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RESEARCH ARTICLE

SMALL BITE, BIG THREAT: ZIKA VIRUS

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ABSTRACT

In recent times Zika virus has dominated the global attention of health care researchers. Though the virus has been known since 1947, it has caused lot of panic and alarm among many countries. It has become the first major infectious disease linked to human birth defects to be discovered in more than half a century and has created such a global alarm that the World Health Organization (WHO)has declared it a Public Health Emergency of International Concern. Though the viral fever caused by it has fairly common signs and symptoms, infection during pregnancy has been known to cause microcephaly and other brain malformations in some babies. Infection in adults has been linked to Guillain–Barré syndrome (GBS). While there is no specific treatment, paracetamol (acetaminophen) and rest may help with the symptoms. Prevention involves decreasing mosquitoes bites in areas where the disease occurs and proper use of condoms. Numerous companies and institutions internationally are developing vaccines against Zika, virus.

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INTRODUCTION

These sentiments have been clearly reflected in an extraordinary occurrence that has triggered a public health threat in recent times, an event that has worried the global medical field and cast a shadow even on the much awaited Rio Olympics. The tiny organism responsible for these worldwide ramifications is the Zika virus. The virus, originally named ZIKV, was discovered for the first time in 1947 in a rhesus macaque in a forest in Uganda known as Zika forest (Petersen *et al*., 2016). Outbreaks were reported from 1951 to 1981 throughout Africa and Asia. Study in 2007 in Polynesia found that 73 percent of the population was infected. However the spread of the virus has been rapid, since the first cases were discovered in Latin America in 2014 (Petersen *et al*., 2016).

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In December 2015, the Pan American Health Organization/World Health Organization (PAHO/WHO) recommended Latin American countries start gearing up to screen for Zika (Petersen *et al*., 2016). Though Zika virus has been known for many decades; it is currently commanding worldwide attention since researchers have found evidence that Zika may be linked to birth defects like microcephaly and Guillain-Barré syndrome in adults. This concern has been reflected by the World Health Organization (WHO), which declared a public health emergency of international concern (PHEIC) on February 1, 2016 as there were more than 4,000 microcephaly cases and neurological *disorders in some areas* affected by Zika virus (ZIKV) (http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/).

Virology

Zika virus is a member of the virus family Flaviviridae and the genus Flavivirus (Malone *et al*., 2016). Zika virus resembles the other members of the Flaviviridae family including the dengue, yellow fever, Japanese encephalitis and West Nile viruses. Zika virus is enveloped and icosahedral in nature. Structurally it has a nonsegmented, single-stranded, positive-sense RNA genome (Hayes *et al*., 2009; Kuno and Chang, 2007). Since the virus has a positive-sense RNA genome, it can be directly translated into viral proteins. The replicated RNA strand is held within a nucleocapsid formed from 12-kDa protein blocks; the capsid is contained within a host-derived membrane modified with two viral glycoproteins. Replication of the viral genome requires creation of an anti-sense nucleotide strand (Pierson and Diamond, 2012). African and Asian are the two lineages for this virus (Enfissi *et al*., 2016). Phylogenetic studies indicate that the virus spreading in the Americas is 89% identical to African genotypes, but is most closely related to the Asian strain that circulated in French Polynesia during the 2013–2014 outbreak (Enfissi *et al*., 2016; Zanluca *et al*., 2015).

Transmission

Before the current pandemic began in 2007, Zika "rarely caused recognized 'spillover' infections in humans, even in highly enzootic areas". The vertebrate hosts of the virus were primarily monkeys in a so-called enzootic mosquito-monkey-mosquito cycle, with only occasional transmission to humans. Though the reason for the pandemic is unknown, dengue, a related arbovirus that infects the same species of mosquito vectors, is known in particular to be intensified by urbanization and globalization (Gubler and Dengue, 2011).

Mosquito

The primary vector for Zika virus is primarily the female Aedes aegypti mosquito, which is active mostly in the daytime. The mosquitos lay eggs while feeding on blood (http://www.cdc.gov/dengue/entomologyecology/). The virus has been known to have an extrinsic incubation period in mosquitoes of about 10 days in some other species (Hayes *et al*., 2009). Since 2015, news reports have drawn attention to the spread of Zika in Latin America and the Caribbean. Research into its ecological niche suggests that Zika may be influenced to a greater degree by changes in precipitation and temperature than Dengue, making it more likely to be confined to tropical areas. However, raising global temperatures allows for the disease vector to expand their range further north, allowing Zika to follow (Carlson *et al*., 2016).

