



Zika Virus Infection, Basic and Clinical Aspects: A Review Article

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Abstract

Background: Zika virus infection has recently attracted the attention of medical community. While clinical manifestations of the infection in adult cases are not severe and disease is not associated with high mortality rates, Zika virus infection can have an impact on fetal development and lead to severe neurodevelopmental abnormalities.

Methods: To gain insight into different aspects of Zika virus infection, a comprehensive literature review was performed. With regard to epidemiology and geographical distribution of Zika virus infection, relevant information was extracted from CDC and WHO websites.

Results: In this review, we discuss different basic and clinical aspects of Zika virus infection including virology, epidemiology and pathogenesis of disease. Laboratory methods required for the diagnosis of disease together with ethical issues associated with Zika virus infection will also be discussed in detail.

Conclusion: Herein, we have tried to provide a multi-faceted view of Zika virus infection, with greater emphasis on disease status in Eastern Mediterranean Region.

Keywords: Zika virus; Flaviviridae; Neurological infections; Fetal development; Microcephaly

Introduction

Flaviviruses are responsible for several important human diseases including Dengue, West Nile and Yellow fevers, Japanese encephalitis, Tick-borne

encephalitis, and Zika fever. The major route of transmission for most Flaviviruses is through arthropod vectors and central nervous system inju-

ry and hemorrhagic fevers represent major clinical outcomes. In recent years, Zika virus infections have attracted the attention of international medical community, chiefly because of their role in causing microcephaly and other neurodevelopmental abnormalities which occur as a consequence of maternal infections (1).

Zika virus was first isolated in 1947 in the Zika forest in Uganda from a rhesus macaque (2). Serological studies performed on human serum samples in Uganda later showed the presence of neutralizing antibodies against the virus, providing the first evidence that the virus can infect humans (3). Later studies revealed the presence of infection in other regions of Africa as well as Asian countries (4). Until 2007 Zika virus infections were mostly considered an infection of limited geographical distribution; however, later outbreaks of infection in Pacific islands and South America, associated with reports that the virus might cause nervous system abnormalities in newborns attracted the attention of a wide range of people in the scientific community. The situation reached its peak in 2015 when a striking increase occurred in reports of Zika virus infections in Brazil (5). The outbreak then spread to other countries in South, Central, and North America. The infections were associated with a significant increase in the number of microcephaly and Guillain–Barré syndrome cases in the infected regions, and this led to the declaration of a Public Health Emergency of International Concern (PHEIC) by the WHO in early 2016 (6-8). Since then, a strong international effort has been started to investigate epidemiological, molecular, pathogenic and clinical aspects of Zika virus infections.

In this review, we have discussed the current knowledge about various aspects of Zika virus infection and recent developments including antiviral treatments for this infection. The first four sections of this review cover virology, epidemiology, pathogenesis, and laboratory diagnosis of Zika virus infection. While Zika virus infection is a global concern, in the epidemiology section we have put some more emphasis on findings from Eastern Mediterranean Region (EMRO) countries. Considering the ethical considerations relat-

ed to feto-maternal and neurodevelopmental dimensions of the Zika virus infection, the final section of this review provides a detailed account of ethical aspects of Zika virus infection (Fig. 1).

Virology of the Zika virus

Zika virus is a new emerging mosquito-borne virus belonging to the *Flaviviridae* family of viruses (9). This family is comprised of 4 genera: Flavivirus, Hepacivirus, Pegivirus, and Pestivirus (10). Zika virus belongs to the Flavivirus genus, which antigenically and phylogenetically is related to the Spondweni virus (9, 11). Many important human pathogens are included in this genus, for instance, Dengue, West Nile, Yellow fever, tick-borne encephalitis, Japanese encephalitis, Murray Valley encephalitis and St. Louis encephalitis viruses. These viruses are associated with a range of infections from asymptomatic or self-limiting febrile infections to some fatal diseases such as hemorrhage, shock, meningitis, and encephalitis (12).

In *Flaviviridae* family, all members have enveloped viruses with a single-stranded RNA genome of positive polarity (10) which contained one open reading frame (ORF) with two flanking noncoding regions (at 5' and 3' end) (13). The genomes are 5' capped without a 3' poly (A) tail.

