Update on Zika

What You Need to Know

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After remaining related to few sporadic cases in limited regions for more than half century since its discovery, Zika virus (ZIKV) was recently introduced into the Western Hemisphere, first in Brazil and then spreading very rapidly in the Americas. Unexpectedly, an increased incidence of microcephaly and other neurologic malformations in fetuses born to mothers infected with ZIKV during pregnancy was reported in Brazil, leading the World Health Organization to declare this situation a Public Health Emergency of International Concern.[1]

The scientific data collected and the lessons learned after ZIKV introduction in the Americas, particularly in Brazil, provided a huge amount of new information and have been crucial in informing a better understanding on several aspects related to the transmission of the virus, its clinical manifestations and neurologic complications, possibility of congenital malformations, potential therapeutic interventions and preventive measures. This review summarizes the current knowledge on the ZIKV infection and provides perspectives on future challenges.

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EPIDEMIOLOGY

ZIKV is an emerging arthropod-borne, single-stranded RNA virus, member of the Spondweni serocomplex (genus *Flavivirus*, family Flaviviridae) and related to other mosquito-borne viruses that cause yellow fever, dengue, West Nile disease, St. Louis encephalitis and Japanese encephalitis. Two major lineages, African and Asian, have been identified through phylogenetic analyses.[2]

After initial identification in 1947 from a rhesus monkey in the Zika forest of Uganda, ZIKV was associated only with few sporadic cases in humans in Africa and Asia over the next 60 years. However, since 2007 when the first outbreak of ZIKV outside Africa and Asia was reported in the Federated States of Micronesia (Yap), it has been identified in subsequent outbreaks in French Polynesia and other Pacific islands.[3] In May 2015, the Ministry of Health of Brazil confirmed autochthonous transmission of ZIKV associated with an outbreak of "dengue-like syndrome" cases in Northeastern Brazil. The ZIKV outbreak continued to evolve, spreading geographically very rapidly in the Americas.[1]

As of November 9, 2016, 75 countries and territories have reported evidence of mosquito-borne ZIKV transmission since 2007, of which 58 countries (from the Americas, Africa and Western Pacific regions) have reported outbreaks of ZIKV from 2015 onwards (Fig. 1).[1]

TRANSMISSION

ZIKV is transmitted to humans primarily by *Aedes aegypti* mosquitoes (and less commonly by other *Aedes* species, like *Aedes polynesiensis*, *Aedes hensilli*, *Aedes africanus* and *Aedes albopictus*), the same vector that can transmit dengue, chikungunya and yellow fever viruses.[4] ZIKV has already been isolated from other non-*Aedes* mosquitoes. However, it is important to emphasize that the isolation of ZIKV from a mosquito is not an evidence that transmission is feasible by this mosquito.

Additionally, nonvector modes of transmission have been identified, including perinatal, in utero, sexual (there is evidence of ZIKV transmission by a man to his sex partners and 1 report of female-to-male sexual transmission), blood transfusion and laboratory exposure.[4] Although ZIKV RNA has been detected in breast milk, transmission through breast-feeding has not yet been demonstrated, reinforcing the current recommendations that mothers with ZIKV infection should continue to breast-feed their infants.[4]

