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Zika: As an emergent epidemic

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ABSTRACT

Zika virus is a new global threat for 2016 that has been swept to almost all Americas and is now posing serious threats to the entire globe. This deadly virus is playing havoc to unborn lives because of its reported association with upsurge of fetal deformation called microcephaly and neuropathic disorders including Guillain-Barré syndrome. Till today, there is no vaccine prospect, antiviral therapy or licensed medical countermeasures to curb the teratogenic outcomes of this destructive viral infection. Diagnosis, treatment, chronicity and pathogenesis are still vague and unsettled. Therefore, this review article addresses all the aspects related to this disease to mitigate the explosive rise in Zika virus infection.

1. Introduction and epidemiology

Zika virus (ZIKV) is a mosquito-borne RNA virus infection related to yellow fever, dengue and West Nile virus. Zika virus is generally transmitted by bite of an infected *Aedes* species of mosquito. Zika virus poses serious threats to pregnant women [1]. An increased risk of Guillain-Barré syndrome and several other congenital neurological abnormalities associated with Zika virus have also been reported [2]. Zika virus infection is clinically similar to dengue fever and cause acute febrile illness, arthralgia, fever, myalgia, rash, headache and conjunctivitis [3,4]. The diagnosis of Zika virus infection is verified by detection of viral genome using RT-PCR genomic amplification and viral isolation [5].

According to phylogenetic studies, Zika virus emerged in East Africa during early 20th century and later on spread to Southeast Asia. The virus was named after the forest in Uganda from where it was first isolated in rhesus monkey in 1947 [5] and identified in 1952 in human beings. Another study confirmed seven patients with serologic evidence of Zika virus infection in Indonesia [6]. In 2007 a small-scale outbreak was documented in Yap, Federated States of Micronesia [7] followed by a larger outbreak in French Polynesia during 2013 with 28 000 cases reported in the first 4 months [8–10]. Since May 2015, Zika virus infection has been escalated to 1.5 million cases in Northeastern Brazil and now the virus has been transmitted to South and Central America and Caribbean [10–12]. Approximately 440 000–1 300 000 cases of Zika virus in Brazil alone were reported during an outbreak of 2015 [2]. Pan American Health Organization confirmed copious cases of Zika virus infection in French Guiana, Venezuela, Paraguay, Guatemala, Mexico, Honduras, El Salvador, Colombia, Panama and Suriname. Besides America, Atlantic island of Cape Verde reported its first Zika outbreak in October 2015 with 4 744 suspected cases by 6 December 2015 [13].

Currently, more than 4 500 microcephaly cases have been identified [14]. In December 2015, Netherlands confirmed the first domestic case of microcephaly (abnormal small size of head) in a neonate whose mother was back from Surinam followed by 20 more cases all imported from Surinam [15]. The average number of live births in Brazil is around 1 242 975 (2009–2013 data) but a considerable increase in microcephaly was reported in Pernambuco state, Northeast Brazil. Since beginning of 2016, 46 deaths in 20 states and the Federal District has been surveyed and this rate is significantly higher than 2010–2014, during which an average of 163 (standard deviation 16.9) cases of microcephaly were reported nationwide (26 states and 1 Federal District) per year [16].

A research based study applied complement-fixation test reactions to 372 samples of serum (43 from humans, 172 from

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domestic animals, 157 from rodents) collected from Pakistan containing eight viruses belonging to family Togavirida followed by identification of antibodies targeted towards each tested virus. The most elevated over-all prevalence rates were: 7.8% West Nile virus, Japanese 3.2% encephalitis virus and 2.4% Zika virus, followed by 1.6%–1.3% Sindbis virus, Chikungunya virus, Uganda virus and Royal Farm viruses [7,17].

2. Virus structure and genome organization

Zika virus is positive-sense single-stranded RNA virus which belongs to the family Flaviviridae consisting of 11 000 bases approximately. The genome consists of 5′ and 3′ untranslated regions flanking a single open reading frame which encodes only one polyprotein that cleaves into three structural proteins, capsid protein, premembrane/membrane protein, envelope protein, along with seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5) [18].