A polyprotein is coded by the ORF then processed into three structural proteins (the envelope (E), the capsid (C) and the precursor of the membrane (prM)) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (1, 12). The ssRNA is held within an icosahedral capsid shaped from 12-kDa protein blocks; the nucleocapsid is surrounded by a host-derived membrane contained two viral glycoproteins (14).

Similar replication strategies are employed by the members of *Flaviviridae* family despite significant differences in tissue tropism, transmission, and pathogenesis (10).

Recent developments in Zika antiviral treatments

At the present time, no effective antiviral treatments are available for Zika virus infection (15).

However, considering rapid geographic expansion of Zika virus, its severe neurological complications and devastating effects on the fetus, investigations are underway to find safe and potent drugs and therapies. These approaches range from repurposing earlier approved drugs to the

screening and testing of in silico designed drugs (16). The antiviral candidates comprise agents targeting cellular components in addition to viral ones (15). All of the steps of Zika virus life cycle in the host cell from entry to release can be targeted by antiviral agents.

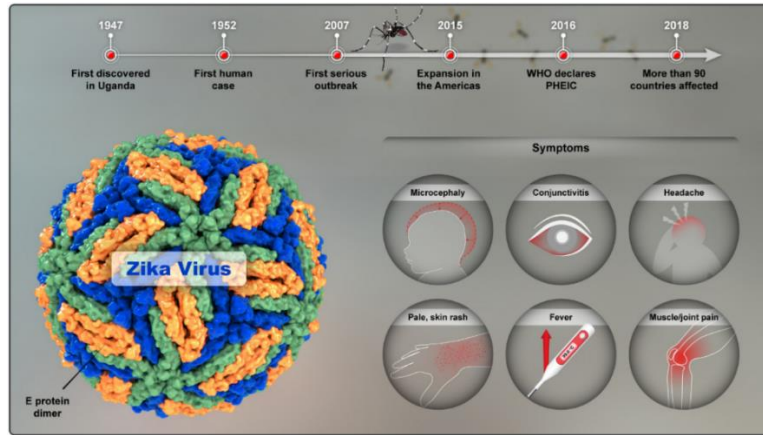


Fig. 1: Zika virus infection at a glance (Original)

In order to block the viral entry into the cell, various strategies have been used. On one hand, blocking the entry by receptor binding agents such as nanchangmycin (previously used as antibacterial and insecticidal agent), ZINC33683341 and ZINC49605556 (both in silico designed) has been proposed. The other approaches include the inhibition of endosomal fusion using compounds that reduce the acidity of endolysosomal vesicles like chloroquine (an anti-malaria drug) and disrupting the electrostatic interaction between cell and virus membrane using squalamine (a cationic chemical) (16).

RdRP activity of NS5 is an outstanding target for antiviral drugs. Therefore, several nucleoside analogues are investigated for their ability to inhibit Zika virus replication. Despite demonstrated efficacy of these drugs in cell cultures and animal models, some of them were unsuccessful in the clinical trials. Sofosbuvir (a nucleotide analog inhibitor) is a RdRP inhibitor approved by FDA for chronic HCV treatment. It also inhibits Zika virus replication (15-17). Zika virus N2B-NS3 protease and NS3 helicase, which play essential roles in virus replication, constitute potential tar-

gets for antiviral drugs (15). As the Zika virus helicase is similar to those of the other members of *Flaviviridae*, antiviral agents targeted their helicase could also be used for the Zika virus (18). Likewise, berberine previously used against dengue virus showed a high binding affinity to the Zika virus NS3 protease (19) as well.

Despite the many studies done on Zika antiviral treatments, so far no FDA approved category A drug has been found safe to use in mothers and fetuses (16).

Epidemiology of Zika virus infection

Before the first large outbreak of Zika virus infection on Yap Island, Federated States of Micronesia (20), only sporadic cases and serological evidence of Zika virus were reported in western and central Africa and south-east Asia (21, 22), but in 2007 Zika Virus emerged as an important human pathogen.

An increased incidence of cases of the Guillain-Barré syndrome (GBS) was reported after a larger epidemic of Zika virus infection in French Polynesia in 2013 (23). In 2014, autochthonous transmission of Zika virus infection occurred in Easter Island (Chile) from Feb until Jul (24).