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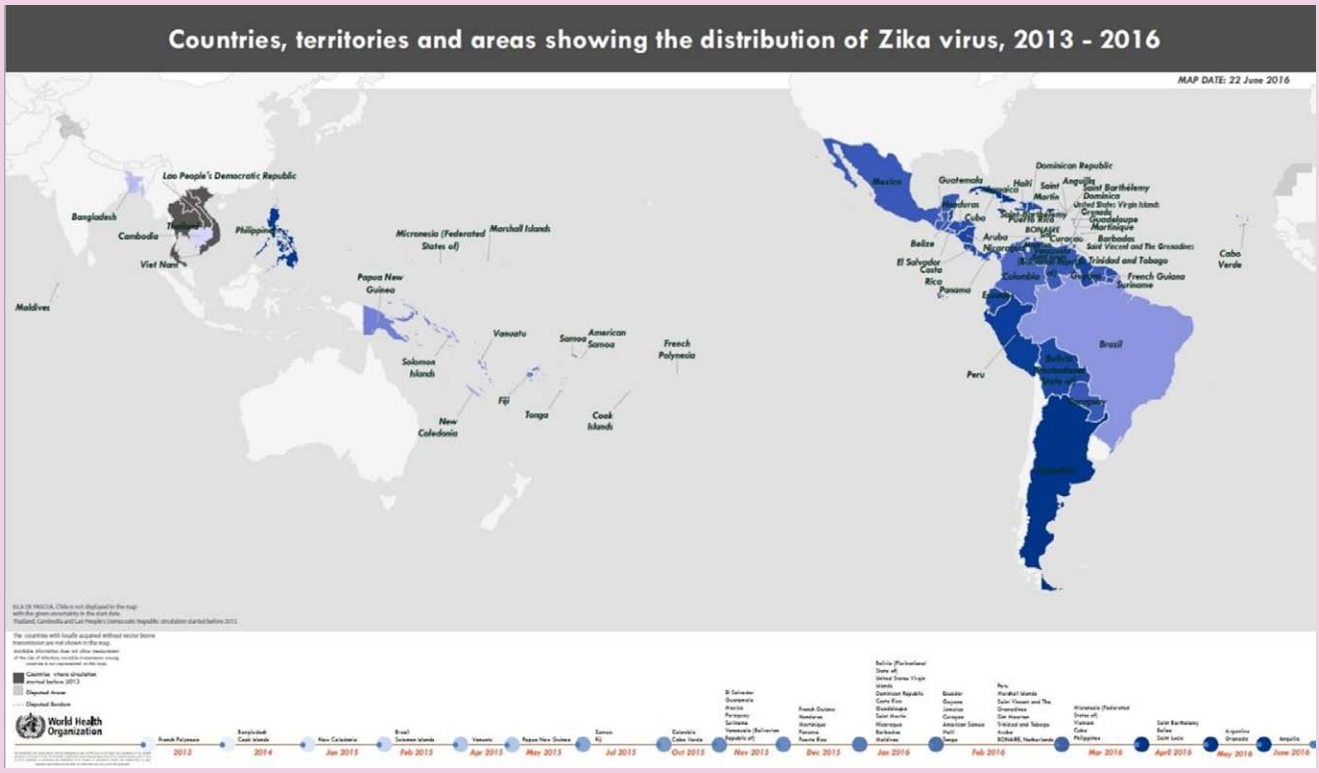

FIGURE 1. Countries, territories and areas showing the distribution of Zika virus, 2013–2016. full color online

Intrauterine transmission of ZIKV was confirmed in Brazil based on the detection of virus genome, by reverse transcriptase–polymerase chain reaction (RT-PCR), in amniotic fluid samples of women with symptoms of ZIKV infection during the first trimester of pregnancy, whose fetuses have been diagnosed with microcephaly, in placental tissues from early miscarriages and also in the blood and brain tissue of infants with congenital neurologic anomalies, including microcephaly.[4–6]

Reports of cases with ZIKV possibly transmitted by blood transfusion are being investigated in Brazil. Interestingly, during the French Polynesian outbreak, 2.8% of blood donors tested positive for ZIKV by RT-PCR, with all of them asymptomatic at the moment of blood donation.[7]

The incubation period in humans before onset of symptoms is thought to be between 3 and 14 days after the bite of an infected mosquito. Infected people, both symptomatic and asymptomatic, can transmit ZIKV to mosquitoes throughout the viremic period that usually ranges from a few days to 1 week.[4]

DIAGNOSIS

Clinical diagnosis is limited by the nonspecific signs and symptoms of ZIKV infection, which are similar to other arboviral infections (eg, chikungunya and dengue), common in endemic areas. Abnormal laboratory findings, including mild thrombocytopenia, leukopenia and elevations in acute-phase markers of inflammation, serum lactate dehydrogenase or liver transaminases, have been observed in symptomatic patients.[4]

ZIKV-specific diagnosis is primarily based on the detection of ZIKV RNA by RT-PCR in serum and/or urine. During the first 5–7 days after onset of symptoms, ZIKV can be diagnosed by performing RT-PCR on serum. However, because viremia decreases and disappears after the first week of illness, a negative test result from serum collected more than 5–7 days after symptom onset does not exclude infection definitively. Recent data suggest that in urine ZIKV RNA can be detected longer, at least 2 weeks after onset of symptoms.[4]