A previously done genetic study based on nucleotide sequences derived from the *NS5* gene unfolded three Zika virus lineages: East African, West African and Asian [19,20].

Till today, scientists were familiar with little knowledge regarding the genetic relationships between strains of Zika virus collected from Africa and Asia. Furthermore, the geographical origins of the strains behind human related Zika virus outbreak on Yap Island in Federated States of Micronesia, and that of Cambodia were undetermined. But genomic comparison revealed several sub-clades exhibiting two major geographically distinct lineages of Zika virus *i.e.*, Asian and African. During the past 50 years, the virus has spread in entire Southeast Asia later on, it invaded Yap Island causing human epidemic in 2007, and proved the etiology of a pediatric case of ZIKV infection in Cambodia during 2010 [20].

3. Potential transmission of Zika virus

Zika virus has the potential to attack any area where the *Aedes* mosquito vector is present [5,21]. Zika virus has been isolated from *Aedes furcifer*, *Aedes africanus*, *Aedes apicoargenteus*, *Aedes luteocephalus*, *Aedes vittatus*, and *Aedes aegypti* (*Ae. aegypti*) mosquitoes [22–25]. It has been reported that *Aedes hensilii* was the most frequently found mosquito species present on Yap island during the Zika virus epidemic of 2007 [7]. Zika virus infection was transmitted by *Ae. aegypti* during the recent outbreak of Brazil [2]. Epidemiological studies in Uganda had suggested that *Aedes africanus* was a vector of Zika virus [26,27].

Studies indicate that the usual mosquito vectors in Asia are *Ae. aegypti* and *Aedes albopictus (Ae. albopictus)* [20]. *Ae. albopictus* is considered as invasive mosquito because of its transportation to other continents through commerce [28]. In Brazil, *Ae. albopictus* was present in 59% of municipalities in 2014 [29] and spread to 24 of 27 states [30]. This vector is able to adapt to both urban and sylvatic habitats, including bromeliads [31] perforated bamboo internodes [32], and tree holes (also with *Ae. aegypti* and *Aedes vittatus*) [33], and is a suspected link for yellow fever virus between preserved and modified environments in the south and southeast regions of Brazil [34]. *Ae. albopictus* species feed on wide range of hosts because it is endophagic and exophagic, as compared to *Ae. aegypti* that usually feeds on humans.

Transmission of Zika virus by blood transfusion has been confirmed during the recent outbreak of Zika virus that invaded 7 Brazilian States [35].

In 2013, French Polynesia faced the largest reported outbreak of Zika virus infection. To escape the Zika virus transmission by blood transfusion, specific nucleic acid testing of blood donors was performed which indicated that 3% (42 out of 1 505) blood donors were positive for Zika virus by PCR from November, 2013 to February, 2014. Therefore, blood safety authorities were recommended to get alert in order curb the risk of Zika virus infection through blood transfusion [36]. During a Zika virus outbreak in French Polynesia, Zika virus was identified and isolated from the semen of a patient in Tahiti when he underwent treatment for hematospermia. This observation supports the fact that Zika virus can also be transmitted by sexual intercourse [4]. In 2008, another evidence of Zika virus infection by sexual intercourse had also been reported in a patient in southeastern Senegal [37]. Zika virus can attack pregnant ladies in all three trimesters [38]. Vertical, maternal fetal transmission or perinatal transmission of Zika virus has also been reported. The possible routes of transmission were breastfeeding, close contact between mother and newborn and transplacental during delivery [9] (Figure 1).

