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Zika Virus: From Obscurity to Potentially Devastating International Threat

Marie Louise Landry[1,*] **[Moderator]**, **Albert I. Ko**[2] **[Expert]**, **Laura D. Kramer**[3] **[Expert]**, and **Nikolaos Vasilakis**[4] **[Expert]**

[1]Departments of Laboratory Medicine and Medicine (Infectious Diseases), Yale University School of Medicine, and Clinical Virology Laboratory, Yale New Haven Hospital, New Haven, CT

[2]Professor and Chair, Department of Epidemiology (Microbial Diseases) and Professor of Medicine (Infectious Diseases), Yale University School of Medicine, New Haven, CT

[3]Director, Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, and Professor, Biomedical Sciences, School of Public Health, State University of New York at Albany, Albany, NY

[4]Associate Professor, Department of Pathology and Member, Center for Biodefense and Emerging Infectious Diseases, Center for Tropical Diseases, and Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, TX

In the fall of 2015, an epidemic of microcephaly in newborn infants was reported in Brazil. Zika virus (ZIKV),[5] a previously obscure arthropod-borne flavivirus in Africa transmitted by the *Aedes aegypti* mosquito, was identified as the suspected etiology. As pictures of severely affected infants began to appear in the news, the virus spread to additional countries in the Western hemisphere, and alarm grew. Travel alerts were issued and in some areas women were advised to defer pregnancy. Within a few months, the link of ZIKV infection to microcephaly was confirmed, and new information began and continues to emerge at a rapid pace. ZIKV is unique for an arbovirus, not only for its devastating outcomes in the fetus, but also for its potential for sexual transmission. Furthermore, it has become apparent that

[*]Address correspondence to this author at: Yale University School of Medicine, PO Box 208035, New Haven, CT 06520-8035. Fax 203-688-8177; marie.landry@yale.edu.

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[5]Nonstandard abbreviations: ZIKV, Zika virus; DENV, dengue virus; CHIKV, Chikungunya virus; GBS, Guillain-Barré syndrome; RT-PCR, reverse transcription PCR; FDA, US Food and Drug Administration; PRNT, plaque reduction neutralization test; NIAID, National Institute of Allergy and Infectious Disease.

obtaining a rapid and accurate ZIKV diagnosis can be challenging. The public health, medical, and research communities are working with great focus and urgency to better understand transmission, pathogenesis, and outcome, to accurately diagnose, and to ultimately prevent human infections. Three experts currently involved in the response to ZIKV have taken the time to share their knowledge and their insights on what we have learned to date, even as the epidemic continues to unfold.

ZIKV was first discovered in primates in the Zika Forest of Uganda in 1947. What changed to facilitate the recent explosive spread in humans?

Laura Kramer

Intercontinental air travel, globalization, and urbanization facilitate spread of viruses and vectors to distant places. Furthermore, global warming and climate change are redefining the geographical distributions of mosquito vectors. In 2007, ZIKV spread from Africa and Southeast Asia to cause the first large outbreak in humans on the Pacific island of Yap, in the Federated States of Micronesia. Before this event, no outbreaks and only 14 cases of Zika disease in humans had been documented worldwide. Although wind-blown mosquitoes can travel distances of several hundred kilometers over open ocean, introduction of ZIKV by travel or trade from Southeast Asia, involving an infected person or mosquito, is considered the most likely source of the 2007 Yap outbreak. Lack of population immunity likely contributed. Nonetheless, previous outbreaks of infection may have been missed due to the clinical similarities of mild illness associated with ZIKV, dengue virus (DENV), and chikungunya virus (CHIKV) infections, and the frequent cocirculation of all 3 arboviruses. After Yap Island, ZIKV spread to French Polynesia in 2013, and then to other Pacific Islands in 2013–2014. ZIKV may have been introduced into Brazil in 2013 during the

Confederations Cup Soccer Tournament or 2014 during the Va'a canoe race. During both events, teams from French Polynesia and other Zika-endemic Pacific island countries competed. Alternatively, Zika may have been introduced during the 2014 World Cup Soccer Match.

