from the Cuban Ae. aegypti surveillance program based on large samples inspection of 100% of the premises. **Conclusions.** With the incorporation of entomological risk stratification as a tool in the surveillance of Ae. aegypti in the current Cuban context, we will be responding to the call made by health managers to

undertake a thorough review of the vector control national program in the country.

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Entomo-virological Surveillance For Different Arbovirus In Urban And Rural Areas Of Colombia

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Abstract— Arboviruses such as dengue (DENV), Zika (ZIKV), Yellow fever (YFV), and Chikungunya (CHIKV) are responsible for endemic, emerging, and re-emerging diseases, with important impacts on human health, having complex life cycles involving different Culicidae mosquitoes. In Colombia, the main vector of these arboviruses is Aedes aegypti, however, Aedes albopictus was introduced in recent years, expanding in most of the country, and there is also the presence of sylvatic mosquitoes. This study aimed to evaluate arbovirus circulation in mosquitoes collected in urban and rural areas of Colombia. We collected adults and immature stages in urban and rural areas of Cundinamarca, Meta, and El Cauca Department. Ae. aegypti was the most common mosquito in the inspected urban and rural areas, being found up to 2100 masl. In general, in the inspected urban and rural areas, the most common infection in this species was for CHIKV, followed by DENV, and in some cases ZIKV, furthermore, in urban and rural areas of La Macarena (Meta), YFV was detected. Ae. albopictus was found at El Meta and El Cauca departments at urban and rural areas and elevations up to 2100 masl, showing infections mostly for CHIKV. The Urban mosquito Culex quinquefasciatus was found infected with CHIKV, DENV, and ZIK. The sylvatic mosquito Haemagogus janthinomys was found infected with YFV, CHIKV, DENV, and ZIKV, also the mosquito Aedes serratus collected at sylvatic areas was infected with YFV, CHIKV, DENV, and ZIKV. Evidence for arbovirus dissemination and vertical transmission was also detected for some of the analyzed mosquitoes. Overall, these results are showing the complex transmission dynamics of arbovirus in Colombia and the importance of arbovirus surveillance targeting different mosquito species. The risk for arbovirus transmission is expanding in the country to rural areas as well as to highest elevations, probably associated with human interventions and mosquito plasticity.

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Field Assessment of VectoMax-G® Larvicide Application For Controlling Aedes Mosquito Populations Using Ovitraps In The City Of Yaoundé, Cameroon

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Abstract— Aedes-borne diseases such as Dengue, Chikungunya and yellow fever are current public health threats in Cameroon regarding frequent detection of cases due to urbanicity and socio-economic conditions of populations. Given the absence of efficient treatment against these diseases, effective vectors management through larviciding intervention could be a way to limit the spread of these diseases. **Methods.** Preliminary studies were conducted from February 2019 to March 2020 in six clusters of Yaoundé: two urban (Obili, Mvan), two peri-urban (Simbock, Ahala) and two rural settings (Lendom, Elig-essomballa). In each study site, 100 ovitraps were installed around habitations and were monitored every 7 days for 04 weeks to determine dynamics of Aedes populations. The current study was carried out from August 2020 to January 2021 in the same sites to assess larviciding approach in Aedes densities reduction. For this, 100 ovitraps (50 treated with 0.25g of vectomax, 50 untreated) were installed

around habitations and monitored using the same methodology. A general linear model was used to assess if there were significant differences of positivity in both categories of ovitraps and among the four treatment periods. Moreover, an estimation of ovitraps positivity index was determine across ecological zones. **Results**. A total of 6241 were Aedes species were collected compared to the initial Aedes populations densities (15323) reported in these study sites. Aedes albopictus (4442) was the most abundant species collected, followed by Aedes aegypti (741), Aedes simpsoni (697) and Aedes contigus (249). Overall, the highest oviposition activity index (81.5%) was registered in rural environment (Lendom). GLM revealed accentuated difference ($p < 2e-16$; F-ratio = 3) of positive treated-ovitraps among study sites; but there was no difference of positivity rate ($df = 1$; $p = 0.058$) between treated (67.66%; 812) and untreated group (63.91%; 767).

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Geospatial Distribution Of Aedes Aegypti Mosquitoes And Bridge Vectors In Mexico

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Abstract— Aedes aegypti is the principal vector of worldwide epidemics of arboviruses such as dengue, Zika and chikungunya, but there are many others of medical or veterinary importance. Arbovirus emergence in the human population typically results from spillover transmission via bridge vectors, which are competent mosquitoes feeding on both humans and wild animals, which make the link between sylvatic and human transmission cycles. Mexico is an endemic dengue country; the autochthonous transmission occurs in 29 out of 32 states. In the last decade, were reported between 69,910 and 268,458 dengue cases annually, with an economic cost of US$149 million to $257 million per year. Mosquito populations are dynamic, constantly changing over time according to factors that regulate their growth, include climatic change. This study was designed to investigate the geographical distribution of mosquito species in urban, rural, and natural areas in Mexico during 2000 to 2020. We investigated changes in mosquito community composition, characterized mosquito habitat and analyzed associations between key vector species and environmental variables like temperature. The information was collected from the Surveillance National System of Health Secretary (SINAVE), Vector Control Program (CENAPRECE), CONABIO, personnel collection, and published data. Approximately, 130,000 registers belonging to 280 different native mosquito species of ten genera were analyzed. The public health importance and ecology of mosquitoes species is also discussed.

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Identification of metabolomic profile of the mosquitoes diet by UHPLC-MS technique in the river basins in Lima - Peru

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Abstract— Malaria is an infectious disease transmitted by mosquito vectors of the family Culicidae of the genus Anopheles. The mosquitoes need a source of nutrients such as carbohydrates, lipids, amino acids to develop physiological activities like parity, longevity, survival and vectorial capacity. The mosquito's crop is part of the digestive system, which plays an important role as a reserve prior to the digestion of consumed sugars. In Peru, the research about the nectarivores diet is limited and does not exist data about the metabolomic profile of the Anophelines species by Ultra High Performance Liquid Chromatography Mass Spectrometry which can separate chemical compounds and identify metabolites with high sensitivity, resolution and accuracy. The aim of the study was to identify and create a metabolomic database of the mosquitoes Anopheles species' diet. The samples were mosquitoes' crop of An. pseudopunctipennis collected in the field, and crops of the bioassay's mosquitoes exposed in a cage to five plants which are Bidens alba, Ludwigia octovalvis, Ludwigia peploides, Bougainvillea spectabilis and Lonicera japónica collected in the field too. Other samples used to compare metabolomic profile and quality control were

methanolic extracts of the five plants and internal standards of sugars; the metabolomic profile of the mosquito's crops were analyzed by UHPLC MS technique. The crops were analyzed by the UHPLC-MS. The results shown a relationship between the compound of plants and compounds found in the mosquito´s crop. It was identified 7 sugars, 10 amino acids, 6 pesticides, 11 lipids, and other natural plant compounds. The results will contribute for future research like feeding preference, design of more efficient traps, study of metabolism pathways and resistance to pesticides.

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Insecticide susceptibility status in Aedes aegypti larval populations from the western, central and eastern regions of Cuba

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***Abstract*— Introduction.** Chemical control of Aedes aegypti remains the principal viable alternative to control dengue, Zika, and chikungunya outbreaks. The insecticides temephos, malathion and cypermethrin have been the most used by the National Aedes aegypti Control Program in Cuba. Its intensive use could generate the occurrence of resistance in populations of Ae. aegypti in the country. **Objective.** To determine the level of susceptibility and/or resistance to the insecticides temephos, malathion and cypermethrin in six Ae. aegypti populations from the western, central and eastern regions of Cuba. **Methods.** The study was realized in six of the fifteen Cuban provinces, namely Pinar del Río and Matanzas (western region); Camaguey and Villa Clara (central region) Santiago de Cuba and Guantanamo (eastern region). The biological material was collected from eggs from ovitraps placed by personnel from the National Control Program for Ae. aegypti and Ae. albopictus during their routine activities following the established sampling protocols during January - June 2022. Two organophosphates (temephos and malathion) and one pyrethroid (cypermethrin) was tested on larvae, using the methodology described by the World Health Organization. **Results.** All populations of Ae. aegypti evaluated showed high resistance to temephos with RF50 values between 19.0 and 68.75. The RF50 values for malathion ranged from 1.38 to 34.51 while the values for cypermethrin ranged from 3.84 to 30. Guantánamo province was found to be susceptible to malathion with an RF50 of 1.38 and to cypermethrin with an RF50 of 3.84. The province of Matanzas expressed moderate resistance to cypermethrin (RF50 = 7.7). **Conclusion.** This study corroborates that the implementation of resistance management in a general way is important to be carried out through the National Program for the Control of Ae. aegypti in Cuba, promoting insecticide rotation policies, to reverse the evolution of resistance or eliminating selection pressure long enough to allow mosquito populations to recover susceptibility. Ideally these strategies could be carried out in a preventive way to preserve the efficacy of the insecticides.

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Larval ecology and host-seeking and biting behavior of Aedes sp in a context of dengue re-emergence in the city of Ouagadougou

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***Abstract*—** Dengue has re-emerged in recent years in Burkina Faso. In the absence of an effective vaccine and treatment, control of the Aedes vector remains the key strategy for responding to epidemics and sustainably controlling disease transmission. The objective of the present study was to investigate the larval ecology and host-seeking and biting behaviour of Aedes sp in the city of Ouagadougou. A total of 60 concessions in the districts of Bogodogo and Nongremasson were visited per month during the period from August to November 2021 for larval surveys and adult collection. All water containers were characterised, inspected and immature stages collected. Adults were captured inside and outside the houses using battery-powered hoovers and double-net human traps. The origin of the blood meal of Ae. aegypti was determined using multiplex PCR. Aedes aegypti was the predominant species and colonised various types of water containers, however abandoned containers and tyres were the most productive sites (df=7; $p<0.0001$). The main entomological risk indices Breteau index, container

index and house index were above the WHO threshold values and varied by month (df=4; p<0.0001). Aedes aegypti was more active outdoors than indoors with humans as the preferred host (83.7%). Host-seeking and biting activity of Aedes was most intense from 6am to 8am and 4pm to 7pm. This activity seemed to be positively associated with high humidity. These results confirm the high risk of arbovirus transmission in Ouagadougou and provide basic information on the bioecology of Ae. aegypti that can contribute to the implementation of a sustainable arbovirus control strategy in Burkina Faso.

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Populations Of Aedes Aegypti Across A Remote Region Of The Peruvian Amazon

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***Abstract*— Historically, dengue virus has been a primarily urban issue in the Peruvian Amazon. In recent years, this threat profile has been shifting, with an increasing number of dengue outbreaks occurring in rural communities. Previous work has shown that the dengue vector, Aedes aegypti, is transported by boat between Iquitos and neighboring riverine communities. However, there has been no characterization of longer distance transport of Ae. aegypti along these fluvial transit routes and little information about the presence of Ae. aegypti populations in more remote communities in the region. Here, we describe the results of the most extensive rural survey of Ae. aegypti in the Peruvian Amazon, including over thirty communities from the Ucayali, Puinahua, Marañon and Amazon Rivers. We found that Ae. aegypti is widespread across the region sampled, including in communities on the Puinahua River, which had no previous reports of Ae. aegypti or local dengue cases. Substantial differences exist between communities in the patterns of Ae. aegypti presence, the household positivity rate, and adult indices. Our results help to quantify the extent of the newly expanding health threat posed by dengue in rural areas in the Peruvian Amazon and can help to guide future deployment of resources, such as diagnostic tests and vector control.

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Spatial And Temporal Analysis On The Impact Of Ultra-Low Volume Indoor Insecticide Spraying On Aedes Aegypti Household Density

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***Abstract*— Introduction.** Aedes aegypti is the primary vector for several arboviruses that can cause widespread epidemics, such as dengue and Zika. This mosquito feeds primarily and frequently on humans, and is well adapted to the urban environment, where it has successfully established itself in many areas throughout the tropics and subtropics. In this study, we characterize the impact of pyrethroid ULV indoor spraying on the Ae. aegypti population within households in relation to the spray events occurring in the proximity of that household in time and space. Improved understanding of the duration and the distance of the effect of a spray intervention on Ae.aegypti populations can better inform planning the coverage and frequency of sprays needed during vector control interventions, as well as modeling efforts that contrast different vector control strategies. **Methods.** This project uses data from two large-scale experiments that involved six cycles of indoor pyrethroid spray applications in the Amazonian city of Iquitos, Peru (Gunning et al. 2018). We developed spatial multi-level models to disentangle the reduction in Ae. aegypti numbers that resulted from (1) recent ULV treatment of a specific household and (2) ULV treatment of households in the neighboring area. We compared the fit of models including different weighting schemes for the spray effect, based on different temporal and spatial decay functions to understand lagged ULV effects. **Results and Discussion.** Our results suggest that the effect of a spray event in a house wanes over time following a gaussian decay. In addition, we found that the reduction of Ae.aegypti density is mainly determined by time since the most recent spray event, with no significant explanatory value added by the cumulative effect of multiple past sprays. Our results also indicate that the reduction of the Ae.aegypti in a house is due to spray events occurring in that same house, with no significant effect of sprays occurring in adjacent houses or the neighboring area. In summary, the reduction of Ae. aegypti in a house is determined mainly by the number of days since the last spray intervention in that same house.

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Vector Competence In Aedes Aegypti (linnaeus, 1762) And Aedes Albopictus (skuse, 1894) From Colombia, Infected To CHIKV And ZIKV

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***Abstract*— Introduction.** The vectorial dynamics of Aedes aegypti and Aedes albopictus against the emergence of Chikungunya (CHIKV) and Zika (ZIKV) viruses continues to be of interest to elucidate aspects of their co-circulation in America. This study analyzed the vector competence in Aedes aegypti and Aedes albopictus from Colombia against infection with CHIKV and ZIKV viruses. **Methods.** Females of the two species were exposed to oral challenges through Hemotek, with blood mixtures and viral inoculum of CHIKV (5×10^5 genomic copies/mL) and ZIKV (1×10^7 genomic copies/mL). Infection in bodies (thorax/abodomen), dissemination in heads (with salivary glands) and potential transmission in saliva (salivary extract in SFB) were evaluated; This was done at 3, 7, 14 and 21 days after exposed (dpe). In pools of four samples, viral RNA extraction and RT-qPCR were performed by Onestep in bodies, while heads and saliva were incubated in cell culture and viral RNA was subsequently obtained from supernatants, followed by retrotranscription and qPCR. **Results.** Aedes aegypti and Aedes albopictus were susceptible to CHIKV and ZIKV infection from 3dpe to 21dpe. Additionally, in Aedes aegypti CHIKV showed dissemination from 3dpe that increased up to 14dpe, while Aedes albopictus spread from 3dpe but with a tendency to decrease genomic copies between 7dpe and 21dpe. Although ZIKV evidenced dissemination from 3dpe, with a gradual increase between 7dpe and 21dpe in Aedes aegypti. In comparison, Aedes albopictus ZIKV spread between 3dpe and 14dpe, increasing at 21dpe. Potential CHIKV transmission was detected from 3dpe, with a similar record at 14 and 21dpe, the report being lower at 7dpe in Aedes aegypti, while in Aedes albopictus it was similar between 3 and 21dpe. Finally, the potential transmission of ZIKV in Aedes aegypti was detected between 7 and 21dpe and in Aedes albopictus from 3dpe to 21dpe, although without registering at 7dpe. **Conclusions** Aedes aegypti and Aedes albopictus from northeastern Colombia are competent to transmit CHIKV and ZIKV 3 days after feeding viremic blood, suggesting the importance of Aedes albopictus as a transmitter in endemic areas of South America.

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Zika Virus Infestation In Mosquito Vectors: Preliminary Results From Viral Surveillance In Goiânia, Goiás - Midwest Brasil

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***Abstract*—** Dengue, Zika, and chikungunya are endemic arboviruses in Brasil and have significant relevance for human infection as they cause epidemics with a high incidence of cases, especially in the state of Goiás. Given the concurrent circulation of these arboviruses and the extensive infestation and spread of mosquito vectors in the region, the importance of viral surveillance in mosquitoes becomes evident in the context of vector control. This study aimed to assess the presence of dengue viruses (DENV 1-4), Zika (ZIKV), and chikungunya virus (CHIKV) in circulating vectors in the municipality of Goiânia-Goiás, located in the Central-West of Brasil. The Zoonosis department of the Municipal Health Secretariat of Goiânia captured mosquitos based on criteria such as neighborhoods with a higher number of suspected cases and environmental characteristics considered favorable for vector proliferation. Mosquitoes were captured during the interepidemic period from June to December 2022 and subsequently sent to the laboratories of the Federal University of Goiás (BIOTEC/FF and LPI/IPTSP), where they were sorted into pools based on sex and species. Viral RNA was extracted from samples of salivary glands and midguts, and then tested in reverse transcription real-

time polymerase chain reaction (RT-qPCR). A total of 402 specimens were processed and divided into 71 pools (60 ♀/11 ♂) comprising mosquitoes of the species Aedes aegypti, Aedes albopictus, Aedes fluviatilis, Aedes scapularis, and Culex sp. Nine pools (12.6%) tested positive for one or more arboviruses, with 66.7% of positive pools comprising non-engorged females and 33.3%, engorged females. Among the nine positive pools, 55.6% were from the species A. aegypti, 33.3% Culex sp., and 11.1% A. scapularis. ZIKV positivity was detected in 88.9% of the pools, identified in all studied species, while DENV-2 was detected in 11.1% of the A. aegypti pools. The identification of DENV confirms the circulation of this serotype in the region. The results revealed an increase in viral positivity and raise concerns regarding ZIKV infestation in Aedes spp. mosquitoes within an epidemiological scenario of decreasing probable cases since 2017, subsequent to the national emergency and epidemic. Therefore, viral surveillance in vectors can contribute to the planning of epidemiological surveillance measures.

Epidemiology — Genomics — Phylogenetics — Modelling — Burden of Disease

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Chikungunya IgG Antibodies Persistence 7 Years Following The Acute Infection In A Cohort In Piedecuesta, Colombia

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***Abstract*—** The Chikungunya virus (CHIKV) was introduced to the Americas in 2014; since then, CHIKV has caused epidemics in the region. Athough acute illness is self-limiting, rheumatism can persist for years. We examine the persistence of CHIKV IgG antibodies in a febrile cohort followed 7 years after the 2014-2015 outbreak in Piedecuesta, Colombia. We evaluated 79 febrile adult patients (median age: 36 years, IQR:21 years; women: 59.5%) whose CHIKV infection was confirmed during the outbreak (2014-2015). CHIKV infection was confirmed by RT-qPCR or IgG/IgM ELISA. In 2022, patients were examined by physicians who had been trained by a rheumatologist. Subsequently, those who reported a non-trauma-related anomaly in GALS musculoskeletal screening were evaluated by a rheumatologist. IgG antibodies were measured in convalescent phase (16.3±3.2 days) and late phase (7.3±0.2 years) samples using IgG ELISA Novalisa®. We evaluated the association of IgG with the clinical outcome 7 years post-CHIK infection using logistic regression. Following clinical evaluation, 11 cases (13.9%) were classified as post-CHIK chronic inflammatory rheumatism (pCHIK-CIR), 32(40.5%) as Non-inflammatory pain likely degenerative (NIP-LD), and 36(45.6%) as recovered. We observed a positive result of IgG ELISA in 30.4% (n=24/79) of the late samples evaluated. This proportion is maintained in the subgroup of patients who have at least one positive IgG ELISA result during the febrile phase (29.7%, n=19/64). The IgG NTU units were also compared according to clinical classification; however, there were no significant differences between the evaluated groups [7 (IQR:7), 8 (IQR:4) and 8(IQR:5) for pCHIK-CIR, NIP-LD and recovered respectively; p=0.347]. Furthermore, there was no association between the IgG NTU units and the clinical stage in the chronic phase adjusted by sex and age (OR = 1.00, 95% CI 0.78-1.29). In chikungunya disease, the humoral immune response, specifically IgG antibodies, tends to disappear over time, regardless of persistent rheumatic symptoms. This should be considered in future research on estimating seroprevalence or its usefulness as a biomarker of immunological protection. These results correspond to a preliminary analysis.

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Co-Circulation Of Serotypes Associated With The Cumulative Incidence Of Severe Dengue And Dengue With Warning Signs In Peru

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Abstract— **Introduction**. Some known risk factors for severe dengue are secondary infection, differences in serotype virulence, and individual patient characteristics. However, the factors that increase the risk of severe dengue at the population level are still unknown. Therefore, secondary population data from the epidemiological surveillance system was used to identify factors associated with the cumulative incidence of severe dengue and/or dengue with warning signs (DS-DCSA). **Methodology**. Ecological and longitudinal study of secondary database analysis. For the analysis, information from the regions of Peru on dengue cases according to diagnosis, identification of serotype types in samples analyzed by the Ministry of Health, precipitation anomaly, poverty index, and total number of primary care facilities was considered. A mixed negative binomial regression model for repeated measures over time was used to estimate crude and adjusted incidence ratios (IRRa) between the cumulative incidence of DS-DCSA and its potential associated factors. **Results.** A total of 13 years of data from 2007 to 2019 were analyzed in 19 regions of Peru, making a total of 247 region-years. Data from nearly 40,000 analyzed samples for serotype identification were analyzed, of which 58.5% corresponded to serotype denv-2, 27.0% to denv-1, 10.2% to denv-3 and only 4.3% to denv-4. Regarding the analyzed dengue cases, 86.4% corresponded to dengue without warning signs, 13.2% to dengue with warning signs and 0.5% to severe dengue. The 4 regions with the highest incidences of DS-DCSA and with the highest identification of serotype types were Loreto, Piura, Madre de Dios, Ucayali. The variables associated with the incidence of DS-DCSA were the circulation of serotype 2 or its combination (IRRa 11.73, CI 5.53-24.84) and multiple circulation of 3 serotypes (IRRa 2.43, CI 1.09-5.39). **Conclusions**. The circulation of denv-2 or its combination, and triple circulation of serotypes in the same period were associated with higher cumulative incidence of DS-DCSA.