In early 2015, several cases presenting a “dengue-like syndrome” investigated in Brazil (a non-Dengue virus and non-Chikungunya virus infection) and Zika virus was detected by reverse transcription polymerase chain reaction (RT-PCR) assay and confirmed by DNA sequencing (25, 26). The Brazil Zika virus strain shares a common ancestor with the Zika virus strain that circulated in French Polynesia (27).

On Jul 2015, in the State of Bahia, Brazil, an increase in the number of Guillain-Barré cases in which half of them had reported symptoms consistent with Zika virus infection (28).

In Jan 2016, an unusually significant increase of GBS was reported in El Salvador. An Emergency Committee was convened by the Director-General of WHO, under the International Health Regulations (2005) on 1 Feb 2016, and finally announced “the recent cluster of microcephaly and other neurologic disorders reported in Brazil to be a PHEIC(28).

Situation and risk analysis in Eastern Mediterranean Region, regional strategic plan

Although no countries of the Eastern Mediterranean Region of WHO have reported importation of Zika virus disease or autochthonous transmission, however, of the 22 countries in the Region, the following 8 have reported dengue outbreaks in recent years and/or have the presence of com-

petent *Aedes* mosquitoes: Djibouti, Egypt, Oman, Pakistan, Saudi Arabia, Somalia, Sudan and Yemen. Some of these countries are particularly vulnerable to emerging of Zika virus, because of the fragility of health systems, weakness of disease surveillance systems, the inadequacy of response capacities, and a suboptimal level of public health preparedness (29-31). Pakistan is the most-at-risk country in the Region, primarily due to a large number of travelers to and from the Americas, dense urban populations, a documented large epidemic of dengue, and the most favorable climatic conditions for the reproduction of *Aedes* mosquitoes (29). In Iran, national committee of *Aedes*-borne diseases recommended enhancement of surveillance after 2016 and updated national guidelines of surveillance and clinical management of suspected cases, especially for travelers returning from at-risk countries. As of Sep 2018, all of the samples from suspected cases of Zika virus sent to the National Laboratory of *Aedes*-borne diseases were shown to be negative. Prevention of *Aedes*-borne diseases needs comprehensive national strategic action plan with "one health" approach and close collaboration between community, academic, and public health authorities. Global map of Zika virus infection demonstrated in (Fig. 2) and areas potentially at risk of Zika in (Table 1).



Fig. 2: Global map of Zika virus infection

Content source: Centers for Disease Control and Prevention, Sep 2018 updated.

(<https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika>)

Table 1: Areas and countries potentially at risk of Zika

Africa	Angola, Benin, Burkina-Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo (Congo-Brazzaville), Côte d'Ivoire, Democratic Republic of the Congo (Congo-Kinshasa), Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Liberia, Mali, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Sudan, Sudan, Tanzania, Togo, Uganda
Asia	Bangladesh, Burma (Myanmar), Cambodia, India, Indonesia, Laos, Malaysia, Maldives, Pakistan, Philippines, Singapore, Thailand, Timor-Leste (East Timor), Vietnam
The Caribbean	Anguilla; Antigua and Barbuda; Aruba; Barbados; Bonaire; British Virgin Islands; Cuba; Curaçao; Dominica; Dominican Republic; Grenada; Haiti; Jamaica; Montserrat; the Commonwealth of Puerto Rico, a US territory; Saba; Saint Kitts and Nevis; Saint Lucia; Saint Martin; Saint Vincent and the Grenadines; Sint Eustatius; Sint Maarten; Trinidad and Tobago; Turks and Caicos Islands; US Virgin Islands
Central America	Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama
North America	Mexico
The Pacific Islands	Fiji, Papua New Guinea, Samoa, Solomon Islands, Tonga
South America	Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Venezuela

Content source: Centers for Disease Control and Prevention, September 2018 updated. (<https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika>)

Pathogenesis of Zika virus infection

Studies on the pathogenesis of Zika virus have shown similarities with the pathogenesis of other Flavivirus infections. Following transmission by the mosquito bite, Zika virus can infect several different cell types including skin keratinocytes, dermal fibroblasts and dendritic cells (DCs) (9). In vitro studies on fibroblasts exposed to Zika virus have shown high infection rates in these cells 24 to 48 h after infection. Flow cytometric analyses of DCs exposed to the virus have also shown that up to 60% of DCs express viral antigens around 24 h after infection (9). Different cell surface receptors have been proposed to mediate Zika infection of permissive cells; including DC-SIGN, AXL and Tyro3 molecules (9) (Fig. 3).

