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## ZIKA VIRUS (ZIKV) - A REVIEW APPROACH

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### ABSTRACT

Zika Virus (ZIKV) is promptly spreading across the America and its disastrous outcomes for pregnant women and infants have driven this earlier ignored pathogen into the eminence. Clinical Symptoms are fever, joint pain or rash and conjunctivitis. The Zika virus (ZIKV), first discovered in 1947, has emerged as a global public health threat over the last decade, with the accelerated geographic spread of the virus noted during the last 5 years. The first outbreak of ZIKV has been reported in the Pacific area in 2007, followed by the second large outbreak in 2013/2014 and subsequently the virus broaden in other Pacific islands. DNA vaccine, recombinant subunit vaccine, ZIKV purified inactivated vaccine, and chimeric vaccines of various such vaccine platforms have shown robustic effect *in vitro* and *in vivo* trials. Numeral drugs such as Sofosbuvir, BCX4450, NITD008 and 7-DMA are ready to set foot in phase-I clinical trial because of approved anti-ZIKV activity. Monoclonal based antibodies had shown a promising result in pregnant women. Without further knowledge of the pathophysiology involved the true risk of ZIKV to the unborn remains difficult to evaluate and remediate. Accurate, portable, and inexpensive point-of-care testing is required to better identify cases and manage the current and future outbreaks of ZIKV, including optimization of preventive approaches and the identification of more effective risk reduction strategies. In addition, much more work needs to be done to produce an effective vaccine. Given the rapid geographic spread of ZIKV in recent years, a coordinated local, regional, and global effort is needed to generate sufficient resources and political traction to effectively halt and contain further expansion of the current outbreak.

**Keywords:** Zika Virus, Emergence, Prevention, *In vitro* and *In vivo* Models

## INTRODUCTION:

Aedes mosquito borne diseases have turned into one of the utmost hazards to human population. Zika virus infection is a mosquito-borne ailment like dengue (DEN) and chickengunya (CHIK) viruses [1]. In several subtropical and tropical countries Aedes mosquito-borne infections continued to be the main cause of deaths [2].

Zika virus was found to be a potential hazard in urban areas. The increase in population in urban areas led to increase in the need for potable water and all storage household practices and perfect making of breeding habitats for ex: Aegypti mosquitoes which otherwise leads to spreading of infections. In 21<sup>st</sup> century, it is going to be hazardous due to this mosquito borne viral diseases.



\*Adapted from <https://www.webmd.com/a-to-z-guides/zika-virus-symptoms-prevention>

## Natural History:

Natural history aids in better understanding to do research and create awareness among the people to control the spread of infection. Men effected with ZIKV may shed ZIKV ribonucleic acid (RNA) in semen for up to 6 months after initial attack [3], and symptomless blood donors who tested positive for ZIKV RNA appear to shed ZIKV in semen with similar manner in those who identified

with clinical diseases [4]. In females, the duration of shedding in genital secretions and breast milk is not known. The research on implicit role of pre-existing immunity to flaviviruses in ZIKV pathogenesis needs to be further developed to dig possible links between Congenital Zika Syndrome (CZS) and neurological illness rigidity and subsisting immunity to flaviviruses [5].

## Epidemiology of ZIKA Virus:

- In 1947, the virus was first isolated from a Rhesus macaque in the Zika Forest of Uganda [6].
- In Nigeria, it was identified for the first time in humans in 1968.
- The first major flare-up, with 185 cases was reported in 2007 in the Yap islands.
- The country which is currently experiencing the largest epidemic ever is Brazil which is recorded with 440,000 to 1,300,000 alleged cases and were reported by Brazilian health authorities and the first cases that were confirmed is in May 2015.
- As of 21<sup>st</sup> October 2015, 3174 cases and 38 deaths were reported from Microcephaly in Brazil.
- The total deaths recorded as of 21<sup>st</sup> October 2015 is 152 [6].

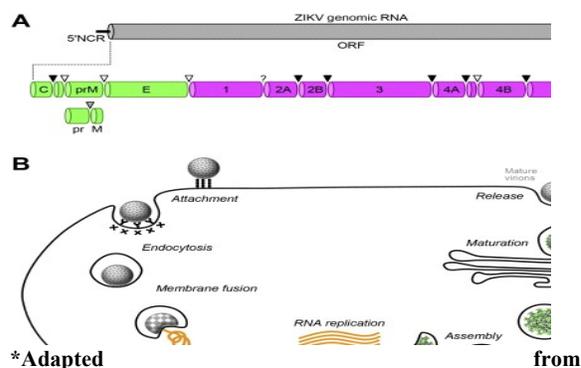
## Virology of the ZIKA virus:

Zika virus is a fresh growing virus which belongs to Flaviviridae family of viruses [7]. This family constitutes of 4 genera: Flavivirus, Hepacivirus, Pegivirus, and Pestivirus [8]. Zika

virus belongs to the Flavivirus genus, which is immunogenically and ontogenetically related to the Spondweni virus [9, 10]. Several chief human microbes are included in this genus, including Dengue, West Nile, Yellow fever, tick-borne encephalitis, Japanese encephalitis, Murray Valley encephalitis and St. Louis encephalitis viruses. These viruses are correlated with a scale of infections from symptomless or self-limiting flushed infections to some lethal illnesses such as haemorrhage, shock, meningitis and encephalitis [11].