4. Pathogenesis of ZIKV

Very little information is known about the pathogenesis of Zika virus but flaviviruses transmitted by mosquitos replicate in dendritic cells near the site of inoculation and later on lead to blood stream and lymph nodes [39]. It is generally considered that *flaviviral* replication occurs in cellular cytoplasm but another study demonstrated that Zika virus antigens might be found in infected cell nuclei [40]. Till today, infectious Zika virus has identified in blood of humans as early as the day of onset of disease but nucleic acid of virus was confirmed as late as 11 d after onset of illness [41]. Human skin is an important portal for the entry of Zika virus and act as major contributor in the induction of antiviral immune responses. It has been reported that immature dendritic cells, epidermal keratinocytes and dermal fibroblasts were responsible for Zika virus outbreak occurred in French Polynesia. There are number of adhesion factors like DC-SIGN (known as CD209), Tyro3, AXL and, to a lesser extent, TIM-1 which support Zika virus entry, with a dominant role attributed to TAM receptor AXL. The use of neutralizing antibody and specific RNA silencing confirmed Zika virus permissiveness of human skin fibroblasts. Zika virus triggered the transcription of Toll-like receptor 3, retinoic acid-inducible gene 1, Melanoma Differentiation-Associated protein and various interferon stimulated genes like MX1, OAS2, and ISG15 characterized by strongly boosted gene expression of beta interferon. Zika virus was sensitive to the antiviral reactions of both type I and type II interferons. The infection of skin fibroblasts lead to production of autophagosomes that were linked with increased replication of virus influenced by a chemical inducer of autophagy *i.e.*, Torin 1, and the specific autophagy inhibitor 3-methyladenine [42].

5. Clinical manifestation of ZIKV

It has been documented that majority (about 73%) of Zika virus infections are asymptomatic [7]. In case of symptomatic

