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Zika Virus as an Emerging Global Pathogen:

Neurological Complications of Zika Virus

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Abstract

IMPORTANCE—Zika virus (ZIKV) is an emerging arthropod-borne virus (arbovirus) in the genus *Flavivirus* that has caused a widespread outbreak of febrile illness, is associated with neurological disease, and has spread across the Pacific to the Americas in a short period.

OBSERVATIONS—In this review, we discuss what is currently known about ZIKV, neuroimmunologic complications, and the impact on global human health. Zika virus spread across Africa and Asia in part owing to unique genomic evolutionary conditions and pressures resulting in specific human disease manifestations, complications, and pathogenesis. Recent data suggest that acute ZIKV infection in pregnant women may result in acute infection of fetal tissue and brain tissue, causing microcephaly and potentially severe debilitation of the infant or even death of the fetus. Cases of acute ZIKV are also associated with Guillain-Barré syndrome. With the increased number of cases, new complications such as ocular involvement and sexual transmission have been reported.

CONCLUSIONS AND RELEVANCE—Zika virus is an emerging viral pathogen with significant consequences on human health throughout the world. Ongoing research into this pathogen is urgently needed to produce viable vaccine and therapeutic options.

Zika virus (ZIKV) is in the family Flaviviridae, a group of positive-sense, single-stranded, enveloped RNA viruses. Members of the *Flavivirus* genus within this family cause widespread human diseases, including yellow fever, dengue, Japanese encephalitis, West Nile virus disease, and ZIKV infections. Flaviviruses have a characteristic RNA genomic organization with a capped, 5′ RNA followed by a short noncoding region, a single open-

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reading frame that codes for a polyprotein, and a 3' noncoding region (Figure 1). The genomic RNA for flaviviruses is typically 10 to 11 kilobases in length, is structured to function in a similar fashion to host messenger RNAs within the host cell, and can quickly undergo translation by host factors.² This allows flaviviruses to form replication complexes and create new RNA genomes and viral proteins.

Discussion

Timeline of the ZIKV Outbreak

Flaviviruses are spread by arthropod vectors, and ZIKV is transmitted by mosquito vectors in the *Aedes* genus. The first isolation of ZIKV occurred in 1947 from the blood of a febrile sentinel rhesus monkey and *Aedes africanus* mosquitoes at the edge of the Zika forest of Uganda as part of a project to collect yellow fever virus isolates.^{3,4} Because it was unclear whether ZIKV could cause human disease, in 1956 a human volunteer who was previously vaccinated against yellow fever virus was inoculated with ZIKV-infected mouse brain suspension; the volunteer subsequently developed a 1-week clinical syndrome of headache, fever, and malaise.⁵ The first reported natural case of ZIKV infection in a human occurred in 1964, in a European man working in Uganda who developed a 5-day syndrome of frontal headache; a maculopapular rash on the face, neck, trunk, and upper arms; fever; and myalgias.⁶ The patient was diagnosed as having ZIKV using acute and convalescent serum samples showing development of neutralizing ZIKV antibodies.⁶ Subsequent serologic data indicated that ZIKV infections occurred in West Africa (Nigeria, Sierra Leone, Gabon, and Senegal) as well as East Africa (Uganda).⁷⁻¹⁰ Zika virus infections were also found in parts of Asia, including Pakistan, Indonesia, and Malaysia.¹¹⁻¹³ Based on nonstructural gene 5 (NS5) gene sequences, 3 lineages of ZIKV were found to represent infection in East Africa, West Africa, and Asia.¹⁴

In 2007, an outbreak of ZIKV disease in the Yap State of the Federated States of Micronesia highlighted the epidemic potential of this virus.¹⁵ During that outbreak, 59 probable cases and 49 confirmed cases of ZIKV infection were reported.¹⁵ Symptoms of acute ZIKV infection included fever, myalgias, rash, and conjunctivitis. An estimated 73% of residents on Yap were infected with ZIKV during the outbreak, with an estimated 900 cases of illness. Surveys and serologic studies indicated that approximately 19% of patients with seroconversion developed clinical illness.¹⁵ Despite the large number of cases, no complications were noted in the study: no hospitalizations, hemorrhagic manifestations, or deaths.¹⁵ Interestingly, no neurologic complications or fetal complications were noted during this outbreak.

