

# Zika Virus: An Emerging Vector Borne Disease

Nanthini Devi P\* and Chitrlekha S

Department of Microbiology, Sree Balaji Medical College and Hospital, Tamil Nadu, India

\*For Correspondence: Nanthini Devi P, Department of Microbiology, Sree Balaji Medical College and Hospital, Tamil Nadu, India, Tel: +919840856073; E-mail: nandhu.smiley7@gmail.com

Received date: 14/12/2017; Accepted date: 21/02/2018; Published date: 28/02/2018

Copyright: © 2018 Devi NP, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

## Review Article

### ABSTRACT

The Zika virus was first isolated in 1947 from Rhesus macaque monkeys and the first human isolation was done in 1952. Though it did not cause any major outbreaks till 2007, it caused an outbreak in 2007 in the French island of Polynesia and thereafter the massive epidemic in Brazil in 2015. The most troublesome part about the epidemic was the clustering of microcephaly cases in new-borns born to pregnant women, that WHO declared it to be a public health problem of international concern. The prevalence of the vector mosquitoes Aedes worldwide, the different modes of transmission, lack of suitable tests for diagnosis, no approved vaccines all make Zika virus a deadly disease. The current review provides an insight into all the aspects of Zika virus.

**Keywords:** Zika virus, Microcephaly, Brazil.

### INTRODUCTION

The Zika virus was first isolated in April 1947 from a pyrexial Rhesus Macaque monkey that was caged in the Zika Forest of Uganda, by the scientists of the Yellow Fever Research Institute by intracerebral and intraperitoneal inoculation [1]. A second isolation was done from the mosquito Aedes africanus in January 1948. The monkey developed fever and the researchers isolated from its serum a "filterable transmissible agent" that was named as Zika virus in 1948 [2]. Though the virus was isolated many years back, it gained prominence only from 2015 onwards when an epidemic occurred in Brazil and continued its spread to the United States and other countries of the world. More important was the clustering of birth defects mostly microcephaly in the affected regions that led the WHO to declare the Zika virus "A public health emergency of international concern". This review focuses on the epidemiology, virology, transmission, pathogenesis, clinical features, lab detection methods, treatment, prevention measures and vaccine strategies for Zika virus.

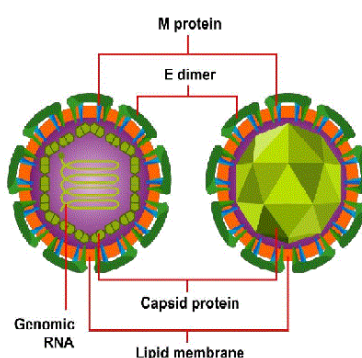
### LITERATURE REVIEW

#### Virology

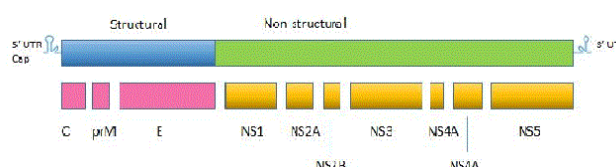
The Zika virus belongs to the family Flaviviridae and the genus Flavivirus and is related to the other viruses of public health importance such as dengue, yellow fever, Japanese encephalitis, and West Nile virus [3]. Zika virus is an enveloped, 10794 kilobase positive-sense RNA genome. It is closely related to the Spondweni virus and is one of the two viruses in the Spondweni virus clade [4].

The virion is approximately 40 nm in diameter and has surface projections of 5-10 nm diameter (**Figures 1 and 2**). The nucleocapsid is 25-30 nm diameters and surrounded by a host-membrane derived lipid bilayer that contains the viral envelope proteins E and M. There are seven non-structural proteins (NS1, 2A, 2B, 3, 4A, 4B, 5). The virus is flanked by two non-coding regions (5' and 3' NCR) and a single long open reading frame encoding a polyprotein: 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3' that is cleaved into capsid (C), precursor of membrane (prM), envelope (E) and seven non-structural proteins (NS) [5]. The E protein (~53 kDa) is the major virus surface protein and is involved in binding and membrane fusion. The NS5 protein (~103 kDa) is the largest viral protein whose C-terminus has RNA-dependent

RNA polymerase (RdRP) activity and the N-terminus is involved in RNA capping due to methyl transferase activity. The 3'NCR of the Zika virus genome contains about 428 nucleotides, including 27 folding patterns that serve various functions like recognition by cellular or viral factors, translation, stabilization of genome and RNA packaging. The structural proteins encapsulate the virus [6,7].



**Figure 1.** Schematic diagram of Zika virus.



**Figure 2.** Genomic structure of Zika virus.

There are three Zika lineages, two African lineages- MR 66 cluster, the Nigerian cluster and the Asian lineage. Phylogenetic studies have revealed that the virus circulating in the American countries is closely related to the Asian strain of the 2013-2014 outbreaks in French Polynesia [8].

## EPIDEMIOLOGY

Though the virus isolation dates back to 1947 in monkeys, Zika virus was first identified in humans in 1952 during an epidemic of jaundice in Eastern Nigeria which was suspected to be yellow fever. The virus was isolated in three patients, one was a 10 year old Nigerian female and the other two were males. It was isolated by mice inoculation with the patient's serum and the other two by a rise in serum neutralizing antibodies [9]. There were no account of deaths or hospitalization due to the virus, but studies have shown human exposure to the virus in the 1960 to 1980s in various countries like India, Indonesia, Malaysia and Philippines [10-12].

In 2007, the first massive outbreak of Zika occurred in the Pacific Island of Yap in the Federated States of Micronesia. Prior to this outbreak, only 14 cases of human Zika virus disease had been reported worldwide that Zika virus was not suspected to be the causative agent. Approximately 73% of Yap residents were infected with Zika virus which showed that there was a lack of immunity in the island's population and *Aedes hensilli* was the causative mosquito species identified [13].

In 2008, US scientist who was working in Colorado State University went to work in Senegal. He was infected with Zika virus and upon his return to Colorado, he infected his wife. This was the first ever documented case of sexual transmission of an arboviral disease [14].

In 2012 researchers identified two distinct lineages of the virus, Africa and Asia. After the first documented Zika outbreak in Yap in 2007, the Asian lineage of the virus reappeared in French Polynesia in October 2013 and caused large outbreaks in New Caledonia (1400 cases), Cook Islands (over 900 cases) and Easter Island. The unique feature of the outbreak was an increased incidence of neurological complications, including 42 cases of Guillain-Barré syndrome [15,16].

In March 2014 in French Polynesia, trans placental transmission was reported in two new-borns [17].

During the of Zika virus outbreak in French Polynesia, 1505 asymptomatic blood donors were reported to be positive for Zika by PCR. These findings led to the conclusion that Zika virus can be transmitted by blood transfusion [18].

In March 2015 Brazil notified WHO of an exanthematous illness in the Northeastern states. From February 2015 to April 2015, there were nearly 7000 mild cases reported with no deaths. Tests for Dengue, Chikungunya were carried out and it turned out to be negative. Tests for Zika virus were not carried out as Zika virus was not suspected at this period.

By November 2015, the number of microcephaly cases continued to increase after the mysterious fever, that Brazil declared it a national public health emergency. Zika virus was detected in the blood and tissue samples of a baby with microcephaly and other congenital anomalies and the baby died within 5 minutes of birth. Brazil reported 3 deaths among 2 adults and a new-born associated with Zika virus infection [19].

Researchers reported the first instance of intrauterine transmission of the Zika virus in two pregnant women in Brazil, whose fetuses were diagnosed with microcephaly and other brain abnormalities, by ultrasound in 2016. Although tests of blood samples from both women are negative, Zika virus was detected in the amniotic fluid [20].

In January 2016, three infants, whose mothers had a history of Zika virus infection were diagnosed with microcephaly and ophthalmological anomalies. One child also displayed neuroretinal atrophy [21].

By the end of January 2016, Brazil reported between 440,000 and 1,300,000 Zika cases in Brazil, 5280 suspected cases of microcephaly, including 108 deaths [22].

In February 2016, the United States reported a case of male to male sexual transmission of Zika infection in Texas. The case report indicated that Zika can be transmitted through anal and vaginal sex [23].

Brazilian health officials confirmed a case of Zika virus infection transmitted from an infected donor through a platelet transfusion that was detected from post donation information [24]. The panic continued to grow as an arboviral disease was found to have various modes of transmission.

Brazilian health officials confirmed a case of Zika virus infection transmitted from an infected donor through a platelet transfusion that was detected from post donation information. The panic continued to grow as an arboviral disease was found to have various modes of transmission.

In March 2016, a case report was published in The Lancet in which a 15-year-old Zika-positive girl in Guadeloupe developed acute myelitis which caused severe back pain, numbness, and bladder dysfunction. This led to the suggestion that Zika virus preferentially affects the nervous system [25]. Chile notified WHO of a confirmed case of sexually transmitted Zika virus. This was the first case of Zika virus infection in a country where Aedes mosquitoes were not prevalent [26].

Contrary to the belief that Aedes mosquitoes transmitted Zika virus, Fiocruz Institute Pernambuco announced that it detected Zika virus in Culex quinquefasciatus mosquitoes collected in the houses in the city of Recife, Brazil. Though the finding was welcomed by WHO, further evidence was needed, to know to what extent Culex was capable of human transmission [27].

In October 2016, Thailand notified WHO of two babies born with microcephaly associated with Zika virus. This was the first such instance of Zika virus infection in the South-East Asian Region [28]. Till date, the US alone have reported a total of 580496 suspected and 221093 confirmed cases, 20 deaths and 3689 cases of congenital transmission [29].

Due to the alarm raised by the Zika virus infection, WHO updated information on vaccine research and development. More than 40 Zika vaccine candidates were in the pipeline and 5 entering Phase I trials, where the vaccine's safety and ability to mount an immune response will be evaluated. The timeline of Zika virus and places that have reported Microcephaly and other CNS malformations associated with Zika virus infection (As of December 2016) (**Figures 3-5**).