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Dengue Disease And Economic Burden In Mexico: An Observational, Retrospective, Database Study

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Abstract— **Background.** Dengue is the most prevalent arboviral diseases globally. The aim of this study was to analyze the epidemic and economic burden of dengue in Mexico from January 2010 to December 2020. **Materials and Methods.** We employed a nationwide public and private healthcare databases (CONAPO, SINAVE /DGE), to extract the probable cases, the laboratory-confirmation and the outpatient, hospitalization and mortality rates, to estimate disease burden. To estimate healthcare resource utilization, an expert Delphi panel was developed, which consisted of 14 specialists in infectology and pediatricians from the public (8 specialists) and private sectors (6 specialists) of healthcare attention in Mexico. **Results**. From 2010 to 2020, the SINAVE/DGE notified 1,620,872 probable cases of dengue, 336,991 laboratory-confirmed cases, 226,554 outpatient cases, 110,437 hospitalized cases and 1,385 deaths. The most affected age-group by dengue infection and severity was the population under 30 years of age. The case fatality rate increased from 0.72 in 2010 to 2.6 in 2020 and the comorbidities associated with hospitalization and death risk are peptic ulcer, liver cirrhosis, diabetes, kidney diseases and hypertension. In 2018 medical care costs for dengue patients were estimated in $23,713,589 USD (pre-outbreak period), $111,851,376 USD (outbreak period) in 2019, and $39,780,809 USD in 2020 (post-outbreak period). **Conclusions.** The cost of Dengue in Mexico is high during epidemic years; in consequence, there is an urgent need for robust methods for treatment and prevention. Health economic analysis could be beneficial to estimate the impact of vaccination against Dengue in endemic areas. Original abstract previously presented at 19 Congreso de Investigación en Salud Pública (19 CONGISP) Mar 16, 2023, Morelos, Mexico.

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Dengue With And Without Complications During 2018 To 2021 In Cusco, Peru

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***Abstract*— Introduction.** Dengue affects more than 120 countries with 100 million infections per year. The SARS-CoV-2 pandemic limited health services provision, reducing focus on other diseases such as dengue. Cusco is located in southern Peru with 1.2 million population, and has highland and jungle areas. The notification rate of dengue in Cusco in 2018 was 1.9 x 10,000 cases. **Methodology**. We report the frequency of dengue notifications pre-pandemic (January 1, 2018 to March 12, 2020) and during the pandemic (March 13, 2020 to December 31, 2021) in Cusco, Peru and we compare the characteristics of patients with dengue with complications and dengue without complications. Dengue with complications was defined as the presence of alarm signs, hospitalization or death until 30 days of the diagnosis. We included all epidemiological surveillance sites in Cusco, Peru and registered age, sex, comorbidities, number of episode of dengue infection and symptoms. Cox proportional hazards models were used to explore differences and the Kaplan-Meir estimator to assess predictors of dengue complications. **Results**. We evaluated 1729 people with confirmed diagnosis of dengue during 2018- 2021. In the pre-pandemic period five districts of Cusco notified 239 cases of dengue, this increased to 13 districts notifying 1430 cases of dengue in the pandemic period. The frequency of dengue complications was 7.0% (21/299) pre pandemic vs 2.4% (34/1430) during the pandemic (p1 episode). **Conclusions**. Despite an increase of dengue cases during the pandemic, the proportion of dengue with complications decreased compared to the pre-pandemic period. Comorbidities were associated to an increased risk of dengue with complications.

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Detection and characterization of arboviral infections in blood donors during two different epidemiological periods in Colombia

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***Abstract*—** Arboviruses pose a challenge in supplying pathogen-free blood components since they are not routinely screened in blood banks, and blood components from asymptomatic donors could be transfused. The present study aimed to detect and characterize arboviral infections in Colombian blood donors. In a cross-sectional study, the prevalence of dengue (DENV), Zika (ZIKV), chikungunya (CHIKV) viruses, and coinfections of blood donors were compared between an endemic period (November 2019-February 2020, n=462) and an epidemic period (November 2021- August 2022, n=1,119). Viral RNA from each donor serum was purified using a commercial kit, and the viruses were detected using a previously standardized Hemi-Nested RT-PCR protocol. Subsequently, fifteen days after the detection of the virus, the positive donors were surveyed to identify clinical characteristics related to the arboviral infection. Prevalences were presented as percentages, and prevalence comparisons between endemic and epidemic periods were performed using a Chi-square test with Bonferroni's test for multiple comparisons ($p < 0.05$; Stata v16). Significantly higher prevalences were found in the epidemic period compared to the endemic period for DENV (14.5% vs. 1.8%), ZIKV (7.7% vs. 0.2%), CHIKV (8.1% vs. 3.3%), and coinfections (4.3% vs. 0.2%). In addition, the survey response rate of positive donors in the two periods was 83/172 (48%). In total, 57% of the donors surveyed were asymptomatic. In symptomatic donors, the most frequent symptoms were headache (26.3%), malaise (11.3%), arthralgia (8.10%), fever/chills (7.8%), and ocular or retro-orbital pain (7.8%). In conclusion, the prevalences observed in epidemic and endemic periods were higher than those reported in other studies in the Americas. The high analytical sensitivity of the RT-PCR used and the sample analysis time (maximum seven days from its collection) could influence these results. Moreover, the high proportion of asymptomatic cases found, in addition to the mild and non-specific manifestations among the symptomatic, may limit the effectiveness of the donor selection criteria used to mitigate the risk of transfusion-transmitted arboviruses. It is necessary to carry out hemovigilance studies in transfusion

recipients to estimate the frequency and impact of the possible transmission of arboviruses by blood transfusion.

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Detection of neutralizing antibodies against West Nile virus in pigeons (Columba livia) from the Northeast of São Paulo state, Brasil

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***Abstract*—** West Nile virus (WNV) is a neurotropic zoonotic arbovirus that causes a dengue-like disease in humans, but in severe cases, neurological impairment. As in humans, some animals presenting with neurological signs lead to suspicion of WNV infection. WNV has been reported in South America since 2003-2006, but no expressive epizootic event has been registered yet in this region. Some orders of birds, such as Columbiformes, may constitute an important sentinel group because their occurring frequency is expressive in many places. In 2020, the Pathology Service of Wild Animals of the School of Agricultural and Veterinarian Sciences evaluated 11 pigeons (Columba livia) from wildlife (3) and captive – racing pigeon (8), from the northeast region of São Paulo state. Clinically, they presented with apparent blindness and neurological disorders (flying difficulty, torticollis, and incoordination). After euthanizing, necropsy was performed, and collection of serum and brain samples was done. Samples were sent to the Virology Research Center at Ribeirão Preto Medical School where brain samples had their total RNA extracted and RT-qPCR was performed using four different primer sets: WNV/SLEV duplex, PAN-Flavi, and PAN-Alpha. Serum samples were inactivated, and the neutralizing antibody tests (PRNTs) were done. Two different cut-offs were used in PRNTs [PRNT50 (screening) and PRNT80 (confirmatory)]. RT-qPCRs were negative for all samples with all primer sets. Nine samples (81.8%) had neutralizing antibodies on PRNT50, with titers varying from 10 to 640. When PRNTs80 were calculated, the WNV PRNT-positive samples were reduced to 6 (54.5%) with a titer ranging from 10 to 40. WNV detection is a challenge in a country with many other circulating flavivirus and due to the cross reaction among them, some serologic tests, such as ELISA, can only be used for screening. Our specific PRNT80 results show the first detection of neutralizing antibodies against WNV in the state of São Paulo, demonstrating that WNV is circulating in the region. Also, our results indicate that serologic and virologic active surveillance must be implemented in some animal populations, as sentinel animals, in order to an early detection of WNV circulation and prevention of future outbreaks by this virus.

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Determinación mediante pruebas diagnósticas de agentes causantes de síndrome febril en sueros de un hospital de tercer nivel. Cali, Colombia, 2023

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***Abstract*—** El dengue es una enfermedad febril de los países tropicales y subtropicales en el mundo, donde, 2500 millones de personas viven en el área de riego para adquirir la enfermedad; anualmente se reporta entre 50-100 millones de nuevas infecciones, 500.000 casos de dengue grave y unas 25.000 muertes cada año (Wallis et al, 2004). La rápida urbanización, el aumento en las poblaciones humanas y la colonización de nuevas áreas geográficas por los vectores que transmiten el dengue (Aedes aegipty y Aedes albopictus), hacen que esta enfermedad pueda ampliarse aún más y generar problemas en regiones donde no se presentaba el dengue (Guzmán, Kouri, 2004). Nos propusimos determinar mediante pruebas inmunológicas los agentes causales de pacientes que consultaron por síndrome febril en una clínica de tercer nivel de la ciudad de Cali, Colombia. Estudiamos un total de 50 muestras de suero, en las cuales determinamos la presencia de IgM contra dengue, hanta virus y virus de hepatitis E(HEV), así mismo se realizaron pruebas rápidas para Ag de HB, HCV, Malaria, Dengue(IgG, IgM, Ns1) y WB Tropical fever para Dengue, Chikungunya, Zika y Flavirirus sp. Encontrando una seroprevalencia del 72% contra Dengue, 14 % para Hanta virus y 0% para HEV en el Elisa de IgM.En las pruebas rápidas solo encontramos pruebas positivas para Dengue: IgG (48%), IgM(28%),

Ns!(40%). Y en el WB: ningún caso fue positivo para Zika, mientras el 6% fueron positivos para flavirirus, el 10% para Chikungunya y 50% para Dengue. Se observo una buena correlación en la positividad de las diferentes pruebas contra el Dengue. También se evidencio en varios pacientes positividad para mas de un agente causal del síndrome febril como IgM positivo para Dengue y Hanta virus en todos los pacientes.en los que la prueba dio positiva para este ultimo, lo mismo sucedió para los paciente con Chikungunya. Podemos observar en esta serie de casos que la infección por el virus Dengue sigue siendo la que ocupa el primer lugar, explicando entre el 72% mediante Elisa IgM y 50 % por WB., pero también hay participación de otros agentes virales tropicales como el Hanta.

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Displacement Of DENV-1 By DENV-2 Serotype And Introduction Of Genotype II - Cosmopolitan For DENV-2 In Argentina, 2023

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***Abstract*—** The four dengue serotypes had been detected in different years and regions in Argentina. DENV-1 has been responsible for the largest number of cases (causing the epidemics of 2009, 2016 and 2020, it was detected in 18 of the 25 years since the disease emergency in the country). The DENV-2 serotype has been detected in Argentina in several provinces, but with a much lower incidence and territorial scope than DENV-1. During 2022 the dominant serotype changed to DENV-2, being the Asian/American genotype detected and mainly limited to the province of Salta with just over 650 cases, In 2022, DENV-1 was also detected in the Central region of the country (Buenos Aires and Santa Fe) but the incidence was very low (just over 200 cases). During 2023, up to EW 22, a total of 102,237 dengue suspected cases and 61 deaths were registered in Argentina. Autochthonous dengue circulation has been confirmed in 18 jurisdictions, DENV-2 circulating in most of them (16/18 jurisdictions), DENV-1 to a lesser extent, and some autochthonous cases of DENV-3 in a restricted area. Until EW 16, genomic surveillance was performed in acute serum samples qRT-PCR DENV-1 or DENV-2 (+) with Ct <28 (14 samples for DENV-1 and 22 samples for DENV-2). For complete genome sequencing, library preparation was performed with Illumina COVID-Seq Assay kit, using specific primers for cDNA generation. The sequencing reaction was performed on MiSeq (Illumina) sequencing instrument using the MiSeq v2 reagent kit on a 300 cycle program. Análisis were made with software Genome Detective Virus Tool and Dengue virus Typing Tool (https://www.genomedetective.com).DENV-1, genotype V and DENV-2, Genotype II - Cosmopolitan were identified. It marked the introduction of a new DENV-2 genotype in the country. The impact of the Cosmopolitan genotype of DENV-2 on the clinical-epidemiological evolution of 2023 outbreak in Argentina is still not clear, but the epidemic was characterized by a wide territorial dispersion of the cases, clinical changes with symptoms of dehydration, more serious cases were reported; all affected age groups and deaths in people without risk factors.

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Entomological Drivers Of Fine-Scale Heterogeneity In Dengue Risk

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***Abstract*—** Dengue virus (DENV) is an arbovirus transmitted primarily by the mosquito Aedes aegypti, and is widely distributed in tropical and subtropical regions. Dengue prevention relies mainly on vector control alone or in combination with vaccination. Improved targeting of high-risk areas by vector control programs could decrease the worldwide disease and economic burden of dengue, but this is challenging due to the highly heterogeneous nature of local dengue risk. Currently, dengue outbreak response is triggered by human case numbers, however this approach lacks timeliness and is a poor predictor of localized dengue risk as the majority of transmission is driven by asymptomatic individuals. Entomological surveillance could improve upon these deficiencies by providing earlier and more spatially accurate risk estimates, however entomological surveillance measuring vector abundance and DENV infection prevalence has thus far provided poor predictions of variability in dengue risk. The poor predictive accuracy of these entomological metrics may be due to imperfect implementation of surveillance strategies that often prioritize coverage

of large areas, thus decreasing the likelihood of detecting highly focal dengue transmission. In this study, we test if dengue detection could be improved through targeted entomological viral surveillance at the neighborhood scale in areas that have had historically higher risk of dengue transmission. To test this hypothesis, DENV infection prevalence was characterized in adult Ae. aegypti female samples that were collected in Iquitos, Peru using backpack aspirators during the DENV-2 outbreak season of 2010-2011 (1). In addition, the environmental and host-associated risk factors associated with DENV infection prevalence and Ae. aegypti abundance increases were explored. DENV infection was detected by RT-PCR in individual female Ae. aegypti samples. Futher, we tested the association between dengue exposure in the human population and the entomological metrics of interest (Ae. aegypti female adults DENV infection prevalence or abundance). For both these objectives logistic regression mixed effects models were used. The findings from this study inform whether monitoring DENV infection prevalence in Ae. aegypti adult females can be an effective tool for dengue risk prediction, and if environmental and behavioral risk factors can improve targeting of entomological surveillance at intermediate spatial scales.

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Epidemiology of dengue fever in Guatemala

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Abstract— Dengue fever occurs worldwide and about 1% of cases progress to severe haemorrhage and shock. Dengue is endemic in Guatemala and its surveillance system could document long term trends. We analysed 17 years of country-wide dengue surveillance data in Guatemala to describe epidemiological trends from 2000 to 2016.Data from the national dengue surveillance database were analysed to describe dengue serotype frequency, seasonality, and outbreaks. We used Poisson regression models to compare the number of cases each year with subsequent years and to estimate incidence ratios within serotype adjusted by age and gender. 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 2005, 2007, and 2013 DENV1 outbreaks, the 2010 DENV2 and 2003 DENV3 outbreaks. Adults had a lower incidence immediately after epidemics, which is likely linked to increased immunity.

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Examining Altitude And Population Density As Key Factors In Dengue Outbreaks In Colombia

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Abstract— Dengue, a prominent vector-borne disease, poses a significant global health challenge. To effectively combat this disease, it is crucial to comprehend the geographic and demographic factors that drive its transmission. This study examines the relationship between altitude, population density, and dengue incidence in ten municipalities of Colombia from 2016 to 2020. The findings reveal a significant positive association between population density and dengue incidence, as well as an inverse association between altitude and dengue incidence. These results underscore the pivotal role of altitude and population density in shaping the dynamics of dengue vectors. Further research is necessary to elucidate how other climatic and social variables contribute to the distribution of dengue within the country.

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Fine-scale Geospatial Modeling Of ZIKV Infection In An Urban Informal Settlement During Its Emergence In 2015

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Abstract— The introduction of ZIKV in Brasil in 2015 caused a nationwide epidemic lasting until 2016, with variations in outbreak timing and disease burden. Prospective studies in vulnerable urban populations found infection rates exceeding 70% early in the epidemic, possibly contributing to transmission decline due to high herd immunity. However, the extent of spatial heterogeneity in infection risk and influencing factors at small scales remains unclear in these high transmission settings. To characterize populations and places of vulnerability to ZIKV infection during the epidemic, we conducted a cross-sectional analysis of an ongoing cohort in an urban informal settlement (0.46 km2) in Salvador, Brasil. A serosurvey was performed in the period after the large epidemic in the city from August to November 2015, using an anti-ZIKV NS1 IgG3 assay (validated to detect recent ZIKV infection). A socioeconomic status index based on family/household factors was constructed using Principal Component Analysis. We used an Integrated Nested Laplace Approximation geospatial model that included a term for the household cluster effect to evaluate the spatial distribution of seroprevalence and evaluate the association of geocoded information on environmental features and household demographic and socioeconomic factors with ZIKV seroprevalence. Among the 1,351(93%) of 1,452 participants who were followed, overall ZIKV seroprevalence was 63.2%. The geospatial model identified significant spatial variation in risk with a range of seroprevalence from 81-27% within the study site. In the multivariable analysis which accounted for continuous spatial and household cluster effects, informal employment (aOR=1.50, 95%CI 1.05–2.14), food insecurity (aOR=1.35, 95%CI 1.04–1.75), and low socioeconomic status (aOR=0.87, 95%CI 0.77–0.98) were associated with a higher risk of seropositivity. We did not identify significant environmental factors that were spatially associated with ZIKV seroprevalence within the study site. Despite ZIKV attack rates leading to transmission decline within a single epidemic season in urban informal settlements, there was notable variation in ZIKV infection risk at small spatial scales. The observed spatial heterogeneity seemed to be driven by differences in social vulnerability rather than specific environmental factors. These findings highlight the importance of directing prevention efforts for mosquito-borne infections towards the most vulnerable segments residing in urban informal settlements.

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First Dengue Outbreak In Balsas, Amazonas - Peru, 2021 – 2022

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Abstract— Recently, Peru has experienced an increase in the number of dengue cases, being Amazonas one of the most affected departments. In 2020, the number of cases reported in Amazonas was 845, while in 2022, this number increased to 3502. The ability of the vector Aedes aegypti to infest new localities and adapt to climate changes has been decisive for the transmission, establishment, and spread of the disease in the region. The community of Balsas is located in the southwest of Chachapoyas province, with a population of 676 inhabitants, and a warm humid climate. Balsas has a temperature that oscillates between 11°C to 35°C and an annual precipitation of 780 cc, presenting ideal climatic conditions for the vector development. In December 2021, Balsas reported a dengue outbreak for the first time, after an earthquake of magnitude 7.5 on the Richter scale, hit the department. In this study, we analyzed serum samples of suspected patients collected by the Regional Directorate of Health during this outbreak in Amazonas. Dengue confirmation was performed by non-structural protein 1 (NS1) antigen ELISA and serotyping was done by multiplex qRT-PCR. A total of fifty-two patients that met the dengue case definition were included in the study, of which 43 (83%) were confirmed as positive by serology (Ag NS1). The most frequent symptoms were fever (93%), headache, and myalgia (90.7%), and 21% of the patients presented severe abdominal pain. Additionally, 60% of the cases were diagnosed during the first two days of the onset of the symptoms. The most prevalent serotype was DENV-2 (92%), and only 8% were DENV-1. Here, we report the first dengue outbreak in Balsas, highlighting the need to improve surveillance and control strategies in areas where the mosquito is circulating. Also, the DENV-2 serotype

has been previously reported as responsible for outbreaks in other parts of Peru, such as Madre de Dios. It is important to further study this serotype and its association with severe cases.

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Health and economic impact of chikungunya vaccination in Asian, African, American and Pacific Island countries: Vaccine impact modelling, cost-effectiveness analysis, and budget-impact analysis

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***Abstract*— Background.** Chikungunya virus was discovered in Tanzania in 1952 and its economic burden has been estimated at $185 billion in the Americas alone. WHO has classified chikungunya as a major public health threat but currently lacks a robust estimate of the health and economic burden of chikungunya. The potential for chikungunya to spread to new countries due to global climate change also means that preventative measures in the form of a vaccine are urgently needed. Therefore, estimating the cost-effectiveness of a potential chikungunya vaccine and its economic benefits would not only fill the current evidence gap, which could accelerate vaccine development and decision-making for its introduction in countries at risk for chikungunya, but also incentivise investment in a potential chikungunya vaccine. **Methods.** We estimated the country-specific pooled estimates of force of infections (FOIs), the rate at which a susceptible individual become infected, by fitting a constant catalytic model and time-varying epidemic models to the age-stratified seroprevalence data collected from the systematic literature review. We used a Bayesian inference framework and Markov Chain Monte Carlo (MCMC) to sample posterior distributions of FOIs. We selected the best-fit model through a comparison of Deviance Information Criterion (DIC) values and categorised endemic and epidemic settings based on the chosen models. To estimate the timing of epidemics, we converted age to years of exposure using threshold functions based on the survey conduction years. Our models incorporated δ parameters to account for multiple epidemics, where δ ranged from 1 to N, and each outbreak was modeled with time-varying FOIs. To account for the uncertainty of epidemic years, we performed random sampling from annual outbreak probabilities derived from inter-epidemic periods. The life-time disease burden and vaccine impact by birth cohort were calculated through a series of 1000 random simulations, taking into account the uncertainties in the timing of epidemic years. **Results and Conclusion.** A total of 61 countries were represented among the included studies. The evidence generated through this project will provide insights for global decision-makers, enabling them to gain understanding of the current health and economic burden posed by chikungunya.

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Interactions Between Rainfall, Water Storage Behavior, And Mosquito Dynamics Drive Variation In Seasonal Dengue Dynamics

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***Abstract*—** Vector control for dengue can vary significantly in its effectiveness, with human behavior being a potentially important cause of that. Compliance with top-down efforts such as spraying campaigns and bottom-up habits around aquatic habitat reduction around households are two examples of behavioral components of successful vector control. One factor that may affect bottom-up habits around aquatic habitat reduction is water storage needs, which influence the relationship between rainfall and aquatic habitat for mosquitoes. Rainfall has been associated with both more and less aquatic habitat—it directly increases standing water outdoors but may lessen intentional water storage. To explore the possible consequences that this relationship may have on the effectiveness of behavioral interventions, we developed a mathematical model for the coupled dynamics of dengue virus and associated container-management behaviors. Preliminary analyses suggest that the effects of different assumptions about the relationship between rainfall and container-management behaviors are most impactful in more temperate climates, where mosquito population dynamics are subject to more seasonal variation than in tropical climates. In a temperate setting, when changes in aquatic habitat more closely tracked seasonal temperature changes, we observed a higher, earlier seasonal peak in dengue incidence than when habitat availability and rainfall trends were dissimilar. This effect was dampened by the transmission of container-management behaviors, which were driven by mosquito density, disease incidence, and social

conformity. Overall, we found that the impact of container-management behaviors is largely dependent on assumptions about what drives mosquito reproduction and aquatic habitat dynamics in a given setting, and suggest this as a focus when tailoring interventions to community-specific conditions and concerns.

