Overview of Zika infection, epidemiology, transmission and control measures

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Abstract The current Zika virus outbreak in the Americas and the proposed link to increases in microcephaly and neurological disorders have prompted the World Health Organization to declare a Public Health Emergency of International Concern on February 1, 2016. The virus is transmitted by Aedes mosquitoes and potentially by transfusion, perinatal and sexual transmission. The potential for spread into countries where Aedes mosquitoes are endemic is high. Previously, cases tended to be sporadic and associated with mild, non-specific symptoms. Prior outbreaks occurred in Yap Island in Micronesia in 2007, the first time Zika arose outside of Africa and Asia, and in French Polynesia in 2013. A birth data review has confirmed that the latter outbreak was followed by an increase in microcephaly cases. A coordinated international response is needed to address mosquito control; expedite development of diagnostic tests, vaccines and specific treatments for Zika; and address the proposed link to microcephaly and neurological diseases.
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History of Zika epidemiology

The ZIKV is a mosquito-borne Flavivirus, of the family Flaviviridae. It was first isolated from a febrile sentinel monkey in the Zika forest in Uganda in 1947 and subsequently from Aedes africanus mosquitoes in the same forest [12,13]. Isolation of ZIKV from Ae. africanus mosquitoes and other Aedes species including Ae. aegypti in Africa and Malaysia over the subsequent decades followed [2]. Many other Aedes species, including Ae. luteocephalus, Ae. albopictus, Ae. furcifer, Ae. vittatus, Ae. taylori, Ae. dalzielli, Ae. hirsutus, Ae. metalicus, Ae. hensilli and Ae. unilineatus have also been implicated as vectors, as have mosquitoes from other genera, including Mansonia uniformis, Culex perfuscus and Anopheles coustani [14,15].

Cases of ZIKV infection of humans arose throughout the 1960s and 1970s in several countries in Africa and Asia [2]. Although the cases were sporadic, they were widespread. The true incidence and prevalence of ZIKV, however, remains difficult to definitively establish because of the lack of simple, reliable laboratory diagnostic tests and the similarity of symptoms caused by ZIKV to those of other arbovirus infections [16].

The first example of human infection by ZIKV outside of Africa and Asia arose in 2007, when there was an outbreak in Yap Island in the Federated States of Micronesia in Oceania. [2]. Yap Island has a population of approximately 7391 people; 49 positive cases and 59 probable cases of ZIKV infection were identified using ELISA to detect IgM antibody against ZIKV or by identification of ZIKV RNA [8]. A household survey suggested prevalence of 75% of the population over the age of three [1,8,17]. Ae. hensilli, the predominant Aedes species on Yap Island, has been identified in laboratory studies as a likely culprit for the ZIKV vector during that outbreak [14].

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The previously largest documented ZIKV outbreak occurred in 2013 in French Polynesia, a French overseas territory in the South Pacific with a population of approximately 268,000 people. Up to 8200 ZIKV infection cases were reported, with an estimated prevalence of 10–11% [4,10,16–18].

Thus far, in the current outbreak, 33 countries have reported autochthonous Zika virus transmission between January 2014 and February 2016, most notably Brazil and Colombia. In addition, six countries are reporting the indirect evidence of transmission of the virus [1,6,11]. ZIKV appears to be spreading globally in a similar manner to other arbovirus family members, including dengue (DENV) and chikungunya (CHIKV) viruses [19].