Zikavirus continued to spread east across the Pacific Ocean, with reported outbreaks in French Polynesia in 2013 followed by outbreaks in the Cook Islands, Easter Island, Vanuatu, and the Solomon Islands.¹⁶ In 2015, ZIKV appeared in Brazil and phylogenetic studies of gene sequences traced the virus to Asian isolates that had appeared in the outbreaks in the Pacific Islands.^{17,18} Since the outbreak in Brazil, ZIKV has rapidly spread through South America, Central America, Mexico, and many of the islands in the Caribbean (Figure 2). The outbreak of ZIKV in Brazil was closely associated with the distribution of *Aedes* mosquitoes, consistent with the known biology of the virus. In early 2015 in Brazil, many of

the clinical symptoms remained unchanged from previously reported symptoms associated with acute ZIKV infection; however, by September 2015, reports of an increase in the number of infants born with microcephaly in ZIKV-affected areas began to emerge.²⁰

ZIKV Complications

Prior to the Brazilian outbreak, ZIKV caused illness in about 20% of patients infected with the virus and was associated with an acute, mild, febrile illness with rare reported complications. By 2014, an increased number of Guillain-Barré syndrome (GBS) cases possibly associated with ZIKV infection were reported in French Polynesia and later in South America.^{21–24} During the outbreak in French Polynesia in November 2013, a Polynesian woman developed GBS 7 days after an acute febrile illness attributed to ZIKV based on serology and the ongoing epidemic on the island.²¹ The patient presented with bilateral paresthesias and ascending muscle weakness. By day 3 of admission, she developed tetraparesis, diffuse myalgia, and bilateral facial, asymmetric peripheral facial palsy. Deep tendon reflexes were absent, and cerebrospinal fluid examination revealed a white blood cell count of 7/mL with albuminocytological dissociation of 1.66 g/L protein. Results of testing for common causes of GBS were negative, but results for ZIKV-specific IgM were positive at 8 and 28 days after the original acute febrile episode. Zika virus seroconversion was confirmed with plaque reduction neutralization tests against ZIKV. Thus, the patient developed GBS associated with acute ZIKV infection.

With the outbreak in Brazil, reports began to surface of possible other complications associated with ZIKV infection. In 2015, the Brazil Ministry of Health established a task force to investigate the possible association of ZIKV with microcephaly during pregnancy.²⁰ Microcephaly is defined as a decrease in head circumference greater than 2 SDs below the mean for sex and gestational age at birth. During a reported outbreak of ZIKV in northeast Brazil in early 2015,¹⁷ the Brazil Ministry of Health confirmed an increase in birth prevalence of microcephaly in the same region compared with previously reported rates of 0.5/10 000 live births.²⁰ Despite likely underreporting of microcephaly prior to the outbreak, the Brazil Ministry of Health established a microcephaly registry and reported an increase in microcephaly cases in areas of the ZIKV outbreak.²⁰ By December 2015, ZIKV RNA was detected in the amniotic fluid of 2 pregnant women whose fetuses were found to have microcephaly and in the brain and tissues of an infant with microcephaly who died immediately in the neonatal period.^{20,25} Additionally, ZIKV RNA was found in amniotic fluid from 2 women pregnant with children with microcephaly.²⁵