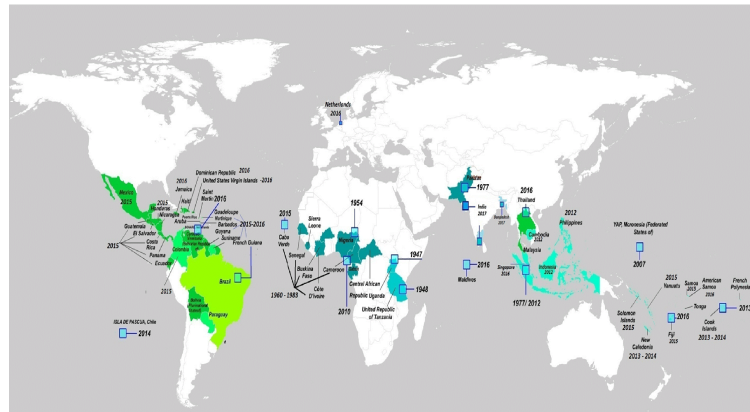


Figure 3. Countries showing the Zika virus outbreaks 1947-2017.

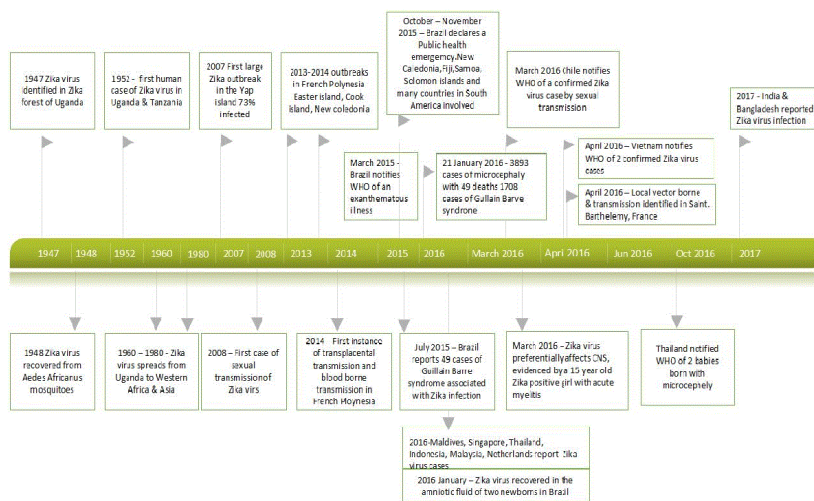


Figure 4. Timeline showing the key events in Zika virus epidemics.

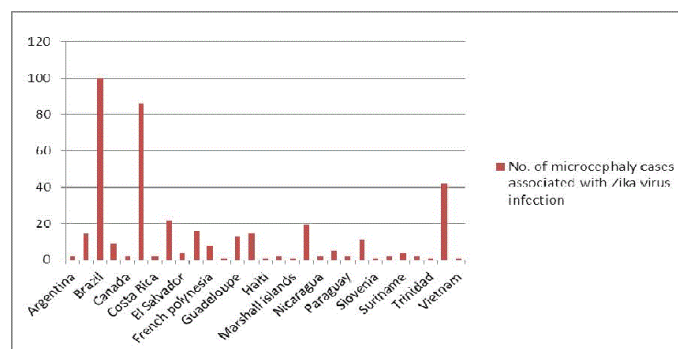


Figure 5. Places that have reported Microcephaly and other CNS malformations associated with Zika virus infection (As of December 2016).

Note: Brazil reported 2366 cases of Microcephaly.

Statistics source: World health organization.

### Host

Apart from human beings, Zika virus antibodies have been detected in different monkey species like Rhesus monkey, African green monkey, County monkeys in Africa and Asia. Epizootics of Zika virus in monkeys were reported in Uganda in 1947, 1948, 1956, 1962, 1963, 1969, and 1970. Another epizootic of Zika virus was reported in the Kedougou region of Senegal in 1973, with *Aedes luteocephalus* and *Aedes furcifertaylori* as the main vectors. However, animal serosurvey data must be interpreted with caution because of the problem of cross-reactivity.

Serosurvey studies also detected antibodies to Zika virus in ducks, goats, cows, horses, bats, rats, carabaos (water buffalo), rodents, sheep and wild orangutans. These data may indicate cross-reactivity with other flaviviruses but it is not clear whether other species can act as reservoir host for Zika virus [30-32].

## ZIKA VIRUS TRANSMISSION

### Mosquito Borne Transmission

In Africa, Zika virus spreads through a sylvatic cycle involving non-human primates and forest-dwelling species of *Aedes* mosquitoes. Several mosquito species, belonging to the *Stegomyia* and *Diceromyia* subgenera of *Aedes*, including *A. africanus*, *A. luteocephalus*, *A. furcifer* and *A. taylori* are enzootic vectors in Africa [33].

In Asia, there is no sylvatic transmission cycle but in urban and suburban environments, Zika virus is transmitted in a human–mosquito–human transmission cycle. Two species in the *Stegomyia* subgenus of *Aedes*- *A. aegypti* and *A. albopictus* are the vectors. *A. hensilli* and *A. polynesiensis*, were also thought to be vectors in the Yap and French Polynesia outbreaks [34-36]. The prevalence of *Aedes* mosquitoes worldwide (Figure 6).

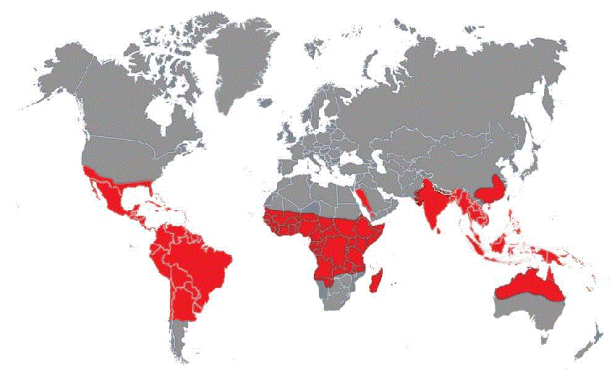


Figure 6. Prevalence of *Aedes* mosquitoes worldwide.

Zika virus has also been identified in other mosquito species, such as *Aedes unilineatus*, *Anopheles coustani*, and *Mansonia uniformis* but vector-competence studies have shown that these species have a low potential for transmission of the virus [37]. Zika virus has been reported only once in *Culex quinquefasciatus* species, which suggests that mosquitoes in this genus have a low vectorial capacity [38].

### Non-Mosquito Transmission

#### Transplacental transmission

Zika virus can be transmitted from the mother to the foetus during pregnancy, as concluded by many studies. Zika virus RNA has been found in the amniotic fluid of mothers, whose foetuses had abnormalities detected by ultrasonography. Viral antigen and RNA have also been identified in the brain tissues and placental samples of children who were born with microcephaly and died soon after birth, as well as in tissues from miscarriages [39].

Two cases of peripartum transmission of Zika virus have been reported in French Polynesia. Zika virus RNA was detected in both infants; one infant had a mild rash with thrombocytopenia and the other infant was asymptomatic. The possible routes of perinatal transmission could be transplacental, during delivery, during breastfeeding and by close contact between the mother and her newborn.

#### Sexual transmission

Sexual transmission also has been reported from the infected male partners working in endemic areas to their spouses. In one case, sexual intercourse occurred before the onset of symptoms, whereas in the other cases sexual intercourse occurred during the development of symptoms and later. Researchers have not determined the risk factors for and the duration of the risk of sexual transmission. The viral RNA load has been found to be high in sperm and it has been detected up to 62 days after the onset of symptoms [40,41].

#### Blood transfusion

During the Zika virus outbreak in French Polynesia, 2.8% of donated blood samples tested positive for Zika virus using an in-house nucleic acid testing [42]. It was further confirmed in Puerto Rico in 2016 with 1.8% of blood donors identified as viraemic [43]. In 2017, Zika virus RNA-positive asymptomatic blood donors were detected in Florida and Texas.

So most blood collection services have asked donors to avoid donating blood for at least one month following travel to a Zika virus infested area, if they have a clinical history consistent with Zika virus disease, or if they have had sexual contact with a person with confirmed or suspected Zika virus infection within the past 3 months [44].

With the lack of accession to proper diagnosis of Zika virus, the number of reported cases due to blood transfusion might be underestimated.

### **Laboratory contamination**

A laboratory staff member developed a febrile illness after yellow fever vaccination (17D vaccine), but Zika virus was isolated from blood taken on the first day of illness. The infection was believed to be laboratory acquired [45]. A volunteer became infected after subcutaneous injection of infected mouse brain suspension [46].

### **Rare Modes of Transmission**

Zika virus transmission in an Australian traveller was documented after a monkey bite in Bali, Indonesia, although mosquito-borne transmission could not be ruled out [47]. Transmission through breast milk has not been documented so far, although there is evidence that Zika virus was present in the breast milk of three women by RT-PCR, who became symptomatic three days after delivery. Though breast milk contained Zika virus in high titre, there was no evidence that it could be transmitted to the infant [48].

## **PATHOPHYSIOLOGY OF ZIKA VIRUS INFECTIONS**

There is very little information about the pathogenesis of Zika virus infection in human beings. It is transmitted by the bite of female *Aedes* mosquitoes. After a blood meal, the virus multiplies in the vector reaches the salivary glands and is inoculated into the human being through bite. The dermal fibroblasts, epidermal keratinocytes and dendritic cells are permissive to Zika virus infection and from the skin; the virus spreads to the draining lymph nodes and causes primary viremia. Then there is dissemination to the various tissues and organs [49].

The cell surface receptors or attachment factors for Zika virus in the skin has been found to be DC-SIGN (dendritic cell-specific intracellular adhesion molecule 3-grabbing nonintegrin), AXL, Tyro-3, TIM-1, trans membrane receptors which interacts with the viral envelope protein [50]. The neural astrocytes and microglial cells also express AXL receptors which explain the neuropathogenesis of the virus [51]. Tyro-3 receptors are expressed on the mid piece of spermatozoa which explains the sexual transmission of the virus [52].

