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Invited Review

Zika virus—a review for clinicians

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Introduction

Zika virus is a mosquito-borne arbovirus in the *Flavivirus* family, which also includes yellow fever, dengue and Japanese encephalitis viruses. It is a single-stranded RNA virus with two reported lineages in Africa and Asia. Zika virus was previously named a neglected tropical disease by the WHO. Before 2013, <50 cases of confirmed Zika virus infections, limited to the tropical regions of Africa and Asia, were reported in the literature. All of the reported cases had mild febrile illness and did not result in any mortality or major sequelae.

However, over the past decade, Zika virus has rapidly swept through Oceania and South America, probably accelerated by human movement and travel. Between January 2014 and February 2016, 33 countries reported autochthonous circulation of Zika virus.[1] Further spread to other countries where the *Aedes* mosquito vector is found, such as southern Europe and Asia, is a real concern.

Seven countries have also reported epidemiological data describing alarming surges in the incidence of microcephaly and/or Guillain–Barré syndrome (GBS) concomitantly with the Zika virus outbreaks. Though the causal relationships are yet to be established scientifically, the WHO has declared the current ongoing Zika outbreak a Public Health Emergency of International Concern (PHEIC) to highlight the urgent need for coordinating international efforts in surveillance and fast-tracked research.

This review aims to summarize the current epidemiology and clinical features of Zika virus infection, with a focus on the contentious issue of Zika virus and microcephaly.

Methodology

References to the publications for this review were identified through searches of PubMed, the WHO

website, the US Centers for Disease Control and Prevention (CDC) website and Promed up to March 6, 2016. The search terms used were a combination of 'Zika' and 'virus'. The full texts of relevant studies were retrieved and reviewed.

A total of 486 articles were retrieved. Articles relevant to epidemiology, pathogenesis, clinical presentation, diagnostics, treatment as well as opinion pieces were included. Articles were excluded if they were repeated or did not directly describe Zika virus infection.

Epidemiology and history

Zika virus was discovered in a pyrexial sentinel Rhesus monkey in 1947, during experiments conducted to study the vectors responsible for yellow fever in Zika forest, Uganda.[2] Serological surveillance of 99 human sera collected for the studies of yellow fever transmission from Uganda in the same year showed that 6.1% had neutralizing antibodies, providing the first evidence that humans were suitable hosts for the virus.[3]

In 1954, the first case series describing Zika viral illness was reported from Nigeria.[4] There were three patients with fever, headache, arthralgia and jaundice. In the ensuing decades from 1950s to 1980s, evidence of Zika virus infections emerged from East and West Africa, Egypt, Pakistan, India, Malaysia, Indonesia, Vietnam, Thailand and the Philippines through serological studies and virus isolation.[5–13]

The initial descriptions of Zika virus disease in humans consisted of small series and case reports, out of which the biggest cohort was from central Java, Indonesia in 1977, with seven patients.[7] The first large-scale outbreak of Zika virus infection took place in Yap, Federation of Micronesia in 2007.[14] This is also the first time that the virus was detected outside of Africa and Asia. The strain responsible is now believed to have originated in Southeast Asia.[15]

In October 2013, another large outbreak with 10 000 registered cases took place in French Polynesia. Increase in the incidence of neurological complications including GBS, meningoencephalitis and autoimmune complications such as thrombocytopenic purpura were also reported concurrently with the Zika outbreak. This outbreak quickly spread to other Pacific Islands including New Caledonia, Cook Islands, Easter Island, Vanuatu and Solomon Islands in 2014.[16]

Imported cases of Zika virus have been reported in Australia, Europe, the USA, Canada and Japan since 2010.[17–23]

Current outbreak

During February to June 2014, a Zika outbreak occurred on Easter Island, Chile. The virus was believed to have been introduced by French Polynesians attending a cultural festival.[24] Within a year, Zika virus had spread to the northeastern states of Brazil. One of these states, Bahia, first isolated the Zika virus in May 2015[25] although molecular genetic analysis suggests that the virus may have appeared in South America as early as 2013. By December 10, 2015, Zika virus had spread to 18 other Brazilian states.[26] From October to December 2015, the Pan-American Healthcare Organization issued alerts reporting the presence of Zika virus in several Latin American countries: Colombia, Surinam, Guatemala, El Salvador, México, Paraguay, Venezuela and Panama. Outside of the Americas, the Atlantic island nation of Cape Verde announced its first Zika virus epidemic in October.[27] Genomic studies showed that the Brazilian strain is the closest to viruses from French Polynesia and the Pacific Islands,[25] belonging to the Asian lineage.