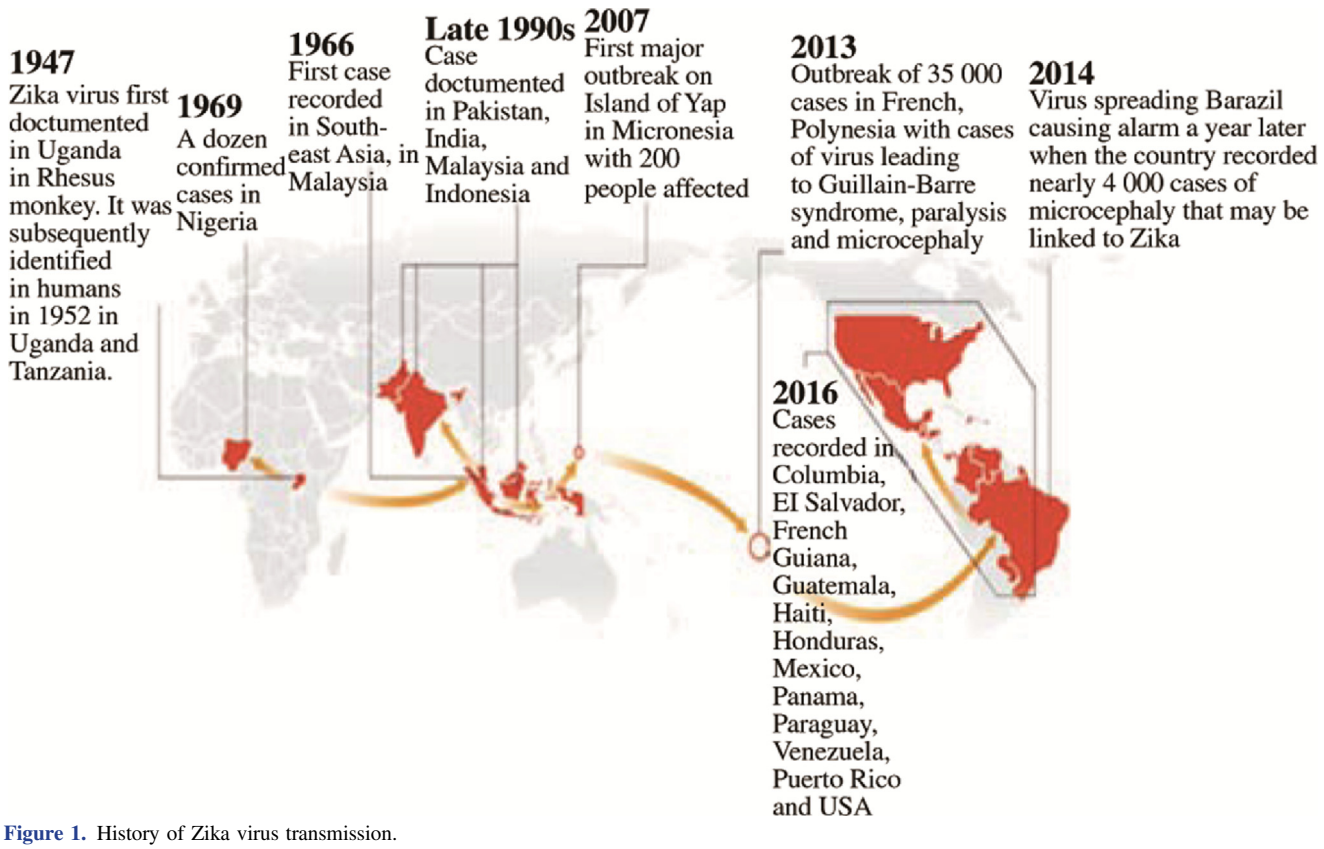

Figure 1. History of Zika virus transmission.

condition, the clinical manifestation is a 'dengue-like syndrome' and is difficult to distinguish from dengue and chikungunya infections. Formation of maculopapular skin rash on the face or trunk that may get more diffused, headaches, low-grade fever, conjunctivitis myalgia and arthralgias are most common symptoms of Zika virus infection [37,43–45]. A study demonstrated that commonly reported symptoms during the most recent outbreak of Puerto Rico that occurred from November 23, 2015 to January 28, 2016, included 77% rash, 77% myalgia, 73% arthralgia and fever 73% [46]. Seven Indonesian patients who were Zika virus positive experienced high fever, diarrhea, stomach aches, malaise, vomiting, dizziness, chills, anorexia, leg pain, lymphadenopathy and hypotension [6]. In many cases abnormal development of small size head or microcephaly has been observed, with hydrocephalus, agyria and multifocal dystrophic calcifications in the subcortical white matter and cortex, along with mild focal inflammation and cortical displacement [47]. According to a research based study, Zika virus epidemics in the Americas and Brazil has unveiled the deleterious effects of virus on fetuses like development of hydrops fetalis, an atypical condition associated with an abnormal accumulation of fluid in the fetus, as well as serious central nervous system defects like hydranencephaly and microcephaly. After the death of fetus, Zika virus RNA was identified in amniotic fluid and tissues of central nervous system. Additionally, this case study provides weighty evidence that besides microcephaly, there may be an association between Zika virus disease and hydrops fetalis alongside fetal demise in some cases [14,48].

Apart from microcephaly, other possible outcomes in neonates positive for Zika virus include ventricular enlargement, brain atrophy, and intracranial calcifications [49]. Several cases of suspected congenital Zika virus infection have been associated with scalp rugae, ocular defects and joint contractures [9,49–51]. Besides, brain abnormalities in newborns, Zika virus RNA has been confirmed in the pathologic tissue specimens of fetal deaths, however, there is no sound evidence which proves that Zika virus causes the fetal demise [52]. Several studies have linked Zika virus with Guillain-Barre syndrome and in early 2014 the first recorded case of Zika virus infection complicated by Guillain-Barre syndrome was identified in French Polynesia [3]. Since then, French Polynesian health surveyors started making efforts to report Guillain-Barre syndrome cases that were considered to be secondary to the primary Zika virus infection [45].

6. Zika virus diagnostics

The detection of Zika virus infection is based on the identification of viral RNA in blood samples. RT-PCR along with confirmation and isolation of virus in the blood samples collected during the early five days of emergence of infection are the reference techniques. The 'pan flavivirus' amplification technique together with sequencing may also be used as an alternative or a substitute [18,53]. The viremic period of humans could be brief, from the third to the fifth day after the onset of Zika virus infection symptoms. Viruria could stay for a longer time than viremia and the real time RT-PCR detection of viral RNA in urine could be a substitute if genetic material in the serum is no longer present [21,54]. In 2014, a case study demonstrated that Zika virus RNA was confirmed by RT-PCR in the urine of Japanese traveller returned from Thailand whereas serological tests showed cross reactivity of IgM against the dengue virus indicating that Zika could be misdiagnosed [55]. Pregnant women infected with Zika virus or exposed to it, must

