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Contemporary Issue

Dengue, chikungunya ... and the missing entity – Zika fever: A new emerging threat

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ABSTRACT

Zika virus (ZIKV), a relative newcomer from the flavivirus group that includes dengue, Japanese encepahalitis and yellow fever, is one of the emerging pathogens that is fast transcending geographical boundaries. It is a vector-borne disease transmitted by the same *Aedes aegypti* and *Aedes albopictus*, which cause dengue and chikungunya. In addition to the vector-mediated transmission of Zika fever, probable human-to-human transmission through exchange of body fluids, including sexual and perinatal transmission and through blood transfusion, makes containment of this new entity more challenging. Moreover, a high index of suspicion by an astute physician is necessary for diagnosis of Zika fever in view of the similarity of symptoms with dengue and chikungunya, especially in areas, where these two diseases are already endemic.

Zika, till recently, has had minimal impact, but its true potential is unfolding with increasing detection of congenital malformities, Guillain–Barré syndrome and other neurological and autoimmune syndromes in patients with recent history of ZIKV infection, or when mothers get infected with Zika during first or second trimester of pregnancy. The association, however, needs to be established, nonetheless it is important that we keep a close vigil on this emerging vector borne disease – the 'ZIKA' fever.

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Introduction

Zika fever, an emerging zoonotic disease is caused by Zika virus (ZIKV), a Flavivirus member of the Spondweni serocomplex, which shares limelight with the other well-known members of the Flavivirus family i.e. dengue, yellow fever, West Nile and Japanese encephalitis (Fig. 1).[1,2] ZIKV is a positive sense single-stranded RNA molecule 10,794 bases long containing a nucleocapsid approximately 25–30 nm in diameter, which is surrounded by a host-membrane derived lipid bilayer that contains envelope proteins E and M and seven

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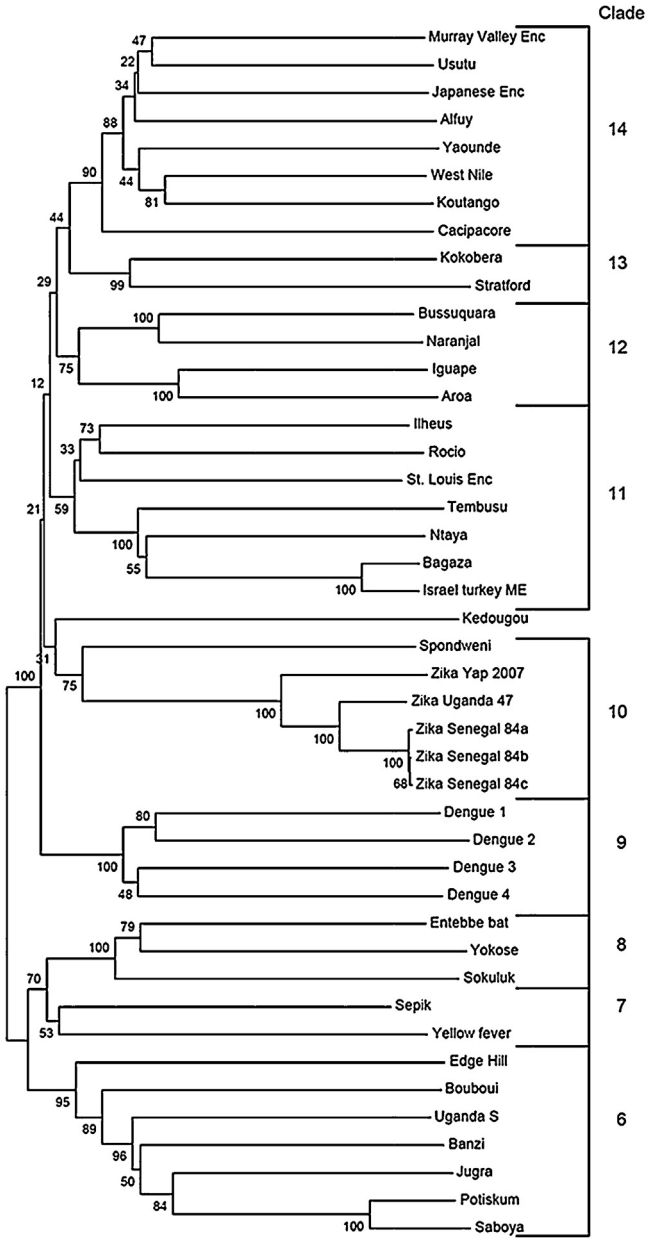