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Measuring the effect of a multicomponent dengue preventive strategy targeting transmission hotspots in Santiago de Cuba, Cuba

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***Abstract*— Introduction.** Aiming to identify efficient dengue control strategies, we evaluated a multicomponent intervention addressing transmission hotspots. **Methods.** A targeted environmental risk reduction intervention, including vector control, syndromic surveillance of suspected dengue cases and community empowerment was set up mid 2017 in two dengue transmission hotspot areas in Santiago de Cuba. Of these, and of two control, areas, routinely collected data from January 2015-December 2019 were used to calculate dengue incidence and House index (HI). An interrupted time series analysis, common trend model and Newey-West method to correct for autocorrelation, was applied to assess intervention effectiveness. **Results.** In the first year after the roll-out of the intervention, the prevention strategy produced a level change on dengue incidence, reducing the risk by 71.6% (95% CI 58.7, 80.5) in the intervention areas. The intervention reduced the HI with a factor 2.35 (95 % CI 1.49, 3.21). The effect at municipality level, measured in the 2018 epidemic wave, resulted in a proportional contribution of dengue cases of intervention areas of 6.1 % vs. 10.7 and 14.1% in the 2015 and 2016 pre-intervention epidemic waves, whereas the contribution in the control areas represented 9.9% in 2018, vs 6.6 and 8.0 % in 2015 and 2016, respectively. The intervention delayed the onset of the epidemic by 4 weeks in comparison to the control areas.**Conclusions.** A multicomponent intervention addressing dengue hotspots could reduce the number of cases by 71.6% compared to the control area and limit the impact and duration of an epidemic wave at municipality level.

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Methodological Approach For Harmonizing Multiple Studies: A Case Of Clinical Data Use From 5 Cohort Studies In The Aedes Network In Colombia

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Abstract— Introduction. Data harmonization promotes efficient data integration, improves data quality, enables more accurate and reliable decision-making, and promotes a unified and comprehensive view of information. Over the years, the AEDES network has conducted several cross-sectional and cohort studies aimed to estimate the burden of dengue, chikungunya and Zika diseases. Here, we report on the strengths and limitations of implementing a methodological approach to integrate, standardize and harmonize retrospective data from five cohort studies conducted in Colombia. **Methodology**. We followed the harmonization methodology developed by Maelstrom Research Group, previously customized to the specificities of the cohorts of the AEDES network in the context of the ReCoDID consortium, an international initiative to harmonize datasets from observational studies.. As a first step, we defined the following research question: Is there a profile of laboratory parameters that allows clinicians to differentiate between dengue, Zika and chikungunya in the first five days of disease? Subsequent activities included identifying and selecting of clinical and laboratory variables from a previously defined master data dictionary. We then prepared, cleaned and extracted the data from each cohort dataset. Once the data sets were centralized and integrated, we compared each variable definition with the master data dictionary to establish the harmonization rules. Finally, we executed the scripts using R programming language generating the resulting harmonized dataset. **Results**. Datasets from different sources and temporalities may have inconsistencies, variations in data collection methods, definitions, and coding

systems, making it challenging to harmonize and integrate effectively. Determining a solid methodology is key in order to gain consistency and high data quality in the resulting dataset. In all, 5046 inpatient and outpatient participants were recruited from 5 cohorts conducted between 2003 and 2019. The timeline included the dengue outbreak in 2010, and the introduction of chikungunya (2015) and Zika (2016). **Conclusions**. Sharing and reconciling data fosters an environment of collaboration that allows not only approaching research questions with higher statistical power but also streamlining the formulation and testing of new ones taking advantage of pooled, harmonized clinical and laboratory data that is particularly relevant in the context of emergent infectious diseases and future outbreaks.

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Molecular epidemiology of imported chikungunya virus in Cuba, 2014

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***Abstract*— Introduction.** Chikungunya fever is an acute febrile illness caused for Chikungunya virus and transmitted to humans by Aedes aegypti and Aedes albopictus mosquitoes. In 2013, the circulation of this virus was reported for the first time in the Americas. For that reason, it was essential to establish the molecular surveillance of Chikungunya virus in Cuba in 2014, to know the imported genotypes into Cuba, their genetic relationship and possible epidemiological implications. **Methodology.** The proposed strategy consisted of the amplification of the E1 region of the viral genome, from serum samples collected in 2014 and positive to Chikungunya virus using the real-time PCR technique. The nucleotide sequencing of the amplified products was carried out by Sanger methodology, and the phylogenetic analysis with the MrBayes program. **Results.** The phylogenetic analysis showed that the nine sequences obtained belonged to the Asian genotype, which was introduced in the Americas since 2013. The close genetic relationship observed between the sequences of this investigation and others from the Americas, was an expected result given the epidemiological context of the region. Also, the mutations detected (A98T, V226A and K211E) have been related to a better replicating capacity of the virus in Aedes aegypti mosquito, and with a possible escape mechanism to the action of neutralizing antibodies. **Conclusions.** This study evidenced the usefulness of the molecular characterization of circulating viruses in Cuba to alert to the National Health System, because this tool provides essential information for the design of effective campaigns for the control of this disease.

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Neurodevelopmental Delay In Preschool Children Exposed To Zika Virus During Pregnancy: An Observational Prospective Study In The Zikalliance Cohort, Colombia

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***Abstract*— Introduction**. Prenatal Zika virus (ZIKV) infection has been associated with neurodevelopmental delay (NDD). We aimed to determine the incidence of NDD and whether it is associated with prenatal ZIKV amongst preschool children from the Zikalliance cohort, Colombia. **Methodology**. We conducted a prospective cohort study among normocephalic children born to mothers exposed (positive RT-qPCR or VNT) and unexposed (negative IgG ELISA in paired antenatal samples) to ZIKV. The Denver Developmental Screening Test II (DDST-II) was administered to children (40-66 months of age) by a trained psychologist who was blinded to exposure. Children were considered at NDD risk when presenting ≥ 1 delays or ≥ 2 cautions in one or more areas, within their age range in the DDST-II. A second evaluation was performed in cases with inconclusive tests at first attempt. We estimated adjusted odds ratios of NDD using logistic regression. **Results**. We evaluated 100 children with a conclusive DDST-II (mean age = 4.7 years [SD = 0.6]; 54% male). Overall, 62.0% (95%CI: 53.3 – 71.7) children were at risk of NDD and 49.0% (95%CI: 39.0 – 59.0) were born to mothers exposed to ZIKV. Exposed and unexposed

children did not differ in maternal age (23.6 vs. 23.3 years), gestational age (39.0 vs. 39.0 weeks), anthropometry (3,220 vs. 3,120 gr [weight]; 34.1 vs. 33.7 cm [head circumference]) or Apgar scores at birth (10.0 vs. 10.0 [10th minute]), and their mothers had similar educational attainment (8.2% vs. 9.8% [more than high school]) and exposure to alcohol (4.1% vs. 3.9%), tobacco (6.1% vs. 0.0%) and recreational drugs (7.0% vs. 4.2%) during pregnancy; however, exposed children were less likely than unexposed children to live in households whose monthly income amounted to ≤ 1 minimum national wage (77.1% vs. 96.0%, p=0.006). Although exposed children were less likely than unexposed children to have NDD (53.1% vs. 70.6%), this difference was not statistically significant after adjustment for the household's monthly income (OR=0.44; IC95%: 0.18 – 1.03). **Conclusions**. Prenatal exposure to ZIKV infection was not associated to NDD among preschool children. This preliminary result should be interpreted with caution, awaiting comparative analysis with other tests administered longitudinally in the same cohort.

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Phylogenetic Analysis Reveals Both Maintenance And New Introduction Of Yellow Fever Virus After The 2017-2018 Outbreak In The Southeastern Region

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Abstract— Yellow fever virus (YFV) is an arbovirus endemic to tropical regions of Africa and South America. In Brasil, a YFV belonging to lineage E within the South American (SA) genotype I was responsible for a massive sylvatic outbreak in the southeastern region between 2017 and 2018, including São Paulo State, which is the most populous one in the country, with the last epizootic event in the metropolitan area of São Paulo being detected in late 2018. YFV genetic findings obtained from a Callitrix spp. found in a green area located in Barueri city in March 2020, and from a Callicebus nigrifons from Águas da Prata municipality in March 2023 revealed a persistence of the SA-I lineage E in the metropolitan area as well as a new introduction from the Center-West region in the State. Sequences generated using NGS from neo-tropical monkeys (NTP) herein were aligned with YFV sequences belonging to SA-I genotype using MAFFT, with a GTR nucleotide substitutuion model used to infer a maximum likelihood tree, and a HKY+G4 nucleotide substitution model and a relaxed molecular clock model for Bayesian analysis, with an underlying lognormal distribution of branch rates with a Bayesian skygrid model. The inferred Maximum Likelihood tree revealed that NTP from Barueri, collected in 2020, clustered within sequences from the 2017-2018 outbreak, revealing persistence of the SA-I lineage 1E in Sao Paulo metropolitan area, while NTP from Águas da Prata clustered within sequences from the Midwest region (Goiás) from 2020, and the Southeast region (state of Minas Gerais. The MRCA of this cluster was estimated to August 2019 [95% HPD=November 2018-May 2020). Ongoing surveillance programs based on NTP remain one the most important actions for understanding YFV circulation in Brasil. As Alouatta spp monkeys act as the main YFV amplifiers hosts, and this population was severely affected by the outbreak, more studies are needed to better understand the role of other NTP species in YF cycle in the southeastern region.

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Recent Circulation Of Dengue Virus Lineages In Northwestern Colombia

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Abstract— Dengue is an important cause of morbidity and mortality in northwestern Colombia. Virological surveillance helps to understand the processes of viral trafficking, changes in the epidemiology and in the virulence of the agents. **Methods**. E-gene sequences of dengue viruses isolated in the period 2010-2022 in the provinces of Choco and Antioquia, northwestern Colombia, were phylogenetically analyzed and compared with sequences circulating in other regions of this, and other countries. **Results**. All isolates were of DENV-1 or DENV-2 serotypes. DENV-1 sequences grouped paraphyletically with strains from Brasil and Peru, within the genotype V. DENV-2 sequences

grouped paraphyletically with strains from Venezuela and other regions of Colombia within the Asian-American genotype. **Conclusions**. These results support the simultaneous circulation of several dengue virus lineages within the same serotypes and suggest frequent trafficking of strains between South American countries and other neighboring regions.

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Results of laboratory surveillance for dengue in Cuba, 2022

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***Abstract*— Introduction**: During the year 2022, several countries in the American region registered increases in the number of cases of dengue, Zika and chikungunya above that was reported in the year 2021. Cuba was not exempt from the increase of cases of dengue. To describe the results of Laboratory Surveillance performed by the Arbovirus National Reference Laboratory of the IPK in 2022 that includes serological, molecular methods and genomic surveillance. **Methodology**: The direct methods used were the isolation and RT- PCR while the indirect method used was IgM Capture ELISA and the genomic surveillance. The samples were collected of all Cuban provinces and the different points in the period studied. The genomic surveillance was performed by a sequence of E gene according to the DTCSQuick Star Master Mix kit (Beckman Coulter, US) and analyzed in a Beckman Coulter automatic sequencer model CEQTM8800 using the raw data analysis procedure for PCR products. Obtained sequences were assembled and edited using the Sequencher program (Sequence Analysis Software, Version 4.10.1, Gene Codes Corporation, US). **Results**: The results of the surveillance show an increase in the number of positive cases of dengue with respect 2021 and the detection of the simultaneous circulation of the four viral serotypes in the country. The serotype 3 was the one that predominated during the year followed by dengue 2 and we observed differences between the different provinces of the country. Neither Zika nor Chikungunya cases were detected in 10% of the dengue-negative samples studied. The genotypes of the different serotypes identified corresponded to those that circulated in the region of the Americas during 2022. **Conclusions**: Laboratory surveillance together with epidemiological and clinical surveillance were essential for the control of the disease in the country during the study period.

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RNA Extraction And Storage (RNAES) Technology And Nanopore Sequencing Of Dengue And Chikungunya Viruses, Implementation Of A Combined Low-Cost Strategy In Paraguay

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***Abstract*—** Arthropod-borne viruses, arboviruses, pose a constant threat to public health in tropical and sub-tropical countries. In Paraguay, dengue virus (DENV) causes large outbreaks every 2-3 years. In 2023, Paraguay also experienced the largest chikungunya virus (CHIKV) outbreak. RNA extraction is essential for molecular detection and viral characterization, but available protocols rely on costly, hazardous commercial reagents and ultra-cold storage (-80°C) conditions that are unsustainable in resource-constrained settings. The aim of this study was to evaluate the feasibility of whole genome sequencing of samples extracted by utilizing the recently developed simple and economical RNA Extraction and Storage (RNAES) technology. Twenty-

three serum samples were selected for the study. Three DENV serum samples (mean Ct=27,8): one collected in 2019 from Central Department and two in 2023 from Caaguazú and Central Department. Twenty CHIKV serum samples (mean Ct=21,7) were collected from Asunción, Amambay, Guairá and Central Departments during the 2023 outbreak. Whole genome amplification was attempted by multiplex PCR as previously described by Quick et al 2017. Barcoding was performed by ligation, adapting the ARTIC SARS-CoV-2 low-cost approach. Successful amplicon primer tilling sequencing was performed by nanopore technology, obtaining mean values of nucleotide coverage of 93.2% for DENV and 96.2% for CHIKV. Consensus sequences of near-complete genomes of DENV were assigned as DENV-1, Genotype V (2 samples from 2023), DENV-4, Genotype II (1 sample from 2019) and all obtained consensus sequences of CHIKV were assigned as East/Central/South African (ECSA) lineage by the Genome Detective tool. Furthermore, genome coverage of DENV-4 RNA extracted by RNAES was slightly improved (92%) comparing to RNA extracted by a commercial kit (91.9%). As mentioned, RNAES technology could be a useful tool to address common barriers in resource-constrained settings. In addition to RNA extraction, it facilitates transport at ambient-temperature to reference laboratories for specialized testing. The combination of RNAES technology and a portable low-cost approach of nanopore sequencing could expand accurate detection and characterization of arboviruses to new key locations and allow for unique studies of arbovirus introduction, transmission, and emergence.

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Series of Dengue-Related Deaths in the Central Region of Brasil, 2015-2022

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***Abstract*—** For over 30 years, dengue has impacted millions of individuals in Brasil, resulting in significant lethality. In the last decade, the central region of Brasil has experienced the highest rates of disease incidence and fatalities. This study aimed to analyze dengue-related deaths in Goiânia, a city located in the state of Goiás, in the Midwest region of Brasil, covering the period from 2015 to 2022. A database was constructed using the records of dengue deaths from the Notifiable Diseases Information System (SINAN), and the data analysis was carried out using R software, version 2022.12.0+353. Our analysis demonstated that the lethality rate of dengue (was 19.9% in 2015, 25.3% in 2016, 33.9% in 2017, 24.7% in 2018, 22.2% in 2019, 27.3% in 2020, 30.0% in 2021, and 46.1% in 2022. The demographic characteristics ($p<0.05$) were as follows: age group 40-49 years (19%), mixed race (40%), and illiteracy (7.6%). The female gender accounted for a higher percentage of deaths (51%). It is noteworthy that there was a disparity between genders throughout the study years. Regarding the age group, a shift towards extreme age ranges was observed in 2016 and 2017 (0-19 years and 80-100 years), and in 2019, above 80 years. Among the comorbidities and symptoms, the most frequent and significant ones ($p<0.05$) were: hypertension (41%), diabetes (18%), fever (86%), myalgia (72%), leukopenia (28%), arthralgia (27%), back pain (22%), and the severe signs of undetectable pulse (37%), convergent blood pressure (22%), capillary refill time (22%), hematemesis (20%). Persistent vomiting (24%), lethargy or irritability (24%), hepatomegaly (12%), and increased hematocrit (11%) were among the alarming signs most strongly associated with deaths. The mentioned results demonstrated that clinical and laboratory signs are associated with dengue fatalities. In 2017 and 2022, similarities in death percentages, particularly regarding race and symptoms, differed from other years. Comorbidities were associated with adult and elderly deaths, showing an increasing trend, particularly in 2022. Therefore, maintaining continuous actions in epidemiological surveillance, case identification, and appropriate clinical management, especially for patients with comorbidities and the elderly, can help prevent fatal outcomes.

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Seroprevalence Of Dengue, Chikungunya And Zika At The Epicenter Of The Congenital Microcephaly Epidemic In Northeast Brasil: A Population-Based Survey

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***Abstract*— Background.** The Dengue viruses (DENV) serotypes 1, 2, 3 and 4 started to be re-introduced in the Northeast Brasil in the 1980's with DENV1 and ending in 2010's with the introduction of DENV4. Zika (ZIKV) and Chikungunya (CHIKV) viruses were introduced in Recife around 2014 and caused large outbreaks of ZIKV in 2015 and CHIKV in 2016. **Methods.** We conducted a stratified multistage household serosurvey among residents aged between 5 and 65 years in the city of Recife, Northeastern Brasil, from August 2018 to February 2019. The city neighborhoods were stratified according to high, intermediate, and low socioeconomic strata (SES). Design-adjusted seroprevalence were estimated by age group, sex, and SES. The ZIKV seroprevalence was adjusted to account for the cross-reactivity with dengue. Individual and household-related risk factors were analyzed through regression models to calculate the force of infection. Odds Ratio (OR) were estimated as measure of effect. **Principal Findings.** A total of 2,070 residents were investigated. The forces of infection for high SES were lower for all three viruses as compared to low and intermediate SES. Overall, DENV seroprevalence was 88.7% (CI95%:87.0-90.4), 81.2% (CI95%:76.9-85.6) in the high SES and 90.7% (CI95%:88.3-93.2) in the low. The overall adjusted ZIKV seroprevalence was 34.6% (CI95%:20.0-50.9), 47.4% (CI95%:31.8-61.5) in the low SES and 23.4% (CI95%:12.2-33.8) in the high. CHIKV seroprevalence was 35.7% (CI95%:32.6-38.9), 38.6% (CI95%:33.6-43.6) in the low SES and 22.3% (CI95%:15.8-28.8) in the high. Surprisingly, ZIKV seroprevalence increased rapidly with age in the low and intermediate SES while in high SES, ZIKV seroprevalence showed only a small increase with age. CHIKV seroprevalence by age was practically stable in all SES. The serological markers of recent infections for ZIKV and CHIKV were 1.5% (CI95%:0.1-3.7) and 3.5% (CI95%:2.7-4.2) respectively. **Conclusions.** Our results confirmed continued DENV transmission and intense ZIKV and CHIKV transmission during the 2015/2016 epidemics followed by ongoing low-level transmission. The study also highlights that a significant proportion of the population is likely still susceptible to be infected by ZIKV and CHIKV.

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Spatiotemporal Dynamics Of Denv-1 Lineages Circulating In Two Colombian Cities Between 2019 And 2021

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Abstract— Dengue virus (DENV) in Colombia has an endemo-epidemic transmission pattern characterized by circulation of multiple serotypes and lineages with a heterogeneous spatiotemporal distribution. It is not clear how inter-epidemic transmission is sustained, and what triggers epidemic seasons in the country. This work aims to understand how a recent dengue serotype 1 outbreak is related to transmission dynamics between near and distant endemic regions of Colombia. We performed sequencing and assembly of 8 complete genomes of dengue virus circulating in La Virginia, Risaralda (2019-2021) and 12 genomes dengue virus circulating Santiago de Cali, Valle del Cauca (2021). Genomes were aligned with sequences

from other regions of Colombia and other countries that were available in GenBank. We performed Maximum-likelihood and Bayesian phylogenetic analyses and identified the best-fit molecular clock and population growth model. Subsequently, we reconstructed the spatial dispersal of the virus using a discrete diffusion phylogeographic model with Markov chains. The lineage of DENV1 that circulated in La Virginia between 2019-2021 is related to the lineages that circulated in the department of Antioquia between 2014-2016 and share ancestry with viruses introduced in the 1990's from Venezuela. By contrast, the lineage detected in Cali in 2021 is more closely related to a lineage that circulated in the department of Santander in 2008. The reconstructed dispersal routes show that distinct DENV1 lineages from two nearby locations experiencing recent DENV1 transmission (La Virginia and Cali) likely derive from different sources. We hypothesize that introduction and re-introduction of new lineages can be related with sustained transmission and epidemics in our region. Our results provide insights about the heterogenous dispersal of DENV1 in Colombia, and its potential impact on transmission dynamics.

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The association of obesity with pediatric dengue virus infection, disease, and immune responses

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Abstract— Obesity is an important risk factor for poor outcomes of multiple infectious diseases and has been associated with reduced antibody responses; however, whether obesity increases risk of dengue virus (DENV) infection and disease or affects DENV antibody responses has not been fully explored. Given that obesity and DENV both pose a substantial and increasing burden in children, we sought to investigate whether pediatric obesity is associated with DENV infection, disease and immune responses. Data were derived from the Pediatric Dengue Cohort Study in Managua, Nicaragua, following ~3,800 children 2-15 years old over 9 years (2011-2019). To study the effect of obesity on anti-DENV antibody responses, we tested serum samples from a subset of 90 participants (n=30 each normoweight, overweight, and obese, defined by BMI z-score) at 6, 18, 30, and 42 months post-infection for anti-DENV antigen-specific antibody binding profiles, neutralizing capacity, and temporal dynamics. To do so, we developed a novel multiplex Luminex system to measure the magnitude of antibodies targeting envelope (E) protein, E domain III (EDIII), and nonstructural protein 1 (NS1) from the four DENV serotypes. We observed that from 2011 to 2019, the prevalence of obesity in the cohort increased 86%, from 7 to 13%. Obesity was significantly associated with DENV infection in the entire cohort and significantly associated with developing symptoms (dengue) in participants infected with DENV. Compared to normoweight, obese participants had 1.30 higher odds (95% confidence interval [CI], 1.11-1.52) of being infected with DENV and 1.88 higher odds (95%CI, 1.38-2.55) of developing dengue given infection. Furthermore, obesity was positively correlated with the quantity of anti-DENV binding antibodies targeting E, EDIII, and NS1 at 6 months post-infection, but not at 18 months post-infection. Conversely, obesity was not associated with antibody quality, measured here as neutralizing titer, at 6 and 18 months post-infection. We are currently analyzing the 30- and 42-month post-infection samples to interrogate the long-term association between obesity and antibody temporal dynamics. In conclusion, our data indicate that obesity is a risk factor for experiencing a DENV infection and developing dengue given DENV infection, and we find that obesity also modulates anti-DENV antibody responses.