All members in the Flaviviridae family have enfolded viruses with a single-stranded RNA genome of positive polarity. It contains one open reading frame (ORF) with two flanking noncoding regions (at 5' and 3' end) [12]. The genomes are 5' capped in absence of 3' poly (A) tail.

A polyprotein coded by the ORF is then progressed into three structural proteins *i.e.*, (the envelope (E), the capsid (C) and the precursor of the membrane (prM)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [12]. The ssRNA is clenched inside an icosahedral capsid shaped from 12-kDa protein blocks; the nucleocapsid is enclosed by a host-derived membrane constituting two viral glycoprote



\*Adapted from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6401583/>

### Transmission:

ZIKV is transmitted chiefly to humans through the bite of infected mosquitoes, but it can also elapse from mother to child during pregnancy and can also drive through sexual contact, breastfeeding, or blood transfusion.

### Vector-Borne Transmission:

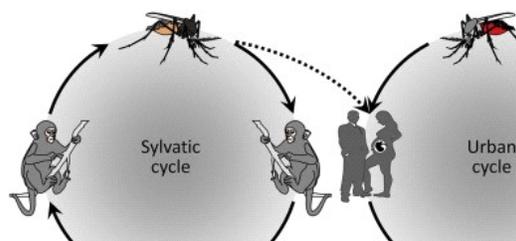
ZIKV is an arthropod-borne virus (arbovirus) that is transferred by mosquito vectors, with two definite transmission cycles [13] a sylvatic cycle and urban cycle.

#### 1. Sylvatic cycle:

Zika virus (ZIKV) emerged and continues to flow in a sylvatic transmission cycle between non-human primate hosts and arboreal mosquitoes in tropical Africa [14]. In recent times ZIKV infested the Americas, where it poses a menace to human health, specifically to pregnant women and their infants. Examining the risk of ZIKV that build a sylvatic cycle in the Americas, aiming on Brazil. Presentation of mathematical dynamic transmission model after reviewing the natural history to evaluate the possibility of development of a sylvatic ZIKV contagious cycle in non-human primates

and/or other mammals and arboreal mosquito vectors in Brazil.

## **2. Urbancycle:**



\*Adapted from

<https://www.sciencedirect.com/science/article/pii/S0165572816304830>

The transference of ZIKV in an urban cycle is trusted to be intervene predominantly by two *Aedes* species mosquitoes: *A. aegypti*, identified by a bright lyre-shaped dorsal pattern with white bands on its legs, and *A. albopictus*, distinguished by a single longitudinal dorsal stripe with white bands on its legs [11]. However, *A. aegypti* is contemplated to be the primary vector linked with ZIKV flare-up, as supported by

- (1) Isolating or determining the virus in a pool of *A. aegypti* mosquitoes [15].
- (2) Showing the sensitivity of *A. aegypti* mosquitoes to infection with the virus [16]; and
- (3) Determining the transmission of the *A. albopictus* has been suggested to be indulged in the urban transmission in Gabon, where five human sera and two *A. albopictus* pools acquired in an urban setting in 2007 were positive for ZIKV [17].

During daylight hours both *A. aegypti* and *A. albopictus* are active [18] and are widely circulated entirely to the tropical and

subtropical regions of the world, with the dwelling of *A. albopictus* expanding further into cool temperate regions [19]. In the US, both *A. aegypti* and *A. albopictus* are present entirely in the southern and south-eastern half of the country), according to a recent assessment by the Centres for Disease.

## **Neurological Complications:**

The data file which contains information on 418 patients with neurological complications and latest chronicle of fevered diseases agreeable with ZVD [20]. Despite of GBS, it also includes other conditions like myelitis and meningoencephalitis shutting out cases with microcephaly and other congenital defects. Four cases in this data file were laboratory-confirmed for ZIKV by reverse-transcriptase PCR [21].

Case study of patients with neurological complications were analysed using case definitions from the Brighton collaboration working batch for GBS, myelitis, encephalitis, and Acute Disseminated Encephalomyelitis (ADEM) [22, 23]. The Brighton Collaboration developed standard case definitions and guidelines for neurologic adverse effects following immunization with the aim of enhancing comparability of vaccine safety data.