Fig. 1 – Phylogenetic relationship of Zika virus to other flaviviruses based on nucleic acid sequence of nonstructural viral protein 5.

non-structural proteins; of which NS5 is the largest. The virion is approximately 40 nm in diameter with surface projections that measure roughly 5–10 nm. The surface proteins are arranged in an icosohedral-like symmetry.[3,4] Zika is one of the emerging arboviral diseases that is transcending geographical boundaries at a very fast rate. The recent reports of Zika activity from across the continents (Fig. 2)[5] have attracted the attention of public health specialists, as it too is a vector mediated disease transmitted by *Aedes aegypti* and *Aedes albopictus*, the vectors of dengue and chikungunya, which are already on a global rampage. The global predicted distribution of *A. aegypti*, the principal vector of Zika is presented in Fig. 3.[6] Till date, the *Aedes* species, which have been implicated in the transmission of ZIKV are *A. aegypti*, *A. vitattus*, *A. furcifer*, *A. africanus*, *A. apicoargenteus* and *A. luteocephalus*. However, *A. aegypti* and *A. albopictus* are considered to be the major players in the transmission of the virus due to their ubiquitous global presence. The evidence so far implicates monkeys and humans as the sole reservoirs of the disease.[7–9]

Outbreaks of ZIKV

ZIKV was first described in April 1947, when a sentinel rhesus monkey fell sick in the Zika Forest of Uganda while participating in a jungle Yellow fever research programme of the United States.[7–9] Report of Human ZIKV was for the first time published in 1964 by a scientist, who described his own

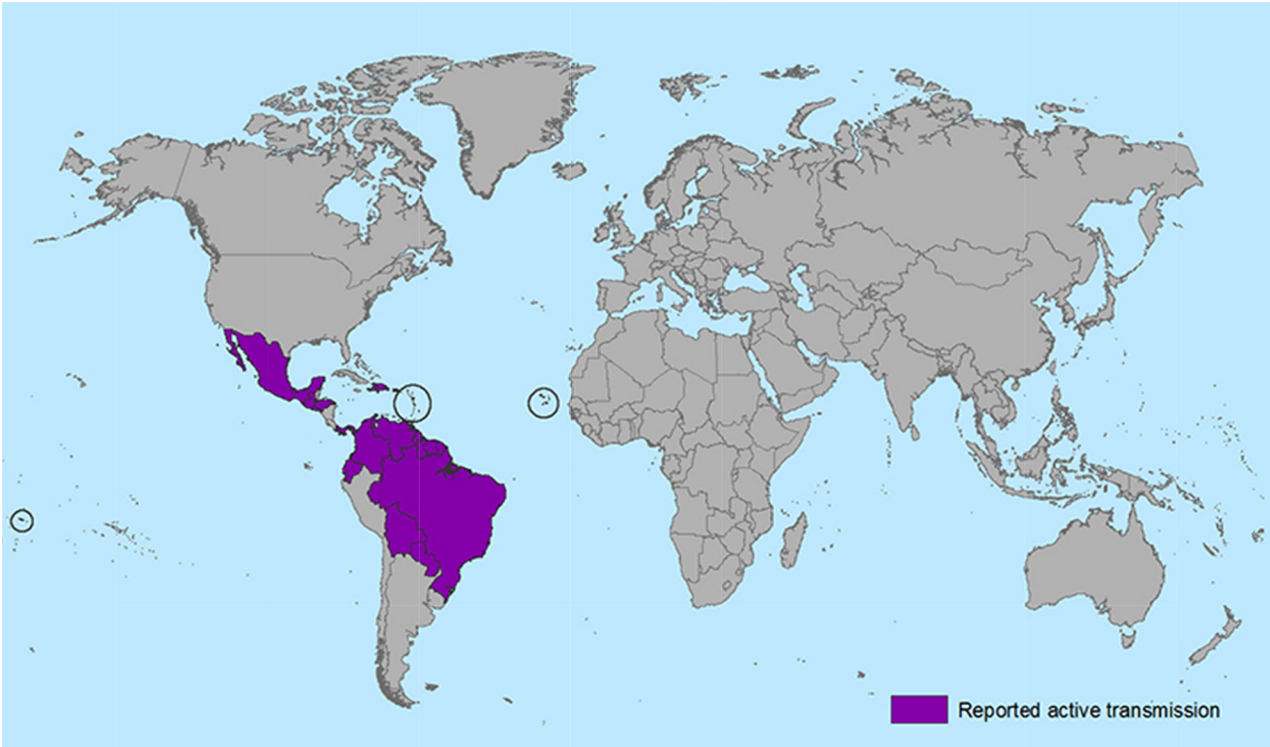

Fig. 2 – World map showing countries and territories with active Zika virus transmission.