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The Impact of Dengue Virus Genetic Diversity on Wolbachia Inhibition

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Abstract— Dengue virus (DENV) is the most widespread arthropod-borne virus worldwide with two-fifths of the world's population at risk of infection. Recently, a new strategy for controlling DENV and other arthropod-borne viruses emerged by introducing Wolbachia into Aedes aegypti mosquitoes. This bacterium inhibits the ability of mosquitoes to transmit viruses and may help to reduce the global health burden in endemic countries. However, studies show that inhibition of DENV by Wolbachia is typically incomplete, resulting in a reduced but incomplete

inhibition of dengue virus transmission. DENV genetic diversity could be one of the factors that influence the levels of Wolbachia inhibition, with some variants being less susceptible than others. However, no studies have comprehensively studied the impact of virus genetic diversity on Wolbachia inhibition. My overall goal is to harness evolutionary genetics tools for identifying pathways that may allow certain DENV strains to persist when challenged by Wolbachia in mosquitoes. This particular project will determine variation in persistence across existing DENV diversity. To investigate a diverse set of viruses, we collected and sequenced DENV isolates from the World Reference Center for Emerging Viruses and Arboviruses, and the Yale Arbovirus Research Unit collections. This collection consists of 60 DENV-1, 68 DENV-2, 17 DENV-3 and 25 DENV-4 isolates. Our study initially focuses on DENV-2, which has one of the highest global impacts. Furthermore, we plan on using Ae. aegypti mosquitoes infected with Wolbachia strains isolated from Drosophila melanogaster (wMel) and Ae. albopictus (wAlbB), two of the most common strains used by control programs, and the uninfected wildtype colony. We will infect wild-type, wMel and wAlbB mosquito colonies with our panel of DENV2 isolated and measure the efficiency of midgut infection and dissemination. We will use phylogenetic and statistical analyses to assess variation in inhibition and determine how the efficiency of infection and dissemination effects cluster by virus genotype. Our results provide important insights into natural variation in DENV2 inhibition by Wolbachia and will inform Wolbachia-based approaches for reducing Dengue global health burden.

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Track to improve the predictive capacity of EWARS signals for early detection of dengue epidemics

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***Abstract*— Background.** The implementation of Early Warning and Response System (EWARS) for dengue outbreaks require the identification of alarm signals with a high sensitivity and positive predictive value (PPV). **Methodology.** We conducted a retrospective analysis of dengue epidemiological, Aedes entomological and climate data (as potential alarm indicators) from the routine surveillance system in Cienfuegos, Cuba (2010—2022). This period was divided into a run period to establish the "pattern of the disease" (2010-2015) and an analysis period to identify sensitivity and PPV of outbreak prediction of individual indicators (2016-2022). **Results.** In Cienfuegos province, the sensitivity of epidemiological alarm signals: fever cases and confirmed dengue cases was 100% with a PPV of 43% and 30%, respectively. Similarly the entomological Breteau index showed a relatively high sensitivity (87%), but a low PPV of 36%. The PPV for climate variables, rainfall, mean temperature and relative humidity was 35%, 31% and 32 % respectively, despite sensitivities being higher than 90% for all of them. The time period between alarm signal and start of the outbreak ranged between 3 weeks (for epidemiological indicators) and 7 weeks for entomological indicators. **Conclusion.** In order to avoid false alarms for dengue epidemics, the predictive capacity of EWARS need to be improved. Calibrating and integrating multiple spatially related indicators and improving thresholds in the definition of an alarm signal, could result in a promising capability of timely disease outbreak prediction.

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Tracking the emergence and spread of new dengue virus variants in Puerto Rico using genomic surveillance

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***Abstract*—** An increase in transmission of the four serotypes of dengue virus (DENV-1-4) has been reported recently in the Americas after a period of low transmission or absence in some areas, including Puerto Rico. The emergence of the Cosmopolitan genotype of DENV-2 in South America, as well as the American variant II of genotype III DENV-3, and the potential to spread across the continent, underscore the need to strengthen surveillance and monitor variants that could present a risk to public health. We developed an amplicon-based DENV-1-4 sequencing method using Illumina technology to integrate genomics into the existing laboratory surveillance program. More than four hundred complete genomes,

including DENV-1, DENV-2, and DENV-3, were sequenced in near real-time from human and mosquito surveillance collected in Puerto Rico and the region between 2019 and 2023. To understand the genomic diversity and evolutionary dynamics affecting Puerto Rico, we reconstructed recent transmission using phylogenetic inference in a regional context. Our analyses revealed that the emergence of the three DENV serotypes in Puerto Rico was caused by virus importation events that progressed to sustain transmission and replace the variants that circulated previously. The emergent DENV-1 is phylogenetically related to contemporary viruses circulating in the Caribbean but distinct from other endemic variants. Wide-spread transmission of this new variant was confirmed in humans and mosquitoes between 2019 and 2023. Similarly, a new variant of the Southeast Asian-American genotype of DENV-2 from South America was first detected in mosquito pools collected in 2021 and continues to circulate across the island. The emergent DENV-3 detected in 2022 is closely related to the American variant II of genotype III that caused the 2022 epidemic in Cuba. This new variant has been recently detected throughout the region, including southern areas of the United States. Our analyses also inferred that the three serotypes circulated on the island before detection by laboratory surveillance, suggesting that cryptic transmission occurred for a period of at least three months. This study highlights the effectiveness of genomic surveillance and epidemiology to enable early detection, tracking the dispersal of imported variants, and to inform public health responses.

Virology – Pathogenesis – Antivirals

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Placenta specific Chromosome 19 microRNA Cluster has antiviral effect against Zika Infection

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***Abstract*—** The largest primate and placenta specific C19MC microRNA cluster has been implicated in the development and function of the placenta, and having an antiviral activity for specific viruses. The 2015 outbreak of the emergent flavivirus Zika in the Americas was associated with microcephalia and other congenital malformations. The risk of having congenital Zika was higher at the beginning of pregnancy, when the expression of C19MC miRNA cluster was low, and then increases during pregnancy and is the highest at term. We hypothesized that, through its antiviral activity, C19MC miRNA could be implicated in the placenta protection of the foetus during pregnancy. We generated a novel Knock-Out (KO) model in vitro from JAR human placental choriocarcinoma cell line using the CRISPR/Cas9 system to demonstrate the antiviral role of the C19MC miRNA cluster against Zika. Through real time quantitative PCR, western blotting, flow cytometry, and immunofluorescence techniques to detect viral infected cells, we showed that C19MC KO cells infected with the Zika virus are more susceptible to viral infections than their wild-type counterparts. Additionally, the TCID50 assay and flow cytometry analysis showed a significantly higher viral infectivity of the supernatants containing virions released from infected KO cells than from WT cells. This antiviral effect of WT supernatants can be transferred to recipient susceptible cells (Vero cell line) by means of extracellular vesicles, suggesting a paracrine antiviral method of action. In conclusion, our study provides a novel KO-model to assess the complex role of C19MC miRNA cluster during viral congenital infections, and whether it can act, in part, via extracellular vesicles. Clear understanding of this cluster's antiviral role is key to the development of new and more reliable prognostic and therapeutic tools for Zika virus and other viruses with a potential congenital effect.

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A Genomewide RNAi screen reveals common host-virus gene

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***Abstract*—** Dengue is the most common mosquito-borne viral disease that in recent years has become a major international public health concern. Dengue is a tropical neglected disease with increasing global incidences, affecting millions of people worldwide, and without the availability of specific treatments to combat it. The identification of host-target genes

essential for the virus life cycle, for which effective modulators may already exist, would provide an alternative path to a rapid drug development of the much needed anti-Dengue agents. For this purpose, we performed the first genome-wide RNAi screen, combining two high content readouts for DENV infection (FLUO) and host cell toxicity (NUCL), against an arrayed lentiviral based shRNA library covering 16,000 genes with a redundancy of at least 5 hairpins per gene. The screen identified 1,924 gene candidates in total; of which, 1,730 gene candidates abrogated Dengue infection, while 194 gene candidates were found to enhance its infectivity in HEK293 cells. A first pass clustering analysis of hits revealed a well orchestrated gene-network dependency on host cell homeostasis and physiology triggering distinct cellular pathways for infectivity, replication, trafficking and egress; a second analysis revealed a comprehensive gene signature of 331 genes common to hits identified in 28 published RNAi host-viral interactions screens. Taken together, our findings provide novel antiviral molecular targets with the potential for drug discovery and development.

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ABSarbo: Aptamer-based Strategies To Create Novel Biotechnological Tools Against Arboviruses

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***Abstract*—** ABSarbo is a new international hub supported by the EU-LAC foundation where several laboratories coming from 6 countries are joining effort with the aim to develop new Aptamer-based strategies and create novel biotechnological tools against Arboviruses. Infections by Arthropod-borne virus (arbovirus) represent nowadays a significant global health problem. In recent years, the world has experienced several epidemics produced by arboviruses. Some of these viruses, such as Dengue, were already known for a long time while others have recently emerged to become a huge health threat like Chikungunya, Zika or Mayaro viruses. In fact, over 100 viruses cause infections and lead to what is globally termed as Arboviral diseases in humans, which annually produce more than 1 million deaths worldwide. Being spread from humans to arthropods, including mosquitos, ticks and flies, these viruses could be potentially transmitted from several vectors to humans triggering the next hemisphere-wide epidemic. Furthermore, the geographical spread and abundance of transmitting vectors, influenced by the consequent climate changes, area of great concern to public health organizations in EU-LAC. All scenarios point to the need of creating new strategies to anticipate the occurrence of a possible new pandemic. Among those, two are of greater importance to enhance our preparedness: i) easy and rapid to deploy Arbovirus detection assays; and, ii) therapeutic/pharmacological candidate at advanced pre-clinical stages. In this context, ABSarbo is focused on the implementation of preventive strategies to rapidly address these emerging diseases by applying state-of-the-art and multidisciplinary aptamer-centered techniques in order to anticipate and minimize arboviruses' impact worldwide, and to develop diagnostic methods able to differentially recognize the most dangerous and abundant arboviruses found in our regions, as well as new treatment opportunities.

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An Ivermectin – Atorvastatin combination impairs host nuclear

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Abstract— Flaviviruses such as Zika (ZIKV) and Dengue (DENV) viruses use cellular nuclear transport machinery to import some proteins into the nucleus. Recent studies have shown that Ivermectin (IVM), an FDA-approved drug, inhibits the nuclear localization of DENV NS5 polymerase through inhibition of the Importin $\alpha/\beta1$ pathway, affecting viral replication. Nevertheless, how this treatment might affect other nuclear-localized viral proteins is not yet known. Atorvastatin (ATV), another FDA-approved drug, can also modulate nuclear transport, but the impact of this cell mechanism on flavivirus infection is poorly understood. Hence, this study aimed to evaluate the effect of treatment with IVM and ATV on nuclear transport during DENV-2 infection. Our findings prove that the nuclear import of NS3 is crucial to replicating DENV-2 since its inhibition prevents the degradation of nuclear pore complex proteins and reduces viral infection. On the other hand, in vivo assays of the combined IVM+ATV treatment increased infected mice AG129 survival time, proving to be a potential antiviral therapy against DENV infection.

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Analysis of the in vitro infectivity of extracellular vesicles from DENV-infected C6/36 mosquito cells

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Abstract— Dengue virus (DENV) is a significant arbovirus transmitted by Aedes mosquitoes with endemic and epidemic transmission cycles that make it a public health problem. The pathogenesis of DENV has been divided into 3 phases: febrile, critical, and convalescent, which in some cases can reach the fatal stage called DENV hemorrhagic fever (DHF). Mechanisms have sought to explain the ability of the virus to trigger DHF, where extracellular vesicles (EVs) are candidates to help elucidate this problem. EVs with heterogeneous content are constantly released by cells as a means of cell-cell communication. They carry proteins and viral genetic material, which could promote cell infection. Depending on the size and protein markers, the classification is into long extracellular vesicles or exosomes (LEVs) and small extracellular vesicles or exosomes (SEVs). LEVs are vesicles > 200nm with histone and Annexin V proteins. Meanwhile, SEVs are <200nm with HSP70, flotillin, and tetraspanins proteins. In the specific case of DENV, it has not been demonstrated that these EVs can play a relevant role in viral infection. With immunoprecipitation, electron microscopy, and confocal microscopy, we will discuss the experimental characterization of LEVs and SEVs secreted by C6/36 mosquito cells infected with DENV serotype 2 (DENV2) and the ability of SEVs to generate infection in mammalian cells.

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Annexin A1-FPR2|ALX Signaling Axis Regulates Acute Inflammation During Chikungunya Virus Infection

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Abstract— Chikungunya (CHIKV) is an arthritogenic alphavirus that causes a self-limiting disease usually accompanied by joint pain and/or polyarthralgia with disabling characteristics. The immune responses developed during the acute phase of CHIKV infection determine the rate of disease progression and resolution. Annexin A1 (AnxA1) is involved in both initiating inflammation and preventing an over-response, making it essential for achieving a balanced

end to inflammation. In this study, we investigated the role of the AnxA1-FPR2/ALX pathway during CHIKV infection. Genetic deletion of AnxA1 or its receptor (FPR2) enhanced inflammatory responses driven by CHIKV. These knockout mice exhibited increased neutrophil accumulation and augmented hypernociception and tissue damage compared to control mice. Conversely, treatment of wild-type animals with the AnxA1 mimetic peptide (Ac2-26) reduced neutrophil accumulation, decreased the local concentration of inflammatory mediators, and alleviated mechanical hypernociception and paw edema induced by CHIKV infection. Changes in viral load were minimal both in genetic deletion and with treatment. Overall, our data suggest that targeting the AnxA1-FPR2/ALX pathway could serve as a potential therapeutic strategy to control acute inflammation and polyarthralgia during CHIKV infection. Indeed, should ideally be used in combination with antiviral drugs.

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Antibody-Dependent Complement Activation And DENV3 Disease Severity

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***Abstract*—** Dengue Viruses (DENV), consists of a family with four serotypes. Dengue is a mosquito-borne agent responsible for causing Dengue Fever (DF), which in a small proportion of cases can further develop into a more severe disease, dengue hemorrhagic fever (DHF), characterized by blood plasma leakage, that can lead to cardiovascular shock and organ failure. Dengue disease overall is an inflammatory-driven pathology, with complement dysfunction having been implicated as playing a role in the progression to DHF. The extent to which antibody-dependent complement activation (ADCA) participates in this process is not fully known. To investigate the ability of dengue antibodies specific to DENV3 NS1 to perform ADCA using a novel bead-based complement assay. In this assay, DENV3 NS1 is bound to fluorescent beads to incubate with patient samples, allowing for the formation of immune complexes. The beads are then incubated with a complement source, allowing for immune complexes to perform ADCA. The beads are then stained with an anti-complement factor 3 (C3) fluorophore-conjugated antibody, and flow cytometry is used to quantify the deposition of C3 fragments. This assay was utilized to quantify ADCA in serial samples from acute and convalescent cases of primary and secondary DENV3 cases, from both DF and DHF. Additionally, antibody endpoint titers were measured by ELISA to determine correlation with ADCA. When comparing the ADCA capacity in the cohort, it is determined that secondary DENV3 infections have higher complement deposition than primary DENV3 infections and that secondary infections with DHF develop the most potent antibodies to activate complement. Antibody titer was shown to have a moderate correlation with C3 deposition and ADCA, as expected. Data utilizing the complement assay supports the hypothesis that secondary DENV3 infections show greater complement deposition, particularly in secondary DENV3 DHF cases during the onset of severe symptoms. Secondary DF cases show the greatest complement activation during the convalescent phase. Further work will be done with this assay to further explore the potential relationship between ADCA and severe outcomes of dengue.

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Antiviral Activity Of Native Plant Extracts From Colombia Against Vector Borne Viruses (DENV, ZIKV And CHIKV)

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***Abstract*—** Arboviruses such as DENV, ZIKV, and CHIKV represent a significant global public health concern due to the absence of specific treatments. Natural products have emerged as valuable sources of pharmacologically active compounds; however, the antiviral potential of Colombia's rich biodiversity remains largely unexplored. In this study, we assessed the in vitro antiviral activity of eight extracts and their components obtained from native plants of the Solanaceae family, found in Colombia's coffee region. Initially, we determined a non-toxic concentration range for antiviral assays using serial dilutions (7.8 µg/mL to 500 µg/mL) in Vero cells, employing the MTT method. Subsequently, we evaluated the antiviral activity against CHIKV/Col, ZIKV/Col, and DENV-2/S16803 in Vero cells, quantifying the infectious

viral particles through supernatant plating. Solanum ovalifolium (CC50 of 300.81ug/mL) exhibited the highest antiviral activity compared to the other methanolic extracts at a concentration of 62.5 µg/mL, demonstrating complete inhibition against all three arboviruses (100% inhibition of infection). We then fractionated the methanolic extract of this plant based on polar affinity and characterized the molecules present in the most active fraction according to the selectivity index (SI), which was calculated by dividing the cytotoxic concentration (CC50) by the antiviral concentration (EC50). The selectivity index values obtained were 39.08 for DENV, 77.26 for ZIKV, and 44.67 for CHIKV. Subsequently, we conducted LC-MS analysis, which revealed the presence of spirostanol/furostanol/spirosolane-type compounds. Furthermore, we isolated and identified two steroidal saponins, coded as E3F3 and S01, which displayed significant inhibitory effects in vitro against DENV (34.8% and 64.08% inhibition, respectively), ZIKV (93.4% and 41.43% inhibition, respectively), and CHIKV (84.95% inhibition for S01). These findings underscore the potential of natural products derived from Colombia's biodiversity in the search for effective antiviral therapies against arboviruses.

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Antiviral effect of different metformin and phenformin analogs

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***Abstract*—** Dengue is the disease caused by dengue virus (DENV), and is associated with significant morbidity, mortality and economic cost worldwide. Despite its relevance, there is currently no specific, safe and effective pharmacological treatment against this disease. Because of this, it is necessary to continue the search, identification and development of new compounds with therapeutic potential. One of the strategies used to achieve this consists of the production of drugs that target host factors that are key to the viral replicative cycle and the pathogenesis of the disease. Our group was able to demonstrate the anti-DENV effect of metformin in both in vitro and in vivo models. However, the therapeutic activity of metformin may be limited by its pharmacokinetics. In this sense, the development of new structural and functional analogues of metformin and phenformin, which seek to improve the lipophilicity of metformin, as well as its physicochemical, pharmacokinetic, pharmacodynamic and toxicological properties, was achieved. In the present work, the effect of 10 different metformin and phenformin analogues on DENV-2 infection was analyzed, all of which showed antiviral effect. The selectivity index was also determined as a predictor of the therapeutic potential of the compounds, where it was found that the compounds named EGL-2, MCC-1 and MCC-6 showed a better cytotoxicity profile and efficiency of inhibition of the infection. Finally, a comparison of the analogues with metformin was performed using various infection parameters, thus showing that; EGL-1, EGL-2, EGL-4, EGL-7 and MCC-3 possess better anti-DENV effect in at least one parameter. Taken together, these results demonstrate that metformin and phenformin analogues are effective antiviral agents in inhibiting DENV-2 infection in Huh-7 cells. Furthermore, the EGL-1 and EGL-2 analogs possess the best therapeutic potential and may represent an alternative to metformin, the only biguanide currently available.

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Antiviral Roles Of Sphingolipid Metabolism

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***Abstract*—** Flaviviruses have previously been shown to alter host cell lipid metabolism to support the extensive membrane morphological changes they induce for virus replication complex assembly. Ceramide, a molecule that belongs to a class of bioactive signaling molecules called sphingolipids, is elevated in both human and mosquito hosts during infection with dengue virus, serotype 2 (DENV2). This sphingolipid forms both a structural component of host cell membranes and is also utilized in cellular signaling pathways. Ceramide is produced by three pathways: the sphingomyelin pathway, the de novo biosynthesis pathway, and the salvage pathway. Alterations in in these pathways have been associated with pathology resulting from flaviviral infections. Preliminary loss of function studies of enzymes in the sphingolipid pathway in human hepatoma (Huh7) cells have identified that 3-Ketodihydrosphingosine Reductase (KDSR) and UDP Glycosyltransferase 8 (UGT8), involved in the synthesis of glycosylated ceramides are antiviral. In addition, each enzyme has been shown to bind to flaviviral non-structural protein 4A (NS4A) in affinity purification-mass spectrometry analysis. We hypothesize that flaviviruses alter expression of enzymes involved in sphingolipid

metabolism to confer an advantage over the host. We validated the loss of function studies using siRNA knockdown in human hepatoma (Huh7) cells and human lung adenocarcinoma (A549) cells followed by infection with dengue virus, serotype 2, and assessed the effects on viral replication. We also overexpressed each enzyme in Huh7 and A549 cells followed by infection with DENV2, and showed a decrease in viral replication, confirming the antiviral phenotype. Confocal microscopy was used to assess co-localization of KDSR and UGT8 with flaviviral nonstructural proteins. We are currently investigating the antiviral mechanisms of these host proteins. These studies will help deepen our understanding of the mechanisms flaviviruses employ to gain an advantage over the host cell during infection.

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Arbovirus Transmission And Disease Pathogenesis In Insulin Resistance And Obesity Murine Model

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Abstract— In the last century, anthropogenic factors such as human movement to new ecotypes, agricultural expansion, and uncontrolled urbanization, have significantly contributed to zoonotic emergence and spillover at a global scale. Arthropod-borne viruses (arboviruses), maintained in nature through transmission cycles involving hematophagous arthropod vectors, are the most important contributors of disease and a public health concern worldwide. Concomitantly, another contemporary public health concern is the prevalence of chronic underlying conditions. According to the World Health Organization (WHO), chronic diseases kill approximately 41 million people each year, with cardiovascular disease, cancer, respiratory diseases, and diabetes accounting for the most deaths. Strikingly, clinical data indicate that patients with preexisting conditions such as diabetes infected with mosquito-borne viruses are prone to severe disease outcomes and mortality. It is reasonable to hypothesize that such conditions will impact the progression of arboviral replication and transmission in and from human hosts. In the present study, we aim to determine how pre-existing obesity/Type 2 diabetes Mellitus (T2DM) alters the pathology, immune response, and kinetics of an arboviral infection. 10-week-old leptin receptor mutant LEPRdb/db, LEPRdb/WT, and wild type C57BL/6J mice were pretreated with IFNAR blocking antibodies to render them permissive to viral infection and then, exposed to Aedes aegypti mosquitoes infected with mayaro virus (MAYV). Following infection, animals were followed for 5 days to assess viral titers, tissue pathology, as well as immune responses. The model demonstrated a predictable pattern of viremia with titers starting to increase at two days post infection (dpi) and the highest titers occurring at 4 dpi. Higher viral titer was observed for C57BL/6J control mice at 2dpi. Most importantly, this model did not demonstrate significant differences in the viral titer between the different genotypic backgrounds at peak viremia. This lack of significant difference in viral titers suggests that different MAYV infection outcomes among the three genotypes might be related to tissue damage and immune response. Further histopathological and cytokine analysis must be conducted to analyze the differences in the MAYV infection outcome related to T2DM and the wild type genotypes.