In an attempt to counteract the infection, the human immune system induces the pattern recognition receptors (PRR) to recognise the pathogen associated molecular patterns (PAMP). This leads to the up regulation of TLR-3 (Toll like receptor-3) and transcription of RIG-1 which recruits TANK binding kinase-1(TBK-1) and phosphorylation of interferon regulatory factor 3 (IRF3) and Type -1 Interferon transcription. This binds to two interferon receptor subunits (IFNAR 1 and 2) and through the Janus kinases (Jak1 and Tyk2) and signal transducers of transcription (STAT1 and STAT2), leads to the induction of IFN-stimulated genes (ISGs) and contributes to antiviral activity [53].

### **Evasion of Immunity**

Zika virus is composed of seven non-structural proteins (NS1, 2A,2B,3,4A,4B,5) which contributes to immunity evasion. NS1 and NS4B suppress type I Interferon signaling by targeting TBK1. Zika virus suppresses JAK-STAT signalling by degrading Jak1 through NS2B3.

The ubiquitin proteasome system which regulates the protein pool by ubiquitination of proteins followed by destruction by proteasome acts as a double-edged sword in viral infections. It promotes anti-viral activity by degradation of viral proteins and it can also be used by the virus to degrade host cellular restriction factors [54]. In Zika virus infection, the NS 5 promotes the proteosomal degradation of STAT 2 and thus interferes with Type 1 interferon production [55]. It was also found that Bortezomib, a FDA approved proteasome inhibitor can prevent Zika infection, which stands as a proof [56].

The nucleic acid of Zika virus is detectable in blood in the first week of infection only upto 10 days after the onset of symptoms, after which there is a decline in titre. But the viral excretion is of a longer duration (>20 days) and higher load in urine when compared to serum [57]. Zika virus RNA shedding has also been found in saliva for upto 29 days after the onset of symptoms and in semen almost 62 days after symptoms onset [58,59]. The appearance of IgM antibodies starts 4 to 7 days after the onset of symptoms followed by IgG antibodies after 2-3 days. IgM persists till 2-3 months while IgG antibodies remains positive for months or years [60].

## Infection of the Foetus

Though the placental syncytiotrophoblast offers considerable resistance to pathogens, infection of the foetus can occur due to free virions or virion antibody complexes or infected Hofbauer cells [61]. One unique mechanism suggested for the transfer of Zika virus across the placenta to the developing foetus is the neonatal Fc gamma receptor (FcRn) which recognises non neutralizing maternal IgG and uptake. These Fc gamma receptors are more expressed in villous endothelium [62]. The immune complex consisting of the virus and non-neutralizing antibody complex binds to the nerve ending through FcγR receptor and transported to the neurons through retrograde axonal transport without any need to cross the blood brain barrier [63]. It has been found that Zika virus infection in the first trimester resulted in microcephaly which was due to the Toll like receptor 3 activation, that resulted in altered expression of genes concerned with neural development, the foetal neural progenitor cells [64]. The abnormalities observed in the second and third trimester could be due to an indirect effect of placental Hofbauer cell invasion or transmission of Zika virus through motile dendritic cell [65].

Garcez et al. used induced human pluripotent stem (iPS) cells cultured as neural stem cells (NSCs), neurospheres, and brain organoids and exposed to Zika virus and found that neurospheres formation was impaired and organoids growth rate was reduced [66].

## Zika Virus and Guillain Barre Syndrome

It has been postulated that autoantibodies against the neural antigen gangliosides is responsible for the causation of GBS by peripheral nervous system damage though there has been no report of molecular mimicry between Zika virus antigens and neural antigens. This was confirmed by an autopsy report of a fatal GBS case where there was no evidence of direct neural invasion suggesting that antibody mediated mechanisms played an important role in pathogenesis of GBS after Zika virus infection [67].

## Animal Models

Various animal models have been used to study the pathogenesis of Zika virus like mice and macaques. Adult mice is resistant to Zika virus, so neonatal mice or mice deficient in Interferon signalling pathway is used.

## Pathogenesis In Immunocompetent Neonatal Mice

Infection of WT C57BL/6 mice with Zika virus resulted in unsteady gait, ataxia and tremors with signs of nervous degeneration [68]. In another study, 1-day old C57BL/6 was injected with 3 different Zika strains through intraperitoneal or subcutaneous and they were found to be lethal to the mice [69].

## Mouse Models

Immunocompetent mice are resistant to Zika virus since NS5 does not degrade mice STAT 2. So mice with deficiencies in the Type 1 IFN pathway or receptor can be used to study the pathogenesis of Zika virus. Mice lacking the IFNAR gene including A129 mice and *Ifnar1*<sup>-/-</sup> C57BL/6 mice, AG 129 lacking both type 1 and 2 interferon receptor genes or mice deficient in *Irf3*, *Irf5*, and *Irf7*.

(*Irf3*<sup>-/-</sup> *Irf5*<sup>-/-</sup> *Irf7*<sup>-/-</sup> triple knockout [TKO]) transcription factors, WT C57BL/6 mice treated with anti-IFNAR 1 blocking monoclonal antibody, STAT 2 <sup>-/-</sup> mice developed limb weakness and paralysis through any route (subcutaneous, intraperitoneal or intravenous inoculation of various Zika virus strains). The lethal effects of the virus were also found to be dependent on the age, with 3 weeks, 5 weeks and 11 weeks mice yielding to infection compared to adult mice [70].

Zika virus infection in humans has been found to cause conjunctivitis and uveitis. *IFNAR1*<sup>-/-</sup> mice infected with Zika virus developed conjunctivitis and panuveitis and the RNA was found in cornea, iris and optic nerve [71]. AG 129 and *IFNAR1*<sup>-/-</sup> mice infected with Zika virus developed infection in the testicle, epididymis, seminal vesicle and prostate, which provides an idea about the sexual mode of transmission of Zika virus [72].

In a study by Tang et al, AG129 mice and *LysMCre*+*IFNAR1*/fl C57BL/6 mice after hormonal treatments were infected by Zika virus by the intravaginal route. There was evidence of viremia and viral replication in brain and spleen and the viral RNA was found in vaginal washes even after 10 days in the diestrus like phase [73].

Infection during pregnancy and fetal pathogenesis. Infection of pregnant *IFNAR*<sup>-/-</sup> mice and WT mice treated with blocking anti-*IFNAR* monoclonal antibody with Zika virus through subcutaneous route led to placental infection, intra uterine growth retardation or fetal demise but no microcephaly was observed [74]. But when a pregnant pigtail macaque was infected with a Zika virus strain, there was evidence of microcephaly, gliosis and axonal damage [75].

## CLINICAL FEATURES

About 80% of Zika virus infections may be asymptomatic. The severity of the disease depends on the immune status of the individual [76]. After an incubation period of 2-14 days, patients present with a low fever, bilateral conjunctivitis, maculopapular rash, headache, retro-orbital pain and arthritis or arthralgia with edema of the tiny joints of hands and feet, myalgia, vertigo and asthenia. The rashes may be erythematous and generalised, pruritic and usually resolves within the first week, but may remain for two weeks. Conjunctivitis in Zika fever is non-purulent and bilateral [77]. Sometimes it can present with sore throat, cough and loose stools [78]. It can also present with sore throat, cervical, submandibular, axillary and inguinal lymphadenopathies. In addition, digestive symptoms may also be present including nausea, vomiting, diarrhoea, constipation, abdominal pain and aphthous ulcers.

Patients with genitourinary symptoms can present with haematuria, dysuria and hemospermia [79]. Haemorrhagic complications are not reported in Zika fever unlike Dengue. Arthralgia in Zika fever is not as severe as chikungunya fever. Because of the varied manifestations, it can be misdiagnosed during the early stages [80].

### Neurologic Complications

A temporal association has been observed between Guillain–Barre syndrome with Zika virus infection preceding the onset in the Pacific and the Americas [81]. Meningoencephalitis and acute myelitis complicating Zika virus infection also have been reported in literature. The Zika virus also was isolated from the cerebrospinal fluid [82-83].

### Adverse Fetal Outcomes

The most alarming feature of the Zika virus outbreak was the presence of congenital anomalies in the new-born, mostly microcephaly. Microcephaly in a live born child is defined as head circumference (HC) adjusted for gestational age and sex <3<sup>rd</sup> percentile at birth, or if not measured at birth, within first 2 weeks of life (Figure 7).

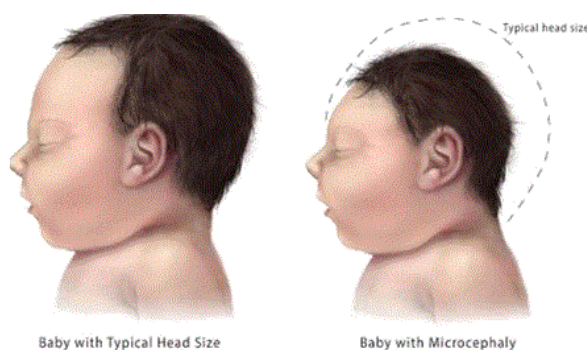


Figure 7. Microcephaly.

In the event of pregnancy loss, it should be calculated as the prenatal HC more than 3 standard deviations below the mean based on ultrasound or postnatal HC [84].

The various brain abnormalities observed were microcephaly, Intracranial calcifications, Cerebral atrophy, Abnormal cortical formation (eg: polymicrogyria, lissencephaly, schizencephaly), Corpus callosum abnormalities, Cerebellar abnormalities, Porencephaly, Hydranencephaly, Ventriculomegaly, hydrocephaly, Fetal brain disruption evidenced by collapsed skull, overlapping sutures, prominent occipital bone, scalp rugae.

Neural tube defects (NTD) observed includes Anencephaly/Acrania, Encephalocele, Spina bifida, Holoprosencephaly and Arhinencephaly [85].

The various ophthalmological abnormalities include Micropthalmia/Anophthalmia, Coloboma, Cataract, Intraocular calcifications, lacunar maculopathy, Chorioretinal anomalies involving the macula (e.g., chorioretinal atrophy and scarring, macular pallor and retinal hemorrhage) and Optic nerve atrophy [86].