## **Risk of Guillain–Barre Syndrome:**

Guillain-Barré syndrome (GBS) is an unusual autoimmune disorder distinguished by ongoing weakness and declined deep tendon reflexes

following infection or, intermittently, vaccination [24]. Increased GBS prevalence has been reported in countries affected by Zika virus [25], a flavivirus transmitted chiefly by species of Aedes mosquitoes [26]. Zika has also been associated to Guillain-Barre syndrome, a condition in which the immune system damages the nerves. It primarily spreads through mosquitoes, despite the fact that some cases of sexual transmission have been reported.

#### **Signs and Symptoms:**

The bulk of people affected with ZIKA virus do not develop symptoms. Symptoms are mild and usually last for 2-7 days which generally includes fever, rash, conjunctivitis, muscle and joint pain, malaise, and headache [27].

#### **Diagnosis:**

This generally involves two diagnosis types. The first one involves the spotting of the antibodies brought on as a result of ZIKV infection. The second type is rooted on detecting viral components or/and whole virus particle. In order to detect the live virus, viral proteins and ZIKV RNA, isolation of virus, immunoassay, and RT-PCR have been developed. ZIKV RNA can be spotted in serum; urine and saliva samples acquired at the acute phase of the infection using RT-PCR with more selectivity, and have low sensitivity results [28]. As early as 3 days of infection, several studies spelled out the detection of anti-ZIKV virus antibodies (IgM) but whereas, recognized by day 8 in most of the

individuals. Neutralizing antibodies evolved within the early 5 days of start of infection [29]. According to documented studies with associated flaviviruses, the evolvement of serological detection assays using ZIKA envelope or NS1 proteins and subviral particles encrypting mutations in the extremely cross-reactive fusion loop in domain II might have increased selectivity of serological assays considerably [56]. RT-PCR test that is done in urine up to 10 to 14<sup>th</sup> day from disease progression or in blood up to the 15<sup>th</sup> day confirms diagnosis [30]. Using amniotic fluid, tissue and blood samples from microcephalic new-born's ZIKV RNA can be diagnosed. Viral antigens were detected in mononuclear cells present in brain tissue of neonates. Ophthalmologic examination discloses visual disabilities and brain imaging discloses brain anomalies. This proves that ZIKV owns enough capacity to pass the placental barrier, effect foetal neural cells, multiply, and cause neurological damage to foetus [31]. Modern development of SYBR Green based one-step real-time RT-PCR assay has proved that real-time assay permits highly specific and susceptible spotting of ZIKV in cell samples. ZIKV titre as low as 1 PFU/mL can easily be noted through this assay [32]. Through transmission electron microscopy ZIKV effected cell lines were evaluated. Like other Flaviviruses particularly dengue virus, ZIKV also confirmed the sensitivity of the

Vero cell line to ZIKV reproduction [33]. Evaluation of tissue samples from a microcephalic aborted fetus provided brief figures at the microscopic, histological, and immunocytochemical levels about neurological destruction persuaded by a congenital infection with ZIKA virus [34].

#### **Treatment:**

There is no verified therapeutic or inhibitory medication obtainable for ZIKV. Patients with asymptomatic or uncomplicated Zika fever doesn't require treatment [35, 38]. Two master plans can be followed for ZIKV antiviral discovery. The first is to refocus on existing clinical medications which have been formerly developed for other disease indications for ZIKV treatment. Antiviral activity has been shown by some drugs in cell culture. The second master plan is to build up obstructers of ZIKV. Therapeutic antibodies are also applicable for treatment of ZIKV. One provocation for the growth of ZIKV antiviral shows higher risk of adverse effects in pregnant women [36]. Treatment of Zika fever is encouraging and includes acetaminophen for fever, headache or myalgia. Because of the risks of bleeding in those with thrombocytopenia, aspirin should be prevented [37] and increasing Reye's syndrome in children [35]. Non-steroidal anti-inflammatory drugs are also not recommended because of the high risk of haemorrhagic syndrome [38]. Enough rehydration for fluid loss should be put

in. For early prescription of intravenous immunoglobulins, it is important to diagnose neurological complications especially GBS [35]. Some of the drugs that focus hepatitis C can have some special effects on ZIKV based advanced studies. Anti-malarial hydroxychloroquine is an autophagy inhibitor and *in vitro* testing has shown prevention of dengue virus. Amodiaquine works through blockage of autophagy and prevents Zika virus infection.

#### **How Can ZIKA be Prevented?**

Measures to be taken to prevent ZIKA virus as follows:

- Usage of mosquito repellent's as per the product labelling.
- Wearing completely covered clothes like full sleeves and long trousers.
- Sleeping in air-conditioned rooms [39].