visit hospital every 4th week for fetal ultrasound scan (USS) examination. This includes asymptomatic patients having symptoms outside the testing window and those who are Zika virus negative by RT-PCR. Microcephaly is an abnormal condition with head circumference less than the 2.5th centile for gestational age and USS has been proved a sensitive screening test for microcephaly apace with other intracranial abnormalities like ventriculomegaly and calcification. Fetal brain MRI has the ability to detect abnormalities not observed on USS. Microcephaly association with Zika virus is just a tip of an iceberg because microcephaly and other intracranial abnormalities may also be caused by a number of disorders unrelated to Zika virus [56]. A case study demonstrated that RT-PCR assay confirmed Zika virus in the brain tissues of fetus that was compatible with observations of electron microscope and the whole genome of Zika virus was recovered from the fetal brain. Calcifications in the placenta and fetal brain were noticed when ultrasonography was performed at 29 weeks of gestation [47].

Generally, for the diagnosis of flavivirus infections acute-phase serum sample must be collected soon after the onset of ailment followed by collection of second sample 2–3 weeks after the first. Therefore, Zika virus is usually diagnosed by PCR test done on acute phase serum samples that detect viral RNA whereas, other tests facilitate the detection of specific antibody against Zika virus in serum. Arboviral Diagnostic and Reference Laboratory of the Centers for disease Control and Prevention (Atlanta, GA, USA) has developed ELISA for detection of immunoglobulin IgM against Zika virus. Samples collected from Yap Island, Federated States of Micronesia exhibited that cross-reactivity in sera from convalescent phase patients occurred more commonly among patients who had previously been exposed to flavivirus infections than among those who were suffering from primary Zika virus infections.

A study elucidated that IgM was detectable during the early three days of onset of infection in some patients but one person previously exposed to flavivirus infection had not developed IgM even at fifth day but had it by eighth day. Neutralizing antibody was developed during the early five days of onset of disease. Immunoassays have ameliorated specificity than plaque reduction neutralization assay; however, the possibility of cross-reactivity in secondary flavivirus infections is always there. Polymerase Chain Reaction tests can be performed on samples collected even at the first day of onset of illness but viral RNA may also be confirmed as late as 11 d after the onset of disease [7,19,21]. A female patient returned from Thailand visited a local emergency department with mild symptoms like fever and papular rash and underwent a test for malaria, measles and dengue. When the conserved region of the non-structural protein 5 gene of the genus *Flavivirus* was amplified, the yielded PCR product exhibited 99% sequence match with Zika virus. Nasopharyngeal swab and urine sample collected for measles research were also positive for Zika virus by RT-PCR. Thereupon, Zika virus was isolated in cell culture, using urine sample. This case study revealed several novel laboratory and clinical findings along with the first and second documentation of ZIKV in Canada and Thailand, respectively [57].

7. Zika virus association with Guillain-Barré syndrome and microcephaly

The current epidemic of microcephaly supported the hypothesis that ZIKV was the most possible cause of this congenital syndrome. A striking increase in the micocephaly cases in newborns has been reported in Brazil in November 2015 [11]. Till day, >4 500 microcephaly cases have been confirmed (Brazil Ministry of Health, 2016). There are number of microcephaly cases identified in 9 Brazilian states so far. By 28 November 2015, 646 cases of microcephaly had been diagnosed in Pernambuco state alone [49].

Death of newborns have been reported because of pathological lesions or ultrasound abnormalities which were limited to the central nervous system [48,49]. Another case study reported in the February 2016 presented symptoms of hydranencephaly that developed hydrops fetalis and death of fetus because of congenital Zika virus infection [14]. In 2015, about 4 000 suspected cases of microcephaly has been reported in Brazil, depicting a 20-increase in prevalence (from 2010 to 2014) [58]. Virus has been detected in the brains of neonates and in the placenta and amniotic fluid of mothers but according to some studies causative link between microcephaly and Zika virus is yet to be established. Whereas, on January 15, 2016, Hawaii documented the first case of microcephaly in a neonate whose mother was just back from Brazil. During next few days, several other cases of microcephaly were reported in Florida, Illinois and Texas after international travel [59]. From October, 2013 to April, 2014, French Polynesia faced the largest Zika virus outbreak and an increased number of cases of Guillain-Barré syndrome were reported during the same period, thus supporting a possible link of Guillain-Barré syndrome with Zika virus. About 42 out of 32 000 Zika virus positive patients were diagnosed with Guillain-Barré syndrome at the Centre Hospitalier de Polynésie Française in Tahiti. The study also revealed that about 88% of the patients with Guillain-Barré syndrome were positive for Zika virus [5,60]. Twenty fold increase in incidence of Guillain-Barré syndrome has been reported [61]. A very recent study has provided sound evidence that proved Zika virus infection as cause of Guillain-Barré syndrome [62,63] (Figure 2).