The virus was believed to have been imported in 2014 during the World Cup[28] or the Va'a World Sprint Championship canoe race in which four Pacific countries (French Polynesia, New Caledonia, Cook Islands and Easter Island) participated[24] although it is not clear whether this was from an asymptomatically infected individual or from mosquito eggs in canoes or other containers. Recent phylogenetic and molecular clock analyses via next generation sequencing of seven Brazilian Zika virus showed a single introduction of Zika virus into the Americas, estimated to have occurred between May to December 2013.[29]

The emergence of Zika in Brazil is particularly concerning since Brazil is the country with the highest number of dengue virus infections worldwide.[30] Chikungunya virus was also recently introduced to South America with >1 million cases diagnosed since 2013 to date.[31] All three viruses share the same vector—members of the *Aedes* genus of mosquitoes most prominently *Aedes aegypti* or *Aedes albopictus*. On February 1, 2016, WHO declared the ongoing Zika epidemic to be a PHEIC based on the clusters of microcephaly and GBS that have been associated with Zika virus outbreaks.[32] The WHO proposed a US$56 m strategic plan focusing on outbreak investigations and accelerated research, including the fast-tracked development of diagnostics, vaccines and treatments (Fig. 1 and Table 1).[33]

Genetic studies

The Zika virus was first sequenced in 2007.[38] It has a positive-sense, single-stranded RNA genome ~11 kb in length. Phylogenetic analyses of five isolates from Cambodia, Malaysia, Nigeria, Uganda and Senegal collected between 1947 and 2010 suggested the existence of two main virus lineages from Africa and Asia.[15]

The current circulating strain of Zika virus was sequenced by Virology Laboratory at the Institut Pasteur in French Guiana in 2016 January.[39] The strain belongs to the Asian genotype and is similar to the strain from French Polynesia in 2013.

Transmission

The other and the first described strain of Zika virus was isolated from *Aedes africanus* mosquitoes in the Zika forest in 1948.[3] Boorman and Porterfield subsequently demonstrated the transmission of the virus by infecting mice and a monkey with experimentally infected *Ae. aegypti* mosquitoes in 1956.[40] The mosquito viral load was found to be high on the day of artificial feeding, dropped to undetectable levels through Day 10, increased by Day 15 and remained high from Days 20 through 60. Therefore, they concluded that the extrinsic incubation period in mosquitoes is ~10 days. Zika virus was further isolated from *Ae. aegypti*, *Ae. apicoargenteus*, *Ae. luteocephalus*, *Ae. aegypti*, *Ae vitattus*, *Ae albopictus* and *Ae. furcifer* mosquitoes.[5,12,17,41,42]

Interestingly, although *Aedes hensilii* was the predominant mosquito species present on Yap Islands during the outbreak in 2007, the investigators were unable to detect Zika virus in any mosquitoes on the island during the outbreak.[14] Only in a later study in 2014, 86% of *Aedes hensilii* on