case of occupationally acquired infection. The virus was however, isolated for the first time in 1968 from human population in studies undertaken in Nigeria.[10] Since then, ZIKV activity has been reported from many African countries i.e. Egypt, Sierra Leone, Gabon, Senegal, Tanzania, Uganda and Central African Republic as well as parts of Asia including Pakistan, Malaysia, Thailand, Vietnam, Indonesia and the Philippines[1] and the latest outbreak and confirmation of autochthonous transmission of Zika in Brazil, which led the World Health Organization (WHO) to declare it as a public health emergency.[11,12]

In 2007, an outbreak of ZIKV occurred on Yap Island in the Federated States of Micronesia. This marked the occurrence of Zika fever, for the first time, outside its known endemicity zone i.e. Africa and Asia. The mosquito – *Aedes hensilli* was implicated as the main vector of transmission as it was the predominant species in Yap during the outbreak. While the route of entry of ZIKV on Yap Island remains obscure, it is

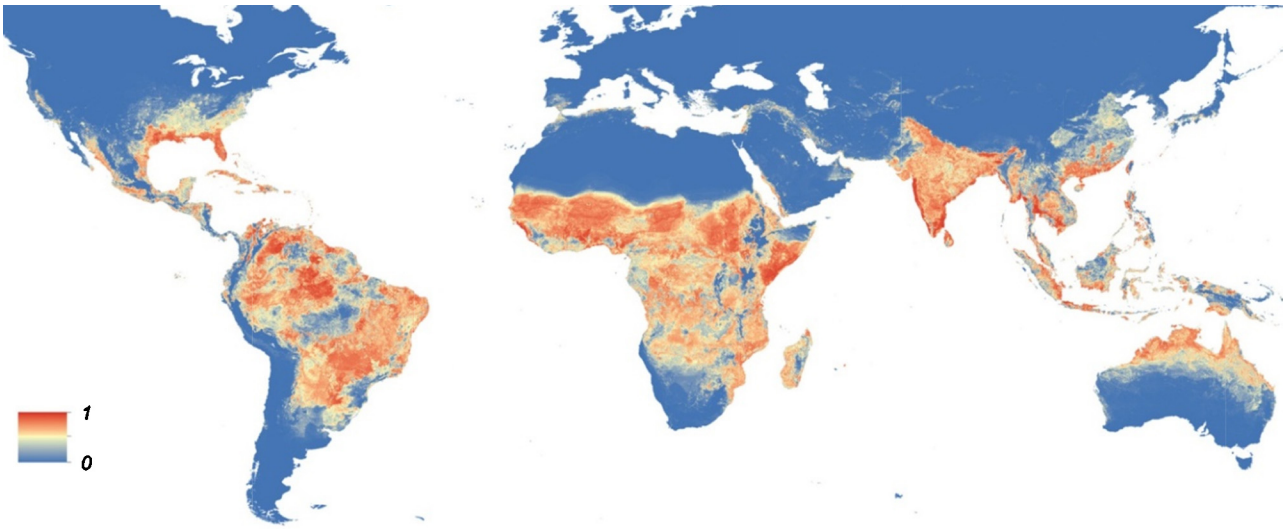

Fig. 3 – World map showing predicted distribution of Zika vector *Aedes aegypti*. *Note*: The map depicts the probability of occurrence (blue, none; red, highest occurrence).

hypothesised that entry of infected mosquitoes or an infected human may be the likely source.[13,4]

ZIKV re-emerged in French Polynesia and the reported outbreak from early October 2013 to March 2014, took a heavy toll with about 8700 suspected cases of Zika. In New Caledonia, 352 confirmed cases of ZIKV were reported from November 2013 up to March 2014. Amongst the reported cases, 244 were locally transmitted with 32 being imported from French Polynesia. The outbreak of Zika in Cook Islands from February to March 2014 reported 648 dengue-like illness cases, with 49 amongst these being laboratory confirmed as Zika cases. In Easter Island, as of March 2014 there have been 40 suspected cases and 1 confirmed case of ZIKV reported.[14–16] In March 2015, Brazil confirmed the circulation of ZIKV in the country following the identification of ZIKV in samples received from an outbreak at Camaçari, Bahia, Brazil.[11]