Out of an abundance of precaution, the Centers for Disease Control and Prevention (CDC) issued a travel advisory for ZIKV-affected regions and on February 1, 2016, the World Health Organization declared the ZIKV outbreak a Public Health Emergency of International Concern.²⁶ The CDC also published guidelines for caring for women with possible ZIKV exposure who are or may become pregnant.²⁶ If a pregnant woman has a history of travel to an area with ongoing ZIKV transmission, then the CDC recommends a ZIKV testing algorithm to evaluate for microcephaly (Figure 3).²⁶

Subsequently, a case report provided additional evidence of microcephaly associated with ZIKV infection.²⁷ A 25-year-old European woman who lived in Brazil became ill during the

13th week of pregnancy with likely ZIKV infection; fetal ultrasonography results at 14 and 20 weeks of gestation were normal.²⁷ By 32 weeks of gestation, ultrasonography of the fetus revealed severe microcephaly. Autopsy of the fetus revealed ZIKV RNA in the fetal brain tissue with 99.7% sequence identity to a ZIKV isolate from the French Polynesia outbreak in 2013. Microscopic evaluation of the fetal brain tissue revealed extensive inflammation, astrocyte and microglial activation, and injury in the cortex and the lateral corticospinal tracts; electron microscopy analysis revealed evidence of flavivirus-like virions in the fetal brain tissue. The complete genome of ZIKV was recovered from the fetal brain tissue and exhibited a high degree of homology to Asian strains and more distant relation to the African strains of the virus.

As the outbreak has continued, other reports of complications and new modes of potential transmission of ZIKV have surfaced. In a small case series of 29 infants with microcephaly born to 29 mothers, 23 of the mothers reported suspected ZIKV symptoms during pregnancy and ocular abnormalities were found in 10 of the 29 children (34.5%) with microcephaly.²⁸ Of these 10 affected children, 7 had bilateral disease; the most common findings were focal pigment mottling of the retina and chorioretinal atrophy in 11 of the 17 eyes with abnormalities (64.7%), followed by optic nerve abnormalities in 8 of the 17 eyes (47.1%). These findings suggest that ZIKV infection in neonates can also cause significant eye disease, and infants born to symptomatic mothers should be screened for eye disease. Additional studies have recently found evidence of human-to-human transmission of ZIKV through sexual transmission.^{29,30} In support of these observations, ZIKV RNA has now been found in saliva,³¹ semen, and urine of infected patients.^{29,32} The duration of infectivity of ZIKV in the semen and urine is not currently known, but in these cases infectious particles of ZIKV were recovered from semen in the absence of viremia,²⁹ implying that infectivity in semen can occur after symptoms and viremia resolve.

There are limited data on the best diagnostic approaches for acute ZIKV infection in humans.³³ Unlike acute West Nile virus infection, patients presenting with acute ZIKV symptoms are viremic for a mean of 3 days following initial symptoms such that the virus can be detected in clinical samples using reverse transcription–polymerase chain reaction assay.³³ The CDC ZIKV assay uses two 1-step real-time reverse transcription–polymerase chain reactions that target the ZIKV premembrane and envelope genes.^{33,34} Other ZIKV gene targets have been used for other polymerase chain reaction assays as well. Clinical samples that have tested positive for ZIKV RNA by polymerase chain reaction include serum or plasma, saliva, urine, semen, and amniotic fluid. Serologic diagnosis using assays measuring ZIKV IgM and IgG production with paired acute and convalescent serum is another important diagnostic method.³³ However, other common flaviviruses such as dengue virus or Japanese encephalitis virus can exhibit a high degree of serologic cross-reactivity to ZIKV serology even with the use of capture enzyme-linked immunosorbent assay. At times more specific tests such as the plaque reduction neutralization tests can differentiate between flaviviruses, but they still can exhibit low-level cross-neutralization with other flaviviruses.³³ Thus, the diagnosis of acute ZIKV infection based on serology alone must be evaluated with caution in regions with other endemic flavivirus infections.