Congenital contractures (eg: arthrogryposis, club foot, congenital hip dysplasia) and congenital deafness documented by postnatal testing were also associated with brain abnormalities [87].

The Zika virus RNA was found in the amniotic fluid of fetuses and in the brain tissue of fetuses and infants with microcephaly. These provide a strong evidence of microcephaly to maternal Zika virus infection [88]. The greatest risk of microcephaly was in the first trimester, mostly between 7 and 13 weeks of gestation, but it can also occur as late as at 18 weeks of gestation [89].



## LABORATORY DIAGNOSIS OF ZIKA VIRUS INFECTION

### Biosafety

Zika virus has been classified as a level 2 pathogen requiring only a biosafety level 2 laboratory by the WHO [90].

### Specimen Collection

Zika virus has been detected in serum, plasma, amniotic fluid, cerebro spinal fluid, urine, semen and saliva. There is evidence that Zika virus persists longer in the semen and urine [91,92]. Specimens suspected of containing Zika virus should be handled with safety as per WHO international regulations [93].

### Clinical Laboratory Testing

The laboratory profile includes leucopenia, thrombocytopenia, albuminemia, C-reactive protein and erythrocyte sedimentation rate have been reported, but their incidence is underestimated as they are common in many viral infections. But a complete blood count is done for all suspected cases of Zika fever for differential diagnosis. In patients with GBS, CSF analysis shows a significant increase in proteins, with normal or little altered cellularity (albuminocytological dissociation) [94].

### Antigen Detection

Immunohistochemistry (IHC) analysis with monoclonal antibodies and PCR analysis can be used to detect Zika virus antigen in autopsy tissues. Zika virus antigens have been detected by IHC in fetal brain in areas of micro calcification and gliosis, fetal retina, and in placental tissues (Hofbauer cells) [95]. The target antigen and its cellular location can be visualised, but it requires technical expertise for specimen processing [96].

Acute phase of dengue and Zika can be diagnosed by the detection of NS1 in blood by rapid immunochromatography approach [97].

### Culture

Isolation of Zika virus from the serum of monkeys and *Aedes africanus* mosquitoes was first performed by mouse brain inoculation. Later chicken embryo yolk sacs, allantoic sacs and chorioallantoic membrane and cell cultures were used [98,99]. Vero, Rhesus monkey kidney and baby hamster kidney cells were also more sensitive for the isolation of Zika virus [100]. Zika virus was also successfully cultured by intrathoracic inoculation of *Toxorhynchites splendens* and C6/C36 mosquito cells [101]. This can be used for viral isolation, production of viral stocks, testing of vaccine and drug targets and to study the viral replication cycle [102].

### Serology

Although serology is the most preferred method for detection of Zika virus because the period of viremia is short lived, there is the problem of cross reactivity between the surface proteins of Zika and other flaviviruses particularly Dengue virus [103]. Serodiagnosis also is essential in cases of Guillain Barre syndrome, in cases of Microcephaly and other congenital syndromes, which all occurs outside the period of viremia [104].

But the chief drawback of serological tests is that their results have to be confirmed by Plaque reduction neutralisation test [105].

### MAC ELISA

IgM antibody capture enzyme-linked immunosorbent assay developed by the CDC Arbovirus diagnostic laboratory is used for the qualitative detection of IgM antibodies in serum or CSF. IgM antibodies start rising on the 4<sup>th</sup> day of infection and persist till 12 weeks or more. Samples collected before 4 days should be tested by RT-PCR or a convalescent serum sample should be collected to check for the rise in antibody titre [106]. The antibody capture ELISA involves the coating of capture antibody (anti-IgM) on microtitre plates followed by the addition of patient's serum and enzyme-conjugated anti-viral antibody sequentially. The calorimetric change occurring due to interaction between enzyme and substrate is detected by an ELISA reader. The test is simple, sensitive, can be used on serum and cerebrospinal fluid samples from humans and animals [107].

The main drawback of the test is serologic cross reactivity which occurs in primary flavivirus infection and titres to the infecting virus are higher. In contrast when there is a secondary flavivirus infection or vaccination, due to anamnestic response, the titres to the previous virus is higher [108].

### **Plaque Reduction Neutralization Test**

Negative or equivocal result to MAC ELISA can be confirmed by PRNT. In this method patient's serum is incubated with a standard dose of the infective virus, then inoculated into cell culture monolayers and then a semisolid medium is overlaid. The virus induced plaques are counted. The endpoint is defined as the titre in which there is 90% reduction of plaques. Although this is a gold standard for the detection of Flavivirus antibodies, it is cumbersome, costly, not easily available and can take 3-4 weeks<sup>[109]</sup>.

### **Molecular Detection**

Acute Zika virus infection can be diagnosed by the use of Reverse transcriptase PCR assay (RT-PCR) which was designed by the CDC using sequence derived from the 2007 Yap Islands epidemic<sup>[110]</sup>. The viremic period is very brief, as proved by case reports in which Zika virus RNA was not detected by PCR at 5 days but IgM antibodies were positive. As flaviviruses are RNA viruses, their amplification needs two steps, RT of genomic RNA into single-stranded DNA (cDNA), followed by conversion to double-stranded DNA and then amplification of the DNA. Most of the protocols described in literature target the terminal portion of the NS5-encoding gene or the 3' NCR of the Flavivirus genome because of highly conserved regions in this part of the genome. It was also successfully detected with Flavivirus RT-PCR assays targeting the E-encoding gene, prM coding gene, the NS1-encoding gene, the NS3- encoding gene and the NS5-coding gene<sup>[111,112]</sup>.

The advantage of PCR is that it can be used for both serum and urine samples. While the duration of detection of Zika virus was 5 days in serum, it has been shown that Zika virus was detectable in urine at higher titres and for a longer period.

After detection of Flavivirus RNA, identification to the species level can be done by nucleic acid sequencing<sup>[113,114]</sup>.

### **Detection of ZIKV In Different Body Fluids**

Diagnosis of Zika fever usually relies on the detection of Zika virus RNA in serum and CSF samples during the first few days after symptom onset.

Saliva, urine, semen and vaginal secretions are the body fluids in which Zika virus was detected. But the duration they can be detected is not well understood still<sup>[115-117]</sup>. This has enlarged the scope of diagnosis of Zika fever.

Viral RNA was detected in urine as early as the first day and as long as 20 days after the onset of illness<sup>[118]</sup>.

The use of saliva as an alternate to serum for the molecular detection of Zika virus during the acute phase was published in a study in which there is higher detection rate in saliva. It is of particular use in children and neonates in whom blood collection is difficult.

### **Diagnosis of Zika Fever In Countries Where It Is Endemic**

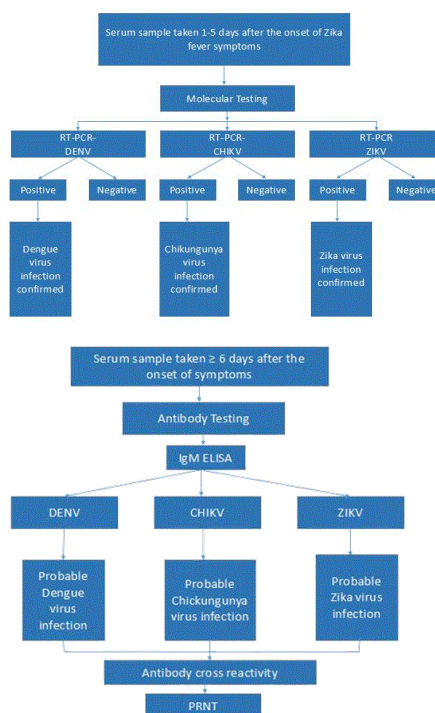
In countries with good laboratory facilities, RT- PCR of Dengue virus, Chikungunya virus should be done and when it is negative, Zika virus RT PCR should be done. In countries where there are no advance laboratory capacities, diagnosis is done by IgM antibody capture ELISA. In all areas where Zika virus is known to be endemic, other arboviruses are also endemic, making the serodiagnosis very difficult, especially for patients with a secondary Flavivirus infection. When dengue is suspected a combined NS1 antigen testing with IgM ELISA should be done<sup>[119]</sup>.

### **Diagnosis of Travelers**

Testing for Zika fever should be done in individuals who have (a) Travelled or resided in a Zika virus affected area in the past two weeks (b) Have 2 of the following features including fever, rash, conjunctivitis or arthralgia<sup>[120]</sup>.

When Zika fever is suspected, a ZIKV RT-PCR assay of serum or saliva should be performed during the acute phase. Otherwise a pan-Flavivirus RT-PCR assay with sequencing of the PCR product should be done if it is positive. Serodiagnosis should be preferred if the patient presents late but because Zika virus IgM testing is not conclusive; PRNT should be performed for confirmation of results.

The following flow charts gives an idea about the laboratory diagnosis of Zika virus infection<sup>[121]</sup> (**Figure 8**).



**Figure 8.** Laboratory diagnosis of arbovirus infection-acute and convalescent phase.

DENV: Dengue Virus; ZIKV: Zika Virus; CHIKV: Chikungunya Virus; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; PRNT: Plaque Reduction Neutralization test.

### Diagnosis of Zika Virus In Pregnant Women

*Testing for pregnant women should be done under the following circumstances*

Category		Tests done
Testing symptomatic pregnant women		RNA nucleic acid test (NAT) testing and Zika virus IgM testing
Testing Asymptomatic Pregnant Women	Asymptomatic pregnant women with ongoing possible Zika virus exposure	Testing three times during pregnancy using RNA NAT testing
	Asymptomatic pregnant women with recent possible exposure to Zika	Testing should be considered based on pretest counselling, risk assessment and patient preferences
Testing pregnant or postpartum women with possible Zika Virus exposure who have a foetus with ultrasound findings of birth defects or an infant with zika virus-associated birth defects		1. RNA NAT testing and Zika virus IgM testing 2. Amniotic fluid for Zika viral RNA testing 3. Placental and fetal tissues testing

**Table 1.** Testing for pregnant women.

History of recent travel to or residence in an area with risk of Zika (during pregnancy or the periconceptional period (the 6 weeks before last menstrual period or 8 weeks before conception) or Sexual intercourse during pregnancy with a person who travelled to or lives in an area with risk of Zika virus [122].