Zika virology and symptoms

ZIKV is an emerging arbovirus member of the Flavivirus genus of the Flaviviridae family. The Flavivirus genus comprises more than 70 viruses, also including DENV, Yellow Fever virus (YFV), Japanese encephalitis virus (JEV) and West Nile virus (WNV). As with the other flaviviruses, ZIKV is a positive, single-stranded RNA virus and has a 10,794 nucleotide genome. Its genome consists of 5’ and 3’ untranslated regions flanking an open reading frame which encodes a polyprotein of three structural proteins, namely the capsid (C), premembrane/membrane (prM) and envelope (E), and seven non-structural proteins, i.e. NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B and NS5 [20,21]. Phylogenetic analysis of ZIKV isolates from Cambodia, Malaysia, Nigeria, Uganda and Senegal, along with other published ZIKV sequences, suggests that there are two main ZIKV lineages, Asian and African. The Yap Island outbreak, for example, was caused by a strain originating in Southeast Asia [21]. Use of real-time PCR on isolates from the 2007 outbreak showed that the envelope protein contained an Asn-X-Ser glycosylation motif at position 154, which is found in many flaviviruses, but not the prototype MR766 ZIKV strain [2,21]. Loss of this glycosylation site may represent a polymorphism among circulating strains but may also reflect passing history of strains [21].

Symptoms associated with ZIKV infection are variable. It is asymptomatic in up to 80% of cases [8,11,22], and when symptoms do occur, they are typically mild and non-specific. These include mild fever, conjunctivitis, maculopapular rash, myalgia and headache [23]. In the Yap Island outbreak, for example, patients suffered from rash, conjunctivitis and arthralgia [2,8]. This leads to the potential misdiagnosis, for example, as DENV infection. However, in the current outbreak, a potential link has been identified between ZIKV infection and an increase in some serious neurological effects, such as GBS and microcephaly. Microcephaly is a rare condition in which the circumference of the baby’s head is smaller than expected, and it is usually a result of the failure of normal brain development in utero. This may be due to toxins or infections. GBS, meanwhile, is an acute neural illness which results in a deficit in lower, bilateral and symmetrical sensorimotor development [1]. Other flaviviruses are associated with neurological disorders. For example, JEV is the main cause of vaccine-preventable encephalitis in Asia and the western Pacific [24].

In Brazil, for example, an average of 163 cases of microcephaly were recorded annually between 2001 and 2014; however, in the year up to January 30, 2016, 4783 microcephaly and/or CNS malformation cases had already been recorded [1]. Brazil, Colombia, El Salvador and Suriname have all also reported sharp increases in GBS cases during the course of 2015 up to the present, concurrent with the ZIKV outbreak [1]. In Brazil, for example, of the 42 patients confirmed to have GBS in Bahia state in July 2015, 62% had previous symptoms consistent with ZIKV infection. Given the putative link between microcephaly and ZIKV uncovered by this outbreak, the French Polynesian authorities have carried out a review of birth data for children born between March 2014 and May 2015, after their outbreak began. Eighteen cases of CNS malformation, including nine microcephaly cases, have been identified as a result, in striking contrast to the usual average annual rate of 0–2 microcephaly cases [1]. While no definitive link has yet been proven, one recent molecular genetics and electron microscopic study demonstrated the presence of ZIKV in fetal brain matter, but not other organs, of an aborted fetus with microcephaly. The mother was a European woman who had been infected with ZIKV at 13 weeks gestation while working in northeastern Brazil [25,26].

The French Polynesia outbreak also yielded the first report of a case of GBS complication of a ZIKV infection [16]. DENV has also been associated with GBS, and prior DENV infection may pre-dispose a ZIKV patient to GBS. Ultimately, the GBS incidence in French Polynesia was estimated to have risen 20-fold following the ZIKV outbreak [16]. Clearly, this potential link between ZIKV and serious neurological disorders has focused attention on viral transmission.
ZIKV transmission