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Biomarker Signature In Patients With Arthralgia During Acute Chikungunya Virus Infection

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Abstract— Chikungunya fever is currently causing severe outbreaks across endemic areas and therefore represents an eminent public health threat to Brasil. Chikungunya virus (CHIKV) is an arthropod borne pathogen that can cause a viral disease characterized by fever and severe joint pain. Arthralgia after several months of infection is a unique feature of this viral infection, which is difficult to manage at long term. The reasons for the acute clinical course even without the presence of virus is still unknown, however, the interaction between the virus and the host may play an important part during acute infection. Therefore, the present study aimed at evaluating soluble systemic biomarkers during acute Chikungunya virus infection according to age subgroups and their network connectivity. For this purpose, patients with Chikungunya fever from the state of Rio Grande do Norte were investigated. A total of 76 patients (25 men and 51 women) testing positive for CHIKV by RT-qPCR were included in the study and serum samples were collected within the first few days of symptoms onset. The highest viral load was found on the first day of infection. Healthy controls matched for age and sex (n=161) were also included in the study. The Luminex assay was performed for the detection of chemokines, pro-inflammatory, regulatory cytokines and growth factors. Our results demonstrated a robust cytokine storm composed of strong chemokine subnets in both children and elderly acute CHIKV patients. Furthermore, a tightly connected pro-inflammatory net was observed in elderly patients as compared to children. While Adult and Elderly patients presented balanced proinflammatory/regulatory networks, children presented weakly connected regulatory subnets. Subnets of proinflammatory biomarkers of elderly presented a unique hotspot of IL-17 hyperconnectivity as compared to children and adults. All in all, the systems biology analysis reveals unique patterns of CHIKV infection in patients of different ages, which brings insight to future tailor-made age-specific therapeutic interventions for acute CHIKV infection.

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Cannabidiol Alters Cellular Membranes With An Impact On The Zika Virus Replication

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Abstract— Cannabidiol (CBD), the main non-psychoactive cannabinoid of the Cannabis sativa plant, is a powerful antioxidant compound that in recent years has increased interest due to causes effects in a wide range of biological functions. Zika virus (ZIKV) is a virus transmitted mainly by the Aedes aegypti mosquitoes, which causes neurological diseases, such as microcephaly and Guillain-Barre syndrome. In this work, we explored the potential antiviral activity of CBD in relevant cellular targets during ZIKV infections. For this, cell cultures were infected and treated for 48 h with CBD (0.6-10 µM). Viral titers of the supernatants were determined and the relative expression of the ZIKV genome was measured by RT-qPCR. The results showed that CBD exhibit a potent antiviral activity against ZIKV in different cell lines

with EC50 values ranging from 0.87 to 5.12 µM. In addition, a reduction in the expression of the intracellular ZIKV genome was observed at concentrations of 5 and 10 µM. Furthermore, we evaluated the CBD antiviral activity against dengue, yellow fever and chikungunya arboviruses; the EC50 in Vero cells were 2.02, 2.13 and 4.63 µM, respectively. To study the possible immunomodulatory role of CBD, Huh-7 cells were exposed to 10 µM CBD during 48 h. CBD-treated cells exhibited a significantly IFN-β increased levels, meanwhile, IL-6 and IL-8 were not induced. Due to cell membranes are a key component in all steps of the ZIKV replication cycle, we study the effect of CBD on the membranes. For this, an immunofluorescence assay was performed, in which cell membranes were labeled with wheat germ agglutinin (WGA). We determined that CBD affects cellular membranes due to a higher fluorescence intensity was observed in CBD-treated cells and lowers intracellular cholesterol levels, which were measured by a fluorometric method. Finally, we demonstrate that CBD inhibits other structurally dissimilar viruses, suggesting that this phytochemical has broad-spectrum antiviral activity.

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Characterization of Mayaro and Una virus infection of an SH-SY5Y human neuroblastoma cell line

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Abstract— The Mayaro (MAYV) and Una (UNAV) viruses are emerging pathogens that belonging to the Alphavirus genus (Togaviridae family) and are transmitted mainly by sylvatic Haemagogus sp. and Psorophora sp. mosquitoes, respectively. MAYV causes a febrile illness associated with long-lasting polyarthralgia, while the symptoms produced by UNAV infection are not defined. Currently, there is limited knowledge about the biology and pathogenesis of these viruses, particularly cell tropism. Although MAYV and UNAV are believed to be arthritogenic viruses, MAYV has recently been shown to propagate efficiently in different human brain cells, including astrocytes, pericytes and neuronal progenitor cells. Furthermore, our laboratory has observed that both MAYV and UNAV are capable of replicating in human microglia cells (unpublished results). These data suggest that these viruses could be implicated in neurological pathogenesis. Therefore, the aim of the present study was to evaluate the potential use of an SH-SY5Y human neuroblastoma cell line as a neuronal model to characterize MAYV and UNAV infection. First, cell viability of MAYV- or UNAV-infected SH-SY5Y cells was determined using the MTT method. Then, the cytopathic effects of MAYV or UNAV infection were evaluated by microscopy. The production of MAYV or UNAV viral particles was also quantified using a plaque-forming assay. Lastly, the expression of viral E1 and nsP1 proteins from MAYV- or UNAV-infected SH-SY5Y cells was analyzed by Western blot. Our results indicate that MAYV and UNAV infection significantly decrease the viability of SH-SY5Y cells and provoke strong cytopathic effects as revealed by our microscopy analysis. Furthermore, we observed a significant time-dependent increase in MAYV and UNAV viral titers for all the multiplicities of infection tested. Finally, we detected a strong expression of MAYV and UNAV E1 and nsP1 viral proteins at 48 hours after infection. Taken together, these findings indicate that SH-SY5Y cells are highly susceptible to MAYV and UNAV infection, suggesting that this cell line could be a useful neuronal model to study the pathogenesis and immune response to these infections. However, more detailed studies are required to further understand the interaction between these viruses and SH-SY5Y cells.

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Development Of A Novel 2-Pyrimidone (SRI-42718) As A Potent Inhibitor Of Chikungunya Virus Infection And Disease

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Abstract— Alphaviruses are arthropod-transmitted RNA viruses, including the NIAID Category B priority pathogens Eastern equine encephalitis (EEEV), Venezuelan equine encephalitis (VEEV) and chikungunya (CHIKV) viruses. In addition, other re-emerging alphaviruses such as Mayaro (MAYV), o'nyong-nyong (ONNV), and Ross River (RRV) viruses represent significant public health concerns. Currently, no FDA-licensed vaccines or antiviral therapeutics are available to prevent or treat alphavirus infection or disease. We identified a small molecule antiviral hit using a screen against CHIKV. Derivatives of the hit were made by medicinal chemistry and we identified a first-in-class, orally available, non-nucleoside small molecule (SRI-42718) that targets a conserved region in nsP4-RdRp. While the binding pocket for this compound is proximal to the catalytic domain of the RdRp, the compound is synergistic with nucleoside inhibitors. The SRI-42718 chemical series blocks both gRNA and sgmRNA synthesis as well as viral protein production. SRI-42718 has limited antiviral breadth (CHIKV) but other chemical analogs in this series have potent activity against all tested alphaviruses (MAYV, UNAV, ONNV, RRV, VEEV and EEEV). SRI-42718 has acceptable solubility and stability in mouse, monkey and human microsomes. The compound has shown no adverse toxicity in mice as repeat dosing at 40 mg/kg, TID, is a well-tolerated treatment for up to 10 days. In vivo PK analysis indicates that the compound has good bioavailability by oral delivery in mice and nonhuman primates, and the compound was distributed to a number of mouse tissues including joints and muscles. Importantly, oral administration of the compound prevents viremia at a dose of 40 mg/kg three times per day (TID) in acutely infected mice. Viral tissue burden and virus-induced foot/ankle swelling (tissue disease) are also significantly reduced in treated animals compared to vehicle controls. A seven-day treatment of mice beginning at 28 days post infection during the persistent phase reduced the viral RNA level in joint-associated tissue. Combined, our data indicate that SRI-42718 is capable of blocking CHIKV replication in vivo during both the acute and persistent phases, which increases the therapeutic potential for this compound. SRI-42718 promises to be an important preclinical candidate and the compound has entered early drug development studies.

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Differences in antiviral responses between Huh-7 and Vero cell lines treated with different dengue virus inhibitors

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Abstract— Dengue is the most important arthropod-borne viral infection of humans. This disease is caused by a single-stranded positive RNA virus belonging to the Flavivirus genus (Flaviviridae family). According to Bhatt et al. (2013) estimated 390 million dengue infections per year based on cartographic approaches (WHO 2009). The DENV genome is a single-positive strand RNA, which is approximately 11kb in length, it single ORF encodes three structural proteins: Capsid (C), membrane (M) and envelope (E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Both structural and non-structural proteins can be potential targets for antiviral intervention. We conducted a comparative study of the antiviral activity of a panel of nine antiviral molecules (ST-148, Celgosivir, ST-610, Ivermectin, NITD-618, 2'CMC, 7D-2'CMA, Ribavirin, T-1105), with has been proven in vitro for anti-dengue virus activity and that act at different stages of the dengue viral life cycle. Their antiviral activity was determined through viral CPE reduction, qRT-PCR and plaque assays. Likewise, the effect of these compounds on cell viability was evaluated by microscopic observation and ATP-lite assays. Both, the human hepatoma cell line Huh-7 and the cell line Vero from African green monkey were used to set up these antiviral assays. In the present study, both Huh-7 and Vero cell lines were sensitive to DENV2 infection, and all compounds were active against DENV2. However, EC50 and CC50 values obtained for each compound and each method showed differences between these cell cultures. In addition, DENV2 response in these cell lines to all compounds did not show a pattern, that is to say none of the cell lines showed to be more sensitive to the action of all compounds against the virus. These variants might be associated to intrinsic heterogeneity in the drug sensitivity of the two cell lines, as well as genetically determined host factors that may affect downstream events and therefore, the specific manner in which DENV2 proteins are processed by Vero or Huh-7 cells, and the antiviral activity of the compounds that were evaluated. This dataset may represent a reference panel to compare the activity of molecules not yet discovered.

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Evaluation Of Amino Acid Determinants Of Reduced Serum Neutralization Of Dengue Type 1 P72-1244

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Abstract— DENV is sustained in two ecologically discrete non-human primate sylvatic and human urban transmission cycles in west Africa and southeast Asia. Sylvatic DENVs form independent clades and are genotypically distinct from other endemic variants within a given serotype, and the full impact of genetic variability on differences in susceptibility to neutralization remains to be assessed. We characterized neutralizing potency of DENV-1 specific primary immune sera against a putative sylvatic DENV1 variant P72-1244 using sera from endemic (n=14) and non-endemic (n=8) subjects 1-30 years post infection, finding that all primary immune sera had reduced neutralization potency with >8-fold differences in neutralization titers between divergent and clinical strains belonging to DENV-1 genotypes IV and V. The main target of neutralizing antibodies is the virus envelope (E) glycoprotein and we identified 4 residues within E that vary between P72-1244 and DENV-1 epidemic strains likely to contribute to differences in the neutralization phen+otypes we observed. To test this hypothesis, we constructed a panel of recombinant DENV-1 West Pac '74 infectious clones containing E residues which vary between DENV-1 strains West Pac '74, Puerto Rico 2006, P72-1244 and Malaysia.36046/05 to recapitulate potential epitope variability between endemic and divergent DENV-1. We interrogated neutralization potency and breadth of endemic and non-endemic primary immune sera against our virus panel using a foci reduction neutralization assay (FRNT50). These results characterize the effects of divergent DENV-1 residue substitutions on viral resistance to neutralization and highlight epitope targets potentially involved in evasion of antibodies elicited by infection with endemic DENV-1.

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Evaluation Of Coinfection Of Dengue And Chikungunya Virus In Primary Culture Cells Model

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Abstract— Dengue (DENV) and Chikungunya (CHIKV) viruses are considered as a global public health threat. DENV causes a broad spectrum of clinical forms, but the severe forms may cause dengue shock syndrome or hemorrhage that can lead to death. Meanwhile CHIKV can cause intense chronic joint pain, probably related with viral persistence. DENV and CHIKV are RNA viruses transmitted to humans through the same vector: Aedes mosquitoes, which are endemic in tropical and subtropical countries. Several studies have reported that Aedes mosquito can transmit both DENV, CHIKV or simultaneously, causing co-infections in humans Co-infections can either facilitate or hinder viral replication and transmission of at least one of the viruses involved and might also affect the immune response of the host. Although DENV-CHIKV co-infection cases have been documented in many countries (including Mexico), little is known about DENV-CHIKV interactions. Thus is it is necessary to addresses the DENV-CHIKV co-infection in skin cells, which are the first cells in contact with the viruses when the mosquito feeds. Therefore, we analyzed the dynamics of DENV and CHIKV infection under two co-infecting scenarios: simultaneously and super infection, using human skin fibroblast as a model. We observed that the viral titers of DENV and CHIKV decreased in both scenarios of co-infection, and it was independent of the cell culture used. Interestingly, CHIKV viral titers were much higher than DENV viral titers, contrary to what has been reported by other researchs. There was also a correspondence with the previous results regarding the number of viral copies, with CHIKV being predominant over DENV in cases of confection. These results suggest a possible suppression of DENV replication by CHIKV.

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Evaluation Of DENV Replication In A Platelet-monocyte Interaction Model

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***Abstract*—** Dengue virus (DENV) and host immune factors are responsible for dengue pathogenesis, that may vary from asymptomatic infection to severe haemorrhagic fever and shock syndrome. DENV can infect different cell types, such as monocytes, macrophages, and endothelial cells. Monocytes can amplify virus replication in antibody-enhanced dengue infection. Platelets from dengue-infected patients are activated, apoptotic, secret inflammatory mediators (PF4, IL-1β and MIF) and express increased P-selectin and phosphatidylserine in their surface. These signals modulate platelet-monocyte interaction and induce inflammatory response by both cells. Recently, it has been shown that platelets sustain DENV genome translation and replication, but not a productive cycle . We aimed to establish and characterize a protocol for dengue replication in primary monocytes and to evaluate if platelets modulate virus replication by transferring newly synthesized viral RNA to monocytes or by regulating monocytes' permissiveness in vitro. Primary human monocytes isolated from healthy donors (HD) by culture plate adhesion were cultured with human serum (HS) or foetal bovine serum (FBS) and infected with DENV-2 without specific antibodies. DENV RNA copies were measured in cell lysate and supernatant by qRT-PCR and, infective viral particles by plaque forming units (PFU) assay. DENV replication was higher in HS-cultured monocytes in terms of PFU and increased linearly overtime. Platelets were isolated from HD, infected with DENV-2 and, after washing out the unbound viruses, cocultured with monocytes. After 24, 48 and 72h of interaction we quantified DENV RNA copies and PFU. We observed increased DENV RNA levels in infected-platelets after 24 and 48h. However, only baseline levels of viral RNA were observed in monocytes after coculture with platelets. Moreover, no infectious viral particles were detected in platelets or cocultures supernatants. Our results demonstrate that platelets do not transfer DENV genome to monocytes in a way it's able to continue viral replication. We will now assess whether platelets modulate the permissiveness of monocytes amplifying or restricting their infection by the virus and, if so, which pathways participate in this modulation.

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Functional And Phenotypical Changes In Circulating Platelets Isolated From Children With Dengue

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***Abstract*—** The Platelets are highly affected by the dengue virus (DENV) infection, and thrombocytopenia is a critical event for diagnosis and following the dengue disease activity. DENV can infect and activate human platelets and surface markers such as the proteins CD41 and CD62P are overexpressing during DENV infection. The relation between the expression and release of platelets-derived factors and the clinical severity of dengue in children is partially known, and it constitutes a limitation for the development of new therapeutic strategies. Here, we evaluated, through multiparametric flow cytometry and ELISA the expression of surface markers and cytokine secretion in primary platelets isolated from children with confirmed DENV infection. First, we compared 3 protocols to purify primary human platelets. The isolation of platelets by ficoll and centrifugation gradient shows the best absolute number, purity, and recovery percentage of these cells. Platelets isolated from children with severe dengue expressed high levels of 62P and CD107a relative to children with dengue, demonstrating increased degranulation activity. The CD40L was secreted by platelets, and this marker was higher in the plasma of children with severe dengue. In summary, platelets-derived factors are increased by DENV infection, and some of them are associated with the clinical severity of the infection in children.

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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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***Abstract*—** 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 Receptor-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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Inhibiting Essential Enzymes In Arboviruses By Combinatorial Biology-Based Strategies

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***Abstract*—** Arboviruses, with a cumulative incidence of 283/100,000 population, pose a substantial epidemiological burden across the Americas. In 2022, the WHO reported over 3.1 million global cases, underscoring the urgent imperative for devising robust strategies to mitigate arboviral pathogenicity. In this context, our investigation seeks to deploy combinatorial biology in the design of inhibitors, targeting critical enzymes implicated in viral replication and immune evasion. Bioinformatic, structural and expression feasibility analysis identified Dengue virus NS5 methyltransferase, and Zika virus NS2B-NS3 and Chikungunya virus nsP2 proteases. All three identified targets are non-structural proteins involved in RNA capping or viral polyprotein cleavage. Through sequential sequence alignment of viral proteomes and structural alignment of available atomic resolution structures, NS5 methyltransferase was found to be the largest and most conserved protein among the four Dengue serotypes with an identity greater than 70%. The proteases exhibited conserved recognition motifs found at the N-terminal residues to the cleavage sites. Based on this information, we are adopting a synergistic approach, amalgamating Phage Display, pharmacophore-based virtual screening, and SELEX for potential inhibitor selection. We will present the candidate molecules, and advances on their in vitro efficacy in inhibiting the target enzymes. Affinity constants, activity and inhibitory mechanisms will be studied by microscale thermophoresis (MST), proprietary FRET based assays, and pre-steady state kinetics. This approach aims to validate the inhibitory potential of the identified molecules, setting the stage for their potential in vivo testing as potential antiviral agents.

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Kinetics Of Mayaro Virus Infection Of New World And Old World Mosquito Vectors

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***Abstract*—** The Mayaro virus (MAYV), a single stranded positive-sense RNA virus that is transmitted by mosquito vectors. MAYV is an Alphavirus belonging to the family Togaviridae. The virus has been classified into three genotypes (D, L, and N); with genotype D having the greatest and only geographical distribution in the Americas and Caribbean. The primary vectors for MAYV in the sylvatic transmission cycle, which includes reservoir hosts such as non-human primates, rodents, and birds, are Haemagogus mosquitoes. However, the virus can also be transmitted in urban transmission cycles by Aedes aegypti and Aedes albopictus. Due to the non-differential presentation of an acute febrile illness, similar to other arboviruses of importance, MAYV infections can cause long-term debilitations in countries such as Brasil, Peru, Argentina, Colombia, and Venezuela. MAYV has also been shown to be

transmissible by Anopheles mosquitoes; further complicating options for vector control interventions. In general, MAYV infection kinetics of mosquitoes after 7 days post-infection (dpi) can support potential horizontal transmission to new vertebrate hosts. To understand the potential expansion of the virus across the Atlantic Ocean, we tested if MAYV (Uruma strain, genotype D) infection kinetics differs between Old World (Anopheles gambiae, KEELE) and New World (Anopheles albimanus, CMAVE/El Salvador) anophelines, as compared to infections in Aedes aegypti (Orlando) mosquitoes from Florida. We were surprised to observe that MAYV disseminated infection of An. albimanus was rapid (2 dpi), which also translated to a high prevalence of infection, as compared to Ae. aegypti. Importantly, we also detected the virus in ovaries, indicating a high potential for vertical transmission and persistence in the environment. To examine this comparable to previous reports, our data corroborated with the theory that An. albimanus can transmit the virus (saliva) after only 2 dpi with a prevalence of 26%, this increases to 80% after 7 dpi. The same occurs in ovaries, wherein infection rates increased from 80% (2 dpi) to 100% (7 dpi). We observed that An. albimanus represents a high risk of transmitting MAYV. We are currently working on proving vertical transmission and measuring the immune response as a key to these differences.

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Modulation Of RHOA GTPase In CD1 Mouse Glial Cells During Zika Virus Infection

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***Abstract*—** Zika virus (ZIKV) belongs to the Flaviviridae family, which includes viruses of medical concern such as dengue virus, both of which are transmitted by arthropod vectors. Currently, there is no vaccine or antiviral treatment against these viruses, which circulates mainly in tropical areas. ZIKV has a tropism for the Central Nervous System, causing Guillain-Barré syndrome in adults and congenital problems such as microcephaly in newborns. In this sense, it has been reported that viruses of the same family, such as the Japanese encephalitis virus (JEV), can alter the RhoA GTPase pathway, which is involved in the signaling of the immune response and in the remodeling of the cytoskeleton. Moreover, the importance of RhoA in the pro-inflammatory and anti-inflammatory profile of microglia cells in neurodegenerative diseases has been described. Therefore, in this study RhoA activity was evaluated during ZIKV infection caused in primary cultures enriched with astrocytes and microglia from CD1 mice. Our results demonstrate overexpression and overactivation of RhoA GTPase during ZIKV infection. Likewise, inhibition of this pathway resulted in decreased viral replication, suggesting that the RhoA pathway may be a therapeutic target during ZIKV infections.

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Multiple Flaviviruses Secrete The Anti-immune Subgenomic Flaviviral RNA In Mosquito Saliva

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***Abstract*—** Among these, West Nile virus (WNV) and Zika virus (ZIKV) together cause about 30,000 symptomatic infections, 50% of them neuroinvasive. There is no efficient treatment nor vaccine against WNV and ZIKV. Epidemic control is restricted to vector interventions, which strategies have little efficacy and sustainability over time. By improving our understanding of the molecular determinants of viral transmission, we will promote the development of new control methodologies. Recently, dengue virus was shown to secrete an anti-immune subgenomic flaviviral RNA (sfRNA) within extracellular vesicles in mosquito saliva to enhance skin infection and thus transmission. Although all flaviviruses produce sfRNA with anti-immune functions, it remains to determine whether the salivary secretion of sfRNA to increase transmission is a conserved mechanism across flaviviruses. We infected Culex quinquefasciatus mosquitoes with WNV-IS98 through oral-feeding. We detected WNV-sfRNA in saliva. To account for the level of infection, we calculated the sfRNA:gRNA ratio (706). To determine whether midgut infection influences the amount of sfRNA, we infected mosquitoes by intrathoracic inoculation. We observed that the sfRNA remained secreted in the was lower (ratio 24.1). To find out if the mosquito species influences the salivary secretion of sfRNA, we inoculated a non-vector of WNV, Aedes aegypti, with WNV-IS98. We observed that sfRNA is similarly

secreted (ratio at 3.22). To assess whether other flavivirus secrete sfRNA into saliva, we infected A. aegypti with ZIKV by intrathoracic inoculation. We detected sfRNA in saliva (ratio 273). Salivary sfRNA could be secreted within extracellular vesicles, which can transfer their cargo to human cells. We quantified sfRNA resistance to RNase with or without triton X-100 permeabilization and found that part of the sfRNA was protected from degradation by a triton-sensitive membrane. We are now studying the increase in infection in human cells by salivary sfRNA that modifies the immune response. We suggest the universality of sfRNA secretion in saliva and we propose a new target for the design of control strategies.