ZIKV Genomic Evolution and Pathogenesis

Owing to the rapidity of sequencing technology, several ZIKV isolates from this outbreak are already reported and available for analysis and comparison with past isolates.^{14,35–37} A recent analysis of the molecular evolution of ZIKV isolates throughout the emergence of the virus in the 20th century revealed several insights.³⁶ Based on phylogenetic analysis, ZIKV likely first emerged in Uganda around 1920 followed by 2 independent ZIKV introductions into West and Central Africa from the eastern portion of the continent and then a third introduction event into Malaysia around 1945.³⁶ During its emergence, ZIKV underwent 13 recombination events, which is an unusual feature for a flavivirus since these viruses are often genetically restricted by the need to replicate in evolutionarily disparate invertebrate (mosquitoes) and vertebrate hosts.³⁶ The unusual evolutionary plasticity of the ZIKV genome is likely due in part to vertebrate host preferences for the virus,³⁸ which may contribute to ZIKV genomic adaptation to specific vector–vertebrate host environments. This likely resulted in an increased frequency of ZIKV activity every 1 to 2 years when compared with other arboviruses, which exhibit an activity frequency of 5 to 8 years for dengue virus and yellow fever virus.³⁶ The unique genomic features of ZIKV likely contributed to the rapid emergence of the virus over a very large geographic area.

Very little is currently known about the biology and pathogenesis of ZIKV. The envelope protein for flaviviruses is largely responsible for host range due to receptor binding and immune responses. The ZIKV envelope gene underwent several selective changes mainly associated with negative selection, implying an important role for this gene in vertebrate host selectivity and emergence.³⁶ Acute ZIKV infection in 6 human cases was characterized by robust, acute polyclonal T-cell activation and acute increases in serum interleukin (IL) responses (IL-1 β , IL-2, IL-4, IL-6, IL-9, IL-13, and IL-17).³⁹ Also, acute infection in these patients was associated with increases in RANTES (regulated on activation, normal T cell expressed and secreted), macrophage inflammatory protein 1 α , and vascular endothelial growth factor.³⁹ These data provide some initial information to characterize the acute inflammatory response in these patients.

Cell culture and mouse models of ZIKV are not well established at this stage. Recent data have shown that human dermal fibroblasts, epidermal keratinocytes, human neurospheres,⁴⁰ and immature dendritic cells are permissive to recent ZIKV isolates and that several entry or adhesion factors (DC-SIGN, AXL, Tyro3, and TIM-1) permitted ZIKV entry, implying that the virus can use a range of host receptors to gain entry into different cell types.⁴¹ During acute infection in cell culture, ZIKV induced transcription of Toll-like receptor 3 (TLR3), retinoic acid-inducible gene 1 (RIG-I), and melanoma differentiation-associated protein 5 (MDA5) as well as several common interferon-stimulated genes, implying that the innate immune response found to be important for control of other flaviviruses also plays an important role in the detection and control of ZIKV.⁴¹ Zika virus was also sensitive to type I and II interferons in these cell culture systems.⁴¹ These data are consistent with what is known about other flaviviruses and help to characterize the role of the immune response for this virus. A better understanding of the mechanisms of immune control of ZIKV will enable targeted vaccination or therapeutic treatments in the future.

A recent mouse model of ZIKV brain infection was developed in interferon signaling-deficient mice.⁴² Work with classic ZIKV isolates in suckling mice revealed neurotropism and 100% mortality by day 10 following intracerebral injection.^{3,43–45} These studies revealed neuronal injury especially in the hippocampus characterized by astrocyte activation and new virions on ultrastructural examination in networks of the endoplasmic reticulum of neurons.⁴³ Adult mice in these studies seemed to be more resistant to peripheral infection with ZIKV but did develop neurologic disease following intracerebral infection.⁴⁴ Further development of animal models for modern ZIKV isolates is needed to understand the complex pathogenesis and immune responses required to prevent disease.