As mentioned, in common with other flaviviruses, ZIKV is transmitted by female mosquito vectors. The mosquito acquires the virus during a blood meal. The ZIKV can then breed in the mosquito, which is not killed by the virus, and it is transmitted to humans during a subsequent blood meal by injection into the skin and infection of permissive skin cells [22,27]. ZIKV is circulated predominantly via a sylvatic cycle involving mainly non-human primates and arboreal mosquitoes, most notably Aedes species, for example, in the forests of Africa and Asia. However, an urban cycle involving humans and species such as Ae. aegypti, Ae. hensilli or Ae. albopictus has now been well established to exist in Africa, Asia, Oceania and the Americas [2,4,8,14,15,17,19]. Transmission by Aedes and other mosquitoes opens up the possibility of spread of ZIKV into any region where such vectors might be present, including Southern Europe and the United States [1,10,11,17,19]. There have been reported cases of importation of ZIKV into Australia by people traveling from Indonesia and the Cook Islands and into Canada by travelers from Thailand [27—29]. Recent cases of importation of ZIKV to Europe have also been reported into France by three travelers to Martinique, Brazil and Colombia [30]; into Italy by travelers to Brazil [31] and French Polynesia [32]; and into Germany by a traveler to Thailand [33].

In another case from January 2016, a baby born with microcephaly in Oahu, Hawaii, was confirmed by the CDC to have been infected with ZIKV. The child’s mother probably acquired the virus in May 2015 in Brazil and passed the infection perinatally to her child [1]. Further evidence of perinatal ZIKV transmission came from clinical and laboratory studies on two mothers and their newborns in French Polynesia in 2014 [4]. Both mothers had ZIKV infections, with confirmation in the newborns by RT-PCR within 4 days of delivery. Transmission most likely occurred either transplacentally or during delivery [4]. The potential for ZIKV transmission via blood transfusion was also demonstrated during the French Polynesia outbreak [5]. This is particularly concerning given that ZIKV infection is so often asymptomatic; therefore, blood donors may not be aware of their potentially infective status.

There is also some limited evidence from case studies that ZIKV can be sexually transmitted. During the French Polynesia outbreak, ZIKV RNA was detected by RT-PCR from semen and urine samples, but not in blood samples, from a patient in Tahiti who had two episodes of symptoms consistent with ZIKV and subsequently presented with hematospermia [3]. The ZIKV was detected in samples taken 1—3 days after he presented with hematospermia; culturing of the samples revealed replicative viral particles in the semen but not the urine samples. These results were consistent with observations in a previous case of a man from the United States who contracted ZIKV while travelling in Senegal [34]. Four days after returning to the US, he had symptoms of hematospermia, while his wife, with whom he had sexual intercourse on the first day of his return, was suffering from ZIKV symptoms. ZIKV infection was confirmed serologically in both spouses, and although the man’s semen was not formally tested, sexual transmission seemed the most likely route. Other probable case of sexual transmission has been noted in Italy in a female partner of a man who had travelled to Thailand [35]. The identification of probable sexual transmission of ZIKV has led to the recent publication by the CDC (February 5, 2016) of recommendations for prevention of ZIKV sexual transmission, especially for pregnant women. It recommends that men who reside in or have travelled to an area of ongoing ZIKV transmission and whose partner is pregnant should abstain from sexual activity or use condoms during sexual activity throughout the pregnancy [36].

