Epidemiology of Zika Virus

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KEYWORDS

• Zika virus • Public health • Neuroepidemiology

KEY POINTS

- Zika virus is an arbovirus belonging to the *Flaviviridae* family, originally isolated in Uganda in 1947, known to cause mild clinical symptoms similar to those of dengue and chikungunya and transmitted by different species of *Aedes* mosquitoes. Direct interhuman transmission occurs perinatally, through blood transfusion, and sexually.
- Recent outbreaks in several regions of the world including Egypt, Easter Island, the insular pacific region, and more recently Brazil, highlight the need for the scientific community and public health community to consider it as an emerging global threat.
- Its clinical profile is that of a dengue-like febrile illness, but recently associated Guillain-Barre syndrome and microcephaly have appeared. There is neither a vaccine nor prophylactic medications available to prevent Zika virus infection.
- Public health recommendation advises pregnant women to postpone travel to areas where Zika viral infection is epidemic, and if not, to follow steps to avoid mosquito bites to avert fetal brain injury associated with early and late intrauterine infection.

HISTORICAL ASPECTS

Zika virus was first identified in a rhesus monkey in the Zika Forest of Uganda in 1947.[1] It was later found in people with febrile illnesses in West Africa in 1954.[2] It then spread to Indonesia,[3] Micronesia,[4] the Philippines,[5] French Polynesia,[6] and Easter Island–South Pacific[7] in 2014. Zika virus infections were not documented on mainland South America until the first report of autochthonous transmission in Brazil in May 2015. The conclusion at that time was that Zika virus was introduced into Brazil during the 2014 World Cup Football.[8] This was not supported due to the fact that no Pacific countries with documented Zika virus infection had competed in the World Cup competition. However, Pacific countries had participated in the August 2014 Va'a World Sprints canoe championship, which was held in Rio de Janeiro, suggesting that introduction of Zika virus into Brazil could have occurred then.[9] Another possibility was the introduction of Zika virus to Brazil by travelers from Chile.[10] Since its introduction into Brazil

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in May 2015, Zika virus infection has subsequently spread rapidly across Brazil and the Americas. As of January 28, 2016, autochthonous cases of Zika virus infection have been reported from 26 countries in the Americas: Barbados, Bolivia, Brazil, Colombia, Curacao, Dominican Republic, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haití, Honduras, Martinique, México, Nicaragua, Panama, Paraguay, Puerto Rico, Saint Maarten, Suriname, Venezuela, Virgin Islands. No autochthonous Zika virus transmission has been reported from European Union countries, and a heightened state of global alert is in place in Europe and the United States to screen for Zika virus in travelers with fever returning from endemic countries. The first travel-associated Zika illness among US travelers was reported in 2007. From 2007 to 2014, a total of 14 returning US travelers had positive Zika testing performed at the US Centers for Diseases Control and Prevention (CDC). In 2015 and 2016, at least 8 US travelers have had positive Zika testing performed at CDC.[11]

INFECTIOUS TRANSMISSION

Mosquito Vectors

Aedes species mosquitoes are present throughout the tropics and are recognized vectors of Zika, chikungunya, dengue, and yellow fever virus.[12–15] Although the main vector associated with transmission of Zika is *Aedes aegypti*, transmission can also occur with *A albopictus*, *A africanus*, *A luteocephalus*, *A vittatus*, *A furcifer*, *A hensilii*, and *A apicoargenteus*. *A Aegypti* mosquitoes live and breed near people and their homes, where they lay eggs in stagnant water and collect in puddles, buckets, flower pots, empty cans and other containers. They bite humans mainly during daytime, outside or inside their houses. *A aegypti* mosquitoes are widely distributed in the Americas, where the climate is suitable breeding condition. Recognizing that *A albopictus* has been found in the United States as far north as New York and Chicago, and in parts of southern Europe, Zika transmission will no doubt increase throughout the Americas, with possibility of local transmission within the United States Moreover, as *Aedes* mosquito species that spread Zika are found in many other locations globally, it is highly likely that outbreaks will spread to new countries.

Sexual Transmission

Whereas Zika virus isolated from semen in returning travelers typically developed up to 6 days after brief travel to Indonesia where Zika was endemic,[16] symptoms in the patient with presumed sexually transmitted infection were noted 10 days after sexual intercourse with the index case.[17,18] Studies are needed to assess how frequently and for how long the Zika virus persists in semen and what precautions should be mandated to prevent sexual transmission of Zika virus short of abstinence during a period of self-imposed quarantine.