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Multi-tissue transcriptomic study in severe dengue disease: In silico analysis of potential drugs for its management

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***Abstract*—** Dengue is a major global health concern given the increase on cases and fatalities, and virus and vector dispersions. Here we analyzed transcriptomic data in the key tissues of liver, spleen and blood profiles. Gene expression evaluation was done by Next Generation Sequencing, using Ion AmpliSeq Transcriptome Human Gene Expression Kit (10.8 million reads per sample) in liver, and spleen of dengue fatal cases and blood tissues of dengue severe cases. It was found upregulation of immune components pathways, like phosphatidylinositol 3-kinase binding, inflammasome and MHC complex; and metabolic components pathways, like insulin-like growth factor binding and cholesterol-binding proteins. It was observed downregulation of STAT protein phosphorylation pathways, RIG I like and type 1 IFN receptor binding, and liver metabolism. Despite transcriptomic differences due to tissue specialization, the common mechanisms of action "Adrenergic receptor antagonist", "ATPase inhibitor", "NFkB pathway inhibitor" and "Serotonin receptor antagonist" were identified as druggable to oppose the effects of severe dengue infection in the studied tissues. These are good candidates for future functional evaluation and clinical trials. Transcriptomics, proteomics and pathogen-host interactomics data is being explored for in silico informed-selection of drugs, prior to its functional evaluation. The effectiveness of this kind of strategy has been put into test in the current COVID-19 pandemic, and it has been paying off, leading to a few drugs being rapidly repurposed as treatment against SARS-CoV-2 infection. Several neglected tropical diseases, for which treatment remains unavailable, would benefit from informed in silico investigations of drugs, as we tried to perform in this work for dengue fever disease.

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NS5 Protein Of DENV-2 Localizes To The Nucleolus And Interacts With Nucleolar Protein B23

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***Abstract*—** The non-structural 5 protein (NS5) is a multifunctional molecule in all flaviviruses. This molecule posses a RNA polymerase activity and participate in RNA Capping. Furthermore this protein is also involved in IFN I blocking. In some flaviviruses, such as DENV serotype 2, this protein is located mainly in nucleus of infected cells, so far, the function of the nuclear form of NS5 is not totally elucidated. In order to provide more information on the role of NS5 protein in the nucleus. We evaluated by IF assays in a HMEC-1 cells model a co-localization of NS5 with nucleic protein LMNA/C and B23, which is nucleolus molecule. Our results revealed a clearly co-localization between NS5 and B23 to evaluate the possible effect of these interactions in the viral life cycle or the host cell. We performed a docking assays, to know the possible interaction sites, first with monomeric NS5 and B23, yielding 15 possible interaction sites all of them on the MTase domain of NS5, another docking was performed with pentameric B23, as in nature, 18 possible interaction sites were determined, again all of them on the NS5 Mtase domain and 3 chains of B23. Given this information we proceed to produce recombinant B23 and NS5 MTase domain, and we

make a surface plasmon resonance analysis, using B23 as the immobile phase and Mtase as the mobile phase, the Kd was determined using two models, Langmuir and two state reaction model, yielding both of them a Kd from the order of 10-9. The exact mechanism through which NS5 and B23 interact remains unknown to this study, we found that NS5s mainly localized in the nucleus of infected cells and specifically appears to target the nucleolus, and MTase domain alone is able to interact with B23 at least in Vitro, further investigation on mechanisms an functionality are required to get to know the advantages that DENV-2 gets with this interaction.

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Participation of the Kinesin Light Chain 1 (KLC1) protein in the secretion of the dengue virus NS1 protein

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***Abstract*—** The molecular processes underlying the activity of dengue virus (DENV) NS1 protein during infection in vertebrate cells have been extensively investigated; in contrast, the intracellular events taking place within mosquito host cells are less well known. Using a non-biased protein-protein interaction assay (Caraballo et al. J. Virol., 2022. doi:10.1128/jvi.00704-22), we previously identified the participation of kinesin light chain (KLC1) within the NS1 interactome in mosquito cells. The KLC1 is a protein that in association with the microtubule cytoskeleton participates in intracellular transport. Thus, the primary aim of this investigation was to validate such interaction and to understand its functional significance during DENV infection. In addition, we seek to gain further understanding of the NS1 secretion process in mosquito cells. The interaction between KLC1 and NS1 in DENV2-infected C6/36 mosquito cells was corroborated through proximity ligation assays (PLA) and co-immunoprecipitation (Co-IP) assays, in cells processed 24 hpi. To gain insights into the involvement of the tubulin cytoskeleton and its accessory proteins in NS1 transport, C6/36 cells infected with DENV-2 were exposed to non-lethal concentrations of the microtubule polymerization inhibitor colchicine. Supernatants collected at 24 hpi from treated cells showed a 50% reduction ($p \leq 0.05$) in the amount of secreted NS1, quantified by ELISA, in comparison with non-treated controls. These findings suggest that the tubulin cytoskeleton, along with its associated proteins, participates in the mobilization and secretion of NS1 in mosquito cells. Currently, our ongoing investigation aims to further investigate these results within the mosquito host, as well as extend our analysis to vertebrate cells.

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Potent antiviral activity of plitidepsin, a natural marine compound, against Mayaro virus and other arboviruses

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***Abstract*—** Arboviruses are transmitted by arthropods, predominantly mosquitoes and ticks. Mayaro (MAYV) is an arbovirus belonging to the Alphavirus genus (Togaviridae family), and it is an emerging pathogen with increasing circulation in the Americas. Despite the potential public health impact of MAYV and other arboviruses, there are no approved vaccines or treatments to combat these infections, resulting in an urgent need to identify new antiviral drugs. Natural products are a rich source of molecules with diverse biological activities, including antiviral properties. Plitidepsin is a marine peptide isolated from the tunicate Aplidium albicans. This compound is currently approved for treating multiple myeloma in Australia, and recent reports have shown that plitidepsin has substantial antiviral activity against the SARS-CoV-2 coronavirus in vitro and in vivo. However, the ability of this molecule to inhibit the replication of arboviruses has not been tested. The aim of this study was to evaluate the antiviral activity of plitidepsin against MAYV and other arboviruses. First, we analyzed the cytotoxicity of plitidepsin in human dermal fibroblasts, microglia and HeLa cells using the MTT method. Subsequently, we quantified the production of viral particles using a plaque-forming assay with supernatants from cells treated or untreated with plitidepsin and infected with several MAYV strains or other arboviruses including, Chikungunya, Zika and Una. We also evaluated the expression of viral E1 and nsP1 proteins in cells treated or untreated with plitidepsin and infected with the MAYV, Chikungunya or Una using immunoblot. All

tested cell lines demonstrated tolerance to plitidepsin doses less than 10 nM. Plitidepsin reduced MAYV viral titers (between 4 and 5 logs) in a dose-dependent manner for all the MAYV strains or human cell lines tested. More importantly, we observed a decrease in Chikungunya, Zika and Una viral titers in cells treated with plitidepsin. Finally, we found that the expression of the E1 and nsP1 viral proteins from MAYV, Chikungunya and Una alphaviruses was strongly reduced by treatment with this compound. Our results demonstrate that plitidepsin has potent antiviral activity at nanomolar concentrations against all the arboviruses tested, which suggests this compound could represent a potential broad-spectrum antiviral drug.

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Primary Versus Secondary Dengue Virus Infection: Morphological Findings In Organs Of Murine Model

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***Abstract*— Dengue is a systemic viral infection and, although most cases are asymptomatic, severe forms of the disease might include multi-organ involvement, hypovolemic shock and lead to death. Epidemiological studies suggest that severe dengue occurs mainly during a heterologous secondary dengue infection and associated to dengue virus (DENV) serotypes -2 and -3. This study evaluated the impact of primary and secondary dengue infection with a non-neuroadptated clinical DENV strain on the morphological profile of liver and lungs of immunocompetent BALC/cJ mice. At two-month-old, by intravenous route, the animals belonging to the primary dengue infection group (PDIG) were infected with 10^4 PFU of DENV-3 and, mice from the secondary dengue infection group (SDIG) were infected with 10^4 PFU of DENV-3 and, two months later, with 10^4 PFU of DENV-2. Five animals were analyzed by time of infection and the negative control group was MOCK-inoculated. After 3, 7, 10, 14 or 21 days after infection (DAI), mice were euthanized and organs were collected and processed for analysis by bright field microscopy. Histomorphometric parameters included: counting uni/binucleated hepatocytes and measuring the luminal area of liver sinusoid capillaries and the thickness of alveolar septa (30 fields per animal). Most alterations were observed in both groups with variations related to its severity and the time of infection they were present. In the liver, alterations included: vascular congestion, hydropic degeneration, hypoxic necrosis and foci of inflammatory infiltrate. In PDIG, the highest percentage of binucleated hepatocytes occured at 7DAI, and the most dilation of sinusoidal lumen, at 10DAI. These values reached the peak at 10DAI in the SDIG, time in which mice showed most hepatic morphological changes. In the lungs, vascular congestion, recruitment of inflammatory cells, bronchiolar and alveolar hemorrhage were observed and, in SDIG, diffuse hemorrhage occurred at 7 and 10DAI, time in which the thickness of the alveolar septa was three times greater than in the negative control group. In PDIG, the morphological changes in both organs were self-limited. Despite the greater severity of certain alterations observed in SDIG, a degree of recovery from the injuries was observed in mice from the last times of infection.

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Small Extracellular Vesicles (sevs) Produced By Eahy 926 Infected With Denv-2, Induced A Protection Effect On Polarized Non-Infected Eahy 926 Endothelial Cells

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***Abstract*— Introduction**. DENV infection can cause endothelial cell (EC) hyperpermeability and vascular leakage. Small EV of infected EC (sEVIC) may participate in the viral spread and regulate EC response to DENV. Still, there is no information on sEVIC cargo and its effect on non-infected EC. This work aimed to characterize the sEVIC and evaluate

their impact on polarized EC. **Methods**. EA.hy 926 were infected with DENV-2, MOI 1. The virus was removed, and serum-free medium was added. Cell viability (Resazurin, LDH) and viral infection (IFI and PCR for DENV E protein) were confirmed. After 48h post-infection, sEV were isolated (ultracentrifugation) and characterized by NTA, DLS, Western blot, LC/MS/MS, and small RNA sequencing. For function assay, sEVIC were pretreated with neutralizing antibody 4G2 or UV exposed after pretreatment. Later, polarized EC were exposed to the different sEV for 24h and then infected. TEER and permeability (Dextran-blue 2KDa) were measured at different times, and IFI for ICAM, E-sele, and actin were made along with qPCR for DENV detection. sEV non-infected cells (sEVNIC) were used as control. **Results**. EC infection induced a high concentration of EV (2x109) with low ALIX expression. Compared to EVNIC, EVIC had 129 increased proteins (mainly of immune response -IR-), 206 were downregulated (cellular adhesion (CA) and developmental processes), and a viral NS5 peptide was found along with sncRNA like YRNA and miRs that mainly regulate CA and IR genes. Interestingly, miRs found exclusively in EVIC may have a proviral effect. Functionally, EVIC induced ICAM and E-sele expression and a protective effect in the polarized EC that maintained a stable TEER (45Ω) a low permeability and reduced viral replication. **Conclusions**. EVIC transported DENV elements and sncRNA that may regulate viral response. Our model suggests that EVIC induced a protection response during infection, reducing viral replication and activating the EC in the first 24h.

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The Role Of Ubiquitin-proteasome System In The Replication Cycle Of Chikungunya Virus Infection: An In Intro Study

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***Abstract*—** Chikungunya virus (CHIKV) is an arbovirus of the alphavirus family that causes febrile illness accompanied by symptoms such as skin rash, polyarthralgia, and myalgia. Although this virus was first isolated in the 1960s, inter-epidemic periods of more than 10 years and its containment within the African and Asian continents for several decades contributed to the fact that research of this virus was scarce; therefore, many aspects of its replicative cycle and the pathogenesis caused remain unknown. Despite multiple global efforts for the development of vaccines and various antivirals, these virus remains a major threat due to their high potential for dissemination. One strategy to find new antivirals is through the identification of cellular factors that are essential during the viral replication cycle. In this study, the role of the ubiquitin-proteasome system during the CHIKV replication cycle was assessed. In this study, we found that during inhibition of the proteasome and deubiquitinating enzymes, both the production of new viral particles and the rate of infection were drastically reduced, at an early stage of infection, therefore, a post-entry event of the viral replication cycle was affected. In addition, treatment with the inhibitors impaired viral RNA synthesis and viral protein translation, which are key events of the replication cycle. On the other hand, a significant change in ubiquitination patterns during CHIKV infection was evidenced. Through proteomic studies, we identified a significant increase in the number of ubiquitinated proteins in infected cells , thus we established that these proteins are involved in different signaling pathways or cellular metabolism, suggesting a modulation of these proteins during CHIKV infection. Finally, we determined that structural and non-structural proteins of CHIKV are targets of ubiquitination. In conclusion, we demonstrated that chikungunya virus requires the different components of the ubiquitin-proteasome system to establish a productive infection. This finding has a strong implication as we found a key cellular factor essential in the viral replication cycle. Therefore, the Ub-P system may be a pharmacological target for blocking CHIKV infection.

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The tetraspanin CD81 is involved in dengue infection of Huh-7 cells

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***Abstract*—** Dengue virus (DENV) is considered one of the most important infectious agents in Mexico and worldwide. Unfortunately, the disease caused by this virus is neglected, and there is no specific treatment or vaccine to prevent infection. In recent years, the relevance of the tetraspanin family in different biological processes in cells, such as cell membrane organization, exosome biogenesis, cell signaling, etc., has become evident. However, these cellular processes are often exploited by viruses at different stages of the replicative cycle to make the replication of infective viral particles more efficient. Some members of the tetraspanins have been directly and

indirectly linked to different viral mechanisms. One of the most studied tetraspanins is CD81, which has been reported to play an essential role in multiple processes, as a viral receptor, in the fusion of endosomal and viral membranes, in genome replication, in the release of viral particles, and in the formation of extracellular vesicles with infectious components. In this study, the role of CD81 in the infection of DENV serotype 2 was analyzed. Huh-7 cells were incubated with anti-CD81 antibodies previous to interaction with exosomes or viral particles. The infected cells were detected at different times by flow cytometry and confocal microscopy. Our results showed a significant reduction in the expression levels of viral proteins in cells treated with antibodies compared to the cells infected in the absence of antibodies. These findings suggest that CD81 may be involved in some of the stages of the replicative cycle; however, the exact mechanism by which CD81 participates in infection remains to be elucidated.

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Unveiling Alpha-1 Adrenergic Receptor Roles During Dengue Virus Infection

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***Abstract*— Dengue virus (DENV) replication involves the activation of lipophagy pathways, resulting in the degradation of triglycerides and the release of fatty acids stored in lipid droplets. This metabolic process is crucial for ATP generation, which is essential for viral multiplication. Hence, targeting these pathways holds promise as a specific antiviral strategy. Transcriptomic analysis of DENV-2-infected hepatocytes revealed differential expression of genes involved in lipid metabolism, including the alpha-1 adrenergic receptor (ADRA1A). ADRA1A has been implicated in mediating sympathetic nervous system responses related to lipophagy regulation and fatty acid β-oxidation. This study aims to assess the interplay between ADRA1A and DENV replication, using ADRA1A antagonists to validate it as a potential therapeutic target against dengue fever. Among commercially available ADRA1A antagonists in Brasil, we selected Tamsulosin Hydrochloride (TH) based on its low cost, minimal hepatotoxicity, and non-psychotropic effects. Initial assays focused on determining the EC50 of TH formulation. Initial assays were conducted to determine the effective concentration (EC50) of the TH pharmacological formulation. Vero and AML12 cells were infected with DENV-2 at an m.o.i of 5 and exposed to 12 concentrations of TH. After five days of culture, the viability was analyzed. AML12 infected with DENV-2 and exposed to 0.3 µM and 0.6 µM of TH demonstrated 57% and 65% higher viability, respectively, compared to the control. Moreover, the EC50 of TH was determined as 9.5 µM for AML12 cells and 31 µM for Vero cells. These preliminary results suggest that TH may confer protection against DENV-2 infection in AML12 cells. By targeting ADRA1A, this study provides insights into the mechanisms underlying DENV replication and highlights the potential therapeutic implications.

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Valproic Acid Exhibits Anti-Dengue Virus Properties Associated With Alterations In The Cellular Cholesterol Levels: An In Vitro Study Using Huh7 Cells

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***Abstract*— Introduction.** Dengue virus infection is a significant public health problem, and there is currently no effective antiviral pharmacological treatment available. Pre-clinical studies suggest that valproic acid (VPA), a known anticonvulsant drug, exhibits antiviral effects during dengue virus infection. However, the exact mechanism of action has not been fully elucidated. Cholesterol is an important lipid in the cellular membrane and facilitates viral replication of the dengue virus inside the cell. Studies with cholesterol-lowering drugs such as ezetimibe have shown that could have an antiviral effect by blocking the Niemman-PickC1-Like 1 (NPC1L1) receptor, responsible for the uptake of extracellular cholesterol. Therefore, this study aims to investigate whether VPA can alter the virus replication, cellular cholesterol levels, and the expression of the NPC1L1 receptor in an in vitro model of dengue virus infection. **Methods.** Huh7 cells were infected with DENV-2 (MOI 1), and 90 minutes post-infection, the cells were exposed for 48 hours to two-fold serial dilutions of VPA (0.25 - 8 mM). The following controls were used: i) untreated and uninfected cells, ii) infected cells without treatment (VPA), iii) non-infected cells treated with VPA. Additionally, a group of cells treated with ezetimibe (25 µM) was included. Cholesterol levels were quantified using the Amplex Red Cholesterol Assay kit following the manufacturer's instructions. Both viral

replication and the expression of the NPC1L1 gene were quantified using qRT-PCR. **Results.** The study found that VPA treatment resulted in a significant decrease in the number of viral copies, a reduction in intracellular cholesterol concentration (30% compared to the control) and an increase in NPC1L1 gene expression (5-Fold) in all cases compared to the untreated infected control. The major changes were observed at 4 and 8 mM after 48 of exposition. **Conclusion.** The study provides evidence that VPA exhibits antiviral effects during dengue virus infection by altering cellular cholesterol levels suggesting a blockage of the NPC1L1 receptor, similar to ezetimibe. Further studies are needed to fully elucidate the mechanism of action of VPA and its potential as a therapeutic option for dengue virus infection.

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Zika Virus Elicits Differential Expression Patterns Of Pro-Inflammatory Transcripts In Infected Pregnant Macaques With Sustained Placental Damage

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***Abstract*— Zika virus (ZIKV) is an arthropod-borne flavivirus that has caused several outbreaks including Brasil 2015, which has been linked to a board range of neurological birth defects known as Congenital Zika Syndrome. How ZIKV causes placental inflammation and how this contributes to fetal pathogenesis is still unknown. Our group is characterizing the immunopathology of placenta in non-human primate(NHP) models to better understand congenital zika infection and immunopathology. We have shown that congenital infection with ZIKV yields seemingly normal fetal growth in NHPs but exhibits abnormal oxygen transport due to placental villous damage and uterine vasculitis. In these placental tissues, ZIKV is present in a limited number of cotyledons at 40 days post infection (dpi) yet placental inflammation is found diffusely even in areas with no ZIKV detection. Herein, we examine the transcriptional changes seen in the villous tissues comparing ZIKV infected animals from ZIKV PCR positive and PCR negative cotyledons versus tissues from mock infected animals. Proinflammatory cytokines downstream of IL-1β, and TNF pathways were upregulated in addition to downregulation of the immune suppression STAT3 pathway in ZIKV+ cotyledons providing a proinflammatory environment. Additionally, many inflammatory cytokines were differentially expressed in ZIKV animals with CCL2, CCL3L3, and CCL4 being higher in expression in the ZIKV-neg cotyledons. As expected, in the ZIKV-neg cotyledons we see a differential expression of type 1 interferon genes when compared to mock, but had a lower differential expression of genes when compared to ZIKV-pos cotyledons. Interesting, endothelial proliferation markers such as endothelian-1 and vasohibin-1 were downregulated in the ZIKV+ cotyledons. This supports the prediction of upstream regulator VEGFA and further, decreased endothelial proliferation and migration. Overall, our results showed that ZIKV infection during pregnancy induces the stable expression of immune signaling pathways and vascular remodeling, which may explain the changes we see to oxygen availability and transport.

Human Behavior & Community Engagement

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Characterization of human mobility to improve dengue prevention strategies

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***Abstract*— Background**: In contrast to the trend expected based on existing prediction models, dengue incidence was historically low in most endemic countries during the COVID-19 pandemic mobility restrictions of 2020-2021. This could indicate that current transmission models do not correctly take into account the human mobility and space where people

reside during day-time transmission moments. Therefor we revisited a recent study that evaluated the association of arbovirus cases with visits to popular/crowded spots. **Methods.** We conducted a retrospective case-control study in Santiago de Cuba and Cienfuegos province between 2018-2019 characterizing 'regular mobility patterns'. For each case, two controls matched on residence neighbourhood, were selected. Cases were selected among the confirmed dengue/Zika cases from the routine surveillance list (IgM ELISA confirmed for dengue, PCR confirmed for Zika). We used a mobility survey, including geolocalization of residences, to characterize regular mobility paths of cases and controls. **Results.** The final sample size was 134/61 cases and 286/144 controls for Santiago/Cienfuegos respectively. The majority of participants are working outside their home, not being different between cases and controls (p= 0.33 for Cienfuegos, and 0.93 for Santiago). Occupation, gender and age were not significantly associated with being a case. In both locations, being a case was associated with regularly visiting during day-time specific 'popular/crowded spots', such as a street frequented for pendular movement (p=0.016) and a school-complex (p=0.032) in Santiago or an 'open square where people are connecting with wi-fi'(p=0.036) and a primary school (p=0.028) in Cienfuegos. **Conclusions.** People spend more time outside their homes than being in their homes, so the focus of epidemiological attention for epidemic control should not only be centered on individuals' homes. Places where many people are gathering during day-time and the movement between such spaces and the residences could be an essential driver of spatio-temporal heterogeneity of DENV transmission dynamics.