Nikolaos Vasilakis

We really do not know the underlying reasons for the observed global spread of ZIKV. However, there are a number of hypotheses that research groups are currently exploring. These include: (i) ZIKV underwent adaptive evolution to enhance infectivity of urban *Aedes* species vectors (such as, *Aedes aegypti* or *A. albopictus*), as recently occurred with CHIKV; (ii) ZIKV underwent adaptive evolution in the human host, leading to higher levels of viremia which would enhance both transmission by the biting mosquitoes and the risk of transplacental fetal transmission; (iii) ZIKV circulation in Asia or Africa resulted in relatively stable levels of human herd immunity that limited the potential for recognized outbreaks; and lastly (iv) the introduction of ZIKV into naïve populations in the South Pacific was stochastic and allowed for sufficient levels of amplification to facilitate the introduction into Brazil fueled by increased global travel, expansion of tropical cities, and susceptible *Aedes* mosquito populations.

DENV and CHIKV viruses are transmitted by the same A. aegypti mosquito vector as ZIKV and so far have had minimal transmission in the continental US. Are there reasons to think Zika will be different?

Albert Ko

Our best guess is that ZIKV will behave similarly to the other *Aedes*-transmitted viruses, where the risk of focal autochthonous transmission occurs in regions such as the Texas–Mexico border and southern parts of Florida. However, there are some important caveats. We do not have a clear understanding of the vectorial capacity, the rate of new ZIKV infections that an *Aedes* mosquito can generate after biting an infected individual, especially for *A. albopictus*, which has a wider distribution within the continental US. Moreover, introduction of the virus at the right place and time, such as during our summer months, may potentially produce a larger impact than what is predicted.

Nikolaos Vasilakis

In the context of continental US, socioeconomic factors would play an important role in limiting the extent and breadth of mosquito transmission. For example, the extensive use of window and door screens and air conditioning, piped water infrastructure, and good drainage significantly reduces available breeding and resting habitats for these mosquitoes. To maintain the likelihood of minimal transmission, vector control and surveillance programs at the federal, state, and community level must continue to enjoy sustainable long-term financial and logistical support.

Laura Kramer

Since 1980, locally acquired US cases of DENV have been confirmed along the Texas–Mexico border, associated with large outbreaks in neighboring Mexican cities. Limited mosquito-transmitted DENV was detected in Key West, Florida, in 2009 and 2010, with single isolated reported cases in other Florida counties in 2010 and 2011, demonstrating the potential in this state. In 2014, 12 locally transmitted CHIKV cases were reported from Florida, and in 2016, one local CHIKV case in Texas. All other CHIKV cases have occurred in travelers returning from affected areas. It is unlikely ZIKV transmission by *A. aegypti* and *A. albopictus* will be different from DENV and CHIKV in the US. Most likely, there will be focal flare-ups of infection, which may go unnoticed since 80% of ZIKV cases are asymptomatic. But once introduced, there may be ZIKV sexual transmission between partners without mosquitoes being involved. Recently, probable ZIKV transmission by oral sex has also been reported. Of all arboviruses, sexual transmission is unique to ZIKV and may be contributing to current outbreaks.

Clearly, the greatest concern for ZIKV is infection in pregnant women. What have we learned so far regarding risk factors for congenital infection and the spectrum of disease in the infant?

Albert Ko

The CDC and the WHO have concluded that ZIKV causes congenital birth defects, including microcephaly. However, we are still uncertain about how large the risk actually is. A small prospective study from Brazil identified severe fetal outcomes among 29% of women who acquired symptomatic ZIKV infection during pregnancy. In contrast, a retrospective study of microcephaly after the outbreak in French Polynesia estimated that microcephaly occurred in roughly 1% of the newborns of women infected during pregnancy. Furthermore, important questions remain unanswered with respect to which period and what type of exposure, symptomatic vs asymptomatic infection, during gestation impart the greatest risk to the fetus. Microcephaly is usually a severe “tip-of-the-iceberg” manifestation for most congenital infections. Case reports have identified central nervous system and ophthalmological lesions in newborn infants who were exposed to ZIKV during gestation but did not develop microcephaly. Yet at present, we do not know the disease burden attributable to congenital ZIKV infection that may be uncovered once infants without microcephaly are systematically investigated.

Nikolaos Vasilakis

We have learned quite a lot in the last 6 months. However, the mechanisms of intrauterine infection remain elusive, as well as the role and mode of sexual transmission in the infection of the placenta and the fetus. Unknown also is the role of asymptomatic infection in the development of congenital infection or whether previous exposure to heterologous flavivirus infection (e.g., DENV) enhances the severity of disease. The spectrum of disease in the offspring varies from fetal demise to various degrees of developmental brain abnormalities that may range from death to various cognitive abnormalities.