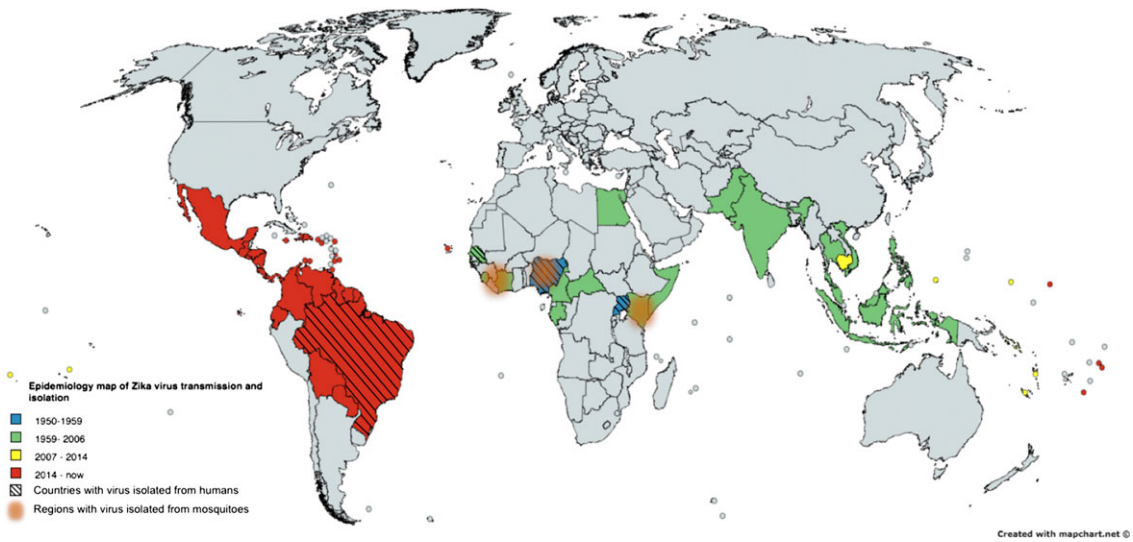

Fig. 1 Epidemiologic map of Zika virus transmission and isolation.

Table 1 Epidemiologic timeline of Zika virus transmission and isolation

Time	Countries
1950–1959	*Africa*
	Uganda,[1,2] Nigeria[4]
1960–1969	*Africa*
	East and West Africa,[6] Egypt[15]
	Asia
	Malaysia,[5] Indonesia,[7] Pakistan, India, Malaysia, Indonesia, Vietnam, Thailand, the Philippines[15]
1970–1979	*Africa*
	Gabon,[8] Senegal, Sierra Leone,[6] Kenya[34]
1980–1999	*Africa*
	Ivory Coast,[12] Central African Republic,[9] Somalia[35]
2000–2009	*Africa*
	Cameroon[36]
	Australia/Oceania
	Yap Islands[14]
	Asia
	Cambodia[37]
2013–2014	*Australia/Oceania*
	Federated States of Micronesia, French Polynesia, New Caledonia, Cook Islands, Easter Island, Vanuatu, and Solomon Islands[16]
2014–now	*South America*[1]
	Aruba, Barbados, Bolivia, Bonaire, Brazil, Colombia, Commonwealth of Puerto Rico, Costa Rica, Curacao, Dominican Republic, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haití, Honduras, Jamaica, Martinique, Mexico, Nicaragua, Panamá, Paraguay, Saint Martin, Saint Vincent and the Grenadines, Saint Maarten, Suriname, Trinidad and Tobago, US Virgin Islands, Venezuela, American Samoa, Marshall Islands, Samoa, Tonga
	Africa
	Cape Verde[27]

Yap Islands were found to carry Zika virus.[43] There is no conclusive evidence of non-primate reservoirs of Zika virus although the virus was first isolated in a rhesus monkey.

Besides mosquito transmission of Zika virus, human sexual transmission was first reported in an American scientist after his return home from Senegal in 2008.[44] Animal contact transmission was reported in an Australian who contracted the virus after a monkey bite in Indonesia.[45] Of note, 42 (3%) of 1505 asymptomatic blood donors had positive Zika virus PCR from November 2013 to February 2014 in French Polynesia.[46] Following this, SP, Brazil saw its first case of Zika transmission via blood transfusion in March 2015.[47]

Clinical presentation

Clinical features of Zika virus disease were reported from the 2007 Yap Island outbreak and the 2013–2014 French Polynesian outbreak (Table 2). Similar to the first case series of Zika virus disease reported since 1954, the commonest presentations include maculopapular rash, fever, arthralgias, conjunctivitis, headache and myalgia. Almost 80% of the cases remained asymptomatic.[14] The course of illness is usually self-limited lasting ~4–7 days.[14] Aside from those who developed secondary bacterial infections, GBS, pregnancy complications, there were no deaths, hospitalizations or hemorrhagic complications with Zika virus illness during both outbreaks. The earlier small case series of Zika virus infections described additional clinical features including jaundice,[4] constipation[7] and hematuria.[7]

Zika virus, dengue virus and chikungunya virus share the same *Aedes* mosquito vectors and therefore overlap in their endemic areas. They present similarly, and it is clinically challenging to distinguish them. Some of the differentiating features between Zika virus and dengue virus are: Zika virus disease is associated with a descending rash that often starts on the face and spreads throughout the body within a median of 6 days, compared to a dengue rash that is usually described as generalized and confluent with islands of sparing;[48] Zika virus disease causes a non-purulent conjunctivitis in up to 60% of the patients, while this is only seen in 15–30% of patients with dengue fever;[49] and lastly, Zika virus usually is associated with a short course of low-grade fever,[50] while dengue fever usually presents with high fevers.[51] However, chikungunya is typically associated with a pronounced bilateral migratory arthralgia,[52,53]