Sexual

Zika can be transmitted from a man to his sex partners. (Oster et al., 2016) As of March 2016, the CDC updated its recommendations about length of precautions for couples, and advised that heterosexual couples with men who have confirmed Zika fever or symptoms of Zika should consider using condoms or not having penetrative sex (i.e., vaginal intercourse, anal intercourse, or fellatio) for at least 6 months after symptoms begin (Petersen *et al*., 2016). The risk factors for and the duration of the risk of sexual transmission have not been determined. Replicative viral particles, as well as viral RNA, often in high copy numbers have been identified in sperm, and viral RNA has been detected up to 62 days after the onset of symptoms (Musso *et al*., 2015).

Pregnancy

Substantial evidence now indicates that Zika virus can be transmitted from the mother to the fetus during pregnancy. Zika virus RNA has been identified in the amniotic fluid of mothers whose fetuses had cerebral abnormalities detected by ultrasonography, and viral antigen and RNA have been identified in the brain tissue and placentas of children who were born with microcephaly and died soon after birth (Oliveira *et al*., 2016; Notes from the field, 2016). Among the few reports of teratogenic effects of flaviviruses, investigators described the brain and eyes as the main targets (Alpert and Fergerson, 2003). No presence of virus and no pathological changes were detected in any other fetal organs apart from the brain, which suggests a strong neurotropism of the virus (Mlakar *et al*., 2016; Besnard *et al*., 2014). The frequency of and risk factors for transmission are unknown (Besnard *et al*., 2014).

Blood transfusion

As of April 2016, two cases of Zika transmission through blood transfusions have been reported globally, both from Brazil (Besnard *et al*., 2014; Vasquez *et al*., 2016), after which the US Food and Drug Administration recommended screening blood donors and deferring high-risk donors for 4 weeks (https://federalregister.gov/a/2016-03393).

Breast milk

Transmission through breast milk has not been documented, although the breast milk of a woman who became symptomatic with Zika virus infection on the day of delivery contained infective Zika viral particles in high titer (Dupont-Rouzeyrol, 2016).

Pathogenesis

Zika replicates in the mosquito's midgut epithelial cells and then its salivary gland cells. After 5–10 days, ZIKV can be found in the mosquito's saliva, which can then infect humans. If the mosquito's saliva is inoculated into human skin, the virus can infect epidermal keratinocytes, skin fibroblasts in the skin and the Langerhans cells. The pathogenesis of the virus is hypothesized to continue with a spread to lymph nodes and the bloodstream (Knipe *et al*., 2007; Chan *et al*., 2016). Flaviviruses generally replicate in the cytoplasm, but Zika antigens have been found in infected cell nuclei (Buckley and Gould, 1988). Very little is currently known about the biology and pathogenesis of ZIKV. The envelope protein for flaviviruses is largely responsible for host range due to receptor binding and immune responses. The ZIKV envelope gene has undergone several selective changes mainly associated with

negative selection, implying an important role for this gene in vertebrate host selectivity and emergence (Faye *et al*., 2014).
A recent mouse model of ZIKV brain infection was developed in interferon signaling–deficient mice (Lazear *et al*., 2016). Work with classic ZIKV isolates in suckling mice revealed neurotropism and 100% mortality by day 10 following intracerebral injection (Dick, 1952).

Clinical aspects

Zika fever (also known as Zika virus disease) is an illness caused by the Zika virus (Zika virus, 2016). The incubation period for Zika virus is unknown, but if it is similar to that of other mosquito-borne flaviviruses, it is expected to be generally less than 1 week (Bearcroft, 1956). Most cases have no symptoms, but when present they are usually mild and can resemble dengue fever (Zika virus, 2016). Symptoms may include fever, nonpurulent conjunctivitis, headache, a maculopapular rash, arthritis or arthralgia, myalgia, retro-orbital pain , edema and vomiting (Zika virus, 2016; Hills *et al*., 2016). Other symptoms that have been noted in association with acute infection include hematospermia, transient dull and metallic hearing, swelling of the hands and ankles, and subcutaneous bleeding (Brasil *et al*., 2016). Symptoms generally last less than seven days (Chen *et al*., 2016). It has not caused any reported deaths during the initial infection (Factsheet for health professionals, 2015).