Blood Transfusion

Given that many infected individuals with Zika virus infection will be asymptomatic and among them blood donors, transmission of Zika virus via blood transfusion is of concern.[19] This has a parallel in the introduction of West Nile Virus in the United States and Canada, which led to careful screening of donated blood.[20] The outbreak of chikungunya virus, which started in Reunion[21] and spread throughout Asia, similarly prompted screening of blood products. After the introduction of chikungunya virus in Italy, systematic screening by blood banks was considered, but a laboratory test for routine testing was not available.[22] Blood donations from people living in the affected municipalities were discontinued, and a 21-day deferral policy was

introduced nationwide for blood donors who visited affected areas even for a few hours. All such stocked blood components collected from donors living in the affected area after the identification of the first case were eliminated. It is likely that blood transfusion-related infection will occur in Zika-endemic areas such that to prevent blood transfusion-related infection, blood donations should also be carefully screened.

ANIMAL AND HUMAN MODELS

Animal and human infectivity have been studied for more than 50 years, revealing the propensity for central nervous system (CNS) involvement. According to Dick and colleagues,[1] Zika virus was first isolated in 1947 from a captive sentinel rhesus monkey caged in the canopy of the Zika forest near Entebbe, Uganda, during the course of research into the epidemiology of yellow fever. The second isolation was made from a lot of *Aedes africanus* taken in 1948 from the same forest. Dick and colleagues[1] carried out cross-neutralization tests indicating that Zika virus was not related to yellow fever, Hawaii dengue, or to Theiler mouse encephalomyelitis virus.

In further experiments of the pathogenicity and physical properties of the virus in experimental animals,[23] Zika virus was found to be highly neurotropic in mice without traces of infection in any tissues other than the CNS at the onset of illness after inoculation. Moreover, the maximum virus titer was present on the first day of signs of illness, with a gradual fall thereafter. After intracerebral inoculation with infected mouse brain homogenates, 1 of 5 experimental monkeys showed mild pyrexia; however, the others showed no signs of infection. Viremia during the first week pi was found in all monkeys tested and antibody was demonstrated by the fourteenth day after inoculation. Among 99 human sera collected for yellow fever studies in Uganda, 6 (6.1%) were considered positive for Zika virus. Antibody was also found in the serum of 1 of 15 wild monkeys tested. The size of Zika virus was estimated to be in the region of 30 to 45 mμ in diameter. The virus was preserved up to 6 months in 50% glycerol and up to 30 months after drying, and susceptible to anesthetic ether; the thermal death point is 58°C for 30 minutes. Neuronal degeneration, cellular infiltration, and areas of softening were present in infected mouse brains sacrificed on the first day of signs of infection and confined to the CNS that was in various stages of infiltration and degeneration including widespread softening, neuronal degeneration, and cellular infiltration in the spinal cord. Minimal inflammatory changes were found in the ependymal membrane. Inclusion bodies of the Cowdry type A were observed in damaged neurons of acutely ill animals, especially in young animals compared with adults. Inclusions were absent from the brains of mice sick for several days or those chronically ill, even though the latter showed extensive round cell infiltration of the brain, and in some, degenerative changes in viscera, although not virus-specific. This histologic picture appeared to differentiate Zika from other neuronotropic viruses.

Two years later, during the investigations of an outbreak of jaundice suspected of being yellow fever in Eastern Nigeria, Zika was isolated from one patient while 2 others showed a rise in Zika virus titers.[2] Patient 1 was a 30-year-old African man with recent cough, diffuse arthralgia, and fever. Patient 2 was a 24-year-old African man who had new onset of fever, headache, and arthralgia. Patient 3 was a 10-year-old girl with recent onset of fever and headache without jaundice. All 3 patients recovered. Acute and convalescent sera from each case were tested by intracerebral protection tests via inoculation into experimental mice, resulting in a mortality rate of 100%. Viral-confirmed neutralization tests using immune monkey serum only in Patient 1, compatible with successful viral isolation. In support of the evidence of viral identification in

that case, it was shown that the serum of a monkey immunized by inoculation of the isolated virus had a log neutralization index of 2.24 against the homologous virus, and 2.94 against Zika virus. That patient had no signs of jaundice, unlike the 2 others, in whom viral isolation was unsuccessful.

Following early epidemiologic studies in Uganda indicating that *A africanus* was probably the Zika vector, Boorman and Porterfield[24] successfully devised a technique employing a mouse skin membrane and heparin-treated blood for infecting mosquitoes. Using this technique, *A aegypti* mosquitoes were infected with Zika virus and their pathogenicity studied. Little or no virus was detected in mosquitoes on days 5 to 10, but thereafter the viral level rose and remained steady from days 20 to 60. Back-feeding experiments through a mouse skin membrane into uninfected mouse blood resulted in transmission in 12 of 20 cases. Successful infection of a rhesus monkey by the bites of 3 infected mosquitoes was demonstrated 72 days after an infected blood meal.