The CDC recommended tests done for pregnant women is given in **Table 1**.

## DIAGNOSIS OF INFANTS WITH POSSIBLE CONGENITAL ZIKA SYNDROME

Laboratory testing for Zika virus should be considered in any infant born to a woman with a positive test for Zika virus infection and for infants with features of congenital Zika syndrome. rRT PCR with the infant’s serum and urine sample should be performed within the first two days of birth. Cord blood is not recommended for the diagnosis because it can yield false positive or negative results through maternal blood contamination. The criteria for the diagnosis of infants with Congenital Zika syndrome is given in the **Table 2**.

**Table 2.** Interpretation of results of laboratory testing of infant's blood, urine and cerebrospinal fluid for congenital Zika virus infection.

Tests		Interpretation for congenital Zika virus
RT-PCR	IgM ELISA	infection
Positive	Positive or Negative	Confirmed infection
Negative	Positive	Probable infection
Negative	Negative	Negative

A positive IgM ELISA should be confirmed by PRNT but PRNT cannot distinguish between mother and infant antibodies. So it is usually advisable to wait for 18 months for the maternal antibodies to decrease and then perform PRNT. When PRNT is positive  $\geq$  18 months, it is conclusive of congenital Zika syndrome [123].

## EVALUATION OF INFANTS WITH CONGENITAL ZIKA SYNDROME

Infants with features suggestive of Congenital Zika syndrome should undergo an extensive,

- (a) Physical examination including size of occipito-frontal circumference, length, weight and evaluation of gestational age and dysmorphic features.
- (b) Assessment of Central nervous system for neurologic abnormalities
- (c) Splenomegaly, hepatomegaly and rash.
- (d) Cranial ultrasound.
- (e) Assessment of hearing by otoacoustic emissions or auditory brainstem response testing.
- (f) Ophthalmic examination [124].

## TREATMENT

There are no specific antivirals available for Zika virus. Management is supportive and includes adequate rest, fluid intake, antipyretics for controlling fever and analgesics. Anti-histamines can be taken to reduce the itching due to rashes. Aspirin and other non-steroidal anti-inflammatory drugs should be avoided until the diagnosis of dengue is excluded to avoid haemorrhage and Reye's syndrome in children [125].

## MANAGEMENT IN PREGNANCY

Only supportive treatment is recommended during pregnancy. Serial ultrasounds should be done to monitor the foetal growth and development. It is mandatory to refer to an infectious disease specialist with specialisation in pregnancy management [126].

## MANAGEMENT IN INFANTS WITH CONGENITAL ZIKA SYNDROME

Infants born with features of Congenital Zika syndrome should undergo a battery of tests like complete blood count, thyroid examination and liver function tests and consultations with various specialists.

- (a) Neurologist for determination of neuroimaging.
- (b) Infectious disease specialist for evaluation of other congenital infections like syphilis, toxoplasmosis, rubella, cytomegalovirus infection and herpes simplex virus infection.
- (c) Ophthalmologist for a detailed eye examination.
- (d) Endocrinologist for evaluation for hypothalamic or pituitary dysfunction.
- (e) Orthopaedician and physical therapist for the management of hypertonia and club foot.
- (f) Pulmonologist for concerns about aspiration.
- (g) Nutritionist, gastroenterologist, or speech therapist for the management of feeding issues.
- (h) Auditory brainstem response to assess hearing should be done during discharge and after few months. At each hospital visit, developmental milestones, feeding and growth, sleep and abnormal movements should be monitored. Eye

examination and auditory brain stem response should be performed and referred to the concerned specialist in case of any abnormalities. Family supportive services should be provided [127].

## NOVEL DRUGS FOR ZIKA VIRUS INFECTION

Currently there are no approved drugs or vaccines to treat or prevent Zika virus infection. Recently an autophagy inhibitor and inhibitor of endosomal TLR activation- Hydroxy chloroquine which is already approved for the treatment of malaria was found to reduce the severe fetal outcomes in pregnant mice [128,129].

Nanchangmycin, a polyether obtained from *Streptomyces nanchangensis* has insecticidal and anti-bacterial properties and blocks Zika virus replication in human osteosarcoma cells *in vitro* [130].

Obatoclox is an anti-cancer and small molecule Bcl-2 protein inhibitor and was found to prevent virus entry into the cell. These antagonists are effective only against viruses that require a low pH for fusion and entry such as Zika, West Nile virus and Yellow fever virus [131].

Chloroquine, which is an anti-malarial drug is an endocytosis blocking agent and inhibits Zika virus infection in human brain microvascular endothelial cells, human neural stem cells, and mouse neuroprogenitor-enriched neurospheres [132].

2'-C-methylated nucleosides were found to be efficient at causing premature termination of nucleic acid synthesis and so has become a promising drug for the future [133].

Sofosbuvir (Sovaldi) is a uridine nucleotide analog that is used for the treatment of chronic Hepatitis C virus infection. Its active metabolite is 2'-fluoro-2-C-methyl-UTP, which binds to the active site of NS5 and inhibited Zika virus replication *in vitro*. It was also found to decrease Zika viremia and delay mortality and morbidity in Zika infected mice [134].

Tetrapeptide-boronic acid effectively inhibited NS2B-NS3 protease. Berberine, luteolin, Epicatechin gallate and epigallocatechin gallate strongly inhibited NS3 and hence Zika virus replication [135].

Mycophenolic acid (MPA) is an Inosine monophosphate dehydrogenase inhibitor and showed inhibition of Zika virus activity in cell culture experiments that was confirmed using quantitative RT-PCR [136].

## DRUG REPURPOSING

New drugs may take many years to be licensed as safe and effective. So nowadays the strategy is to use FDA approved drugs for treatment of Zika infection. This drug repurposing has already been applied successfully to Ebola virus disease, Giardiasis, *Exserohilum rostratum* infection, Hepatitis C. Emricasan, a pan-caspase inhibitor inhibited caspase 3 activity and protected human cortical neural progenitors [137].

A variety of drugs like Azathioprine, Bortezomib, Cyclosporine A, Dactinomycin, Daptomycin, Digoxin, Mefloquine, Mebendazole, Micafungin, Sretraline, Pyrimethamine, Thioguanine [138]. But their safety profile should be considered in conditions like pregnancy.

## USE OF CONVALESCENT SERUM

The use of convalescent serum for treatment of Zika infection has been observed in Albino mice. It was found to reduce the infected brain cells, reduced caspase activity and reduced brain progenitor cell death. So this has a better scope for the treatment of pregnant women infected with Zika virus [139].

## USE OF MONOCLONAL ANTIBODIES

Monoclonal antibodies has been in research which was found to neutralize Zika virus E protein and thus reduced Zika virus replication and maternal fetal transfer [140].

## ALTERNATE MEDICINE TREATMENT

Ayurveda and Homeopathy are ancient medical sciences which claims to have remedies for many diseases without any side effects. *Eupatorium perfoliatum*, *Atropa belladonna*, *Rhus tox* may be used to relieve Zika virus symptoms [141]. *Atropa belladonna* has already been tried to treat Japanese encephalitis virus infection [142].

In Ayurvedic management of Zika virus, Giloya (*Tinospora cordifolia*), *Tulsi swarasa*, Turmeric, Ginger was found to have antipyretic, antibacterial and immunomodulator properties and could be used to reduce the symptoms of Zika fever [143].

## PREVENTION

There are no licensed vaccines for Zika prevention. So the prevention of Zika virus infection lies in the control of vector mosquitoes and personal protection measures.

## CONTROL OF VECTORS

The WHO recommends the Integrated Vector Management which consists of elimination of *Aedes aegypti* mosquitoes breeding sites. Buckets, tires, flower pots, planters and pools should be cleaned or covered or emptied to prevent the breeding of mosquitoes. Use of biological larvicides and Monomolecular films and oils can eliminate the mosquito larva [144,145].

Control of adult mosquitoes can be achieved by spraying of insecticides and mosquito traps, mosquito nets and air-conditioned rooms, fumigation of cargoes at ports [146-150]. Wolbachia infected mosquitoes and sterile insect technique can also be applied to the control of Zika virus [151,152].

## PERSONAL PROTECTION MEASURES

The best way to eradicate Zika infection is by following personal safety precautions which includes wearing long-sleeved shirts and long pants, usage of permethrin-treated clothing and gear, insect repellents, use of mosquito mesh on windows and doors, indoor insect spray and sleeping under a mosquito bed net [153].

Zika virus infection during pregnancy can have adverse outcomes on the baby. Therefore pregnant women should not travel to areas with high risk of Zika virus and take proper preventive measures to avoid mosquito bites. Male partners who are infected with Zika virus or travelled to a Zika endemic region should abstain from sexual intercourse or use condoms for about six months [154].

People who have travelled to or living in a Zika infected area should avoid blood donation. In an endemic region, blood should be screened for Zika virus by Nucleic acid amplification tests [155].

## ZIKA VACCINES

There has been difficulty in designing a suitable vaccine against Zika virus. There are approved vaccines for other flavivirus like Japanese encephalitis and Yellow fever. But there is the problem of cross reactivation of antibodies between Zika and other flaviviruses. Antibodies can lead to exacerbation of secondary flavivirus infections by Antibody dependent enhancement. The vaccine should be suitable for women of reproductive age and pregnant women. More than 38 Zika vaccine candidates have been reported to the WHO but none have been licensed so far. There are various vaccine approaches including live attenuated, inactivated, subunit, DNA, mRNA, proteins and viral vector vaccine platforms all of which express Zika virus structural proteins [156].

Shan et al. described a live attenuated vaccine with deletions in the 3'untranslated region of the Zika virus genome that prevented viral transmission during pregnancy and testis damage in mice. Live vaccines have the advantage of single dose efficacy, low cost and long duration of immunity which will be highly beneficial in low income countries, where the Zika virus is endemic [157].