Neurologic Complications

A temporal and geographic relationship has been observed between Guillain–Barré syndrome and Zika virus outbreaks in the Pacific and the Americas (Thomas *et al*., 2016; Broutet *et al*., 2016; https://en.wikipedia.org/wiki/Zika_virus). GBS is an uncommon sickness of the nervous system in which a person's own immune system damages the nerve cells, causing muscle weakness and sometimes paralysis (Cao-Lormeau *et al*., 2016). A case–control study in French Polynesia revealed a strong association (odds ratio, >34) between Guillain–Barré syndrome and previous Zika virus infection; the findings from electrophysiological studies were compatible with the acute motor axonal neuropathy subtype of Guillain–Barré syndrome. Meningoencephalitis and acute myelitis complicating Zika virus infection also have been reported (https://en.wikipedia.org/wiki/Zika_virus).

Adverse Fetal Outcomes

Microcephaly is a clinical finding of a small head size for gestational age and sex and is indicative of an underlying problem with the growth of the brain (Woods, 2013). The lack of consistent and standardized case definitions has challenged the accurate monitoring of microcephaly during the current Zika virus outbreak (Victora, 2016). Centers for Disease Control and Prevention (CDC) guidance has recommended that microcephaly be defined as an occipitofrontal circumference below the third percentile for gestational age and sex (Oster *et al*., 2016). Microcephaly can occur as a result of fetal brain disruption sequence, a process in which, after relatively normal brain development in early pregnancy, collapse of the fetal skull follows the destruction of fetal brain tissue (Corona-Rivera *et al*., 2001). Initial case reports from Brazil have suggested that some of the infants with microcephaly related to Zika virus infection have a phenotype consistent with fetal brain disruption (Mlakar *et al*., 2016). The timing of the Zika virus and microcephaly epidemics in Brazil and French Polynesia indicate that the greatest risk of microcephaly is in the first trimester. In case reports of microcephaly, documented maternal Zika virus infection most often occurred between 7 and 13 weeks of gestation, but in some cases it occurred as late as at 18 weeks of gestation (Petersen *et al*., 2016). Early fetal loss and fetal death have been noted in association with maternal infection that occurred between 6 and 32 weeks of gestation. Ocular anomalies have been reported among infants with microcephaly in Brazil (Petersen *et al*., 2016).

Diagnosis

The mainstays of the routine diagnosis of Zika virus infection are the detection of viral nucleic acid by RT-PCR and the detection of IgM antibodies by IgM-capture enzyme-linked immunosorbent assay (MAC-ELISA) (Petersen *et al*., 2016). There are limited data on the best diagnostic approaches for acute ZIKV infection in humans (Waggoner *et al*., 2016). The CDC ZIKV assay uses two 1-step real-time reverse transcription–polymerase chain reactions that target the ZIKV premembrane and envelope genes (Waggoner *et al*., 2016). Other ZIKV gene targets have been used for other polymerase chain reaction assays as well. Clinical samples that have tested positive for ZIKV RNA by polymerase chain reaction include serum or plasma, saliva, urine, semen, and amniotic fluid. Serologic diagnosis using assays measuring ZIKV IgM and IgG production with paired acute and convalescent serum is another important diagnostic method (Waggoner *et al*., 2016)
However, other common flaviviruses such as dengue virus or Japanese encephalitis virus can exhibit a high degree of serologic cross-reactivity to ZIKV serology even with the use of capture enzyme-linked immunosorbent assay. Thus, the diagnosis of acute ZIKV infection based on serology alone must be evaluated with caution in regions with other endemic flavivirus infections (Waggoner *et al*., 2016).

Limited data suggest that Zika virus RNA can be detected longer in urine than in serum; if verified, this would extend the period during which a definitive diagnosis of Zika virus infection can be established by RT-PCR (Korhonen *et al*., 2016). Another large study that compared RT-PCR results in serum and saliva samples indicated that RT-PCR had higher sensitivity in saliva than in serum, although samples from some patients were positive in serum but not saliva, and testing of saliva did not extend the duration of detectability of viral nucleic acid after the onset of illness (Musso *et al*., 2015).