In the same year, Bearcroft[25] inoculated a 34-year old Nigerian male volunteer with an Eastern Nigerian strain of Zika virus that was comprised of 0.25 mL of 10-3 brain suspension representing 265 mouse lethal dose (LD50) given subcutaneously into the arm and specimens of blood drawn on day 4, 6, and 8 after inoculation. Following an incubation period of 82 hours, a mild, short-lived febrile condition occurred without evidence of involvement of any particular tissue or organ. Zika virus was isolated from the blood during the febrile period, accompanied by a rise in serum antibody to Zika virus by mouse protection and hemagglutination inhibition tests. Both adult and infant mice receiving undiluted serum from the patient between days 4 and 6 died. Histologic examination of portions of the brain showed encephalitis suggestive of viral infection.

CLINICAL ASPECTS

The classic clinical picture of Zika virus infection resembles that of dengue fever and chikungunya and is manifested by fever, headache, arthralgia, myalgia, and maculopapular rash, a complex of symptoms that hampers differential diagnosis. Although the disease is self-limiting, cases of neurologic manifestations including Guillain–Barré syndrome (GBS) have been described in French Polynesia and in Brazil during epidemics. One prototypical 40-year-old Polynesian woman[26] suffered from an influenza-like syndrome with myalgia, febricula, cutaneous rash, and conjunctivitis suspicious for Zika virus infection before development of flaccid paraparesis accompanied by dysautonomia and acquired demyelinating neuropathy. There was no evidence of systemic inflammation. Cerebrospinal fluid showed albuminocytologic dissociation with 1.66 g/L proteins (norm: 0.28–0.52) and 7 white cells/mL (normal <10). Reverse transcriptase polymerase chain reaction (RT-PCR) was negative on blood samples 8 days after the beginning of influenza-like symptoms (corresponding to Day 1) prior to the administration of intravenous immunoglobulin. Blood samples taken at 8 and 28 days after the beginning of the influenza-like syndrome were both positive for Zika-specific immunoglobulin M (IgM) and immunoglobulin G (IgG), assessed by enzyme-linked immunosorbent assay (ELISA). Antibody specificity was determined by plaque reduction neutralization test (PRNT). She slowly improved concomitant with the administration of 2 g/kg body weight of intravenous polyvalent immunoglobulin over 1 month.

A major concern associated with Zika virus infection has been the increased incidence of microcephaly in fetuses born to mothers with Zika virus infection. Ultrasonography in suspected fetuses shows the first signs of fetal anomalies and growth retardation, including a head circumference that is below that expected for fetal age

and development. There may be blurred brain structures and calcifications in spite of normal fetal, umbilical, and uterine blood flow on Doppler ultrasonography. A well-studied fetus aborted at 32 weeks from a Brazilian woman[27] had a head circumference in the first percentile as the only external anomaly. However, neuropathologic examination showed agyria, internal hydrocephalus, calcifications, and otherwise normal subcortical development. The most prominent histopathological features included

- Filamentous, granular, neuron-shaped calcifications
- Diffuse astrogliosis; activated microglial cells expressing human leukocyte antigen (HLA)DR
- Scattered perivascular infiltrates of T cells and B cells in subcortical white matter
- Wallerian degeneration of long descending tracts, especially the corticospinal tract
- Granular intracytoplasmic reaction indicative of possible location of the virus in neurons

Electron microscopy showed enveloped structures with morphologic characteristics of *Flaviviridae* virus, and microbiologic investigation was positive for Zika on RT-PCR assay in the fetal brain sample alone. Analysis of the genome showed the highest identity with Zika strain isolated from a French Polynesian patient in 2013 (KJ776791), consistent with an emergence from the Asian lineage. The presence of 2 major amino acid substitutions in nonstructural proteins NS1 and NS4B represented, in all likelihood, an accidental event or adaptation of the virus to a new environment.

MICROBIOLOGIC AND SEROLOGIC CONSIDERATIONS

According to Petersen and colleagues,[28] the CDC recommends specific diagnostic algorithms for Zika virus diagnosis in adults and children.[29,30] The diagnosis of Zika virus can be confirmed by RT-PCR amplification of the viral genome, but it is expensive and prone to contamination. Commercial diagnostic tests for Zika detection are under development but not yet available. The Zika outbreak in the Americas generated renewed interest in development of new rapid diagnostic methods, drugs, and potential vaccines. Ongoing efforts in diagnostics include the standardization of real-time RT-PCR (rRT-PCR) methods for comparative purposes to detect viral RNA; development of rapid specific serologic tests for clinical and epidemiologic studies; determining the role of viral load in pathogenesis; and in utero transmission and validating the use of non-blood specimens. Due of the kinetics of Zika viremia, the clinical utility of rRT-PCR is limited to testing blood samples collected less than 1 week after onset of symptoms. Because Zika virus is excreted for a longer time in urine, such samples are useful for up to 3 weeks after onset of viremia. rRT-PCR can also be performed on amniotic fluid, although the positive and negative predictive values for fetal infection and development of fetal pathology are not well understood. Serum total antibody testing is not reliable because of extensive cross-reactivity against dengue fever and yellow fever, 2 diseases that collocate geographically. This diagnostic limitation to demonstrating seroconversion to Zika in pregnancy hampers the retrospective investigations into the temporal relationship between the Brazilian epidemic and increase in congenital malformations.