ZIKV transmission and mass gatherings

The ready transmission of ZIKV via mosquito bites, combined with the possibilities of sexual and perinatal transmission, have raised fears regarding up-coming mass gatherings in areas where the virus is currently endemic and other areas where local Aedes mosquito populations provide potential vectors for new outbreaks. Potentially most significant is the summer Olympics, which will take place in Brazil from August 5—21 2016 and is likely to attract millions of people both from within Brazil and from other countries, along with the Paralympics, due to take place between September 8 and September 13 [6,7]. The type of young and healthy, middle-to-high-income group of international travelers most likely to attend the Olympics are less likely to have been previously exposed to ZIKV or other arboviruses [7]. They are also perhaps less likely to be aware of basic precautions against mosquito bites, such as wearing suitable protective clothing, optimal use of EPA-approved insect repellents and use of mosquito nets while sleeping. They may also be potentially more vulnerable to possible sexual transmission of ZIKV [7]. This has serious implications for Public Health Preparedness in the context of the Olympics, and underscores the
need for a coordinated international response to surveillance, prevention and limiting the spread of disease at events associated with mass gatherings [6]. In terms of the Olympics, the organizers have implemented plans to control vectors in the vicinity of the Games [6,7]. However, a combination of personal and community measures is necessary, as well as a coherent public health policy, to minimize risk of ZIKV transmission either by mosquito bites or other means [7]. It is recommended that travelers to the Olympics consult their family doctor and/or travel medicine providers in advance of travelling and acquaint themselves with all up-to-date health information and relevant precautions, including on ZIKV, by consulting agencies such as the Latin American Society for Travel Medicine (SLAMVI) or their own country’s public health agencies. Given the status of ZIKV as a blood-borne disease, it is vital that local medical facilities are prepared, with basic infection control strategies in place, including rigorous hand hygiene practice; contact precautions; safe disposal practices for sharps, blood and other body fluids and disposable instruments; and adequate sterilization practices for non-disposable instruments [6].

The CDC has already issued interim guidelines pertaining to the ZIKV to pregnant women [22]. They have recommended that pregnant women or those considering pregnancy postpone travelling to countries with ongoing ZIKV transmission. If this is not possible, they must follow the strictest of steps to avoid mosquito bites both outdoors and indoors, especially during the day, when the Aedes mosquitoes are most likely to bite [6,22].

The timing of the summer Olympics, which is actually during the time of winter temperatures in Brazil, means that mosquito populations will be lower than at other times of the year, helping to mitigate the potential risk of ZIKV spread. However, there are other mass gatherings scheduled to occur elsewhere in the world in which Aedes populations present a potential new outbreak vector. For example, in Saudi Arabia, the Umrah (June to September, 2016) and Hajj (8th–13th September, 2016) pilgrimages are due to take place. These events will attract upwards of 7 million pilgrims from nearly 200 countries, including thousands from Latin America [7]. The profile of these pilgrims will tend to be older adults from lower-income countries, and they therefore present different Public Health Preparedness challenges compared to the Olympics attendees [7]. Saudi Arabia is well-acquainted to preparing for the public health challenges posed by such pilgrimages and routinely convenes annual international public health consultations to address relevant disease-specific issues; this year, ZIKV will feature high on this list. It is routine to provide health advice on an individual level to pilgrims in advance of traveling, with reinforcement of advice at points of both departure and arrival, and arranging for distribution of methods of prevention, such as mosquito nets and insect repellent [7]. Such measures would be directly relevant in Brazil during the run-up to the Olympics, with issues including shortages of insect repellent to be met in advance through international collaboration. Saudi Arabia has experience in the application of public health measures for prevention of disease in the context of mass pilgrimages in the past, for example, during the H1N1 pandemic in 2009.

Control measures

The WHO has identified Aedes mosquitoes, for example Ae. aegypti, as particularly tenacious pests, which have readily adapted to changing environments such as increased urbanization [37,38]. Unfortunately, reductions in mosquito control programs during the 1960s led to consequent loss of expertise, resources and infrastructure. Now there is a tendency toward emergency-driven reactions, for example, reactive spraying/fogging with insecticides. However, this type of approach is neither sustainable nor effective in the long-term, particularly as mosquitoes develop resistance to more insecticides [37].