Laura Kramer

Severe microcephaly and intracranial calcifications appear to occur following infection in the late first or early second trimester. ZIKV infections that occur later in pregnancy have been associated with poor intrauterine growth, fetal death, or in some pregnancies, defects visible on prenatal imaging. Suspected “congenital Zika syndrome” includes such clinical features as low birth weight, reduced fetal movement, redundant scalp skin, fetal anasarca, polyhydramnios, and arthrogryposis, and may be associated with miscarriage. Neurological findings include microcephaly, polymalformative syndromes, brainstem dysfunction, and absence of swallowing. Ophthalmological findings include cataracts, asymmetrical eye sizes, intraocular calcifications, macular alterations, optic nerve abnormalities, iris coloboma, and lens subluxation. Thus, viral infections in early pregnancy can affect brain growth and lead to microcephaly, but infections later in pregnancy can cause less obvious but still significant pathology.

Zika infections in the normal host are 80% subclinical, or are associated with a self-limited rash illness. Have any complications been identified in nonpregnant patients?

Albert Ko

Case reports have described severe complications associated with an acute ZIKV infection, such as a sepsis-like presentation, thrombocytopenia, acute respiratory distress syndrome, and death. These complications are rare given that millions have been exposed to the virus during the recent epidemic in the Americas. The risk of ZIKV-associated Guillain-Barré syndrome (GBS) was first recognized during the outbreak in French Polynesia and was estimated at approximately 1 case per 1000 infections. The risk of GBS may therefore be higher than observed for many infectious agents, but lower than what has been observed for *Campylobacter jejuni*. In addition to GBS, the virus has been implicated to cause another immune-mediated disorder, acute demyelinating encephalomyelitis. However, ZIKV has been detected in cases that have presented with meningoencephalitis, myelitis, and encephalopathy, indicating that this neurotropic virus may mediate direct pathogenic effects in adults as well as in fetuses.

Nikolaos Vasilakis

We now have evidence that, in patients with underlying morbidities or compromised immunity, ZIKV infection may result in death. Several reports also indicated transient auditory abnormalities (e.g., hearing loss), as well as neurologic complications such as GBS, brain ischemia, myelitis, and meningoencephalitis. Because of the inherent limitations of serologic diagnosis, we can only estimate the force of infection, including the incidence of GBS.

Laura Kramer

Disease symptoms may include arthralgia, especially the small joints of hands and feet, and conjunctivitis (nonpurulent). Other commonly reported clinical manifestations include myalgia, headache, retro-orbital pain, and asthenia. Less commonly observed symptoms and

signs include abdominal pain, nausea, diarrhea, and mucus membrane ulcerations. In addition to GBS, scientists in Brazil uncovered a brain disorder associated with ZIKV infections in adults, an autoimmune syndrome called acute disseminated encephalomyelitis, or ADEM, that attacks the brain and spinal cord.

What are the challenges in diagnosing patients who have been exposed to ZIKV?

Albert Ko

There are key diagnostic needs in responding to the Zika epidemic, which include (i) diagnosing acute symptomatic infections in the clinical setting, (ii) detecting exposures, whether symptomatic or inapparent, among pregnant women, (iii) identifying prior infection in utero at the time of birth, and (iv) screening for past exposure in women of childbearing age, who may presumably have immunity to reinfection during a subsequent pregnancy. At present, it is not clear whether current molecular detection approaches, such as reverse transcription PCR (RT-PCR), have adequate performance in diagnosing symptomatic infections. The limited number of validation studies has hampered a clear understanding of the clinical application and potential limitations of these assays. Screening for ZIKV exposure, whether before or during pregnancy in women or in newborn infants, will likely need to rely on a serologic assay. However, a particular diagnostic challenge is detecting ZIKV exposures in settings where a significant proportion of the population have been previously exposed to flaviruses, such as DENV, West Nile virus, and yellow fever (via immunization), and have cross-reactive antibodies to ZIKV. Anti–whole ZIKV IgM detection assays have been employed as part of routine diagnostic workup but may not have adequate specificity in such settings due to differentiating ZIKV infection from exposures to other flaviviruses.

