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The challenge of conducting arbovirus surveillance in Venezuela

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I was born in Caracas, Venezuela, to a mother from Kansas City, Missouri and a Venezuelan father with German roots. Growing up, I watched my parents navigate, negotiate and resolve vast cultural differences that challenged their worldviews every day. For several years, we lived with my grandparents, who co-founded the Foundation for the Conservation of Nature (FUDENA), Venezuela's major conservation NGO. My grandfather managed a cattle ranch and biological research station that has given rise to more than 500 peer-reviewed publications and dissertation projects conducted by Venezuelan and international students of ecology, animal behaviour and animal husbandry.

At home in Caracas, we had three large artificial ponds with pet turtles and Orinoco crocodiles, and three fenced areas with capuchin monkeys and other animals that were sometimes brought from the ranch for veterinary examinations or because they could no longer survive in the wild. I grew up with a sense that all creatures have realities of their own, many of which I would spend my life trying to understand. I've known for as long as I can remember that I would pursue a career in biology. In recent years, I have discovered that my love for biology – and for science – is inextricably tied to my love for my home country, whose rich tropical wealth is being eclipsed by its oil wealth and corruption. What has taken me by surprise is that I would someday be studying viruses, which aren't quite alive, and that I would be finding these viruses in the rubble of an unprecedented public health crisis in Venezuela.

Arthropod-borne viruses in Latin America

In Latin America, arthropod-borne viruses (arboviruses) such as Dengue, Chikungunya, Yellow Fever, West Nile, Zika and Mayaro viruses are becoming increasingly widespread (1). Clinical symptoms of these diseases range from mild febrile illness to haemorrhagic fever, neuroinvasive disease, and death. Substantial international efforts have been made to monitor and control the spread of arboviruses in Latin America. From the 1950s to the 1980s, Venezuela was a leading example in the surveillance and control of arboviruses. However, in recent years, the country's public health system collapsed amid social, political and economic turmoil. Conducting arbovirus surveillance and control in the country has become increasingly challenging, yet the need for effective interventions is greater than ever.

International collaboration for arbovirus surveillance in Venezuela

During my graduate studies at the University of Florida (UF), I was given the opportunity to study arboviruses in Venezuela. I embraced the opportunity to participate in this project, knowing that the circumstances were going to be challenging. The collaborative project was initiated by myself together with Dr. Glenn Morris, Jr., director of the UF Emerging Pathogens Institute (EPI), Dr. John Lednicky, a virologist at the UF Dept of Environmental and Global Health of the College of Public Health and Health Professions and EPI, Dr. Juliet Pulliam (then at the UF Department of Biology and EPI), and Dr. Alberto Paniz-Mondolfi, who at that time was practicing medicine at the Hospital Internacional Barquisimeto in Cabudare, Venezuela (now at Instituto Diagnóstico Barquisimeto). This project was designed to monitor the spread of arboviruses across international borders: Venezuela and the United States are in close proximity, and there is a substantial degree of travel from Venezuela to my resident state of Florida. Our project was funded by a National Science Foundation grant (PI: Juliet Pulliam) and by private donors through a crowdfunding campaign.

Differential diagnosis of arboviruses is required

In early 2016, during the start of our project, Brazil was reporting an increase in microcephaly and Guillain-Barré syndrome. This trend was associated with the spread of a virus that was relatively new to the Americas: *Zika virus* (ZIKV), the causative agent of Zika fever (ZF). One aim of our study was to establish the occurrence of ZIKV in Barquisimeto, Venezuela, as no laboratory-confirmed information was available at the time. This information would be useful for physicians trying to diagnose arbovirus infections in areas where dengue- and chikungunya viruses co-circulate endemically. Noteworthy, the clinical symptoms of ZF – mild fever, conjunctivitis, maculopapular rash, and arthralgias - resemble those of other arboviruses. Thus, as for other arbovirus infections, clinical diagnosis alone cannot be relied on to diagnose ZF. Because ZIKV can interfere with foetal development and can cause a range of congenital problems that include microcephaly and delayed neuromuscular development, differential diagnosis of ZF and detection of ZIKV infections in otherwise asymptomatic patients is essential, especially for women who are pregnant. Diagnosis of suspected ZIKV infections is most accurate ("gold standard") when the virus is isolated from the patients, but is typically attempted using some type of ZIKV nucleic acid detection test, such as by using by reverse-transcription polymerase chain reaction (RT-PCR). Evidence of ZIKV infection is also attained through serology (detection of specific IgM antibodies as evidence for new infection and serum IgG for convalescence or previous exposure). However, serology tests have been shown to be problematic for patients who live in areas endemic for viruses related to ZIKV, because the antibodies against the viruses can be cross-reactive; this results in frequent false-positive test results.