The WHO Emergency Committee on ZIKV made various recommendations on how the spread of this virus could be potentially be controlled, which is particularly important in the current absence of a vaccine or specific treatment [1,37]. Some of the recommendations pertain to personal and household protection, such as the measures mentioned above to avoid mosquito bites, including the use of EPA-approved insect repellents; wearing light-colored protective clothing to keep as much as possible of the body covered; using physical barriers such as screens and closing doors and windows; using mosquito nets to sleep under, especially when the mosquitoes are most active in the daytime; and avoiding collection of stagnant water in containers such as buckets, used tires, water barrels or flower pots [1,17,37]. Other control measures include the type of travel advice issued by the CDC to pregnant women, or those considering pregnancy [11,22]. However, other recommendations have addressed the need to improve mosquito control programs, including the use of larvicides for treatment of standing water that cannot be otherwise cleaned, covered or emptied [1,37].
The WHO, overall, recommends an integrated approach, in which the mosquitoes are targeted at all life stages, not just via fogging of adults with insecticides such as Deltamethrin [17]. Fogging would only be recommended during emergencies and should be conducted around dawn and dusk, when mosquitoes are most active [37,38]. More innovative, newer tools are also being considered by a WHO Vector Control Advisory Group. These include the recommendation of further field trials and risk assessment of the OX513A transgenic mosquito. Introduction of male OX513A mosquitoes gained some success in the reduction of the Aedes aegypti population in the Cayman Islands and in a suburb of Juazeiro, Bahia, Brazil [39]. Other approaches under consideration include the mass release of radiation-sterilized males, which will mate with females who will lay non-viable eggs [40,41]. Yet another approach could exploit the naturally occurring Wolbachia bacteria, by super-infection of Aedes mosquitoes with strains of Wolbachia [42]. If a female mosquito mates with a Wolbachia-carrying male, she will lay non-viable eggs. In El Salvador, meanwhile, there are plans to introduce fish that eat mosquito larvae into water storage containers. Thus, beyond conventional mosquito control strategies, the WHO is encouraging the development of potential new strategies that do not rely on pesticides [1,37].

It is more important than ever, therefore, that research continues on innovative methods for potentially reducing mosquito populations. In one recent study, for example, researchers identified a Chromobacteria family member termed Csp_P in the gut microbiota of Aedes aegypti mosquitoes [43]. When mosquitoes ingested Csp_P bacteria, Csp_P biofilms colonized the mid-gut. This colonization reduced mosquito susceptibility to DENV and Plasmodium falciparum infection and reduced the lifespan of both mosquito larvae and adults. The Csp_P also had direct in vitro anti-DENV and anti-P. falciparum activities in the absence of mosquitoes. Such studies open up the possibility of exploiting mosquito gut microbiota elements and using them against the mosquito, as well as potentially for use as direct therapies against viruses spread by mosquitoes, including ZIKV. Another approach, which was first proposed as a theory more than 60 years ago, is to induce sex distortion in the mosquito population, to tip the balance toward the non-biting male mosquitoes, thus reducing disease spread and ultimately depleting mosquito populations. For example, in one study, a slime mold endonuclease called I-Ppol selectively shredded the X chromosome of the malaria mosquito vector, Anopheles gambiae [44]. Male mosquitoes were genetically engineered to heritably produce I-Ppol during spermatogenesis and hence produced hardly any X-chromosome-containing sperm. By introducing these sex-distorted males, the researchers were able to achieve dramatic reductions in female mosquitoes. Ultimately, there was a complete loss of the population over six generations [44]. While such an approach is still some years away from ultimate field deployment, if such an approach could be adapted to Aedes mosquitoes it nonetheless presents a promising potential way forward in mosquito control and hence control of diseases such as ZIKV. Interestingly, many commentators suggest that targeting the Y chromosome would be more effective in the long-term.

**Diagnosis and treatment of ZIKV**

Diagnosis of ZIKV infection is complicated by the fact that it is asymptomatic in up to 80% of cases. Symptoms that do occur tend to be mild and non-specific, including headache, fever and rash [8,23]. Similarity in symptoms to those of other flavivirus infections, such as DENV further complicates diagnosis. Dating of the onset of symptoms tends to be complicated by the fact that there is no abrupt clinical onset [9]. There is, as yet, no gold standard laboratory diagnostic method available. Diagnosis during the acute phase of the illness is by detection of viral RNA in serum by reverse transcription PCR (ZIKV RT-PCR) [10]. For example, during the outbreak in Yap Island, a real-time RT-PCR test targeting the viral envelope gene was used [2]. Another envelope targeting test using degenerate primers was designed based on samples from West Africa [45]. A more recently developed test, which has the potential to be converted to a real-time platform, was designed to target the highly conserved NS5 gene [46].