Conclusions

Zika virus has rapidly emerged throughout the 20th century and now is causing a large epidemic of disease in the Americas that has concerning links to possible intrauterine infection of the brains of developing fetuses. There is no current therapy or vaccine for this infection and the best approach to avoid complications from ZIKV is to avoid exposure to mosquitoes by using insect repellent, wearing long-sleeved shirts and pants, and using air conditioning and window screens to keep mosquitoes outside. For women traveling to regions undergoing a current outbreak of ZIKV, cautions must be undertaken in the case of pregnancy as outlined by the CDC.⁴⁶ For men and women living in the endemic region of the ZIKV outbreak, we need urgent solutions and study of this virus to produce interventions that can prevent birth defects now linked with acute ZIKV infection in pregnant women.

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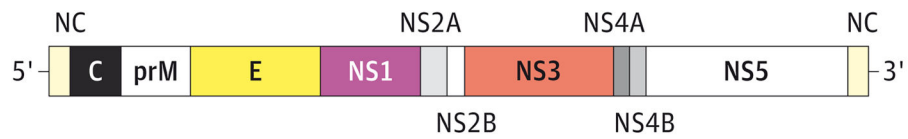


Figure 1. Organization of the Flavivirus Genome

Flaviviruses have capped RNA genomes with 5' and 3' noncoding (NC) regions. Following the 5' NC region, flavivirus genes are translated as a single polyprotein and cleaved into structural proteins (capsid [C], premembrane [prM], and envelope [E]) and nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Adapted from Petersen and Roehrig.¹



Figure 2. Map of Current Regions in the Americas Experiencing Active Zika Virus Infections
Adapted from the Centers for Disease Control and Prevention.¹⁹

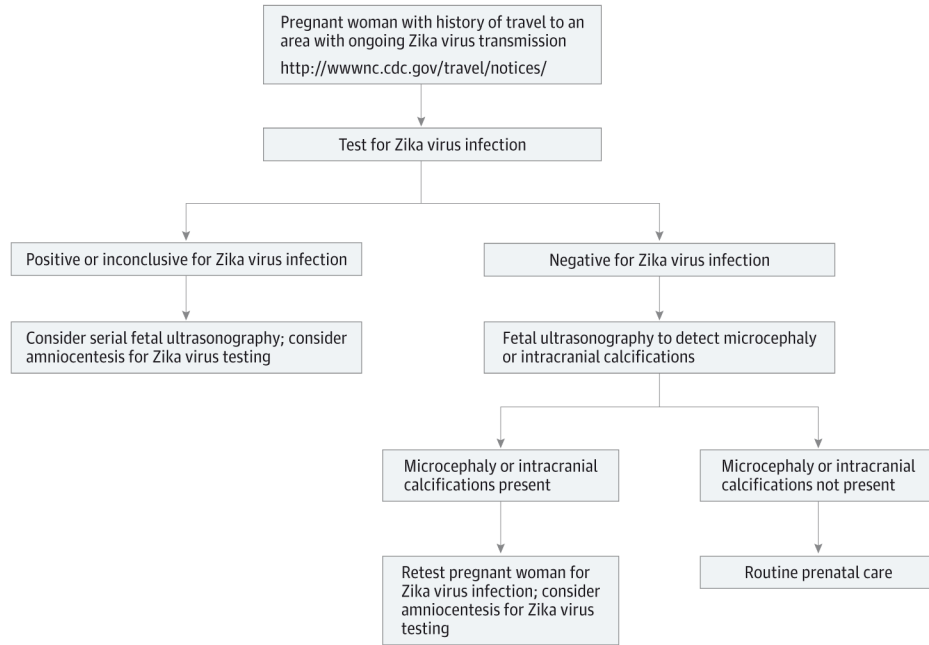


Figure 3. Centers for Disease Control and Prevention Algorithm for Evaluation of Pregnant Women With a Possible Exposure to Zika Virus
Adapted from Oduyebo et al.²⁶