ZIKV-specific immunoglobulin M (IgM) and neutralizing antibodies can be detected by enzyme-linked immunosorbent assay in serum specimens collected by the end of the first week of illness. IgG antibodies develop within days after IgM and can be detected for months to years. However, false-positive results because of cross-reaction with related flaviviruses (eg, dengue and yellow fever viruses) are commonly observed. During the outbreak of ZIKV infection in the Yap state, an analysis of patient samples showed that low levels of cross-reactive IgM were present in all subjects with secondary flavivirus infection.[8]

Positive results in primary flavivirus infections should be confirmed with a 4-fold increase in the titer of neutralizing antibodies to ZIKV with plaque reduction neutralization test (PRNT). In endemic areas, where a great proportion of the population may have been previously infected with other flaviviruses or vaccinated against a related flavivirus (ie, secondary flavivirus infection), neutralizing antibodies might still yield cross-reactive results in these individuals.[4,8]

CLINICAL MANIFESTATIONS

It is estimated that approximately 80% of persons infected with ZIKV are asymptomatic. When symptomatic, the infection is considered to be associated with a mild, self-limited disease, lasting few days and characterized by low fever, pruritic rash edema of extremities, conjunctivitis, headache and myalgia. Less common manifestations include gastrointestinal symptoms, retroorbital pain and lymphadenopathy. Clinical manifestations in infants and children with acquired infection are similar to the findings observed in adults with ZIKV infection. The presence of arthralgia in infants and young children is difficult to detect and

can manifest as irritability, limited moving or refusing to move an extremity. During outbreaks of ZIKV, cases were reported in all age groups, with higher incidence rates in adults compared with children.[3,4] World Health Organization developed interim case definitions with the purpose of providing global standardization for classification and reporting of ZIKV cases: patient with rash and/or fever with at least one of the following signs and symptoms: arthralgia, arthritis or conjunctivitis (nonpurulent conjunctival hyperemia). A confirmed case is a suspected case with laboratory confirmation of recent ZIKV infection [presence of ZIKV RNA or antigen in serum or other samples (eg, saliva, tissues, urine, whole blood) or IgM antibody against ZIKV positive and $PRNT_{90}$ for ZIKV with titer ≥20 and ZIKV $PRNT_{90}$ titer ratio ≥4 compared with other flaviviruses; and exclusion of other flaviviruses].[1]

NEUROLOGIC COMPLICATIONS

Neurologic complications, such as Guillain–Barré Syndrome (GBS), meningitis, acute disseminated encephalomyelitis and myelitis, mainly in adults, have been reported after ZIKV infection. French Polynesia, Brazil, Colombia, Venezuela and several other countries from Central America and the Caribbean reported an increase in the rates of GBS during the recent ZIKV outbreak. The reported incidence of GBS was higher among males and consistently increased with age, with males over 60 years having the highest rates, findings that are in line with previous reports on the epidemiology of GBS.[9] This epidemiologic situation reinforces the hypothesis of a link between ZIKV infection and the occurrence of GBS, highlighting that ZIKV should now be included in the list of potential infectious pathogens that can trigger the development of GBS.[9,10]

CONGENITAL SYNDROME

The most striking finding during the ZIKV outbreak in Brazil, however, was the strong cumulative evidence that provided the basis to establish a relationship between ZIKV infection during pregnancy and congenital abnormalities. A wide range of congenital malformations was described, characterized predominantly by central nervous system alterations and associated symptoms: microcephaly (with significant cranium–facial disproportion), spasticity, convulsions, marked irritability and brainstem dysfunction including feeding difficulties. The results of preliminary neuroimaging studies suggest that intrauterine ZIKV infection is associated with severe brain anomalies, such as cerebral calcifications, hydrocephalus, lissencephaly with agenesis of the corpus callosum, pachygyria, cerebellar dysplasia and white-matter abnormalities.[4,6,11,12] The severity of the neurologic alterations appears to be related to the period of gestation when the women are infected, that is, the earlier the infection during pregnancy, the more severe the neurologic outcomes to the fetus. Arthrogryposis, neurosensorial hearing loss, microphthalmia, funduscopic alterations in the macular region and optic nerve abnormalities were also described in infants with suspected congenital ZIKV syndrome.[4,10–12]