The short viremic period of ZIKV limits the utility of molecular diagnostic techniques on serum samples to a window of approximately 3–5 days following the onset of infection. ZIKV can also be detected in saliva samples by ZIKV RT-PCR at a higher rate than for blood samples, but it does not extend the window of time during which the virus can be detected [9,10]. Saliva samples provide an alternative or additional sample to blood, which could be particularly useful in circumstances in which blood sample collection is difficult, for example with infants and young children [9]. ZIKV can also be detected by RT-PCR on urine samples, which may allow an extension of the detection window. ZIKV appears to be detectable in urine for more
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than 10 days after the onset of disease [3,10,47]. ZIKV diagnosis can also be achieved by detection of ZIKV-specific IgM and/or IgG antibodies via ELISA or immunofluorescence, starting from day 5 or 6 after the onset of symptoms [10]. However, results need to be interpreted carefully due to cross-reactivity in patients with previous flaviviral infections, particularly DENV [2,8,10,23]. Recently, the release of a commercial kit for serological detection of ZIKV by ELISA and indirect immunofluorescence is available by EUROIMMUN. This is in keeping with the global response strategy outlined by WHO, which prioritizes development of a reliable, affordable and rapid diagnostic test [1,48].

There is currently no vaccine available against ZIKV, nor is there any specific prophylactic treatment. Prevention, by following the steps mentioned above for avoiding mosquito bites, is the best approach. Symptom treatment, including getting sufficient rest, drinking plenty of fluids to avoid dehydration and taking medication for pain relief and fever reduction, such as acetaminophen, is recommended by the CDC and the ECDC [10,11]. Treatment with acetylsalicylic acid and non-steroidal anti-inflammatory drugs is to be avoided, as they have been associated with a potentially increased risk of hemorrhagic syndrome in other flavivirus infections. They have also been associated with the risk of Reye’s syndrome following viral infection in children and teenagers [10]. Fast-tracking of development of a ZIKV vaccine and specific antivirals is a public health priority. It is critical to restoring confidence and security to people who wish to have children who reside in or visit areas in which Aedes mosquitoes circulate.

Future perspectives

The WHO Emergency Committee has identified fast-tracking of research into the possible association of ZIKV infection with the etiology of microcephaly and neurological diseases such as GBS, as well as development of vaccines and antivirals, as key elements of the global response strategy mitigate the impact of ZIKV infection [1]. Little is currently known about the pathogenesis of ZIKV, as until now it has received relatively little attention compared to other flaviviruses. A recent study identified human skin cells including dermal fibroblasts, epidermal keratinocytes and immature dendritic cells as permissive to the ZIKV isolate that caused the outbreak in French Polynesia [27]. The phosphatidylinerse receptor AXL was identified as playing a major role in ZIKV entry into cells, while cellular autophagy enhanced viral replication. An antiviral innate immune response was initiated, with production of type I interferons; ZIKV was sensitive to the antiviral properties of type I and II interferons [27]. Such studies cast light on the interplay between ZIKV and the human host, and suggest potential targets for directing therapies to thwart the virus. Meanwhile, at least 15 research groups and companies are currently researching ZIKV vaccines, although most projects are at an early stage. A DNA vaccine under development by the pharmaceutical company Inovio Pharmaceuticals, Inc. in the USA has been recently reported to induce robust immune responses in mice in preclinical studies [49]. Additionally, Bharat Biotech in India has filed a global patent for an inactivated vaccine that they claim could be finished with pre-clinical testing within 5 months [50]. In the meantime, the best ‘cure’ is prevention via avoidance of mosquito bites while the world awaits definitive answers on the link between ZIKV and serious neurological diseases.

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