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Fidelidad a la guía de atención clínica integral del paciente con dengue y apropiación de un curso para la implementación de la misma, por los médicos de Urabá Antioquia, 2022-2023

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***Abstract*— Background.** El dengue es una enfermedad viral prevenible y constituye la arbovirosis más importante a nivel mundial en términos de morbilidad, mortalidad e impacto económico; en la región de Urabá exhibe tasas de incidencia superiores a las del promedio departamental, a pesar de asumirse que la mortalidad por dengue es evitable en el 98% de los casos y está estrechamente relacionada con la calidad en la atención del paciente. Por lo anterior se implementó un curso que permitiese la apropiación de la guía para el manejo del paciente con dengue, que incida en la disminución de la tasa de letalidad en la región. **Objetivos.** El objetivo de este estudio fue describir la apropiación de un curso que permita el fortalecimiento de capacidades en los médicos de la zona, para el abordaje y manejo de los casos de dengue según las guías de práctica clínica, y comparar la fidelidad a la guía de atención clínica integral del paciente con dengue, antes y después del curso mencionado, en los municipios del eje bananero durante un periodo de 16 meses. **Métodos.** Se trató de un estudio de implementación de métodos mixtos, a nivel cuantitativo dado por una fase antes-después y cualitativo acoplado. **Resultados.** Se evaluó un total de 353 historias clínicas, 257de ellas previas al curso, y 96 posterior al mismo, para una razón de historias 7:3. Se realizó una homogeneización de los datos, dándoles un tratamiento ecológico. Al realizar la comparación entre ambos grupos, no se encontraron diferencias con significancia estadística, sin embargo, al momento de analizar las medianas de mejora, con el paso del tiempo estas diferencias clínicamente son significativas para el aumento en la fidelidad a la guía de atención clínica integral del paciente con dengue, posterior al curso. **Conclusiones.** El curso no demostró diferencias con significancia estadística en los grupos, sin embargo, se encontraron diferencias en la atención médica. Mejorar el grado de fidelidad a la guía de dengue es factible, las intervenciones de implementación podrían generar diferencias en el manejo y abordaje clínico de los pacientes atendidos en la región.

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Mega-Operativo De Descacharrización (MOD) Como Medida De Prevención De La Transmisión Del Dengue Con Participación Social En Una Comunidad Del Municipio De Coatlan Del Rio, Morelos México

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***Abstract*— La** participación de la comunidad en las acciones de prevención del dengue tiene como propósito sensibilizar y capacitar a familias y diferentes actores sociales, con el objetivo de reducir y eliminar criaderos de Aedes aegypti (CAa) a nivel comunitario. Metodología: El MOD incluido en la ley del dengue del estado de Morelos es un proceso que comprende estrategias de organización comunitaria, educación ambiental y educación para la salud, que permite a la población identificar y controlar los criaderos en viviendas y espacios públicos. Se realizó en la comunidad de Cocoyotla un MOD de octubre-noviembre 2022, con la participación social para promover cambios sustentables y sostenibles para la prevención y el control de CAa, así como el mejoramiento del ambiente de la comunidad. Resultados: previa gestión, capacitación y perifoneo, el día del MOD se eliminaron 2 toneladas de cacharro y 36 llantas con el apoyo de 2 brigadas para el levantamiento de cacharro y 7 brigadas para la verificación casa por casa. Resultados: Se visitaron 78 casas de las cuales 50(64.10%) fueron intervenidas, 19(24.35%) casas abandonadas y 9(11.5%) casas cerradas, sin contar los lotes baldíos. Se verificó exterior de la vivienda, los indicadores entomológicos muestran un Índice de Casa Positiva a larvas de 78%, Índice de Recipiente Positivo a larvas de 49.67% y un ÍNDICE DE BRETEAU de 306%. Conclusión: Los indicadores fueron altos y era de esperarse, por ser una comunidad que nunca se había intervenido, aun así, es de resaltar la participación y buena disposición de la comunidad.

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Perception Of Dengue Disease And Acceptability Of Dengue Vaccines In University Students: A Worldwide Survey

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***Abstract*— Background.** Dengue is the most prevalent arbovirus at present. Almost half of the global population are at risk of dengue virus infection along with 50-60 million people each year with clinical manifestations. Currently, there are two licensed dengue vaccines. Other vaccine candidates are at different stages of clinical development. Acceptability studies of dengue vaccines in university students are scarce. The objective of this study was to understand the level of awareness about dengue disease and dengue vaccines in university students of science, commerce, and humanities worldwide. **Method.** An online survey containing questions regarding demography, dengue disease history, acceptance for a future vaccine and rationale for vaccine hesitancy, was launched and circulated in different social media platforms: WhatsApp, Facebook, LinkedIn, Twitter, and Instagram. It was open for 6 weeks to the targeted population- University students. **Result.** 225 students from 50 countries took the survey. Due to the number of responses from each country, they were grouped into 8 clusters (Africa, Western Europe, Eastern Europe, South Asia, Asia Pacific, Latin America, North America, and Bangladesh). 1/3rd of the respondents had personal exposure to dengue. Overall, 80% (n=147) of the sample were willing to take any dengue vaccine, however, the key reason behind the reluctance of 20% of the respondents was inadequate information about dengue vaccine. **Conclusion.**: Results of the survey provides an overview of the perception on dengue and dengue vaccines in university students around the world, grouped by regions of different endemicity levels. This study can inform future education needs and help design future studies and surveys in this population.

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Política Pública En La Prevención Y Control De Las Enfermedades Transmitidas Por Vector (ETV) Dengue, Zika Y Chikungunya En El Estado De Morelos México

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***Abstract*— Introducción.** La Gestión de Políticas Públicas (GPP) y la investigación son procesos dinámicos que se complementan y presentan puntos de convergencia. La GPP busca resolver problemas concretos y la investigación orienta cambios sociales benéficos. La influencia potencial de las investigaciones sobre las decisiones políticas depende

de múltiples factores, por lo que debe promoverse un mayor contacto entre los investigadores y los tomadores de decisiones. **Objetivo**. Contribuir a resolver las limitaciones que tiene la Secretaria de Salud en cobertura y efectividad, en las actividades de vigilancia prevención y control de las ETV mediante la Ley. **Metodología**. Mediante un proceso de investigación/gestión que culminó en la Ley para la Prevención y Control de las ETV en el Estado de Morelos, que propone un nuevo enfoque de política pública . Esta ley es de orden público, interés social y observancia general en todo el estado de Morelos. Comprende nueve capítulos: I DISPOSICIONES GENERALES, II COORDINACIÓN, III PROGRAMA EMERGENTE DE PREVENCIÓN Y CONTROL DE LAS ETV EN MORELOS, IV PLANEACIÓN ESTRATÉGICA Y RENDICIÓN DE CUENTAS. V PARTICIPACIÓN CIUDADANA. VI SISTEMA DE INFORMACIÓN, VII DENUNCIA CIUDADANA, VIII SANCIONES, IX RESPONSABILIDADES DE LOS SERVIDORES PÚBLICOS. **Resultados.** la publicación de la ley que establece acciones de coordinación entre el Gobierno Estatal, Municipios y la sociedad, respaldada por evidencia científica, con una metodología, un reglamento y un presupuesto aplicable a todo el estado de Morelos. **Conclusiones**. La Ley generada propone un nuevo enfoque de política pública para atender el problema de las enfermedades transmitidas por vector.

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

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***Abstract*— Introduction.** A challenge that prospective studies face, is the difficulty of recruiting the required number of study participants within a pre-specified 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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Space-Time Distributions Of Aedes Aegypti Outbreaks Associated With Cases Of Dengue In Chincha For Partnership Educational Actions Brasil – Peru As A Model Of Epidemiological Containment

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***Abstract*— According to WHO, in 2022, dengue cases in Latin America were 90,1% (2.803.096) of arboviruses and the highest incidences were observed in Brasil (2.383.001), Nicaragua (97.541) and Peru (72.844). In 2023 the cases have increased. In Peru has been detected an outbreak mainly on the north coast of Peru and Amazon region, border with Brasil, and the increase each year has been around 62% since 1984 becoming endemic. Furthermore, in the last years a spread of dengue has been observed as in the desert region of the Peruvian coast where there is little or any incidence of rain, what makes the fact worrying. Peru has a population around 31 millions of citizens and that 79,3% live in the urban area and 20,7% live in the rural area, in which 82,9% speak Spanish, 13,9% speak quéchua and had 71,7% of the economically active population. Most of the population is in a situation of economic and health vulnerability and attrition of public health care services due to the COVID-19 pandemic. The purpose of this paper is to determine the current space-time of Aedes aegypti and the cases of dengue in Chincha directing educational actions for it`s epidemiological containment serving as a model for other regions of the country. The methodology is mapping the cases from the data provided by Peruvian epidemiological surveillance working in partnership with Federal University of Ouro Preto (UFOP) located in Ouro Preto, MG, Brasil. The educational actions are being implemented in schools, community groups and health units with pedagogical material from UFOP and translated to Spanish, quéchua language, beyond interpreting International Sign language. The results have been encouraging with the maps and graphics produced from the database produced and the educational actions to be carried out, will surely have the same success as in Brasil in projects running since 2016. Considering that there isn't still implementation of the vaccine against dengue in the Americas, concluded that the proposed model of educational actions is important for the sanitary control of the proliferation of Aedes aegypti subsidizing the containment of epidemiological surveillance in Peru.**


Takeda

Late Breaker Posters

Diagnostics — Prognostics — Clinical

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Correlation Between Obesity And CD11b Expression In Classical Monocytes And Prevalence Of Anti-DENV IgM In Serum

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***Abstract*— Obesity and dengue are two common health issues in certain regions, particularly in the Americas. Research indicates that chronic diseases, including obesity, may contribute to a worse prognosis and increased severity of dengue. The objective of a study conducted between September 2017 and June 2018 was to clarify the relationship between obesity and dengue. The study involved 49 adult individuals, including 15 with a normal weight and 34 classified as obese, and aimed to investigate the association between obesity and recent asymptomatic dengue. Various data points were collected during the study, including measurements of adiposity, metabolic and endocrine markers, immunological factors, and serum tests for the detection of DENV NS1 and IgM. The findings of the study did not demonstrate any association between adiposity, metabolic and endocrine data, and asymptomatic dengue. No positive tests for NS1 Anti-DENV were detected. However, the results did reveal that 44.1% of obese individuals and 33.3% of those with a normal weight tested positive for Anti-DENV IgM. When comparing the relationship between DENV and body fat index, no significant association could be established (OR = 1.32, 95% CI = 0.59—2.98, p = 0.48). However, the study did observe a higher expression of CD11b in classical monocytes (CD14++ CD16−) in individuals with obesity (1103.0 ± 311.3 mean fluorescence intensity) compared to those without obesity (720.3 ± 281.1 mean fluorescence intensity). Additionally, recent asymptomatic dengue was more prevalent in individuals with a higher body fat index (14.7 ± 3.1 kg/m²) compared to those with a lower body fat index (12.7 ± 2.1 kg/m², p = 0.04). These findings suggest a positive correlation between CD11b expression in classical monocytes and the presence of Anti-DENV IgM in obese individuals, indicating a potential association between obesity and the immune response to dengue virus infection.**

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Mayaro Virus And Chikungunya Virus IgM And IgG Testing In Parallel Improves Diagnosis Of Acute Mayaro Virus Infections

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***Abstract*— Introduction.** Mayaro virus (MAYV, genus Alphavirus) is primarily transmitted by Haemagogus mosquitoes found in Central and South America. Infections are characterized by flu-like symptoms that are similar to related arthropod-borne virus infections, such as the co-circulating chikungunya virus (CHIKV). Anti-MAYV IgM antibodies appear 3-8 days post symptom onset (dpso) while anti-MAYV IgG develop between 4-10 dpso. The close antigenic relationship between MAYV and CHIKV can affect serodiagnostics due to cross-reactive antibodies. This study analyzes the value of the simultaneous measurement of anti-MAYV and anti-CHIKV IgM and IgG in patients with suspected acute MAYV infection to support improved differential serodiagnostics. **Methods.** Two consecutive samples from 14 MAYV-infected patients

(pre-characterized using in-house IFA or ELISA, validated by NAMRU-SOUTH, Lima, Peru) were collected at 1-4 dpso and 10-43 dpso. The samples were re-analyzed using an Anti-Mayaro Virus ELISA (IgM, IgG, EUROIMMUN, CE marked) as well as an Anti-Chikungunya Virus ELISA (IgM, IgG, EUROIMMUN, CE marked). All borderline results were considered positive. Additionally, the titers of MAYV- and CHIKV-specific IgM and IgG were determined using IFA (EUROIMMUN, RUO). Here a ≥ 10-fold titer difference between MAYV- and CHIKV-specific antibodies indicates the respective virus. **Results.** Overall, of the 14 samples at 1-4 dpso, 3 were positive for anti-MAYV IgM and 3 for IgG, 0 were positive for anti-CHIKV IgM and 2 were positive for anti-CHIKV IgG. After 10-43 dpso, anti-MAYV IgM and IgG positivity was found in all 14 samples, indicating seroconversion in 11 individuals. Additionally anti-CHIKV IgM was positive in 1 and anti-CHIKV IgG in 10 samples. IFA confirmed MAYV infection in 12 samples, and 2 samples could not be clearly differentiated. **Conclusion.** In this study, IgM or IgG seroconversion and increase were detected in all patients with acute MAYV infection using the Anti-MAYV ELISAs. Regarding the differentiation from CHIKV, sequential samples were either anti-MAYV IgM positive and anti-CHIKV IgM negative or an anti-MAYV IgG seroconversion was detected. Thus, the combination of the ELISAs used supported the diagnosis of acute MAYV infection and the differentiation from CHIKV. In almost all cases (86%), the interpretation of results was confirmed by IFA.

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Molecular Cloning Strategy To Obtain Recombinant Clones Expressing The Zika Virus NS1 Protein

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***Abstract*— Introduction.** Although the Zika virus (ZIKV) is a self-limiting infection, the neurological complications described in fetuses and newborns of infected woman, such as Guillain-Barré syndrome, have been an important public health issue. Currently, early differential diagnosis is limited by several factors such as similar clinic symptoms with other flaviviruses and cross-reactivity to antibodies against them, especially in populations with immunity to them. In this sense, studies carried out identify the non-structural protein NS1 of the Zika virus as a potential candidate to be used in the serological diagnosis of this infection. **Materials and methods.** The ZIKV ns1 gene was amplified by RT-PCR from viral genome of Zika strain 9661/2016 and initially inserted into the pGEM-T vector to maintain its availability during the work, and later into the pQE30 and pET50b+ expression vectors. A cloning strategy using a restricción enzymes was carried out with both vectors. Recombinant clones were selected by PCR and enzymatic digest assay. Protein expression analysis was made by SDS-PAGE and Western blot techniques. **Result** Four recombinants clones of genetic construction ns1-ZIKV/pGEM-T and eight from genetic constructions ns1-ZIKV/pQE30 and ns1-ZIKV/pET50b were obtained. However, not all clones were able to express efficiently the ZIKV NS1 protein when they were detected with specific antibodies by WB. **Conclusions.** The molecular cloning strategy used in this study allowed us to obtain, recombinant clones capable of efficiently expressing the ZIKV NS1 protein in different Escherichia coli strains. This is the first step into the development of a serological system based on recombinant protein and monoclonal antibodies for the diagnosis of ZIKV infection.

Human Behavior & Community Engagement

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NS3 Protein As Potential Vaccine Candidate Against Dengue Virus

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***Abstract*—** La transmisión del dengue es determinada por una compleja y diversa interacción de factores biológicos, sociales, económicos, demográficos y ambientales. Objetivo: Evaluar la efectividad de una estrategia de control integral del dengue durante tres periodos administrativos (2013- 2015, 2016-2018, y 2019-2021) en el municipio de Xochitepec, Morelos. Materiales y Métodos: Investigación Acción Participativa con análisis epidemiológico (casos confirmados), entomológico (patios controlados) y actividades de control integral del vector. Aplicación

de peces para el control biológico del vector, saneamiento físico y químico, promoción de la salud, educación ambiental, coordinación intersectorial y municipal (sala situacional). Resultados: Participación y formación de 3100 promotores comunitarios. Eliminación de 298, 371 y 281 toneladas de cacharros en cada trienio de gobierno, respectivamente. Incremento en cada periodo de patios sin riesgo entomológico de 76% a 97.6%, de 93% a 97.9% y de 94 a 98%, así como disminución de presencia de larvas de 25.49% a 7.58%, de 14.6% a 3.1% y de 13% a 2%, respectivamente. Los casos confirmados de dengue no grave en cada periodo fueron 111, 9 y 82 y de dengue grave 75, 1 y 4 respectivamente en cada trienio, sin reporte de defunciones por dengue. Reducción sostenida del uso de insecticida en 50% y de 25% de larvicida, con reducción progresiva del control biológico. Conclusiones: Se demuestra la efectividad de alternativas de control integral con participación social y comunitaria bajo la coordinación municipal en el control efectivo del dengue y enfermedades transmitidas por Aedes aegypti, a pesar de los cambios de administración municipal.

Vector Biology — Ecology — Control

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Dynamics Of Degue, Zika And Chikungunya Viruses Through Entomo-Virological Surveillance In Four Arbovirus Endemic Areas Of Mexico During The Period 2018-2022

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***Abstract*—** The region of America is an area of sustained high endemicity of arbovirus infection in which the dengue virus (DENV) stands out. In addition, the introduction and rapid spread of chikungunya virus (CHKV) and Zika virus (ZIKV) has been added. The factors involved in its transmission are complex, since in addition to viruses, they involve factors of the vector, the host as an individual and as part of the population, as well as environmental conditions. These elements highlight the need to understand the dynamics and interactions of transmission between mosquito populations and humans. PAHO has emphasized carrying out virus monitoring in mosquito populations; the need to establish this technology is due to the search for new entomological indicators, as well as having outbreak early warning systems, which will help us identify regions with transmission, new circulating viruses and the vector capacity of mosquito populations. In this study, DENV-ZIKV-CHIKV was monitored in mosquitoes from four states of Mexico between 2018-2022. 10,972 pools were evaluated with a total of 44,379 mosquitoes composed of Aedes aegypti, Ae. albopictus and Culex sp. It was possible to observe the dynamics of ZIKV and CHIKV in field mosquitoes and how it correlates with cases in humans, a competition of these viruses with respect to DENV was observed when they were present. High infection rates were obtained in the first months of the year and with cases of recidivism in the same human population sectors throughout the samplings. The infection rate (MLE and MIR) of Ae. albopictus was higher compared to Ae. aegypti, so the vectorial capacity and molecules of the immune response of a wild population of this species of mosquito were evaluated, obtaining high infection rates and efficient dissemination to saliva in an incubation period less than Ae aegypti, focusing on the participation of this mosquito in the dynamics of DENV transmission in Mexico. This study has contributed to the entomo-virological surveillance of the transmission of VBD in Mexico, with the intention of an anticipatory diagnosis.

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Monitoring wMel Introgression With Oviposition Traps As An Alternative To BG-Sentinel Trapping Of Adult Aedes Aegypti During Wolbachia-Based Vector Control

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Abstract— Wolbachia-based vector control interventions have shown to be effective in reducing the risk of arboviral infection and are being evaluated for WHO prequalification. However, release of Wolbachia (wMel)-positive Aedes aegypti requires labor- and cost-intensive monitoring with BG-Sentinel traps (BG-traps) of adults to evaluate long-term introgression into local populations. Less costly approaches, such as with monitoring eggs or hatched larvae from oviposition traps (ovitraps), have not been fully evaluated. Ae. aegypti eggs and adults were collected from 124 ovitraps and 209 BG-traps, respectively, between March and April 2023 from 12 clusters in the EVITA cluster-randomized control trial that is evaluating the effectiveness of a Wolbachia-based intervention in Belo Horizonte, Brasil. We tested a sample of L3-L4 stage larvae (up to 29 per trap), reared from eggs, and adults (up to 10 per BG-trap) in a wMel qPCR detection assay. We used multivariate models to compare cluster-level wMel prevalence in larvae, which were adjusted by trap-specific Ae. aegypti counts, and in adults, which were unadjusted because of the need to ensure blinding. Among 2,434 larvae that were reared from ovitraps and tested, overall wMel prevalence was 0.69 (SD0.15) and 0.03 (SD0.03) in intervention and control clusters, respectively. Among 974 adults tested, overall prevalence was 0.69 (SD0.14) and 0.07 (SD0.02) in intervention and control clusters. There were no significant differences between the unadjusted cluster-level wMel prevalence in larvae and adults (mean difference= 0.007, p=0.8474). However, there was a small but significant difference between the adjusted wMel prevalence in larvae and unadjusted prevalence in adults (mean difference= -0.15, p=0.01). Cluster-level estimates for adjusted wMel prevalence in larvae and unadjusted prevalence in adults were highly correlated (Pearson r=0.65, 95% CI=0.33-0.83, p=0.00065). Multivariate analysis did not identify trapping approach (ovitraps vs BG-traps) as a significant predictor of prevalence. These findings indicate that Wolbachia prevalence in larvae reared from ovitrap-derived eggs are highly correlated with estimates obtained for BG-trapped adults. Given the similar predictive precision, ovitrap-based monitoring represents a low cost, more efficient approach to evaluating introgression as the Wolbachia-based interventions are scaled up and implemented broadly in high burden regions for Dengue and other arboviral diseases.

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Determinants Of Aedes Aegypti And Culex Spp. Landing Activity On Human Bait In A Northern Ecuador

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Abstract— **Background.** Mosquitoes vector several infectious diseases; ascertaining what contributes to their biting patterns in distinct contexts enables us to tailor public health interventions. We aimed to establish the human landing patterns of mosquitoes in a peridomestic context in northern Ecuador and to explore their variability across the year in two human communities of different size. **Methods.** Starting in June of 2021, mosquitoes were collected during three-hour periods, thrice daily for five consecutive days each month. Collections occurred in two communities - Santa María, a small, riverine community with less infrastructure and the more populated Borbón, a regional hub for commerce and travel. We used two methods concurrently to measure mosquito landing activity. The first was to place a researcher sitting on a chair inside a tulle tent (2 x 2 x 2 m) under a roofed area in an inhabited home. The second method was to collect mosquitoes landing on the exposed legs of a person sitting immediately outside the home. We also assessed environmental variables. **Results.** Of the total mosquitoes (n = 18,408), 17,525 (95%) were of the genus Culex, and 354 (2%) were Aedes aegypti. More total mosquitoes were found in Santa María (16,723 or 94%), which was driven by Culex spp., with 16,691 mosquitoes. There were statistically significant temporal and environmental preferences for both Culex and Ae. aegypti in Borbón, with Culex being mainly crepuscular and Ae. aegypti landing throughout the day. Aedes aegypti showed landing activity peaks at 06:00 - 08:00, 11:00—13:00, and 17:00-19:00, while Culex quinquefasciatus and Cx. nigripalpus showed a small activity peak from 06:00-08:00 and a larger peak late afternoon from 18:00-20:00. There were also differences between the dry and rainy seasons. **Conclusions.** The differences in mosquito behavior are relevant in the context of climate change and vector-control programming. Capturing the heterogeneity across diverse communities can inform site-specific interventions.