As of October 29, 2016, after investigation and classification, 2,106 confirmed cases of microcephaly and/or central nervous system malformation compatible with a congenital infection were reported in Brazil, of which 405 had laboratory-confirmed ZIKV infections. The cases were concentrated in the areas where ZIKV peaked in 2015. Seventy-three fetal or neonatal deaths with laboratory confirmation for ZIKV were also reported.[13]

The true burden of the congenital disease associated with ZIKV is probably underestimated assuming that it is likely that a significant proportion of the affected newborns have subclinical manifestations at birth, without microcephaly, preventing these infants from being diagnosed by the current ascertainment methods, at least until later stages of childhood/adolescence when cognitive, developmental and/or visual limitations can be detected.

The unique characteristics of the ZIKV outbreak in Brazil, where the population was completely susceptible (naive) to the virus, affecting highly populated urban areas with high density of *A. aegypti*, and the established surveillance reporting system are possible reasons to explain why the role of the ZIKV as a potential cause of congenital disease has only been recognized after circulation in Brazil. Furthermore, if ZIKV infection is associated with lifelong immunity, it is expected that in endemic places in Africa and Asia, where the virus is circulating for years, many women, once they reach childbearing age, have already been infected and are immune.

It is also possible that the more severe outcomes of ZIKV infection observed in Brazil and other countries may be related to mutation in virulence characteristics of the ZIKV circulating strain or even immune interaction between consecutive flavivirus infections. Interestingly, after the reports from Brazil raised a causal relationship between ZIKV infection in pregnancy and microcephaly and other congenital malformations, a retrospective study performed in French Polynesia found an association between ZIKV and microcephaly.[14] As of November 9, 2016, 26 countries or territories have reported microcephaly and other central nervous system malformations potentially associated with ZIKV infection, or suggestive of congenital infection.[1]

TREATMENT AND PREVENTION

We currently do not have any available vaccines to prevent the disease or specific antiviral treatment for patients with ZIKV disease. Only supportive care is indicated, including rest, fluids and symptomatic treatment (acetaminophen to relieve fever and antihistamines to treat pruritus). Aspirin and other nonsteroidal antiinflammatory drugs should be avoided to reduce the risk of hemorrhagic complications. One recent study showed that chloroquine exhibited antiviral activity against ZIKV in Vero and human brain microvascular endothelial and neural stem cells. In this study, the authors were able to demonstrate that chloroquine reduced, in vitro, the number of ZIKV-infected cells, virus production and cell death promoted by ZIKV infection without cytotoxic effects.[15]

Several ZIKV vaccine candidates, based on plasmid DNA or purified inactivated virus, showed promising results in animal models and are now tested in trials in humans.[16] In the context of the ongoing Public Health Emergency of International Concern, protection of women at childbearing age, to prevent fetal congenital malformations after in utero infection, is a high priority. World Health Organization recommended that vaccination strategies should be prioritized to target women of childbearing age (including adolescent and preadolescent girls 9 years of age or older), and boys/men of reproductive age 9 years or older (to prevent sexual transmission).[17]

Prevention and control currently rely on personal strategies to avoid mosquito bites and community-level programs to reduce vector densities in endemic areas. Personal measures include using insect repellent containing diethyl-meta-toluamide, picaridin, oil of lemon eucalyptus or IR3535. Permethrin-treated clothing and gear can repel mosquitoes.[4]

FUTURE RESEARCH AND CHALLENGES

Despite the advances that were recently achieved on the understanding of several aspects related to ZIKV infection, it is important to acknowledge that we still have many research gaps and unanswered questions about ZIKV. Crucial areas of future research include the need of a better understanding of the full spectrum of fetal outcomes resulting from fetal ZIKV infection; evaluation of potential risk factors for vertical transmission (viral load, coinfections, timing, virulence of the circulating strain); development of more specific diagnostic tests; the role, if any, of non-*Aedes* mosquitoes in the transmission, as well as other potential modes of nonvector transmission; and the pathogenesis of neurologic and autoimmune complications after ZIKV infections.

Finally, novel methods of vector control and the development of specific antiviral drugs and vaccines will be of paramount importance to control the disease and decrease the burden of ZIKV infection.

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