Following cell entry, Zika virus induces strong interferon responses in infected cells. Studies on Zika virus-infected human primary fibroblasts have shown strong upregulation of interferon-beta transcripts 24 to 48 h after infection (9). A milder response has been reported for interferon-alpha. Upregulation of intracellular "pattern recognition receptors" (PRRs) involved in sensing non-self-nucleic acids seems to be a crucial

part of the initial immune response to the virus (9). Studies on Zika virus-infected fibroblasts have revealed strong induction of RIG-I and MDA-5 transcripts. Both of these molecules are capable of initiating a signaling process following the detection of intracytoplasmic viral RNA molecules. Innate immune responses are followed by adaptive immune events including the activation of T cells; Zika virus-infected DCs migrate to regional lymph nodes where they stimulate T cell proliferation, differentiation and cytokine production (32).

Productive infection of dermal fibroblasts and dendritic cells together with insufficient control of infection by innate and adaptive immune mechanisms usually leads to Zika virus viremia, which underlies the non-specific clinical symptoms which might last for a few days. In a pregnant woman, maternal viremia might lead to fetal viremia. It is not well known how the virus is transmitted to developing nervous system following the establishment of fetal viremia. While in circulation, Zika virus can infect fetal monocytes and these monocytes can carry the infection to the developing nervous system. Studies on fetal brain tissue derived from Zika virus-infected

mothers who have undergone abortion have shown viral particles in neural cells, indicating that the virus can proliferate inside neural cells in a developing brain (5). While inside the CNS, Zika virus can exert direct neurovirulence, similar to other Flaviviruses. There is also evidence that Zika virus might cause indirect neurovirulence through the activation of immune mechanisms including microglial activation and macrophage

infiltration (5). Indeed, in adults Zika virus might be an etiological factor for “Acute Disseminated Encephalomyelitis” (ADEM) (33), an immune-mediated disease of the CNS, which occurs subsequent to varieties of viral and nonviral infections (34). Whether an ADEM-like process might affect developing brain remains an open question.

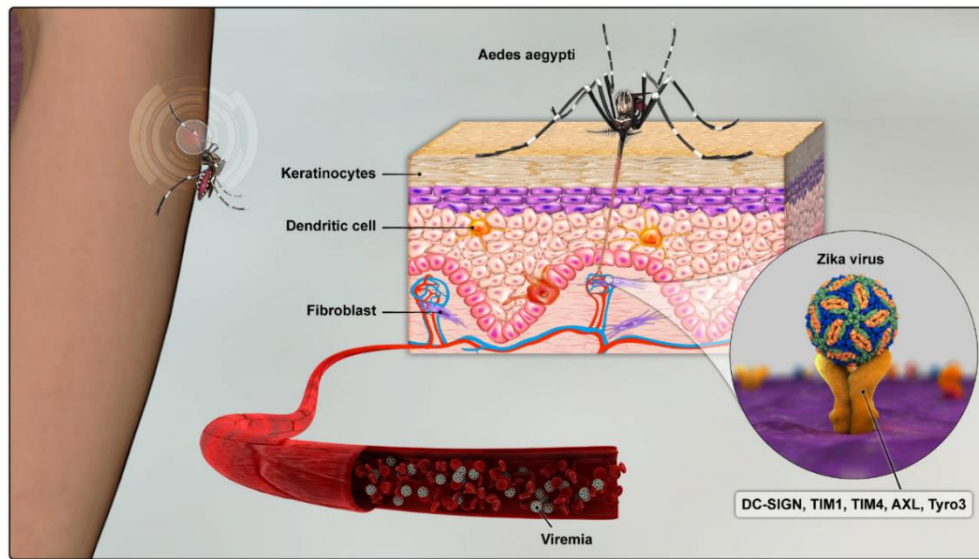


Fig. 3: Immunopathogenic pathways and virus-host cell interaction in Zika virus infection. Infected vectors (*Aedes aegypti* and *Aedes albopictus*) introduce Zika viruses into the host during their blood meal. A variety of cells (keratinocytes, fibroblasts, and immature dendritic cells) can be infected through receptor-mediated endocytosis using flaviviruses E glycoprotein. Zika virus entry into these cells is mediated by several receptors including DC-SIGN (CD209), TIM-1, 4 (T-cell immunoglobulin and mucin domain-1, 4), AXL and Tyro3 (cell surface receptor tyrosine kinases, part of the TAM family) (Original)