The 01 December 2015 publication of epidemiological alert of the Pan American Health Organization (PAHO) and the WHO reports an increasing number of cases of congenital anomalies (especially microcephaly), when mothers are infected with Zika in the first or second trimester of pregnancy or Guillain–Barré syndrome and other neurological and autoimmune syndromes in areas, where ZIKV is circulating suggestive of possible relation to the virus. The occurrence of these complications has enforced an alert from PAHO for surveillance of neurological syndrome and congenital malformities in areas, where ZIKV is circulating.[17] Similar surveillance should also be instituted in other countries, which are reporting Zika activity so that association of Zika infection and neurological syndrome can be conclusively evidenced.

Symptoms of ZIKV infection

Zika fever is very similar to dengue and is generally mild and self-limiting and lasts for 4–7 days, with an incubation period of 3–12 days. However, as Zika infection also causes rash like measles or dengue, these diseases need to be ruled out before suspecting Zika. Thus the diagnosis of Zika is primarily based on the presenting symptoms, history of travel and exclusion of diseases with similar symptoms i.e. measles, rubella and dengue.[1] It is estimated that only about one in five people carrying the virus actually develop symptoms of Zika fever.[18] The common symptoms of ZIKV infection are low-grade fever (between 37.8 °C and 38.5 °C), arthralgia of small joints of hands and feet with occasional swelling of joints, maculopapular rash, headache, retro-ocular headaches, conjunctivitis, myalgia and post-viral asthenia.

Other less common symptoms reported include anorexia, dizziness, constipation, abdominal pain, diarrhoea, mucous membrane ulcerations (aphthae) and pruritus. Unlike dengue, there is no evidence of Zika infection preferentially affecting pregnant women or children, however, there are few cases reported in the literature.[19]

Pathogenesis

Although vector mediated transmission of Zika fever is the most common mode of transmission, human-to-human transmission through other routes such as sexual and perinatal[20] and by blood transfusion[21] makes the containment of this new entity – Zika fever more challenging than dengue.[22–24]

The virus is hypothesized to first infect dendritic cells near the site of inoculation, and then gradually it spreads to the lymph nodes and finally into the bloodstream. Flavivirus generally replicates in the cytoplasm, however, ZIKV antigens have been detected in infected cell nuclei.

Diagnosis

Diagnosis of ZIKV infection is confirmed by viral genome detection by genomic amplification (RT-PCR) and viral isolation, whereas serological tests (IgG and IgM) are employed to detect specific antibody against ZIKV. The serological test samples are drawn in a similar manner as for dengue. The specific IgM antibodies test is positive in sera from day 5 post onset of fever. RT-PCR is the diagnostic method of choice for detection of ZIKV in acutely ill patients.[25] It is important to remember the well-documented fact that serological cross-reactions with closely related flavivirus especially dengue occurs, thus necessitating stringent clinical discrimination.[26] As of now rapid diagnostic kits are not available commercially, ready to use real-time PCR kit are available, however they require real-time thermocycler, utracentrifuges and other accessories, which are available only in well-established laboratories. In India, National Institute of Virology, Pune and National Centre for Disease Control, New Delhi are the nodal agencies identified for testing and outbreak investigation as per Ministry of Health and Family Welfare guidelines published on their website.[27] The RT-PCR test available with NIV has been standardised from published primers. India has valiantly stood up to the challenge and has brought out details of the diagnostic algorithm as well as other important aspects on prevention and has issued guidelines and instructions for stepping up *Aedes* surveillance.[27]

Treatment

As in cases of dengue, there are no specific treatments available. Only symptomatic treatment in the form of non-steroid anti-inflammatories and non-salicylic analgesics are administered. Presently, there is no established immuno/chemoprophylactic agents against these two *Aedes*-borne flavivirus infections (dengue and Zika), recent claims of a possible dengue vaccine notwithstanding.

Prevention

Till date no vaccine is available for Zika, although initiative to develop a vaccine has been taken by various Institutes notable amongst them being the National Institute of Allergy and Infectious Diseases, USA (NIAID). The NIAID is currently pursuing several vaccine approaches viz. a DNA-based vaccine that uses a strategy similar to an investigational flavivirus vaccine for West Nile Virus, live-attenuated investigational

Zika vaccine building on a similar vaccine approach for the closely-related dengue virus and Zika vaccine that uses a genetically engineered version of vesicular stomatitis virus – an animal virus that primarily affects cattle.[28] However, its important to first understand the immune response to the infection, before a vaccine is contemplated.