Laura Kramer

ZIKV is circulating in countries where related flaviviruses, including DENV, Japanese encephalitis, Saint Louis encephalitis, West Nile fever, and yellow fever viruses also are active. These flaviviruses produce nonspecific symptoms early in infection that are largely indistinguishable such as headache, rash, myalgia, and fever. While virus is detectable in the blood during the first week of symptoms, the utility of molecular methods such as RT-PCR for the detection of viral RNA is limited by the short duration of the viremia. Following the viremic phase of infection and a slightly longer period of viruria, diagnosis must be made with serological methods, which is challenging. Flavivirus serology is complex, due to extensive cross-reactivity between antibodies made in response to different flavivirus infections or vaccination. Furthermore, secondary infections increase the difficulty of diagnosis where titers to the originally infecting virus increase following a heterotypic secondary flavivirus infection. The importance of accurate diagnosis of ZIKV infections in pregnant women is increased considerably by the risk of serious consequences to the fetus. Since approximately 80% of infections are asymptomatic, if a woman has traveled to a ZIKV endemic area during pregnancy, or has a sexual partner who has, testing should be recommended even in the absence of symptoms.

Diagnostic testing is currently confined to public health laboratories. When will ZIKV testing be more widely available?

Albert Ko

A number of commercial kits have been developed, using a spectrum of molecular detection and serologic approaches. The rate limiting step will be the ability to recruit well-characterized patient populations and samples to perform validation studies. Confirmation with serology may unfortunately be limited to regions where transmission of other flaviviruses has not occurred, owing to the issue of high serologic cross-reactivity between members of this family.

Nikolaos Vasilakis

The only reliable test is based on the molecular detection of the ZIKV genetic signature. Albeit in principle it is highly sensitive and specific, it has a short window of detection in serum, saliva, and urine samples. While the use of serology-based tests as confirmatory is not recommended as yet because of their cross-reactivity with other flaviviruses, these serologic tests are highly accurate in monotypic infections,; therefore, context is important—for populations, such as American, that are not routinely exposed to such infections serologic testing may be applicable, but not for populations that reside in hyperendemic settings, where repeated flavivirus exposure is the norm.

Laura Kramer

The availability of ZIKV testing in the US at non–public health laboratories has been delayed by several factors. In addition to the usual time needed for developing and implementing laboratory developed tests, there has been very limited availability of clinical samples for validation testing. Additionally, the US Food and Drug Administration (FDA) requests review of validation data. A few commercial and hospital laboratories have submitted validation data to the FDA for Emergency Use Authorization of molecular tests for the detection of viral RNA in serum, and in some cases urine, and a few have been approved. The number of facilities offering such testing will likely increase in the coming months as more tests obtain FDA approval.

Commercial serologic assays are also in the process of FDA submission and approval, so market availability is expected soon. The development of serological assays for ZIKV reactive antibodies has been slower than that for molecular assays because of cross-reactivity with antibodies to other similar flaviviruses. Developing screening tests that are technically simple to perform and detect ZIKV reactive antibodies with a high level of specificity, while maintaining an acceptable level of sensitivity, has proven challenging.

In many US states, confirmation by the state laboratory is required in the event of detection of an arbovirus infection. For samples from these states, RNA-positive samples must therefore be forwarded to the state public health laboratory for confirmation. The confirmation of commercial serologic assays (IgM ELISA) by plaque reduction neutralization tests (PRNT) is strongly recommended until assays are fully validated. However, performing labor-intensive PRNT, which uses infectious virus, to confirm large

numbers of IgM ELISA test results from clinical and commercial laboratories, may be very challenging for public health laboratories whose resources have already been strained by response efforts.

Can you comment on standard preventive measures, as well as the novel vector control strategies being developed?

Laura Kramer

The best approach is integrated pest management incorporating multiple approaches to prevent further spread of the virus. To decrease mosquito population density is difficult with *A. aegypti* because they frequently rest indoors. But using physical barriers such as screens, closed doors, and windows in homes is highly effective. State and local mosquito control efforts can assist with source reduction, which involves eliminating or covering containers with standing water. Discarded automobile tires, which can become mosquito habitats, should be collected and properly disposed of. Distribution of biological controls such as fish is possible. Larvicides, with selective action and moderate residual activity, can be added to aquatic habitats when immature stages are concentrated in the breeding sites and before the adult forms emerge and disperse. People can protect themselves against mosquito bites by using insect repellent and wearing clothes that cover as much of the body as possible. A third approach involves decreasing the lifespan of *A. aegypti* through release of mosquitoes infected with a rickettsial symbiont, *Wolbachia*. Another novel approach to control is through engineering male mosquitoes genetically so that following mating, the progeny larvae die before emerging as adults.