Laboratory diagnosis of Zika virus infection

Depending on the purpose of the investigation, laboratory diagnosis of Zika virus can be conducted by virus isolation, antigen detection, viral RNA detection with molecular assays and anti-Zika virus antibodies detection with serological assays (35).

Virus Isolation

The first isolation of Zika virus was performed by intracerebral mouse inoculation considered as the reference assay for arboviruses isolation (25, 36).

Among human clinical specimens, Zika virus can be cultured from blood (37), urine (38), saliva (39) and semen (40).

Antigen Detection

Antigen detection is a valuable assay to approved Zika virus infection in autopsy tissues. Using immunohistochemistry (IHC) technique, Zika virus antigen has been detected in brain and placental tissues from congenitally infected newborns with microcephaly and miscarriages (41, 42). Recently, new assays for detection of Zika virus antigen including NS3 protein identification by flow cytometry in whole blood (43), aptamer-

based ELISA assay for targeting of Zika virus NS1 protein (44), NS1 protein based competitive ELISA (45), NS1 protein-based rapid tests (46) have been developed.

Molecular Assays

Zika virus RNA is detectable in different types of body fluids such as blood (serum or plasma) (47-50), urine (49, 51), saliva (52) semen (40), breast milk (49), conjunctival fluid (53) and amniotic fluid (54); and brain and placental tissues of congenitally infected fetuses (41, 42).

Reverse transcriptase PCR (RT-PCR) is highly sensitive and specific and known as the “gold standard” for ZIKV diagnosis (55). Regarding Zika virus-specific RT-PCR, several conventional and real-time assays targeting prM, E, NS1, NS3, NS4, and NS5 genes have been developed (50, 56-59). However, to the best of our knowledge, the Food and Drug Administration (FDA) has approved only one commercial assay e.g. Cobas Zika test (Roche) which is a qualitative nucleic acid test for screening Zika virus RNA in blood donors. Additionally, FDA has authorized the use of several molecular assays under an Emergency Use Authorization (EUA) (60) all of them are based on RT-PCR (such as Triplex Real-Time RT-PCR, a multiplex assay for detection of Zika virus, Dengue virus, and Chikungunya virus (CDC) (61), Zika virus RNA qualitative real-time RT-PCR (Quest Diagnostics Infectious Disease, Inc.) and RealStar Zika virus RT-PCR kit (Altona Diagnostics, GmbH)) expect Aptima Zika virus assay based on Transcription-Mediated Amplification (TMA) Technology (60).

Molecular diagnosis of Zika virus infection in human usually performs on plasma or serum specimens within the first week after onset of clinical symptoms (60). Although, there are several lines of evidence for advantage of urine for Zika virus RNA detection because of the long duration of viral shedding in this easily collectable specimen (55, 12, 62), interesting findings indicating the shorter persistence of ZIKV RNA in urine

versus serum have been observed (60, 63, 64).

Serological Assays

Despite the fact that the molecular diagnosis possesses high sensitivity and specificity, short period of viremia can negatively affect the Zika virus RNA detection (65). Therefore, detection of anti-Zika virus antibodies by serological assays could be an advantageous option for a wider diagnostic window.

Detection of anti-Zika virus antibodies can be performed by different serological tests including complement fixation, haemagglutination inhibition, immunofluorescence (IF) assay, ELISA and neutralization tests (13, 35). Typically, anti-Zika virus IgM antibody develops within the first week after the onset of symptoms and is detectable from day five to 12 wk of illness. Anti Zika virus IgG antibody rises few days after IgM and is traceable for months to years.