Efficient vector management remains the key to prevention and containment of ZIKV – carrying mosquitoes *A. aegypti* and *A. albopictus*, which are container breeders – artificial/natural. The commonly employed measures, including covering of all water containers, emptying of refrigerator defrost trays, air coolers, proper disposal of tyres and discarded containers, observing dry day once a week, releasing larvivorous fish in ornamental water tanks and vegetation management to deny shelter to adult mosquitoes seeking shade, are well established and effective if implemented energetically. The use of *Aedes* attracticide and insecticide combination product developed by Defence Research and Development Establishment, Gwalior would also additionally prove effective in the management of vector population.

Other interventions, namely barrier clothing (full sleeves shirts and trousers), screening of windows and doors, use of insect repellent, vaporizers and mats/coils/fast cards etc. during day (as *Aedes* is a day biter with peak biting time as dawn and dusk), use of mosquito nets or insecticide treated nets, while sleeping or resting during daytime and fogging in high-risk areas to kill mosquitoes, have also paid rich dividends in *Aedes* mosquito control. The Armed Forces personnel may also use treated uniforms (repellent/insecticide) to protect themselves besides topical application of repellent on the exposed areas of the body. It is thus important to interrupt man-mosquito-man transmission cycle to prevent further spread of the virus.

Reasons for spread of ZIKV

The spread of ZIKV from Africa and Asia to newer areas is disconcerting. The reasons for its trans-continental spread have been attributed to increased international travel, illegal cross border migration, inadequate housing, inefficient waste management and poor vector control activities. Further, impact of El Nino Southern Oscillations and La Nina conditions have been implicated in causing changes in household water storage practices and surface water pooling, leading to increased availability of breeding areas for the mosquito vectors.[17]

A recent study phylogenetically analysed the nucleotide sequence of ZIKV from 1947 onwards and found that the strain responsible for the Yap and Cambodian epidemic originated from SE Asia.[29] Genetic studies undertaken by Faye et al. have revealed that ZIKV has evolved into 3 distinct genotypes: West African (Nigerian cluster), East African (MR766 prototype cluster), and Asian. It has been postulated that the virus originated in East Africa and then spread into both West Africa and Asia ≈50–100 years ago.[2] Further studies on phylogeny indicates that Asian genotype viruses have been gradually evolving and spreading geographically throughout Asia and the Pacific Islands since at least 1966; the tree suggests that the Malaysia 1966 ZIKV is representative of an ancestral genotype.[30] Investigation of isolates of ZIKV from 1968 to 2002 in Africa and six other countries, revealed that these were two

