2024 CREID Pilot Program Awardees

Principal Investigator	**Henry Nelson Puerta-Guardo, PhD** **Universidad Autonoma de Yucatan (UADY)**
Title	***Understanding the genetic variation and the spatial and temporal distribution of dengue viruses in children and mosquito populations in urban areas: the case of Merida, Yucatan***
Pathogen Focus	Arboviruses, Dengue
Country	Mexico
Collaborating CREID Research Centers	A2CARES, EpiCenter
Abstract	Aedes-borne viruses (ABVs) such as the dengue serotypes pose a major public health burden worldwide. Model projections estimate an average of 390 million DENV infections occur per year, of which 96 million manifests clinically. Given the heavy global burden of dengue, and in the absence of efficacious vaccines or other therapeutic options, the dissemination of DENV continues to expand. Changing viral and vector transmission factors will likely results in more frequent outbreaks, potentially in regions of the world previously unaffected. In Mexico, 54,406 symptomatic cases occurred in 2023, and 19% were confirmed in Yucatan, involving all four DENV serotypes. The goal of this proposal is to define the spatial and temporal patterns of DENV infections in a cohort of children in Merida, Yucatan and characterizing viruses collected from mosquitos in infected participants´ homes. We aim to understand the infection distribution dynamics in endemic urban areas. Importantly, this proposal will complement an ongoing Clustered Randomized Controlled Trial (CRCT) of targeted Indoor Residual Spraying on Aedes-borne diseases in Merida (clinicalTrials.gov: NCT04343521). Using banked human samples and Aedes collected from participants´ houses during a DENV outbreak in 2023, we will describe the genetic diversity of Aim 1) DENV causing symptomatic infections in children living in Merida; and Aim 2) DENV infecting Aedes mosquitoes from households where cases have been identified. With these data, this proposal also pursues to Aim 3) analyze geographic patterns of genetic variation of the DENV infecting children and Aedes mosquito populations using ad hoc qualitative data analyses. Dr. Puerta-Guardo is an associate researcher in the Virology Laboratory (CIR-Biomedicas) and Campus of Biological and Agricultura Sciences, UADY. He will be mentored by Dr. Angel Balamaseda of the National Virology Laboratory, Centro Nacional de Diagnóstico, y Referencia and Instituto de Ciencias Sostenibles, Managua, Nicaragua along with several other mentors from UC Davis, UC Berkeley, Emory University, and UADY.

Principal Investigators	**Jacob Van der Ende, MD, MSc, Hospital San Miguel** **Paul Cardenas, MD, PhD, MSc, Universidad San Fransisco de Quito**
Title	***Bridging gaps between arbovirus field research in the Ecuadorian Amazon and molecular diagnostics with RNAES technology***
Pathogen Focus	Dengue Virus, Arboviruses
Country	Ecuador
Collaborating CREID Research Center	A2CARES
Abstract	The opening of Hospital San Miguel in November 2021 brought the possibility of molecular diagnostics to the village of Puerto el Carmen in the Putumayo Amazonia region of Ecuador on the border with Colombia. The hospital serves more than fifty communities along the Putumayo and San Miguel rivers, in which dengue virus (DENV) and other arboviruses are likely widespread but the epidemiology of acute febrile illness is poorly understood. Access to these communities through medical outreach creates a unique opportunity and imperative to characterize emerging and endemic pathogens in the region. In 2023, a DENV outbreak spread through Putumayo. Monitoring of such an outbreak relies on molecular diagnostics, but reliable RNA extraction and storage is difficult and expensive in Ecuador. Therefore, widespread molecular testing for RNA viruses is not feasible in our communities. For this reason, we propose to evaluate RNA Extraction and Storage (RNAES) technology, developed by A2CARES/CREID member Dr. Jesse Waggoner, as an affordable and locally sustainable solution for RNA extraction, stabilization, storage, and transport between study sites. The study will be divided into two aims. In Aim 1, RNAES will be compared to a commercial RNA extraction kit using a biobank of characterized clinical samples available at Hospital San Miguel. RNA will be tested in real-time RT-PCRs and whole virus genome sequencing protocols. In Aim 2, we will evaluate RNAES feasibility and RNA stability with protocol implementation in remote Amazon communities. Based on initial experience, current protocols will be iterated to optimize workflow in these remote locations. Drs. Van der Ende and Cardenas will be mentored by Dr. Jesse Waggoner of the School of Medicine, Division of Infectious Diseases at Emory University and Dr. Josefina Coloma of the Division of Infectious Diseases and Vaccinology, School of Public Health at UC Berkeley.