8. Prevention and treatment

Pregnant women should stay out of travelling to areas which are exposed to Zika virus outbreaks and must visit travel health specialist and incase, travelling is unavoidable because of any circumstances they must consult travel health specialist and should regularly apply mosquito repellants [56]. Zika virus has the ability to invade newer areas where the *Aedes* mosquito vector is present and may become the risk for entire globe therefore, Zika virus control practice like mosquito vector eradication is necessary and must follow standard mosquito protection advice [5]. United Kingdom has advised a guideline that any pregnant woman back from Zika-affected country must pay a visit to obstetrician or general physician. Travellers must avoid getting pregnant in an affected area and for 28 d after returning to United Kingdom males are recommended to use condoms [64]. Pregnant women positive for Zika virus RT-PCR need to consider serial fetal ultrasounds every 3–4 weeks [52] and must visit a fetal medicine service for follow up including amniocentesis from 15 weeks to identify Zika virus alongside other neonatal infection in the amniotic fluid [56]. To treat the symptoms of Zika virus, Centre for Disease Prevention and Control has issued some guidelines like taking plenty of rest, increased fluid intake to prevent dehydration, fever and pain relief medicines, avoid taking aspirin and non-steroidal anti-

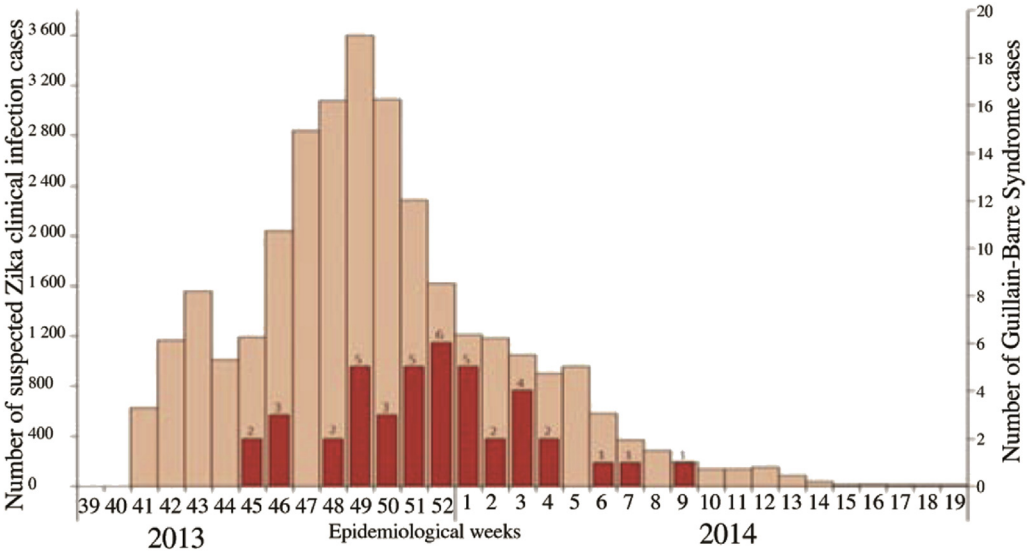

Figure 2. Graph representing suspected cases of Zika virus infections and Guillain-Barre Syndrome [63].