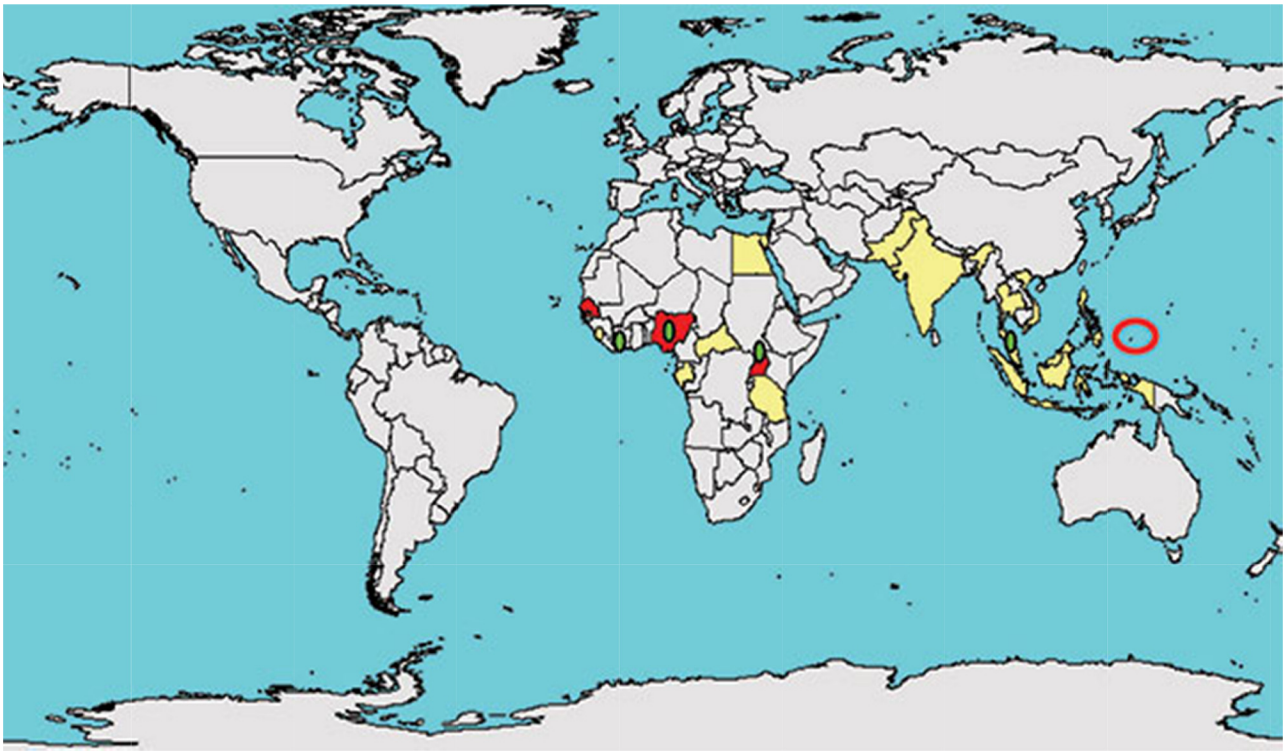

Fig. 4 – Approximate known distribution of Zika virus, 1947–2007. *Note*: Red circle represents Yap Island. Yellow indicates human serologic evidence; red indicates virus isolated from humans; green represents mosquito isolates.

independent introductions of ZIKV strains in the 20th century and that these viral lineages were not strictly restricted to mosquito vectors and that ZIKV has possibly undergone recombination in nature with loss of N154 glycosylation in *Aedes dalzeili* vector, which is a significant finding.[2]

Zika – the missing entity in India

The serological evidence of presence of ZIKV in India has been documented and cited.[1] The Approximate known distribution of ZIKV since 1947–2007 (Fig. 4) clearly marks the presence of serological evidence of Zika activity in India, although these claims are unsubstantiated by any independent publication from India.[1] It is perplexing and difficult to accept the claim of serological evidence of Zika in India, as till date no serological tests for the same are available in India, although the possibility of its existence cannot be ruled out due to the widespread existence of the vector, favourable environmental conditions and practices followed in our country.

Ironically, Zika fever does not figure in the list of differential diagnosis while dealing with cases of fever with rash during dengue/chikungunya outbreaks. Often, cases present with symptoms of dengue/chikungunya, but serological tests remain negative for these infections. Could these cases be of Zika fever? Are we missing something vital in our investigation? An emerging arboviral infection is in our midst, a high index of suspicion for Zika will be necessary in unravelling the epidemiology of this lesser known, yet emerging entity – Zika fever.

There is a need to undertake detailed study to determine the association of the infection to development of neurological complications and congenital deformities and the related pathways of pathogenesis. Little is known about the reservoirs of Zika, studies should be undertaken to unravel the existence of any other insect/animal reservoirs of this emerging pathogen. Is there any association of ZIKV lineages to virulence, neurological complications or potential to cause an outbreak? Does prior infection with dengue reduce the severity of infection with Zika? What is the fate with concomitant dengue and Zika infection? These vital questions need to be addressed a priori in order to understand this missing information on Zika, which would greatly assist in formulating effective preventive algorithms (management and vaccine development) for Zika prevention and control.

Conclusion

The clinical presentations and complications of Zika are not well researched as of now. There is a need first to establish the link of Zika with the reported microcephaly and neurological complications. However, given the varied modes of transmission, vector borne and non-vector borne through body fluids viz. sexual, perinatal, blood transfusion, and its potential to spread to any *Aedes* infested area, control of this virus may be more difficult than other *Aedes* borne diseases. ZIKV has traversed outside its endemic zone – Africa and Asia, to hitherto naïve areas in the last few years, especially since 2007 and its reported arrival in China sounds a warning bell (although no autochthonous transmission has been reported) and thus India needs to keep up the surveillance in order to prevent an outbreak and related consequences of the disease. It is thus the need of the hour to maintain a close vigil on Zika, an emerging vector-borne disease, which has surpassed all other vector borne diseases in its magnitude of impact on human health.

Conflicts of interest

The authors have none to declare.

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