Principal Investigator	**Jean-Paul Carrera, PhD** **Gorgas Memorial Institute of Health Studies**
Title	***Unraveling cross-reactivity role in Alphavirus epidemics***
Pathogen Focus	Alphaviruses
Country	Panama, US
Collaborating CREID Research Centers	CREATE-NEO, PICREID
Abstract	The alphavirus encephalitis viruses (*Alphavirus*, *Togaviridae*) are ARN arthodpod borne transmitted zoonotic viruses, and include the Venezuelan (VEEV), Eastern (EEEV), Madariaga (MADV) Western (WEEV) equine encephalitis viruses. The sporadic emergence of VEEV IAB epizootic subtypes in South America from enzootic subtypes leads to significant epidemics. Our epidemiological observations in Panama suggest that VEEV may play a role in limiting the potential emergence of MADV. Studies on hamsters inoculated with an attenuated VEEV strain experience a 37% and 59% reduction in mortality when subsequently exposed to WEEV and EEEV respectively. We therefore hypothesize that heterologous alphavirus cross-reactivity modifies host susceptibility, impacting epidemic potential. This study aims to investigate the impact of endemic alphavirus cross immunity, specifically VEEV (TC-83, ID and IE), MADV and Everglades virus (EVEV), in both murine and computational models on the emergence of epidemic alphaviruses throughout the Americas by **1)** Assessing cross-reactivity among selected alphaviruses (VEEV (TC-83, ID and IE), MADV and EVEV) using murine model and serological assays and **2)** analyze age-stratified serological data generated from three population level cross-sectional studies from Panama to fitt catalytic force of infection models in order to reconstruct historical alphavirus dynamics and to simulate the epidemic course of an alphavirus introduction accounting for baseline conditions of multiple alphavirus circulations. The proposed study will provide insights into whether pre-existing immunity to endemic alphaviruses, such as VEEV and shape the introduction, emergence, and transmission of novel alphaviruses. Dr. Carrera is a virology and biotechnology researcher with Gorgas Memorial Institute of Health Studies and will be mentored by Shannon Rossi with University of Texas Medical Branch and Simon Cauhemez with Institut Pasteur.

Principal Investigator	**Jesus Miguel Torres Flores, PhD** **Escuela Nacional de Ciencias Biológicas – Instituto Politécnico Nacional (ENCB-IPN)**
Title	***Ecological dynamics of sandfly-borne viruses in Chiapas, Mexico***
Pathogen Focus	Sandfly-borne viruses
Country	Mexico
Collaborating CREID Research Center	CREATE-NEO, CREID-ECA
Abstract	In Mexico, entomovirological surveillance focuses on dengue, Zika, and chikungunya viruses therefore targeting *Aedes* mosquitoes. However, approximately 40% of the suspected cases of viral febrile infections that occur annually in the country remain of unknown etiology, suggesting other hematophagous arthropods may play an active role in transmitting both known and previously undiscovered viruses. While sandflies in Mexico are primarily studied as vectors of human Leishmaniasis, emerging evidence points to their clinical relevance as vectors of members of the *Phlebovirus* genus in the Americas. Yet, despite the rich diversity of sandflies in Mexico's southern border, particularly in Chiapas with 36 reported species, there has been limited research dedicated to characterizing their potential as vectors for mammalian viruses, including phleboviruses and other yet-to-be described viral agents. We *hypothesize* that human influence has impacted the distribution, abundance, and diversity of sandfly populations in the state of Chiapas, creating new areas of overlap between human populations, the enzootic reservoirs of vast diversity of phlebotomine transmitted viruses, and sandfly species willing to feed on both, thereby elevating the potential for spillover of these viruses. To test this hypothesis, we have the following aims 1) Characterize the ecological distribution of sandfly populations in Chiapas across an environmental gradient encompassing peri-urban, rural, and sylvatic areas using DNA barcoding and Ecological Niche modelling 2) Determine the host range of the hematophagous sandfly species identified in Aim 1 across an environmental gradient by single sandfly metatranscriptomics, and 3) Describe the RNA Virome of Sandflies Across Diverse Ecological Niches using RNAseq. Dr. Torres Flores is an investigator in the National Laboratory of Vaccinology and Tropical Viruses at ENCB-IPN and will be mentored by Dr. Kathryn Hanley, Regents Professor of Biology at New Mexico State University and Dr. Stephanie Seifert, Assistant Professor, Paul G. Allen School for Global Health, College of Veterinary Medicine at Washington State University.