Zika purified inactivated vaccine was developed by the Walter and Reed Army Institute of Research. In a study by the National Institute of Allergy and Infectious diseases the vaccine produced antibodies which provided protection to rhesus macaque monkeys against the Brazil and Puerto Rico strains of Zika virus [158].

DNA vaccines are safe, thermostable, simple to manufacture and multiple candidate antigens can be used. Larocca et al. developed a Zika vaccine expressing the M and Env immunogens based on the Brazil BeH815744 strain [159]. But DNA vaccine was found to be less potent than inactivated vaccine in a study by Abbink et al. [160].

Richner et al. designed a mRNA vaccine in which lipid nanoparticles encapsulate modified mRNA encoding Zika virus structural genes. The advantage of mRNA based vaccine is that it does not integrate into chromosomes which can lead to mutagenesis and oncogenesis unlike DNA vaccines. Two doses of immunization with modified mRNA encoding Zika Virus prM-E vaccine induced high levels of neutralizing antibodies that protected three mice strains [161].

Chahal et al. developed a modified dendrimer nanoparticle (MDNP)-based RNA replicon vaccine which induced anti-Zika virus IgG responses in C57BL/6 mice. They also identified a Class I MHC-restricted 9-mer peptide to which a CD8+ T cell response was induced in immunized C57BL/6 mice [162].

Pardi et al. designed a single low-dose intradermal immunization with lipid-nanoparticle-encapsulated nucleoside-modified mRNA (mRNA-LNP) encoding the prM and E glycoproteins of a strain from the Zika virus outbreak in 2013, that elicited durable neutralizing antibody responses in mice and macaque monkeys [163].

## DISCUSSION

Recombinant viral vectors are emerging as vaccine carriers due to their safety, easy to manufacture and capability of mounting a good immune response. An adenoviral-based Zika vaccines expressing the Zika virus envelope gene (E) fused to the T4 fibrin foldon trimerization domain (Efl) was delivered intradermally through microneedle array (MNA) in C57BL/6 mice. Pups born to the mice were protected against Zika virus with no neurological signs and weight loss <sup>[164]</sup>.

A single-shot recombinant rhesus adenovirus serotype 52 vector vaccine, expressing Zika virus premembrane and envelope, produced neutralizing antibodies and protected rhesus monkeys completely against Zika virus infection. Recombinant vesicular stomatitis virus (rVSV) expressing either the Zika virus envelope or precursor membrane was found to be a highly effective vector for vaccine purposes. The rVSV-ZIKV constructs showed immunogenicity in murine models and also maternal protective immunity in newborn mice born to female mice vaccinated with VSV vectored-Zika Virus <sup>[165]</sup>.

Virus like particles are structures resembling viruses where the immunogens have the ability to mount immune response and hence are potential vaccine candidates. Boigard et al. designed a Zika virus like particle expressing both the structural and non-structural proteins and demonstrated their vaccine efficiency in mice. The mice produced neutralizing antibodies even higher than the antibody titre of a person who recovered from Zika virus infection in Brazil in 2015 <sup>[166,167]</sup>.

Using immunoinformatics, linear and conformational B-cell epitopes and a cytotoxic T-lymphocyte (CTL) epitopes for Zika virus Envelope (E), N S3 and NS5 proteins were predicted. The binding interactions between the groove of class I major histocompatibility complex (MHC I) and peptides were studied. The stability of the peptide-MHC I complex was investigated by molecular modeling simulations. This study on peptide vaccines presents a set of peptides for future vaccine development against Zika virus <sup>[168]</sup>. An investigational vaccine which is designed to protect against multiple mosquito borne diseases called AGS-v was developed by the London based 86 triggers an immune response to four mosquito salivary proteins. It is evaluated in a Phase 1 clinical trial and is said to provide protection against Dengue, Zika, West Nile fever and Malaria <sup>[169]</sup>.

## CONCLUSION

Zika virus is a potential threat not only to the currently involved American territories but globally. Though it is an arboviral disease it is found to spread through sexual contact and blood transfusion. Much advance has been made about the virus in recent times, yet there are many knowledge gaps which needs to be filled. It leads to neurological complications and when pregnant women are infected, it can infect the foetus causing a plethora of manifestations. Laboratory testing and diagnosis. There are no effective anti virals against Zika virus discovered so far. Though many vaccines are in the pipeline, none of them are licensed for human use so far. So the only effective way to put an end to this disease is by following prevention measures such as control of the vector mosquitoes, safe blood transfusion and protected sex.

## REFERENCES

1. Dick GWA, et al. Zika Virus (I): Isolations and serological specificity. *Trans R Soc Trop Med Hyg.* 1952;46:509-520.
2. Musso D, et al. Zika Virus: Clinical Microbiology Reviews. 2016;29:487-524.
3. Plourde AR, et al. A literature review of Zika virus. *Emerg Infect Dis.* 2016;22:1185-1192.
4. Lanciotti RS, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis.* 2008;14:1232-1239.
5. Kuno G, et al. Full-length sequencing and genomic characterization of Bagaza, Kedougou and Zika viruses. *Arch Virol.* 2007;152:687-696.
6. Faye O, et al. Molecular evolution of Zika virus during its emergence in the 20th century. *PLoS Negl Trop Dis.* 2014;8.
7. Chambers TJ, et al. Flavivirus genome organization: expression and replication. *Annu Rev Microbiol.* 1990;44:649-688.
8. Vorou R, et al. Zika virus, Vectors, reservoirs, amplifying hosts and their potential to spread worldwide: What we know and what we should investigate urgently. *Int J Infect Dis.* 2016;48:85-90.
9. Macnamara FN. Zika virus: A report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg.* 1954;48:139-145.
10. Olson JG, et al. Zika virus: A cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg.* 1981;75:389-393.

11. Marchette NJ, et al. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. *Am J Trop Med Hyg.* 1969;18:411-415.
12. Hayes EB. Zika virus outside Africa. *Emerg Infect Dis.* 2009;15:1347-1350.
13. Duffy MR, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med.* 2009;360:2536-2543.
14. Hastings AK, et al. Zika virus and sexual transmission: A new route of transmission for mosquito-borne flaviviruses. *Yale J Biol Med.* 2017;90:325-330.
15. Haddow AD, et al. Genetic characterization of Zika Virus strains: Geographic expansion of the Asian Lineage. *PLoS Negl Trop Dis.* 2012;6:e1477.
16. Roth A, et al. Concurrent outbreaks of Dengue, Chikungunya and Zika virus infections -An unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012–2014. *Euro Surveill.* 2014;19:pii-20929.
17. Besnard M, et al. Evidence of perinatal transmission of Zika virus, French Polynesia. *Euro Surveill.* 2014;19: 20751.
18. Musso D, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia. *Euro Surveill.* 2014;19:20761.
19. Heukelbach J, et al. Pamplona de Goes Cavalcanti L. Zika virus outbreak in Brazil. *J Infect Dev Ctries.* 2016;10:116-120.
20. Calvet, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: A case study. *The Lancet Infect Dis.* 2016;16:653-660.
21. Ventura CV, et al. Zika virus in Brazil and macular atrophy in a child with microcephaly. *Lancet.* 2016;387:228.
22. Heukelbach J, et al. Zika virus outbreak in Brazil. *J Infect Dev Ctries.* 2016;10:116-120.
23. Deckard DT, et al. Male-to-male sexual transmission of Zika virus-Texas. *MMWR Morb Mortal Wkly Rep.* 2016;65:372-374.
24. Barjas-Castro ML, et al. Probable transfusion-transmitted Zika virus in Brazil. *Transfusion.* 2016;56:1684-1688.
25. M̃charles S, et al. Acute myelitis due to Zika virus infection. *Lancet.* 2016;1387:1481.
26. <http://www.who.int/csr/don/15-april-2016-zika-chile/en/>.
27. Guedes RD, et al. Zika virus replication in the mosquito *Culex quinque fuscatus* in Brazil. *Emerg Microb Infect.* 2017;6:e69.
28. Wikan N, et al. Zika virus from a Southeast Asian perspective. *Asian Pac J Trop Med.* 2017;10:1-5.
29. [http://www.paho.org/hq/index.php?option=com\\_content&view=article&id=13201&Itemid=2291](http://www.paho.org/hq/index.php?option=com_content&view=article&id=13201&Itemid=2291).
30. Darwish MA, et al. A sero-epidemiological survey for certain arboviruses (Togaviridae) in Pakistan. *Trans R Soc Trop Med Hyg.* 1983;77:442–445.
31. Olson JG, et al. A survey for arboviral antibodies in sera of humans and animals in Lombok: Republic of Indonesia. *Ann Trop Med Parasitol.* 1983;77:131-137.
32. Wolfe ND, et al. Sylvatic transmission of arboviruses among Bornean orangutans. *Am J Trop Med Hyg.* 2001;64:310-316.
33. Diallo D, et al. Zika virus emergence in mosquitoes in south-eastern Senegal. *PLoS One.* 2014;9:e109442-e109442.
34. Grard G, et al. Zika virus in Gabon (Central Africa) 2007: A new threat from *Aedes albopictus*. *PLoS Negl Trop Dis.* 2014;8:e2681.
35. Ledermann JP, et al. *Aedes hensilli* as a potential vector of Chikungunya and Zika viruses. *PLoS Negl Trop Dis.* 2014;8:e3188.
36. Petersen LR, et al. Zika Virus. *N Engl J Med.* 2016;374:1552-1563.
37. Zanluca C, et al. Zika virus: An overview. *Microbes Infect.* 2016;18:295-301.
38. Guo XX, et al. *Culex pipiens quinquefasciatus*: A potential vector to transmit Zika virus. *Emerg Microbes Infect.* 2016;5(9):e102.