Nikolaos Vasilakis

In the absence of effective vaccines and antivirals, the best methods for controlling ZIKV rely on reducing contact between the vector and susceptible humans. In resource-poor settings where piped water supply is nonexistent, elimination or protection of water containers that fill with rainwater and serve as larval habitats is an effective method and relies on community engagement and personal responsibility. Another method will be to apply larvicides to these sources and effectiveness is dependent on sustainable application. Lastly, indoor spraying including the application of residual insecticides that have repellant activity can be extremely effective but this method is quite expensive and labor-intensive.

Recently several new technologies have been developed that show promise in controlling *A. aegypti* populations. The first involves the release of genetically modified male mosquitoes that express a dominant lethal gene, resulting in the death of all offspring from mating with wild females. While this approach has been quite successful in reducing mosquito populations on a small scale, logistical, technical, and financial scale up challenges have not been adequately addressed. Thus, its impact in controlling mosquito populations in the sprawling and chaotic megacities of the tropics where ZIKV and other arboviruses circulate cannot be ascertained. The second approach is to release *A. aegypti* infected with *Wolbachia,* which are endosymbiotic bacteria that spread through natural populations and suppress viral transmission by interfering with replication in the mosquito. While this approach has been quite effective in controlled releases throughout the tropics, potential

limitations such as the need to release these mosquitoes over wide geographic ranges to overcome their limited flight range, as well as the possibility that arboviruses will rapidly evolve mechanisms to overcome their inhibitory effects, remain to be addressed. Lastly, the use of lethal traps, which are inexpensive and relatively maintenance free has been shown to reduce *A. aegypti* populations substantially.

Perhaps most importantly, how close are we to having a ZIKV vaccine?

Albert Ko

Several candidate vaccines are in development with projections for Phase II trials to begin by 2017. If successful, we may potentially have an efficacious vaccine as early as 2018. However, these efforts may encounter several challenges. We do not know at present whether natural infection with ZIKV confers immunity to reinfection and thus have limited insights into the immunological correlates that would aid development of a vaccine. The phenomenon of Zika-associated GBS adds an additional complication. The specific mechanism and ZIKV moiety that elicits this immune-mediated process is not well understood. Although GBS is a rare manifestation of ZIKV infection, clinical trials will presumably need to evaluate whether immunization with vaccine candidates elicits this potential adverse event. Finally, the epidemic and unpredictable nature of ZIKV transmission is a challenge with respect to planning vaccine efficacy trials that can enroll sufficient numbers of participants that are at risk for ZIKV infection.

Laura Kramer

The process of developing, testing, and commercializing a vaccine takes years to ensure products are safe and effective. Several teams of scientists are working on ZIKV vaccines. The National Institute of Allergy and Infectious Diseases (NIAID) had created a trial vaccine for a related flavivirus, West Nile virus, that is being repurposed for ZIKV. The Jenner Institute has conducted trials with mice and aims to hold clinical trials by 2017. The latter is based on a simian adenoviral vector being developed in Oxford to determine if it is immunogenic and can be taken to clinical trials. The NIAID vaccine will go into Phase I trial in September 2016 to determine its safety profile. Phase 2 and Phase 3 trials would then have to be designed, conducted, analyzed, and reported before a company intent on licensing the vaccine could apply to the FDA for approval of the vaccine. If all goes well, the vaccine would be employed in 2018. Other approaches include a DNA vaccine, a whole inactivated [virus] particle vaccine, and a live chimeric inactivated vaccine that will not go into phase I until the middle of 2017.

Nikolaos Vasilakis

There are several platforms that have been developed and licensed as efficacious flavivirus vaccines, including live attenuated virus [yellow fever virus (17D strain) and Japanese encephalitis virus (14–14-2 strain)], inactivated-virus vaccine [Japanese encephalitis virus (14–14-2 strain)], and chimeric virus [17D vaccine strain of yellow fever virus containing the structural membrane and envelope genes from DENV] that was recently approved for clinical use for DENV in Mexico, Brazil, and the Philippines. Other approaches, such as subunit vaccines representing ZIKV proteins, DNA vaccines expressing viral proteins, and

other viral vectors expressing viral antigens could be explored as potential vaccine delivery platforms for ZIKV vaccine development. Whereas preclinical development is a rather “quick” process, clinical evaluation for safety and efficacy to obtain licensing for human use is a time consuming process that may take up to 15 years and requires hundreds of millions of dollars in financial commitments. Although subunit vaccines may exhibit better safety and shorter development time, a live attenuated virus vaccine may elicit stronger humoral and cellular immune responses for better protection. Therefore, complementary approaches should be explored simultaneously to further advance vaccine ZIKV candidates.