Challenges posed by Venezuela's complex social and political landscape

In Venezuela, by 2016, most diagnostic laboratories and pharmaceutical companies had gone bankrupt and were no longer operational. Publication of Venezuela's epidemiological bulletin had ceased. Unfortunately, access to basic medical supplies, equipment and reagents for diagnosis of viral diseases was extremely limited. The country's healthcare system was overburdened and under-resourced. In February of 2016, at the UF EPI, we prepared specimen collection boxes containing blood collection tubes, syringes, cryovials and other basic supplies to provide diagnostic support and to track the virus during this time of need.

I was able to solicit help from local friends and family to transport supplies from Caracas, the capital, to Barquisimeto, a city that is four hours away. Upon arrival to the hospital, we were greeted by a deluge of doctors, patients and interns who had observed symptoms of ZF but were unsure of whether the virus was indeed in Venezuela. Reports of ZF in neighbouring countries (Colombia and Brazil) suggested the illness would be present in Venezuela as well. Physicians, policymakers and local government officials were operating with little information, limited supplies, frequent power outages, and misleading information from government authorities.

We convened with a group of clinicians, students and interns to begin efforts to identify the agent(s) of presumed arbovirus-caused illnesses in Barquisimeto. Once the inclusion criteria and hospital protocols were established, we trained volunteers to collect and store urine, plasma and saliva specimens in a liquid nitrogen tank that we had purchased from a local cattle rancher. The specimens were shipped on dry ice to the University of Florida, where they were screened for ZIKV RNA by RT-PCR, and attempts were made to recover ("grow") the virus in cell cultures.

Zika virus and other viruses were confirmed in patients

In early 2016, Venezuela was experiencing a long drought. We therefore expected that transmission rates of arboviruses would be low, however, because of widespread water shortages, people were storing water in containers which then became breeding grounds for mosquitos such as *Aedes aegypti* and *Aedes albopictus,* which are common vectors of chikungunya, dengue and other viruses (including Zika).

Unofficial reports based on clinical diagnoses estimate that the incidence of ZIKV infections in Venezuela in 2016 was 2,057 per 100,000 (2). In our ongoing studies, one of the patients who was infected with ZIKV was a woman who was breastfeeding her five month-old child. Both the mother and the child were positive for the virus, including the mother's breast milk. Phylogenetic analysis of the genomic sequences of ZIKV isolated from both patients suggests that breastfeeding is a possible route of transmission, as both sequences were essentially identical (3).

The incidence of dengue virus infections in Venezuela has increased in the last decade: Venezuela has one of the highest numbers of Dengue Fever (DF)-like febrile illness cases in the American continent (4). In our study, several patients clinically diagnosed with ZF instead had DF. For example, we detected *Dengue virus* type 2 in a patient with haemorrhagic fever, and *Dengue virus* type 4 in two patients diagnosed with ZF.

Madariaga virus, a member of the South American *Eastern Equine Encephalitis virus* complex, was detected in one of the patients with suspected ZF (5). As in a previous outbreak, this case was detected in the context of an outbreak of equine encephalitis that was occurring in the region due to a shortage of vaccines against Venezuelan Equine Encephalitis Virus (VEEV). The symptoms of MADV and its close relatives range from mild febrile illness to encephalitis and death (6). Little is known about the transmission dynamics and epidemiology of *Madariaga virus* in humans; however it is suspected that the incidence of *Madariaga virus* is higher than previously thought.

Arbovirus surveillance: broadening the scope of the search

Because we utilize both molecular diagnostic techniques and virus isolation in cell cultures, we are able to broaden the scope of our search and identify other possible causes of arboviral disease in Venezuela. This combined approach is difficult and expensive to implement in most resource-strapped diagnostic laboratories; however it is a valuable tool for developing a more accurate picture of the potential cause(s) of an arbovirus epidemic. In many cases, concurrent epidemics due to more than one type of arbovirus occur during what is thought to be a single epidemic, as exemplified by cases of DF and ZF at the same location at the same time period in Barquisimeto. Moreover, in some cases, co-infections by more than
1 type of virus occur, further complicating the diagnosis without confirmation through laboratory tests.

As shown by our example, international collaborations can be helpful when in-country public health and pathogen surveillance systems are in disrepair. Our findings shed light on the complexities of arbovirus outbreaks, and confirmed the presence of ZIKV infections in Barquisimeto. The international community should always take notice; uncontrolled outbreaks in one country potentially cause a domino effect, spreading to surrounding countries.

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