39. Panchaud A, et al. Emerging role of Zika virus in adverse fetal and neonatal outcomes. *Clin Microbiol Rev.* 2016;29:659-694.
40. Foy BD, et al. Probable non-vector-borne transmission of Zika Virus, Colorado, USA. *Emerg Infect Dis.* 2011;17:880-882.
41. Atkinson B, et al. Detection of Zika Virus in Semen. *Emerg Infect Dis.* 2016;22:940.
42. Bierlaire D, et al. Zika virus and blood transfusion: The experience of French Polynesia. *Transfusion.* 2017;57:729-733.
43. <https://www.sciencedaily.com/releases/2016/12/161202150851.htm>.
44. Blood donation and Zika virus. *Editorial Lancet Infect Dis.* 2016;3:E398.
45. Filipe AR, et al. Laboratory infection with Zika virus after vaccination against yellow fever. *Arch Gesamte Virusforsch.* 1973;43:315-319.
46. Simpson DI. Zika virus infection in man. *Trans R Soc Trop Med Hyg.* 1964;58:335-338.
47. Leung GH, et al. Zika virus infection in Australia following a monkey bite in Indonesia. *Southeast Asian J Trop Med Pub Health.* 2015;46:460-464.
48. Colt S, et al. Transmission of Zika virus through breast milk and other breastfeeding-related bodily fluids: A systematic review. *PLoS Negl Trop Dis.* 2017;11:e0005528.
49. Barzon L, et al. Zika virus: From pathogenesis to disease control. *FEMS Microbiol Lett.* 2016;363:pii-202.
50. Hamel R, et al. Biology of Zika virus infection in human skin cells. *J Virol.* 2015;89:8880–8896.
51. Meertens, et al. Axl Mediates ZIKA virus entry in human glial cells and modulates innate immune responses. *Cell Reports.* 2017;18:324-333.
52. Bagasra O, et al. Cellular targets and receptor of sexual transmission of Zika Virus. *Appl Immunohisto chem Mol Morphol.* 2017;25:679-686.
53. Wu Y, et al. Zika virus evades interferon-mediated antiviral response through the co-operation of multiple non-structural proteins *in vitro*. *Cell Discovery.* 2017;3:17006.
54. Seissler T, et al. Hijacking of the Ubiquitin/Proteasome Pathway by the HIV Auxiliary Proteins. *Viruses.* 2017;9:322.
55. Grant A, et al. Zika virus targets human STAT2 to inhibit type I interferon signaling. *Cell Host Microbe.* 2016;19:882-890.
56. Xin QL, et al. Quantitative proteomic analysis of mosquito C6/36 cells reveals host proteins involved in Zika virus infection. *J of Virol.* 2017;91:e00554-17.
57. Gourinat AC, et al. Detection of Zika Virus in urine. *Emerg Infect Dis.* 2015;21:84-86.
58. Calvet GA, et al. ZIKABRA study team: Study on the persistence of Zika virus (ZIKV) in body fluids of patients with ZIKV infection in Brazil. *BMC Infect Dis.* 2018;18:49.
59. Mansuy JM, et al. Zika virus in semen and spermatozoa. *The Lancet Infect Dis.* 2016;16:1106-1107.
60. Lesser J, et al. Times to key events in the course of Zika infection and their implications: A systematic review and pooled analysis. *Bull World Health Organ.* 2016.
61. Noronha L, et al. Zika virus damages the human placental barrier and presents marked fetal neurotropism. *Mem Inst Oswaldo Cruz.* 2016;111:287–293.
62. Ishikawa T, et al. FC gamma receptor IIb participates in maternal IgG trafficking of human placental endothelial cells. *Int J Mol Med.* 2015;35:1273-1289.
63. Tsunoda I, et al. Neuropathogenesis of Zika Virus Infection: Potential roles of antibody-mediated pathology. *Acta medica Kinki University.* 2016;41:37-52.
64. Dang J, et al. Zika Virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3. *Stem Cell.* 2016;19:1-8.
65. Collantes-Fernandez E, et al. Infected dendritic cells facilitate systemic dissemination and trans placental passage of the obligate intracellular parasite *Neospora caninum* in mice. *PLoS ONE.* 2012;7:e32123.

66. Garcez PP, et al. Zika virus impairs growth in human neurospheres and brain organoids. *Science*. 2016;352:816-818.
67. Dirlikov E, et al. Postmortem findings in patient with Guillain-Barré syndrome and Zika virus infection. *Emerg Infect Dis*. 2018;24:114-117.
68. Manangeswaran M, et al. Zika (PRVABC59) Infection is associated with T-cell infiltration and neurodegeneration in CNS of immunocompetent neonatal C57Bl/6 Mice. *PLoS Pathog*. 2016;12:e1006004.
69. Li S, et al. Zika virus fatally infects wild type neonatal mice and replicates in central nervous system. *Viruses*. 2018;10:49.
70. Morrison TE, et al. Animal models of Zika virus infection, Pathogenesis, and Immunity. *J of Virol*. 2017;91:e00009-17.
71. Miner JJ, et al. Zika virus infection in mice causes panuveitis with shedding of virus in tears. *Cell Rep*. 2016;16:3208-3218.
72. Clancy CS, et al. Comparative histopathologic lesions of the male reproductive tract during acute infection of Zika virus in AG129 and IFNAR<sup>-/-</sup> Mice. *Am J Pathol*. 2018;pii:S0002-9440.
73. Tang WW, et al. A mouse model of Zika virus sexual transmission and vaginal viral replication. *Cell Reports*. 2016;17:3091-3098.
74. Miner JJ, et al. Zika Virus infection during pregnancy in mice causes placental damage and fetal demise. *Cell*. 165:1081-1091.
75. Adams Waldorf KM, et al. Fetal brain lesions after subcutaneous inoculation of Zika virus in a pregnant nonhuman primate. *Nature Med*. 2016;22:1256-1259.
76. Vasilakis N, et al. Zika virus: Diagnosis, Therapeutics and Vaccine. *ACS Infect Dis*. 2016;2:170-172.
77. Blum J. Zika without symptoms in returning travellers: What are the implications. *Travel Med Infect Dis*. 2016;14:16-20.
78. Tappe D, et al. First case of laboratory confirmed Zika virus infection imported into Europe 2013. *Euro Surveill*. 2014;19:20685.
79. Chan JF, et al. Zika fever and congenital Zika syndrome: An unexpected emerging arboviral disease. *J Infect*. 2016;72:507-524.
80. Moghadam SR, et al. Zika virus: A review of literature. *Asian Pac J Trop Biomed*. 2016;6:989-994.
81. Krauer F, et al. Zika Virus Infection as a cause of congenital brain abnormalities and Guillain–Barré syndrome: Systematic review. *PLoS Med*. 2017;14:e1002203.
82. Schwartzmann PV, et al. Zika virus meningoencephalitis in an immunocompromised patient. *Mayo Clin Proc*. 2017;92:460-466.
83. Carteaux G, et al. Zika virus associated with meningoencephalitis. *N Engl J Med*. 2016;374:1595-1596.
84. Millichap JG. Zika virus infection and microcephaly. *Pediatr Neurol Briefs*. 2016;30:8.
85. Melo AS, et al. Congenital Zika virus infection: Beyond neonatal microcephaly. *JAMA Neurol*. 2016;73:1407-1416.
86. De Paula Freitas B, et al. Ocular findings in infants with microcephaly associated with presumed Zika virus congenital infection in Salvador, Brazil. *JAMA Ophthalmol*. 2016;10:1001.
87. Linden VV, et al. Congenital Zika syndrome with Arthrogryposis: Retrospective case series study. *BMJ*. 2016;354:l3899.
88. Martines RB, et al. Notes from the field: Evidence of Zika virus infection in brain and placental tissues from two congenitally infected new-borns and two fetal losses-Brazil, 2015. *MMWR Morb Mortal Wkly Rep*. 2016;65:159-160.
89. Meaney- Delman D, et al. Zika virus infection among US pregnant travellers- August 2015 to February 2016. *MMWR Morb Mortal Wkly Rep*. 2016;65:211-214.
90. [http://www.who.int/ihr/publications/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/](http://www.who.int/ihr/publications/WHO_CDS_CSR_LYO_2004_11/en/).
91. Lamb LE, et al. Advantage of urine based molecular diagnosis of Zika virus. *Int Urol Nephrol*. 2016;48:1961-1966.