The CDC has developed an ELISA technique to detect specific anti-Zika IgM, but the frequency of cross-reactions with other flaviviruses such as dengue and yellow fever make the diagnosis difficult. In the early phase of infection, the rate of IgM and IgG can be low, making confirmation of the diagnosis challenging. The detection of specific antibodies should be confirmed by a complementary seroneutralization assay

employing PRNT to demonstrate a fourfold increase of the antibody titer initially found. No commercial kit is currently available for the detection of antibodies specifically related to Zika virus. rRT-PCR is an appealing option as a rapid, sensitive, and specific method for detection of Zika in the early stage of infection. So far, only 1 rRT-PCR assay has been described in the context of the outbreak in Micronesia in 2007. Faye and colleagues[31,32] described a 1-step rRT-PCR test for Zika to detect a wider genetic diversity of Zika isolates from Asia and Africa, including Zika RNA (NS5) and the envelope protein coding region (360 bp) in tissue samples. Next-generation sequencing, complete-genome Zika sequences, multiple-sequence alignments and neighbor-joining phylogenetic trees can be constructed to show phylogenetic relationships for epidemiologic research purposes.

THE HIDDEN TOLL OF ZIKA

The potential hidden toll of Zika virus infection is unknown. Illnesses that occur early in utero can cause fetal wastage, developmental defects, and serious malformations with brain calcification. However later in utero infection can act as a trigger for other CNS sequelae. There has been no research into the long-term sequelae of in utero Zika virus infections. If the rubella epidemic of the United States in the mid-1960s that infected an estimated 12 million Americans and affected 20,000 newborns with significant early and late CNS sequelae including autism and learning and behavioral disabilities is a good analogy, then with an estimated 500 million people residing in countries of Latin America and the Caribbean, Zika spread, there could be devastating consequences on already frail health care systems.

TREATMENT

There is no specific treatment or vaccine. Treatment is symptomatic, combining acetaminophen and antihistaminic drugs. Prevention against the infection relies upon antisectorial protection combining the avoidance of mosquito bites and the eradication of mosquitoes. Prevention at the community level consists in decreasing the number of mosquitoes by decreasing the number of egg-laying sites in potted plant saucers, moats, and water reservoirs by drying them, isolating them, and treating them with insecticides. Individual protection includes wearing long and light-colored clothes and using skin repellents and mosquito bed nets, especially for the protection of babies and bedridden patients, to avoid mosquito bites. There is a role for intravenous immunoglobulin in neurologically affected patients.

PUBLIC HEALTH RECOMMENDATIONS

Adequate public health preventive measures, including public education and mosquito bite prevention, should be implemented quickly after the diagnosis of an imported case. Other control measures include the isolation of the patient during the viremic phase and vector control activities centered on the case's residence, including spraying adult mosquitoes and destruction of larval breeding sites. The roles of clinicians are crucial including the early diagnosis of imported arboviruses such as Zika infection and the timely notification of public health authorities. Clinicians should be aware of current outbreaks in parts of the world that are popular tourist destinations. This is especially important for newly emerging and possibly devastating diseases with specific public health implications. Imported cases should be suspected in travelers who develop compatible symptoms within 1 to 2 weeks after returning from endemic areas. Cross-reactive dengue viral serology (IgG or IgM) during Zika infection may be

used as a screening test to identify subjects, since commercial serologic tests for dengue are widely available. Taking into account possible cross-reactions among different viruses belonging to the *Flavivirus* family when using current serologic tests, an approach combining direct and indirect detection techniques, as well as neutralization assay for confirmation, should be utilized. Public health experts highlight the need of improving pretravel advice and consultation for travelers planning to visit countries in which various arboviruses are endemic. Such advice should include effective preventive measures of mosquito bites and avoidance of the use of acetylsalicylic acid, which is contraindicated in suspected or confirmed dengue fever due to the increased risk of bleeding. The explosive spread of Zika in Brazil poses challenges for public health preparedness and surveillance for mass gathering that will occur during the 2016 Brazil Olympic Games and Paralympics in Rio De Janeiro this year. Termed a public health emergency of international concern, the Olympic games constitute an extraordinary event and a public health risk to other countries through the potential for international spread of disease, and as such, will require a coordinated international response.

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