inflammatory drugs [65]. To prevent from Zika virus, people must wear long-sleeved shirts and pants and must ensure the use of doors and windows with intact screens alongside EPA (environmental protection agencies) registered insect repellants and permethrin-treated clothing [46]. The transmission of ZIKV through blood transfusion was reported in epidemic that hit French Polynesia and the inactivation of pathogen present in blood is the most powerful strategy to mitigate transfusion-transmitted ZIKV infection. A study demonstrated the efficiency of ZIKV inactivation by ultraviolet A illumination and amotosalen. Both amotosalen and ultraviolet a light combined thus, lead to an inactivation of Zika virus in fresh-frozen plasma. This process of inactivation control plasma transfusion-transmitted Zika virus infections [66]. There is no antiviral therapy developed yet and care is a supportive therapy to resolve symptoms [58]. Effective and safe Zika virus vaccine will probably be developed in next 3–10 years even with speedy research. In late 2015, the National Institutes of Health started an initiative for development of Zika virus, and Brazil has accelerated vaccine development. Insecticide spraying of mosquito habitats is an effective strategy to avoid Zika [59]. Eliminate water reservoirs and containers because stagnant water acts as breeding site for Aedes mosquito that is a vector for Zika virus [67]. Hydroxychloroquine which is an anti-malarial drug acts as inhibitor of autophagy. *In vitro* testing elucidated the control of dengue virus infection through accumulation and induction of reactive oxygen species along with mitochondrial antiviral signaling protein. Amodiaquine another autophagy inhibitor and hydroxychloroquine have been proved safe even in pregnancy. Amodiaquine acts as inhibitor of Ebola virus. It has been reported that in preliminary cell culture studies, amodiaquine has been confirmed as an agent that curbs the Zika virus pathogenicity at similar concentrations to those already recorded for Ebola virus (unpublished results of Drs. V Soloveva and S Bavari) [68].

9. Future prospects

Medical countermeasure strategies like neutralizing antibody preparations, development and testing of antiviral drugs along with medicines aiming to impede Fc receptor interactions must be evaluated during the first and second trimesters of pregnancy because pregnant women are at a greater risk of acquiring Zika virus infection [69–71]. Institute Butantan which is Brazilian biomedical research center, is working on anticipated Zika vaccine projected for accomplishment in next three to five years. Previous researches and experience suggests that this is the most rational approach and optimistic plan for vaccine development and licensure, which may take more than 20 years of clinical development alongside testing [68,72].

Production of prophylactic vaccine targeting Zika virus-induced disease will need significant time and heedful safety evaluation and efficacy for the population. This is true for vaccine designed to target virus responsible for teratogenic effects and autoimmune disease (GB Syndrome) of neurologic disorders caused due to Zika virus infection [73,74].

There is no vaccine currently available, therefore development of therapeutics and prophylactics must be probed and studied thoroughly. Development of targeted immunotherapeutic strategies may inhibit Zika infection including GB Syndrome, and ADE (Antibody Dependent Enhancement) that has been demonstrated with Zika virus. Monoclonal antibody based therapies for arbovirus infections is a promising approach that must be evaluated carefully given the potential challenge of Antibody Dependent Enhancement. Therapeutic and prophylactic use of cross-reactive neutralizing monoclonal antibodies for flavivirus infections has been observed potent and efficacious in animal models. The production of *De novo* antibodies is possible, and these antibodies attack Zika-specific epitopes. Appropriately engineered and de-risked monoclonal antibodies possess the ability to mitigate Zika virus infection, but monoclonal antibody product should be highly potent if it is to yield sufficient number of doses at reasonable cost.

10. Conclusions

Because of current explosive rise in Zika virus, there is a dire need to carry out research based study to comprehend this life-threatening disease and develop medical countermeasures. Zika virus is a complex challenge than Ebola as it may infect more lives especially unborn fetus and is invading the entire globe very rapidly. Currently, there is not enough epidemiological and

clinical data to fully analyze how much of a threat Zika poses. Garnering this information must be the priority. Gaining ground is the most rational approach that it would be a relatively affordable insurance policy to develop experimental treatments especially vaccines, against an array of potential threats. The short-term actions must be to combat the vector directing to reduce the vector density and long-term action is the development of anti-viral therapy or vaccine to alleviate the blooming risk of Zika virus.

Conflict of interest statement

We declare that we have no conflict of interest.

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