So far there is no FDA approved a serological test for Zika virus. However, FDA has authorized the use of five serological assays with emergency use authorization for detection of anti-Zika virus IgM antibody including IgM antibody capture ELISA (Zika MAC-ELISA) (CDC) (61), ZIKV Detect IgM capture ELISA (InBios International, Inc.), Liaison XL Zika capture IgM assay (DiaSorin Incorporated), ADVIA Centaur Zika test (Siemens Healthcare Diagnostics Inc.) and DPP Zika IgM system (Chembio Diagnostic Systems, Inc.) (60).

Because of cross-reactivity with other Flaviviruses such as dengue and yellow fever viruses, and the possibility of nonspecific reactivity, results of IgM detection assays should be interpreted with caution and positive or equivocal results must be confirmed by plaque-reduction neutralization testing (PRNT) (66).

Laboratory Biosafety

Zika virus has been classified as a risk group 2 human pathogen. Therefore, diagnostic laboratory tests should be performed in a Biosafety Level 2 (BSL-2) facility (13). The virus can be inactivated by Ultraviolet (UV) radiation, temperatures above 58 °C, solutions of pH under 6.2 above < 7.8, ether, and 5% potassium permanganate (67-69).

Ethical aspects and challenges of Zika virus infection

Addressing ethical questions and moral conflicts of Zika virus infection may be essential to develop a comprehensive approach to control this public health crisis (70). Therefore, the health authorities commitment to allocating a fair portion of health resources for responding to this situation is morally required (71). The government should priorities paying the noticeable amount of money for preventive measures in order to control the Zika virus spread (72). This priority setting is usually the most difficult step while encountering such situations, especially is the absence of enough scientific evidence to measure the real burden of disease and also the presence of social and media pressure, amplified by 2016 Olympic games in Brazil (73).

National and international health authorities should observe the principle of veracity, by clear and transparent informing system for raising the public awareness regarding the real situation (74). At the international level, the “Cosmopolitan Solidarity” for encountering such universal pattern of disease spread seems to be inevitable (75). Like Ebola outbreak, responsiveness to actions required by responsible international bodies especially WHO is another responsibility of states to form a global health governance network (76). In mosquito control, governments should try to keep the moral obligation of observing biosafety and protecting the ecosystems in the mind while trying to restrict the insect spread and reproduction (77). During policy-making for disease control, deliberative ethical evaluation is required in order to protect of population basic civil and human rights such as people’s free mobilization or reproductive rights in case that border or birth control are among the solutions (78, 79). Similarly, confidentiality of patients’ health information and protecting people against stigmatization must be observed when policies for reporting the probable cases to health authorities are discussed (80).

The fear raised from high probability of congenital problems, mainly microcephaly, of neonates delivered by mothers with Zika virus infection in

their first trimester of pregnancy (81), puts abortion at the center of moral concerns (82). The position of various schools of ethics regarding abortion has a range from strict prohibition to complete moral justification based on the moral status they consider for the fetus in each development stage. Accordingly, abortion laws are still different in various countries according to the social, political, religious and cultural context (83, 84). In Iran despite of general prohibition of abortion by Criminal Law, the “Therapeutic Abortion Act” of 2005 allowed performing abortion in presence of a “definite diagnosis of retardation or malformation of the fetus that is unbearable for the mother...” only before 4 months (19th wk) after conception and any attempt for abortion after this gestational age is illegal and even criminal (85).

Conclusion

Together with Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), Zika virus infection qualifies as a “newly emerging” infectious disease, with the potential to cause serious public health issues. Unlike the other “newly emerging” infections which can lead to severe morbidity and mortality in infected adults or pediatric hosts, Zika infection does not pose a significant threat to infected adults and its risk is more due to the potential to cause fetal abnormalities, provided that the infection occurs during pregnancy. Indeed, among the four recent PHEIC declarations by WHO (i.e. 2009 Swine flu declaration, 2014 Polio and Ebola declarations and 2016 Zika virus declaration), Zika is unique in the sense that it is a member of the TORCH group of infections; i.e. the group of pathogens with the ability to lead to congenital infections/anomalies. This might influence both disease prevention and management strategies as well as raising ethical and sociological issues. Thus, increased awareness of the medical community together with improvements in vector control and disease surveillance systems are of utmost importance for controlling any potential Zika virus-related threats in different countries.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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