92. Atkinson B, et al. Presence and persistence of Zika virus RNA in semen, UK, 2016. *Emerg Infect Dis.* 2017;23:611-615.
93. [http://apps.who.int/iris/bitstream/10665/204671/1/WHO\\_ZIKV\\_LAB\\_16.1\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/204671/1/WHO_ZIKV_LAB_16.1_eng.pdf).
94. Xavier AR, et al. Clinical and laboratory diagnosis of Zika fever: An update. *J Bras Patol Med Lab.* 2017;53:252-257.
95. Bollweg BC, et al. Optimization of commercially available Zika virus antibodies for use in a laboratory developed immunohistochemical assay. *J Path Clin Res.* 2017;4:19-25.
96. Kim SW, et al. Immunohistochemistry for pathologists: Protocols, Pitfalls, and Tips. *J of Pathol Transl Med.* 2016;50:411-418.
97. Irene Bosch, et al. Rapid antigen tests for dengue virus serotypes and Zika virus in patient serum. *Sci Transl Med.* 2017;9:eaan1589.
98. Taylor RM. Studies on certain viruses isolated in the tropics of Africa and South America: Their growth and behavior in the embryonated hen egg. *J Immunol.* 1952;68:473-494.
99. Way JH, et al. Comparative studies of some African arboviruses in cell culture and in mice. *J Gen Virol.* 1976;30:123-130.
100. Nikolay A, et al. Propagation of Brazilian Zika virus strains in static and suspension cultures using vero and BHK cells. *Vaccine.* 2017:S0264-410X.
101. Buathong R, et al. Detection of Zika Virus infection in Thailand (2012-2014). *Am J Trop Med Hyg.* 2015;93:380-383.
102. Barreto-Vieira DF, et al. Structural investigation of C6/36 and vero cell cultures infected with a Brazilian Zika virus. *PLoS ONE.* 2017;12:e0184397.
103. Priyamvada L, et al. Human antibody responses after dengue virus infection are highly cross-reactive to Zika virus. *Proc Natl Acad Sci USA.* 2016;113:7852-7857.
104. [http://www.paho.org/hq/index.php?option=com\\_docman&task=doc\\_view&gid=36616&Itemid=270&lang=en](http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=36616&Itemid=270&lang=en).
105. Panning M. Zika Virus Serology: More diagnostic targets, More reliable answers. *E Bio Med.* 2017;16:12-13.
106. Landry ML, et al. Laboratory diagnosis of Zika virus infection. *Arch Pathol Lab Med.* 2017;141:60-67.
107. <https://www.cdc.gov/zika/pdfs/zika-mac-elisa-instructions-for-use.pdf>.
108. Keasey SL, et al. Antibody responses to Zika virus infections in environments of flavivirus endemicity. *Clin Vaccine Immunol.* 2017;24:e00036-17.
109. Shan C, et al. A rapid Zika diagnostic assay to measure neutralizing antibodies in patients. *E Bio Med.* 2017;17:157-162.
110. Waggoner JJ, et al. Zika virus: Diagnostics for an emerging pandemic threat. *J Clin Microbiol.* 2016;54:860-867.
111. Faye O, et al. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught Mosquitoes. *J Virol.* 2013;10:311.
112. Calvo EP, et al. Easy and inexpensive molecular detection of Dengue, Chikungunya and Zika viruses in febrile patients. *Acta Trop.* 2016;163:32-37.
113. Gu SH, et al. Whole-genome sequence analysis of Zika virus, Amplified from urine of traveller from the Philippines. *Virus Genes.* 2017;53:918-921.
114. Guevara C, et al. Full-genome amplification and sequencing of Zika viruses using a targeted amplification approach. *J Virol Methods.* 2017;248:77-82.
115. D'Ortenzio E, et al. Evidence of sexual transmission of Zika virus. *N Engl J Med.* 2016;374:2195-2198.
116. Musso D, et al. Detection of Zika virus in saliva. *J Clin Virol.* 2015;68:53-55.
117. Prisant N, et al. Zika virus in the female genital tract. *Lancet Infect Dis.* 2016;16:1000-1001.
118. Bingham AM, et al. Comparison of test results for Zika virus RNA in urine, Serum and Saliva specimens from persons with travel associated Zika virus disease- Florida. *MMWR Morb Mortal Wkly rep.* 2016;65.
119. Fry SR, et al. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis.* 2011;5:e1199.

- 120.<https://www.cdc.gov/zika/hc-providers/testing-guidance.html>.
- 121.<http://iris.paho.org/xmlui/handle/123456789/18602>.
- 122.Oduyebo T, et al. Update: Interim guidance for health care providers caring for pregnant women with possible Zika virus exposure- United States (Including US Territories). *MMWR Morb Mortal Wkly Rep.* 2017;66:781-793.
- 123.Russell K, et al. Update: Interim guidance for the evaluation and management of infants with possible congenital Zika virus infection, United States, August 2016. *MMWR Morb Mortal Wkly Rep.* 2016;65:870-878.
- 124.<http://www.who.int/csr/resources/publications/zika/assessment-infants/en/>.
- 125.<https://www.cdc.gov/zika/symptoms/index.html>.
- 126.Petersen EE, et al. Interim guidelines for pregnant women during a Zika virus outbreak, United States. *MMWR Morb Mortal Wkly Rep.* 2016;65:30-33.
- 127.<http://pediatrics.aappublications.org/content/pediatrics/137/1/e20153908.full.pdf>.
- 128.Kuznik A, et al. Mechanism of endosomal TLR inhibition by antimalarial drugs and imidazoquinolines. *J Immunol.* 2011;186:4794-804.
- 129.Cao B, et al. Inhibition of autophagy limits vertical transmission of Zika virus in pregnant mice. *J Exp Med.* 2017;;214:2303-2313.
- 130.Rausch K, et al. Screening bioactives reveals nanchangmycin as a broad spectrum antiviral active against Zika virus. *Cell reports.* 2017;18:804-815.
- 131.Varghese F S, et al. Obatoclox inhibits alphavirus membrane fusion by neutralizing the acidic environment of endocytic compartments. *Antimicrob Agents Chemother.* 2017;61:e02227-16.
- 132.Delvecchio R, et al. Chloroquine, an endocytosis blocking agent, Inhibits Zika virus infection in different cell models. *Viruses.* 2016;8:E322.
- 133.Eyer L, et al. Nucleoside inhibitors of Zika virus. *J Infect Dis.* 2016;214:707-711.
- 134.Reznik SE, et al. Sofosbuvir: An anti-viral drug with potential efficacy against Zika infection. *Int J Infect Dis.* 2017;55:29-30.
- 135.Munjal A, et al. Advances in developing therapies to combat Zika virus: Current knowledge and future perspectives. *Frontiers in Microbiol.* 2017;8:1469.
- 136.Goebel S, et al. A sensitive virus yield assay for evaluation of Antivirals against Zika Virus. *J Virol Methods.* 2016;238:13-20.
- 137.Xu M, et al. Identification of small molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nature med.* 2016;22:1101-1107.
- 138.Barrows NJ, et al. A screen of FDA-approved drugs for inhibitors of Zika virus infection. *Cell host & microbe.* 2016;20:259-270.
- 139.Wang S, et al. Transfer of convalescent serum to pregnant mice prevents Zika virus infection and microcephaly in offspring. *Cell Res.* 2017;27:158-160.
- 140.Sapparapu G, et al. Neutralizing human antibodies prevent Zika virus replication and fetal disease in mice. *Nature.* 2016;540:443-447.
- 141.Saxena SK, et al. Zika virus outbreak: An overview of the experimental therapeutics and treatment. *Virus Dis.* 2016;27:111-115.
- 142.Bandyopadhyay B, et al. Decreased intensity of Japanese encephalitis virus infection in chick chorioallantoic membrane under influence of ultradiluted Belladonna extract. *Am J Infect Dis.* 2010;6:24-28.
- 143.Sanju K, et al. Ayurvedic approach of Zika virus infection: A new challenge. *WJPMR.* 2017;3:127-129.
- 144.[http://www.paho.org/hq/index.php?option=com\\_content&view=article&id=11605:2016-paho-statement-on-zika-transmission-prevention-&Itemid=41716&lang=en](http://www.paho.org/hq/index.php?option=com_content&view=article&id=11605:2016-paho-statement-on-zika-transmission-prevention-&Itemid=41716&lang=en).
- 145.<https://www.cdc.gov/zika/vector/vector-control.html>.
- 146.Sharma A, et al. Zika Virus: Transmission, detection, control and prevention. *Frontiers Microbiol.* 2017;8:110.

147. Anderson AL, et al. Effectiveness of mist-blower applications of malathion and permethrin to foliage as barrier sprays for salt marsh mosquitoes. *J Am Mosq Control Assoc.* 1991;7:116-117.
148. Kitron U, et al. Quantifying the spatial dimension of dengue virus epidemic spread within a tropical urban environment. *PLoS Negl Trop Dis.* 2010;4:e920.
149. Williams CR, et al. Field efficacy of the BG-Sentinel compared with CDC backpack aspirators and CO<sub>2</sub>-baited EVS traps for collection of adult *Aedes aegypti* in Cairns, Queensland, Australia. *J Am Mosq Control Assoc.* 2006;22:296-300.
150. Rather IA, et al. Prevention and control strategies to counter ZIKA epidemic. *Frontiers in Microbiol.* 2017;8:305.
151. Jiggins FM. The spread of *Wolbachia* through mosquito populations. *PLoS Biol.* 2017;15:e2002780.
152. Dyck, et al. *Sterile insect technique: Principles and practice in area-wide integrated pest management.* Springer. 2005;1-4.
153. <https://www.cdc.gov/zika/prevention/prevent-mosquito-bites.html>.
154. <https://www.cdc.gov/zika/prevention/sexual-transmission-prevention.html>.
155. Marano G, et al. Zika virus and the never-ending story of emerging pathogens and transfusion medicine. *Blood Transfusion.* 2016;14:95-100.
156. Lagunas FA, et al. Current trends in Zika vaccine development. *J Virus Erad.* 2017;3:124-127.
157. Shan C, et al. A live-attenuated Zika virus vaccine candidate induces sterilizing immunity in mouse models. *Nat Med.* 2017;23:763-767.
158. Abbasi J. First inactivated Zika vaccine trial. *JAMA.* 2016;316:2588.
159. Larocca RA, et al. Vaccine protection against Zika virus from Brazil. *Nature.* 2016;536:474-478.
160. Abbink P, et al. Protective efficacy of multiple vaccine platforms against Zika virus challenge in Rhesus monkeys. *Science.* 2016;353:1129-1132.
161. Richner JM, et al. Modified mRNA vaccines protect against Zika Virus infection. *Cell.* 2017;169:176.
162. Chahal JS, et al. An RNA nanoparticle vaccine against Zika virus elicits antibody and CD8<sup>+</sup> T- cell responses in a mouse model. *Cell.* 2017;168:1114-1125.
163. Pardi N, et al. Zika virus protection by a single low dose nucleoside modified mRNA vaccination. *Nature.* 2017;543:248-251.
164. Kim E, et al. Preventative vaccines for Zika virus outbreak: Preliminary evaluation. *E Bio Med.* 2016;13:315–320.
165. Betancourt D, et al. Cutting edge: Innate immune augmenting vesicular stomatitis virus expressing Zika virus proteins confers protective immunity. *J Immunol.* 2017;198:3023-3028.
166. Shirbaghaee Z, et al. Different applications of virus-like particles in biology and medicine: Vaccination and delivery systems. *Biopolymers.* 2016;105:113-132.
167. Boigard H, et al. Zika virus-like particle (VLP) based vaccine. *PLoS Negl Trop Dis.* 2017;11:e0005608.
168. Usman MM, et al. Towards peptide vaccines against Zika virus: Immunoinformatics combined with molecular dynamics simulations to predict antigenic epitopes of Zika viral proteins. *Scientific Reports.* 2016;6:37313.
169. <https://www.nih.gov/news-events/news-releases/nih-begins-study-vaccine-protect-against-mosquito-borne-diseases>.