Prevention

Prevention involves decreasing mosquitoes bites in areas where the disease occurs and proper use of condoms (Chen *et al*., 2016; Oster *et al*., 2016). Efforts to prevent bites include the use of insect repellent, covering much of the body with clothing, mosquito nets, and getting rid of standing water where mosquitoes reproduce (https:// en. wikipedia. org/wiki/Zika_virus). and using insect repellent containing DEET, IR3535 or icaridin. Special attention and help should be

given to those who may not be able to protect themselves adequately, such as young children, the sick or elderly. Travelers and those living in affected areas should take the basic precautions described above to protect themselves from mosquito bites. Communities should support local government efforts to reduce mosquitoes in their locality. Health authorities may also advise that spraying of insecticides be carried out (Petersen *et al*., 2016; https://en.wikipedia.org/wiki/Zika_ virus).

Treatment

While there is no specific treatment, paracetamol (acetaminophen) and rest may help with the symptoms. Admission to hospital is rarely necessary (Chen *et al*., 2016; Factsheet for health professionals, 2015).

Vaccine development

Despite a limited number of available full-length Zika virus sequences, the molecular data are sufficient to reveal patterns of viral evolution and movement (Faye *et al*., 2014). The current data suggest that any vaccine product developed against any strain of Zika virus should be protective against all strains. The very nature of the close relatedness among the flaviviruses is responsible for the challenges in developing diagnostic algorithms for distinguishing among these viruses (Petersen *et al*., 2016). World Health Organization experts have suggested that the priority should be to develop inactivated vaccines and other non-live vaccines, which will be safe to use in pregnant women and those of childbearing age (http://www.who.int/ mediacentre/news/notes/2016/research-development-zika/ en/). Bharat Biotech International (India) reported in early February 2016, that it was working on vaccines for Zika using two approaches: "recombinant", involving genetic engineering, and "inactivated", where the virus is incapable of reproducing itself but can still trigger an immune response with animal trials of the inactivated version to commence in late February (Bagla *et al*., 2016).

As of March 2016, 18 companies and institutions internationally were developing vaccines against Zika, but none had yet reached clinical trials (http://www.who.int/ mediacentre/news/notes/2016/research-development-zika/ en/). Recently researchers have found that protection against ZIKV challenge can be achieved by single-shot subunit and inactivated virus vaccines in mice and Env-specific antibody titers represent key immunologic correlates of protection. Their findings suggested that the development of a ZIKV vaccine for humans will likely be readily achievable (http://dx.doi.org/ 10.1038/nature18952 (2016)

WHO response

To control Zika virus disease WHO has outlined the "Zika Strategic Response Framework (https://apps.who.int/ *iris/ bitstream/10665/246091/ 1/WHO-ZIKV-SRF-16.3-eng.pdf*). According to this a comprehensive approach has been planned with convening experts and partners. This includes defining and prioritizing research into Zika virus, enhancing surveillance of Zika virus and potential complications. In addition it is planned to strengthen capacity in risk communication to engage communities to better understand risks associated with Zika virus as well as to strengthen the capacity of laboratories to detect the virus. The strategic response plans to support health authorities to implement vector control strategies aimed at reducing Aedes mosquito populations. It has also been planned to prepare recommendations for the clinical care and follow-up of people with complications related to Zika virus infection, in collaboration with experts and other health agencies (https://apps.who.int/iris/bitstream/10665/246091/1/WHO-ZIKV-SRF-16.3-eng.pdf). Expanding capacities of the health care facilities has been focused on in the revised Zika Strategic Response Plan. This would help in better prevention and management of medical complications caused by Zika virus infection. The major target is to enable a wider reach of information and facilities for the vulnerable population including pregnant women, their partners, households and communities to ensure they have the information they need to protect themselves (https://www.who.int/emergencies/zika-virus/response/en/)

Future Outlook and Directions

Even though the Zika virus was known for decades, the causes for its sudden re-emergence remain a mystery. In recent times other infections like dengue, chikungunya which resemble Zika virus by having A. aegypti as the primary vector have increased and spread. These observations hint towards a possible common mechanism like globalization and urbanization (Wilder-Smith and Gubler, 2008). Other possible explanations include viral mutations affecting transmission or virulence and viral introduction to previously unexposed populations leading to epidemic spread. However what is of paramount importance is the need to rapidly and systematically address identified research gaps. Efforts have to be made to completely understand the complete gamut of effect of this virus on the affected off springs, develop new vector control products and strategies, effective therapeutics, and vaccines to protect humans against the disease. "Much has been learned about Zika virus infection...the response now requires a unique and integrated strategy that places support for women and girls of child-bearing age at its core." Dr Margaret Chan (WHO Director-General) (https:// www.who.int/emergencies/zika-virus/response/en/)

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