Epidemiology — Genomics — Phylogenetics — Modelling — Burden of Disease

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Dengue In Amazonas: Spatiotemporal Dynamics And Its Relationship With Climate Variables

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***Abstract*—** Peru is suffering the worst dengue outbreak that the country has ever had. Amazonas is one of the departments that has been severely affected in five out of its seven provinces, reporting 3502 dengue cases in 2022. In this study, we analyzed the spatiotemporal dynamics of dengue and the effect of climate variables in Amazonas for the last 23 years, with a database provided by Dirección Regional de Salud (DIRESA)-Amazonas. Inhabitants estimation and geospatial vector datasets were obtained from Instituto Nacional de Estadística e Informática (INEI) and Instituto Geográfico Nacional (IGN), respectively. Climate data was acquired from NASA MERRA-2, a global high-resolution dataset for atmospheric variables. Statistical analysis was performed using R software v.4.3.1. According to the data, a major dengue outbreak was reported in Bagua, Utcubamba, and Condorcanqui provinces between 2008 and 2011. Then, in 2020, cases increased considerably in Amazonas, spreading to Bongará and a district called Balsas in Chachapoyas. Spearman's correlation indicated that no significant relationship exists between incidence and temperature, relative humidity, and precipitation in Bagua, Condorcanqui, and Utcubamba. Nevertheless, principal components analysis (PCA) showed that climatic variables do strongly influence these provinces, unlike incidence, which is related to the endemicity of the disease in these provinces of Amazonas. On the other hand, Chachapoyas (Balsas) presented a positive weak association between incidence and minimum temperature, relative humidity, and precipitation (rho = 0.17, p = 0.03; rho = 0.15, p = 0.05; rho = 0.18, p = 0.01, respectively) with a lag of 0-1 month. The PCA showed that climatic variables did not influence Balsas, suggesting that additional factors play a more important role in dengue dynamics. This dengue outbreak occurred after a 7-degree earthquake generated favorable conditions for the spread of the disease in the district. In conclusion, the higher incidence of dengue and its spread to different provinces of Amazonas seem to be associated with geological hazards rather than climate variables, which implies that local programs for control and prevention should act immediately after natural disasters that contribute to the establishment and expansion of both the vector and associated arboviruses.

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Estimating the effect of extreme hydrometeorological events on the incidence of dengue in rural and urban settings: A case study of Peru between 2018-2023

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***Abstract*—** Dengue is a vector-borne disease of public health importance worldwide whose incidence and geographic spread have increased markedly in recent years as environmental and climate conditions have changed. Tropical regions, such as Latin America, face heightened vulnerability to these changes, as demonstrated by the unprecedented outbreak that occurred in the region this year. Higher temperatures and rainfall have been associated with increased risk of dengue in many areas; however, these findings are not consistent throughout different regions and over time. In this study, we aimed to estimate the non-linear and lagged effects of extreme hydrometeorological hazards on dengue incidence risk in recent years in rural and urban areas in Peru. We used weekly and district-level confirmed dengue

case reports from the Ministry of Health and climate data from ERA5 and CHIRPS databases to create a distributed lag non-linear model to determine the exposure-lag-response association between dengue incidence and precipitation and maximum temperature, accounting for urbanization and for sociodemographic factors including education, income, and access to basic services. The dataset included 149890 cases of dengue reported in all 1851 districts within Peru between 2018 and April 2023. Risk of dengue was highest 7-12 weeks after high temperatures (maximum RR: week 9, 36.5°C, 2.55 [Confidence Interval (CI) 2.28-2.86]), and 4-11 weeks after 40-60mm rainfall (maximum RR, week 7, 48mm, 1.66 [CI 1.26-2.19]). However, average precipitation higher than 60mm led to a decrease in dengue RR. Similar patterns in the effect of maximum temperature were not observed; the highest recorded temperatures led to increased RR. We hypothesize that increased temperatures can lead to increased dengue risk by shortening development times of Aedes aegypti larvae, increasing viral proliferation in vectors, and changing their behaviors. Moderately increased precipitation can create larval habitats leading to proliferation of vectors, but high precipitation can wash away mosquito breeding sites, thus reducing risk. Previous studies conducted in Peru at lower geographical resolution have found an association between dengue and high temperature but not precipitation. Our findings can inform the timely implementation of outbreak prevention measures in response to extreme hydrometeorological events.

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Epidemiological Profile Of Notified Cases Of Dengue Infection In A Brasilian City Between 2019 And 2020 And Their Characteristics In The Context Of Overweight And Obesity

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***Abstract*—** Epidemiological evidence suggests that obesity can increase the risk in viral infections, but still little is known about the obesity impact on dengue. Therefore, this study evaluated the epidemiological profile and the prevalence of overweight and obesity amongst the dengue virus (DENV) cases reported in Diamantina-MG, Brasil, in 2019 and 2020. The study was approved by the ethics institutional review board (CAAE: 42613321.3.0000.5108). Data was collected from the records of dengue cases reported in the local healthcare network system in 2019 and 2020. The number of the reported DENV cases was 1,734 (1,141 in 2019 and 593 in 2020). So, the incidence of the disease was 364/10,000. Young adults (18-29 years-old) and women were amongst the majority of DENV cases, corresponding to 34.4% and 56.9% of the cases, respectively. There were 25 notifications of dengue with alarm signals, all in 2019. Five of those patients progressed to severe dengue, but no death was reported. Of the 1,734 registered notifications, 246 cases (14.2%) were traced through electronic medical record and interview, allowing the frequency of obesity/overweight to be estimated with maximum error of 5.7%. So, amongst the DENV cases reported, 39% of the individuals were eutrophic, 35% overweight and 26% had obesity. The most common symptom was headache (87%), regardless the nutritional status. On average, 5 ± 2 of the alarm signs were present in 3.2% of the eutrophic and in 5.8% of the patients with overweight. At least 6 ± 2 of the alarm signs were present in 4.6% of the patients with obesity. Dengue notifications was higher in 2019, similarly to the Brasilian scenario. In 2020, the number of dengue notifications in the first 4 months almost doubled when compared to the same period in 2019. However, from March onwards, there was a gradual decline in records, probably due to underreporting, as this period was marked by the installation of CoViD-19 in Diamantina. So far, we could not establish the association between obesity and the aggravation of DENV infection. The study was limited by missing information in medical records and the underreporting of dengue in 2020.

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A Research History Of Arboviral Infections In Peru From 1993-2022

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Abstract— Arboviral infections are a nearly global public health burden, which are especially problematic to U.S. and Peruvian military service members. These viruses are found in austere settings and may cause a significant threat to force health protection, particularly in deployed settings. Therefore, establishment of arbovirus identification , especially in tropical areas, has become an important part of the U.S. Naval Medical Research Unit (NAMRU) SOUTH mission. In 1993, NAMRU SOUTH initiated febrile illnesses characterization in Iquitos, Loreto, later expanding to 13 health facilities in different regions of Peru: Northern coast, Amazon region, Central jungle, and Southeastern region. In 1993 in Iquitos alone, we identified 192 cases of dengue virus (DENV), 47 Venezuelan equine encephalitis virus (VEEV), 68 Oropouche virus (OROV), and 4 Mayaro virus (MAYV). These viruses were not previously described in this region. In 2003, OROV and Guaroa virus (GROV) were detected in Iquitos and Chanchamayo. In 2006, Saint Louis encephalitis virus (SLEV) was detected for the first time in Iquitos. In 2008, when more detection assays were incorporated, this surveillance allowed us to observe the DENV-4 expansion through northern Peru and Loreto. In addition, we detected yellow fever virus (YFV) in Iquitos, Cusco, and Yurimaguas. Additionally, in 2014 we identified an outbreak of GROV in a rural area of Iquitos associated with Plasmodium vivax infection. Furthermore, we identified 6 new viruses through virus isolation and next generation sequencing: Maguari virus in 1998, Maldonado virus in 2004; Saint Louis Encephalitis in 2006; Itaya virus in 2006; Iquitos virus in 2006; and Zungarococha virus in 2008. During this period of time NAMRU SOUTH has regularly identified emerging and re-emerging arbovirus circulating in Peru and has been used to inform the U.S. and Peruvian militaries of biological threats that can affect warfighter health.

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Transmission Dynamics Of Dengue Virus In Large And Small Population Centers In Northern Ecuador Using A Phylogenetic Analysis

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Abstract— Although dengue is considered an "urban" disease, rural communities are also at high risk. To further our understanding of dengue virus (DENV) transmission in settings with characteristics generally considered rural (e.g., lower population density and remote), we conducted a phylogenetic analysis in 6 communities in the northwestern province of Ecuador that have distinct landscape, ecological and social variables that we identify as contributors to transmission risk. During household-based active fever surveillance, we collected 488 serum samples with suspected dengue from participants between 2019 and 2021. One hundred and twenty one had detectable DENV RNA by PCR. Twenty seven samples with CT under 30 were selected for whole genome sequencing (MinION nanopore technology) and phylogenetic analysis that included available DENV sequences from Ecuador and South America. Our data confirmed that DENV-1 circulated from May 2019 to March 2020 and DENV-2 from December 2020 to July 2021. Combining locality and isolation dates, we found strong evidence that DENV entered Ecuador into the northern province of Esmeraldas from neighboring country Colombia, and that viral isolates were related to Colombian and Venezuelan DENV. Phylogenetic patterns suggest that within this province communities with larger populations and commercial centers were more often the source of DENV but that smaller remote communities also play an important role in the regional transmission dynamics acting as sources or sinks of DENV.

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Prevalence Of Dengue In The Northern Ecuadorian Amazon Basin

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Abstract— The burden of mosquito-borne infectious disease is rising within the Americas. Historically, the Ecuadorian Amazon has reported Malaria (P.vivax and P.falciparum) as the main etiologic agent of fevers due to mosquito bites. Hospital San Miguel, is a new NGO-run a full-service health delivery center located in the most remote location in the province of Putumayo, serving mostly indigenous communities that reside along the Colombian border. When

processing negative samples for malaria and previous reports in northern coastal Ecuador and elsewhere of observed transition from malaria to arbovirus fever etiologies, we screened samples for dengue, using dengue, chikungunya and Zika, ZDC, triplex PCR assay. We implemented the full protocol from RNA extraction to RT-qPCR in a basic laboratory established as a key service of San Miguel Hospital. Over 90% of processed samples were positive for dengue. We have thus started a longitudinal screening for arboviruses protocol for dengue-like fevers in the remote village of ¨Puerto el Carmen¨ where Hospital San Miguel is, and surrounding communities alongside the San Miguel river and Putumayo river in the Ecuadorian Amazon Basin. These results will give insight into the prevalence of dengue for the first time in this part of the Amazon.

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Chikungunya Virus East-Central-South-African (ECSA) Lineage Linked To Fatal Cases In The Northwest Of São Paulo, Brasil

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Abstract— Since the chikungunya virus (CHIKV) was first detected in Brasil in 2013 (Asian lineage), recurring outbreaks over the subsequent years illustrate the endemicity of the disease in the country. Currently, Brasil and other countries in Latin America are facing an increase in CHIKV cases, mainly linked to the East-Central-South-African (ECSA) lineage. Our molecular surveillance of CHIKV in serum samples collected from patients with dengue-like symptoms revealed increased virus detection since February 2023. Until June 2023, the number of confirmed chikungunya cases (n=12) was double that of confirmed cases from 2020 to 2022 (n=6). We sequence, using NGS, the whole genome of CHIKV from seven cases, four females and three males, with ages ranging from 34 to 73 years old (five over 60 years old). The most common symptoms were fever, myalgia, and fatigue. Two female cases with comorbidities, aged 34 and 79, evolve to death. Phylogenetic analysis classified all of the genomes belonging to the ECSA lineage, grouping with other CHIKV Brasilian sequences mainly collected from 2021 to 2022. Our findings demonstrate a slight increase in CHIKV cases in the northwest region of São Paulo and linked CHIKV-ECSA strains with two fatal cases. We reinforce the critical role of differential diagnosis of arboviruses due to overlapping symptoms and molecular/genomic active surveillance in predicting outbreaks.

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Studying The Genetic Diversity Within Zika Virus Genome Sequences From Puerto Rico, 2016-2017

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Abstract— An increasing number of outbreaks caused by mosquito-borne viruses, arboviruses, have been affecting tropical regions world-wide, especially the Caribbean islands where the Aedes aegypti mosquito is predominant. The zika virus (ZIKV) is one of the most prevalent arthropod-borne viruses in this region. By 2013 and 2014, the outbreak was reported in French Polynesia and in the Easter Island of Chile, respectively. Puerto Rico (PR) reported the first positive case of ZIKV (genus flavivirus) in late 2015. Studies have demonstrated evolutionary patterns in ZIKV and genomic diversity during the epidemic of 2016-2017. Non-synonymous mutations influence the genetic diversity in ZIKV and have an effect on protein function. Our hypothesis states that non-synonymous mutations emerged in the ZIKV Puerto Rican population during the epidemic of 2016-2017. To address this, we performed a phylogenetic analysis to identify the genetic changes present within ZIKV whole genome sequences from PR isolates that occurred during the epidemic. We were able to identify non-synonymous mutations in our ZIKV sequences from PR and high frequency mutations. We found mutations in PR isolates that were previously known. Interestingly, understudied mutations were also identified. Our study has characterized the genetic variability within the ZIKV population in PR during the epidemic of 2016-2017. These data can lay as a foundation for future genomic epidemiologic and molecular-based studies to determine the effects of these mutations on the virus phenotype, impact on ZIKV infectivity, viral fitness, and severity of infection.

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Factors Associated With Deaths In Patients With Severe Dengue

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Abstract— The study objective was to identify factors associated with deaths in patients with severe dengue. A case-control study was performed in Espírito Santo state, Brasil, including data from the Information System for Notifiable Diseases of patients with severe dengue reported between 2009 and 2019. For each case (death), one control (survival) was randomly selected. The study included 53 deaths and 53 patients who survived. Age in those who evolved to death (median = 53, interquartile range = 29 - 69.5) was significantly higher than in those who survived (median = 37, interquartile range = 23.5 - 60) (p-value = 0,041). Some clinical manifestations were significantly less frequent in deaths than in survival, such as myalgia (36 vs. 46, p-value = 0.02), headache (27 vs. 39, p-value = 0.016), exanthema (3 vs. 11, p-value = 0.042), retro-orbital pain (7 vs. 23, p-value = 0.001), progressive increase in hematocrit (27 vs. 39, p-value = 0.016), and hematemesis (30 vs. 40, p-value = 0.040). On the other hand, some manifestations were significantly more frequent in deaths than in survival, such as arthralgia (25 vs. 15, p-value = 0.045), liquid accumulation (30 vs. 13, p-value = 0.001), weak or undetectable pulse (40 vs. 13, p-value = 0.000), late-stage of arterial hypotension (39 vs. 11, p-value = 0.000), alteration of consciousness (24 vs. 10, p-value = 0.004), severe organ damage (25 vs. 8, p-value = 0.000), and epistaxis (16 vs. 6, p-value = 0.017). The binary logistic regression model showed that headache (OR = 0.340, 95% CI = 0.117 - 0.992), retro-orbital pain (OR = 0.167, 95% CI = 0.046 - 0.663), liquid accumulation (OR = 4.669, 95% CI = 1.626 - 13.404), severe organ damage (OR = 7.239, 95% CI = 2.094 - 25.023), epistaxis (OR = 11.837, 95% CI = 2.563 - 54.679) and age (OR = 0.967, 95% CI = 0.946 - 0.989) were significant predictors of progression to death [X2 (1) = 53.468; p-value = 0.000; R2Nagelkerke = 0.528]. These findings can help improve patient outcomes by enabling early recognition and targeted management of high-risk individuals.

Virology — Pathogenesis — Antivirals

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Global And Cell Type-Specific Immunological Hallmarks Of Severe Dengue Progression

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Abstract— Approximately 1 in 20 symptomatic dengue patients progresses within days of symptom onset to severe dengue (SD). Yet, SD pathogenesis in humans is incompletely characterized, and no effective measures to predict or prevent it exist. To investigate the cellular and molecular determinants of

SD progression, we integrated virus-inclusive single cell RNA-Seq 2 (viscRNA-Seq 2) with functional assays in patient-derived PBMCs obtained from our Colombia cohort. We demonstrated that beyond myeloid cells, B cells harbor replicating DENV capable of infecting permissive cells. Alterations in cell type abundance, gene and protein expression and secretion, and cell-cell communications, suggest a disjointed immune response preceding SD. Increased plasmablasts and enhanced migration and pro-inflammation signatures were prominent in myeloid cells from SD progressors, yet antigen presentation and interferon responses were impaired, potentially due to suppressive Tregs and uncoordinated NK cell interactions. These findings define DENV target cells in human blood and provide insight into immunological hallmarks of SD progression.

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Impact of Immune Cell Infiltration in Testes Following ZIKV Infection in Mice

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Abstract— Zika virus (ZIKV) is a sexually transmitted arbovirus which has caused devastating outbreaks worldwide and has the capacity to emerge again. The mechanism for testicular infection and persistence facilitating sexual transmission is not fully characterized. Further, there are currently no FDA-licensed vaccines or antivirals to block or treat ZIKV infection. As the testes is an 'immune-privileged' organ, identifying the location of infected cells is critical to investigating a druggable target with minimal damage to the testes. Here, we use a combination of cell culture and murine models to characterize acute (7 days post infection [dpi]) and persistent (23 dpi) ZIKV infection. Sertoli cells, which form a layer within the seminiferous tubule and comprise the blood-testes-barrier (BTB), are susceptible to ZIKV infection. Testicular damage, including destruction of the seminiferous tubules and testicular atrophy, was observed in some ZIKV-infected A129 mice at 23 dpi. Cytokine analysis of these testes revealed high levels of pro-inflammatory cytokines and chemokines, including CCL-3 and CCL-5, which were not observed in the sera, suggesting testicular cells are still responding to infection and producing signals for the recruitment of immune cells. Flow cytometry to detect neutrophils (CD11b+ Ly6G+), macrophages (CD11b+ F480+), dendritic cells (CD11c+), CD4+ T-cells (CD44hi CD62Llo CD4+) and CD8+ T-cells (CD44hi CD62Llo CD8+) in the spleen, inguinal and mesenteric lymph nodes and testes revealed large quantities of these cell in all tissues on days 7 and 23 dpi. In contrast to CD4+ T-cells, CD8+ T-cells were more abundant in ZIKV-infected testes on day 7 than day 23, suggesting a role in early infection and likely critical to the establishment of infection. To test this, CD8 knockout (KO) transgenic C57Bl/6 mice treated with anti-interferon antibody, infected with ZIKV and analyzed at 23 dpi. Compared to uninfected CD8 KO mice, more ZIKV-infected mice showed alterations in testicular architecture, reduced testes size, reduced spermatogenesis and high levels of ZIKV antigen within the testes, including within the seminiferous tubule lumen. More studies to examine the interplay of macrophages and T-cells are needed, as well as determining the cells persistently infected with ZIKV.

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Obesity And Metformin Modify Macrophage Response To Dengue Virus Infection

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Abstract— This study is investigating the response of macrophages from mice with obesity to Dengue virus (DENV) infection. Obesity was induced by the treatment of C57Bl/6 mice (8 weeks-old) with a high fat diet (60% of lipids - HFD) for 10 weeks. Control mice (CNT) was maintained in standard chow diet for the same period. Peritoneal macrophages were

elicited at the 10th week of HFD treatment by the injection of 3% thioglycolate and collected 4 days later. Macrophages were infected, in vitro, with DENV-2, at a multiplicity of infection (MOI) of 1 or 0.1 virus per cell. Non-adherent virus particles were removed after 2 h and macrophage nitric oxide (NO) production and viral load were evaluated 48 h later. Morphologic evaluation of macrophages, before DENV infection, evidenced extensive cytoplasm vacuolization of macrophages from HFD-treated mice. NO production by LPS-stimulated macrophages was not affected by obesity. However, macrophages from HFD-treated mice produced more NO in response to DENV infection (2.9 times higher) compared to CNT animals. HFD treatment also resulted in increased macrophage viral load (4,098 +- 3,345 vs 26,611 +- 16,867, CNT vs HFD). To investigated whether obesity effects can be modified by drugs used on obesity management, macrophages were treated, before DENV infection, with metformin (5 and 10 uM). NO production in response to DENV infection by macrophages from HFD-treated mice was partially reduced by metformin. Also, viral load in macrophages from mice with obesity was reduced in response to metformin treatment (from 26,611 +- 16,867 to 9,567 +- 7,773). We are currently investigating the involvement of AMP-activate kinase and intracellular lipid content in the modification of macrophage response to DENV by obesity.

Human Behavior & Community Engagement

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Determining Factors Of Hesitancy Towards Fumigation, Focal Treatment, And Collection Of Breeding Sites For The Control Of Aedes Aegypti In Homes: A Mixed Study In Two Dengue-endemic Regions Of Peru

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***Abstract*— Introduction**. Dengue is a public health problem in Peru and vector control (including fumigation, focal treatment, and collection of breeding sites) is the principal way to contain the vector population. However, people are often reluctant to employ these strategies, and thus the aim of this study was to investigate factors associated with this hesitancy. **Methodology**. A mixed study was conducted in dengue-endemic regions of Piura and Loreto (Iquitos). First, we conducted focus groups stratified by age and sex to explore the constructs associated with hesitancy. Next, representative sample surveys were applied to quantify these constructs and determine other associated factors. The qualitative information was transcribed, and a codebook was created using the deductive method of a conceptual framework of vaccine hesitancy (confidence, complacency, convenience). Levels of hesitancy (not hesitant, sometimes hesitant, and always hesitant) were measured for each vector control activity and crude and adjusted prevalence ratios (RPa) were estimated using ordinal logistic regression. **Results**. 16 focus groups were conducted with 147 participants (53% women). Participants expressed distrust towards fumigation, specifically regarding the efficacy of the product and reliability of health personnel. Focal treatment was more accepted due to its regular usage within these communities and perceived confidence in the personnel. The collection of breeding sites was more irregular, and participants expressed difficulty in getting rid of old objects that can serve as breeding sites. 883 surveys were applied and the variable that best explained the levels of hesitancy was the perception of the effectiveness of the product or intervention. Impressions that the product used in fumigation/focal treatment was effective were associated with a lower risk of being hesitant towards these activities (RPa 0.46 CI 0.29-0.73 for fumigation; RPa 0.28 CI 0.15-0.53 for focal treatment). Similarly, having the opinion that collecting breeding sites works was associated with a lower risk of being hesitant to this activity (RPa 0.65 CI 0.55-0.77). **Conclusion.**: Efficiently demonstrating the effectiveness of vector control activities can reduce population hesitancy.