Table 2 Clinical characteristics of patients with confirmed Zika Virus Disease on Yap Island during the 2007 outbreak and French Polynesia during the 2013–14 outbreak

Sign or symptom	No. of patients (%)		Overall %
	Yap Island[14] ($n = 31$)	French Polynesia[55] ($n = 297$)	$n = 328$
Macular or papular rash	28 (90)	276 (93)	304 (93)
Fever*	20 (65)	214 (72)	234 (71)
Arthritis or arthralgia	20 (65)	193 (65)	213 (65)
Non-purulent conjunctivitis	17 (55)	187 (63)	204 (62)
Headache	14 (45)	137 (46)	151 (46)
Myalgia	15 (48)	131 (44)	146 (45)
Edema	6 (19)	140 (47)	146 (45)
Retro-orbital pain	12 (39)	50 (17)	62 (19)
Malaise		232 (78)	
Vomiting	3 (10)	86 (29)†	
Upper respiratory signs		71 (24)	
Lymphadenopathy		48 (16)	
Mouth ulcers		12 (4)	

*Cases of measured and subjective fever are included.
†Diarrhea, nausea and vomiting.

affecting mainly the small joints of the extremities. This can persist, lasting years after the initial infection.[54]

Routine laboratory test are not helpful in discriminating between the different viruses and commonly show abnormalities typical of nonspecific viral infections. Complete blood counts may show hemoconcentration, mild leukopenia and thrombocytopenia associated with activated lymphocytes.[56] Liver function tests may reflect elevated serum transaminases. Blood urea nitrogen and creatinine may be elevated with occasional hyponatremia and hypoglycemia.[57]

Zika virus and pregnancy

Vertical transmission of Zika virus has been a contentious issue with conflicting epidemiological evidence since November 2015.

We found 37 articles in our literature review discussing the relationship between Zika virus and pregnancy. The reported epidemiology link and subsequent isolation of Zika virus in neonates and amniotic fluids provided evidence for vertical transmission of Zika virus and a causal relationship with birth defects. Replicative Zika virus was not detected in the breast milk of two mothers tested positive for Zika virus.[58]

Following the emergence of Zika virus in early 2015, the Brazilian Ministry of Health announced a 20-fold increase in the incidence of neonatal microcephaly in November 2015.[59] By November 30, 2015, 1248 cases (99.7/100 000 live births) of microcephaly, including 7 deaths, were reported in 14 states of Brazil.[60] In the same month, the French Polynesian health authorities also reported an increase of fetal central nervous system malformations during 2014–15, coinciding with the Zika virus outbreak on the island. Seventeen malformations were registered, including brainstem dysfunction and the absence of swallowing.[61]

However, the reported surge in microcephaly in Brazil during late 2015 and February 2016 is not without controversy. Of the 4783 suspected cases of microcephaly reported from mid-2015 to January 2016,[62] 1103 cases had clinical, laboratory and imaging examinations, and only 404 were classified as confirmed cases of microcephaly. Among the confirmed cases, brain abnormalities were detected by imaging in 387 babies and Zika virus was detected

in 17, including in 2 fetal losses.[63] The discrepancy between numbers is probably due to inconsistent reporting and unclear definition for microcephaly.[64]

The baseline rate of microcephaly in Brazil is also controversial. A retrospective analysis of head circumference from 2012–15 by Paediatric Cardiology and Perinatology Network from Paraíba in northeast Brazil suggested that the incidence of microcephaly in Paraíba, even with conservative criteria, was more than 2000/100 000 births since late 2012[65] before even the earliest predicted Zika virus infection in Brazil. Numerous other agencies including the CDC, European Surveillance of Congenital Anomalies and the Latin American Collaborative Study of Congenital Malformations estimate the true baseline incidence to be 10–28.5/100 000 in Brazil. It is noteworthy that during this large outbreak in Brazil where an estimated 11% of the population was infected with Zika virus, there was no overall increase in number of fetal deaths or premature births.[58] This questions the clinical significance of milder cases of microcephaly, which account for the majority of the reported cases.

There has yet to be a confirmed increase in birth defects and microcephaly reported from other Zika-affected territories although there have been reports in travelers from Brazil to the USA and in Panama. In French Polynesia, there were only 17 cases of fetal central nervous system malformations, 4 were found positive by IgG serology assays for *Flavivirus*,[61] which are not specific for Zika virus. Furthermore, more than 6000 pregnant women were recently infected with Zika virus in Colombia, but there has not been increase in cases of microcephaly observed according to published reports to date.