Principal Investigators	**Kristopher M. Smith, PhD, Washington State University** **John Gachohi, PhD, Washington State University, Global Health Kenya**
Title	***Adapting to Rift Valley Fever: Identifying, assessing, and upscaling what works in diverse ecologies***
Pathogen Focus	Rift Valley Fever virus
Country	Kenya, US
Collaborating CREID Research Center	CREID-ECA, A2CARES
Abstract	Rift Valley fever (RVF) virus and other climate-sensitive diseases cause large epidemics affecting livestock and humans in sub-Saharan Africa. As climate change intensifies, RVF outbreaks are expanding into ecological areas in which RVF was not previously observed. In regions where RVF was historically observed, preventative adaptations – such as migration or mosquito nets – are likely to be in use but taxed by longer epidemic intervals; in regions where RVF is relatively new, preventative adaptations are likely to not be in use. This project synthesizes expertise in biomedical and social sciences to understand how communities in different ecologies in Kenya are adapting to RVF and other climate-sensitive diseases, and to scale up locally-led, effective adaptations across regions. Using interviews, discussion groups, and surveys, we will assess what adaptations communities in arid lowlands and agriculturally-rich highlands have used to prevent RVF, who uses these adaptations, and identify barriers to adopting adaptations. We will use longitudinal data available to CREID-ECA and surveys for an initial assessment of how effective adaptations are at preventing RVF. After identifying effective adaptations, we will support between-ecology sharing of what works by bringing members from different regions together to discuss acceptability and feasibility of new adaptations to RVF. This lays the groundwork for future work to scale up locally-led adaptations and track their effectiveness. This project will elucidate adaptations that prevent RVF transmission, how these adaptations are affected by climate change, and help build adaptive capacity for at-risk communities to respond to the challenge of climate-sensitive diseases. Dr. Smith is a Postdoctoral Research Associate in Anthropology and Dr. Gachohi is an Epidemiologist in the Global Health Program Kenya both with WSU and both will be mentored by Dr. Kariuki Njenga, PI of CREID-ECA, WSU and Anne Pisor of WSU, and by Dr. Josefina Coloma of UC Berkeley.