Before the current debate on Zika virus and microcephaly, perinatal transmission of arboviruses has been reported for dengue virus,[65–69] chikungunya virus,[70,71] West Nile virus[72,73] and yellow fever virus.[74,75] There were rare reports of associated perinatal complications including encephalopathy and hemorrhagic fever in chikungunya virus[71]; fetal death, fetal anomalies, prematurity and acute fetal distress during labor in dengue virus.[65,67] However, transplacental transmission of *Flaviviruses* has not been proven conclusively.

Numerous studies have attempted to establish the transplacental route of transmission of Zika virus. The first evidence was from French Polynesia in 2014 where Zika virus RNA was found in two mothers as well as in serum from their newborns collected within 4 days post-delivery.[58] However, one newborn was tested negative on the day of delivery, and the other was not tested on the delivery day. In November 2015, the Brazilian Ministry of Health detected Zika virus genome in the blood and tissue samples of a microcephalic neonate from the state of Pará.[76] There were no clinical or biochemical details about the mother.

In another study in November 2015, Zika virus RNA was found in the amniotic fluid of two women carrying microcephalic fetuses in November 2015.[77] Both women reported signs and symptoms suggestive of Zika virus infection earlier in pregnancy. In February 2016, Slovenia and the CDC reported three cases of positive Zika virus RNA in the aborted microcephalic fetuses' brain and placental tissue.[78,79] The USA saw nine pregnant women with positive travel history who were tested positive for Zika RNA up till February 2016. Six of them reported symptoms in the first trimester. Two of the six had miscarriages and two delivered an infant with microcephaly. Zika virus RNA was detected in the specimens from both cases with early pregnancy loss. One of the pregnancy terminations had evidence of brain atrophy on fetal ultrasound and MRI, as well as Zika virus RNA on amniocentesis.[80]

The first prospective study on Zika virus in pregnancy was published in March 2016.[50] This study followed 42 pregnant women positive for Zika RNA. Twelve were found to have abnormal fetal ultrasound scans, and there were two fetal deaths after 30 weeks of gestation. However, there were some limitations in the study, in particular the limited follow-up and lack of controls.

The pathogenesis of microcephaly can be primary, which results from a developmental insult during neurogenesis early in pregnancy or secondary due to injury to a previously normal brain from a reduction in the number of dendritic processes

and synaptic connections.[81] Zika virus has been found to be neurotropic in two early studies by Dick[3] and Bell[82] in mouse models. Bell observed the virus to infect both neurons and glia, producing intracytoplasmic inclusions or autophagy. *Flavivirus* infections are known to hijack the autophagy process for viral replication.[83] Zika virus was observed to have this capability in experimentally infected skin fibroblasts.[84]

Ocular abnormalities have also been reported in microcephalic neonates during the current Zika outbreak. However, Zika infections were presumptive in most of the reported cases.[85,86]

Since the beginning of 2016, pregnant women have been warned against visiting Zika-affected territories through a series of advisories and guidelines from the CDC, the European Centre for Disease Prevention and Control (ECDC), the American Congress of Obstetrics and Gynecology (ACOG) and the Society of Maternal Fetal Medicine (SMFM).[61,87–93] While this has gathered a lot of international attention and concern, this is similar to travel advice issued by the US CDC for pregnant women traveling to malaria-affected areas.

Neurological complications

Neurological complications of Zika viral disease were first reported from French Polynesia in 2013. Of note, 74 patients presented with neurological syndromes of autoimmune etiology after developing signs and symptoms of Zika virus infection.[61] Of these, the majority[59] were GBS. Other syndromes included meningitis, encephalitis, meningoencephalitis and myelitis. A case–control study[94] compared 42 inpatient cases of GBS recruited from October 2013 and April 2014 against two control groups. The first consisted of 98 matched inpatients with non-febrile illness; the second was made up of 70 patients with acute Zika virus disease and no neurological symptoms. The study found that 41 (98%) GBS patients had anti-Zika immunoglobulin M (IgM) or IgG, and all (100%) had neutralizing antibodies against Zika virus compared with 54 of 98 (56%) in the Control Group 1($p < 0.0001$). Thirty-seven (88%) GBS patients experienced symptoms consistent with Zika virus infection about 6 days before the onset of neurological symptoms. Sixteen patients (38%) required intensive care for respiratory failure, but there was no mortality. Interestingly, none of the GBS patients were tested positive for Zika RNA, while 100% of the 70 controls in Group 2 was tested positive suggesting an immunological response rather than directly invasive viral pathology.

A similar increase in the incidence of neurological syndromes was reported in Brazil. Up to July 13, 2015, 76 patients with neurological syndrome had been identified, of which 55% were confirmed as GBS. Of note, 62% of those with GBS had symptoms consistent with Zika virus.[95] In January 2016, El Salvador also reported a 3-fold increase of GBS since early December 2015.[96] The association of GBS with Zika seems a little more widespread than the association of microcephaly, and it has been noted with a number of viral infections in the past.[97–99]

Diagnosis

The diagnosis of Zika virus infection is challenging due to low and transient viremia[100] and the low specificity of Zika antibodies. The available methods include reverse transcription polymerase chain reaction (RT-PCR) and serology.

Detection of viral genome via RT-PCR can be used in early infection, from serum, urine, saliva, amniotic and semen samples.[56,101] The CDC Zika virus assay comprising the two one-step real-time RT-PCR is one of the most studied methods.[102] Viremia lasts for ~4–8 days after symptom onset, with a mean of 3.3 days.[56,101] Therefore, the CDC recommends a 5-day cutoff for performing RT-PCR. Zika virus was more frequently detected in saliva compared to blood.[101] Urine samples were reported to have a higher viral load and carry the virus for a longer duration than in serum for 10 to >20 days.[56] This makes it the most useful option for returning travelers.

Commercial real-time RT-PCR assays are likely to soon be available. However, these have yet to be validated in the literature with clinical specimens.[102]

Serologic testing is an option after the acute phase. However, it is limited by the considerable

cross-reactivity between Zika virus and other *Flaviviruses*, such as dengue, which are endemic in a geographic distribution similar to Zika virus. Confirmatory neutralizing antibody testing is required to differentiate recent Zika Virus infection from other viruses.[103] Dengue IgM assays including the Focus Diagnostics DENV IgM Capture[104,105] and the SD BIOLINE Dengue Duo 215 NS1 Ag + Ab Combo[20] have shown false-positive results with Zika virus infection. A positive test for dengue IgM antibodies in the absence of dengue NS1 antigen or dengue virus RNA should prompt further investigations for Zika virus. Zika Virus IgM is detectable in the serum by at least 4 days after symptom onset but may not become positive until 2 weeks after the onset of infection. It is unclear how long the Zika Virus IgM persists in serum, but experience with other *Flaviviruses* would suggest that the IgM could remain positive for up to 6 months post-infection.[106]

Zika virus can be cultured via intracerebral mouse inoculation[2,107] or cell lines such as the African green monkey (Vero) and Rhesus monkey kidney (LLC-192 MK2). These culture methods are used in public health and research laboratories, and are not generally available for clinical purposes.

Treatment

It is important to rule out other infections or co-infections such as malaria, chikungunya and dengue when assessing a patient presenting with symptoms suggestive of Zika virus infection. As there is no specific antivirals or vaccination for Zika virus, the treatment is mainly supportive with fluids and pain management. Aspirin and other non-steroidal anti-inflammatory drugs are discouraged in numerous guidelines until dengue can be conclusively ruled out to reduce the risk of bleeding.

Travelers, in particular pregnant women, have been advised to avoid Zika-affected areas. If travels are unavoidable, travelers should wear long-sleeved shirts and pants, stay in air-conditioned areas, use insect repellent and sleep under mosquito nets. *Aedes* mosquitoes are most active at dawn and dusk. Vector management, mainly in reducing mosquito-breeding sites, is critical to controlling the outbreak.

Conclusion

Sixty years after its initial discovery, we are just beginning to understand how Zika virus affects humans. It has proven itself to be a pathogen with the potential for rapid dissemination across borders. Given the association with severe outcomes such as congenital birth defects and debilitating neurological syndromes, more collaborative efforts are needed to carry out larger and higher quality studies to better define the risk of complications in what is otherwise a more benign disease than dengue fever. Clinicians in non-endemic areas or areas where the *Aedes* mosquitoes are not found should pay particular attention to fever in returned travelers, especially those with prominent myalgia, rash and conjunctivitis. While the WHO has again been criticized for perhaps overreacting to a generally benign disease with rare complications, the mobilization of resources in vaccine development and vector control can only benefit the populations of tropical countries affected by many neglected tropical diseases and, by extension, travelers from the rest of the world.

Conflict of interest statement

The authors have no potential conflicts of interest.

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