Principal Investigators	**Aunji Pradhan, PhD, Tribhuvan University** **Anurag Adhikari, PhD, Kathmandu Research Institute for Biological Sciences**
Title	***Dengue virus seroprevalence and genetic diversity: Investigation of recent dengue outbreaks in Nepal***
Pathogen Focus	Dengue virus (all 4 serotypes)
Country	Nepal
Collaborating CREID Research Centers	CREID-ESP
Abstract	Dengue virus (DENV) was first detected in Nepal in 2004; since then, outbreaks have occurred regularly and become progressively more severe. The outbreaks are marked by changes in clinical symptoms and disease severity; shifts in dominance of the four serotypes (DENV1–4); and increases in geographic spread, suggesting that there is high chance dengue becomes hyperendemic in Nepal. However, little is known about how DENV genetic evolution may be associated with altered viral and epidemiological dynamics in successive outbreaks. Here, we propose to fill this knowledge gap by testing our hypothesis that the genetic evolution of DENV shapes the serotype dominance, seroprevalence, and, possibly, clinical manifestations, using blood samples collected from 853 Nepalese patients during the 2019 and 2022 outbreaks. In Aim 1, we will examine the distribution of DENV NS1 antigen, anti-DENV IgG, and anti-DENV IgM (by ELISA); anti-DENV neutralizing antibodies (by PRNT assay); and DENV serotype prevalence (RT-qPCR) across various demographic parameters. In Aim 2, we will perform whole-genome sequencing of DENV in RT-qPCR-positive samples (Aim 1) to identify and track genomic changes (Illumina MiSeq platform). The results of this study will increase our understanding of how genetic changes in DENV may have influenced the serotype distribution, seroprevalence, and key clinical features during the 2019 and 2022 outbreaks in Nepal. This pilot study may also help to guide health authorities in developing targeted mitigation strategies against DENV, bolstering the local healthcare infrastructure, and creating a vital biobank for continued DENV Dr. Pradhan is a postdoctoral fellow in the Central Department of Biotechnology at Tribhuvan University and Dr. Adhikari is the Group Leader for the Department of Infection and Immunology at KRIBS. Both will be mentored by Dr. Sujan Shresta at La Jolla Institute of Immunology and Dr. Krishna das Manandhar from the Central Department of Biotechnology at Tribhuvan University.

Principal Investigators	**Momoh Mambu, PhD, Kenema Government Hospital** **Nell Bond, PhD, Tulane University**
Title	***Cellular immune responses to rVSVDG-ZEBOV-GP vaccination in Ebola survivors in eastern Sierra Leone***
Pathogen Focus	Filoviruses
Country	Sierra Leone
Collaborating CREID Research Centers	WAC-EID, WARN-ID, CREID-ECA, CREATE-NEO
Abstract	The devastating 2013-2016 Ebola virus disease (EVD) outbreak in West Africa was the largest in history with over 28,000 cases and 11,000 deaths. This outbreak left a large cohort of EVD survivors, many with persistent health concerns following resolution of disease, who have since participated in research studies investigating adaptive immunity to EBOV and viral persistence. Cellular immunity studies have shown that strong, diverse, T-cell responses are associated with EBOV clearance and survival. However, EBOV persists in immunologically privileged sites in survivors much longer than initially thought: the recent outbreak in Guinea was traced back to an EVD survivor who had no known exposure over five years after recovery. Recent reports suggest that EBOV IgG levels cycle within survivors over time, which suggests a potential role for periodic exposure to antigen sequestered in immunologically privileged sites. These data suggest significant risk for recurrent disease as natural immunity wanes over time. Given these risks, it is imperative to identify methods to improve EBOV specific immune responses in survivors and prevent such events from occurring in the future. Immunization of EVD survivors with the rVSVΔG-ZEBOV-GP vaccine is one such strategy. In this Pilot Program study, Drs. Momoh and Bond will leverage a unique cohort of vaccinated EVD survivors and naive, vaccinated controls to understand the role of vaccination on the strength, quality, and durability of EBOV specific cellular and systems level humoral immune responses in this population. This study will lay the groundwork for developing improved strategies for protecting EVD survivors and their communities from recurrent disease. Dr. Momoh is a scientist in the Viral Hemorrhagic Fever Research Lab at Kenema Government Hospital and Dr. Bond is a post-doctoral research fellow in Microbiology and Immunology at Tulane School of Medicine. They will be mentored by researchers with complementary expertise in immunology and vaccinology, clinical trials, immunity assays, and bioinformatics from four CREID Research Centers representing three different institutions, including UTMB, Washington State University, and Tulane School of Medicine.