#### **Control Strategies:**

##### **Mechanical Control Measures:**

These are the age old techniques, which have been adopted and rehearsed out in various countries; as they are easy and cost-effective methods for the control of mosquito residents. Mechanical control measures include removal of stagnant water which otherwise leads to tendency of mosquito breeding. Streets, buildings and housing units must be maintained properly and cleaned. Personal and community hygiene is the prime thing to prevent mosquito breeding. Mosquito-proof

water storage options and use of mosquito nets on windows should be encouraged.

#### **Chemical Control Measures:**

Chemical treatment involves the use of pyrethroids, organochloride, and organophosphorus, which chiefly acts on the nervous system of the vector [40]. In various mosquito species, insecticides like Imidacloprid, Thiachloprid and Thiamethoxam have larvicidal and adulticidal efficacies. The use of fogging with insecticides is acquired in the outdoor environment to deal with the vector inhabitants though this can lead to development of defiance among them [41, 42]. The growth of resistance and bioaccumulation are major problems linked with the use of chemicals to manage mosquitoes [43]. The wide range of toxic action upon usage of these chemical products effects several organisms, in some cases to all arthropods or even the rest of aquatic fauna as well as mammals and birds.

#### **Biological Control Measures:**

In order to combat with mosquito population, biological control measures have been exploited as an alternative to the use of chemicals. Various biological measures, such as the use of bacteria, fungi, plants, and fish engaged to control the extension and propagation of the mosquito inhabitants.

#### ***In vitro* and *In vivo* models for studying ZIKA virus biology:**

##### ***In Vitro* Models:**

##### **Cell Lines:**

Viral improvement kinetics in cell culture is advantageous to distinguish various viral strains, and also to recognize and test drug and vaccine candidates. Commonly, cell lines are contemplated to be genetically and phenotypically homogenous, but biological variations among the same cell line from various laboratories might occur and researchers should be conscious of this probability. ZIKV replicates well in Vero and C6/36 cells like other viruses, which are extensively used for virus isolation from both clinical and mosquito samples [44, 45]. The susceptibility of distinct human and animal cell lines has been systematically distinguished. Here we will recapitulate a few studies using various cell lines. Human cell lines acquired from placenta (JEG-3), neurones (SF268), muscle (RD), retina (ARPE19), lung (Hep-2 and HFL), colon (Caco-2) and liver (Huh-7) permitted productive ZIKV replication and exhibited cytopathic effects (CPE). In opposition, cell lines arise from prostate (LNCaP), testes (833KE), cervix (HELA), endometrium (HOSE6-3) and kidneys (HEK) supported viral reproduction but did not show CPE. Among animal cell lines, those of nonhuman primates (Vero and LLCMK2), swine (PK-15), rabbit (RK-13), hamster (BHK21) and chicken (DF-1) origin allowed productive ZIKV duplication [46]. Guinea pig lung fibroblast cells (JH4) are also prone to infection [47]. Recently, a human

neuroblastoma cell line (SH-SY5Y) has been shown to be very advantageous for the assessment of antiviral drugs in opposition to ZIKV [48].

ZIKV reproduces at a low rate in human monocytic leukaemia cells (THP1), but triggers strong antiviral inherent cytokine reactions. The infection of first trimester human extra villous trophoblast cells (HTR8) manifested that ZIKV reproduces effectively in these cells and persuades strong inflammatory cytokine and chemokine production [49]. ZIKV can also infect U87-MG (human glioblastoma) cells and generates NLRP3 inflammasome stimulation and IL-1b release post infection. Upsurged gene expression for superoxide dismutase 2 (SOD2) and heme oxygenase (HemeOX), two important antioxidant enzymes widely used to evaluate oxidative stress, has also been seen. This implies that ZIKV infection can cause oxidative stress and inflammasome stimulation, which can lead to cell death through pyroptosis and CNS destruction [50].

Mosquito cell lines were used to exemplify basic biological questions. For example, Varjak *et al.*, used the *Aedes aegypti*-derived Aag2, AF319 and AF5 cell lines to explain ZIKV–mosquito RNAi interlinkages [51]. C6/36 cells were also engaged to look into the cyto architecture of ZIKV during reproduction, and the authors advised that these cells are good models for this kind of study [52]. To demonstrate ZIKV prevention by Wolbachia

strains in mosquito cell cultures, Schultz *et al.*, used *Aedes albopictus* C710 and C/ wStri cells (derived from C710 cells). In this case, this study affirmed foregoing data acquired using in vivo models [53].

#### **Reverse genetics systems for ZIKV:**

Reverse genetics is a significant tool that permits essential viral properties such as duplication, virulence, cell penetration, transmission, host range and the purpose of coding or non-coding genomic regions to be studied. The process is tiring and the complexities caught in duplicating such clones in bacterial cells can cause viral progression to be unsteady and can even cause toxicity in bacterial hosts [54, 55]. Upon the evolution of ZIKV in the Americas, this technology was engaged by various groups using the classical and epidemic strains [56-60]. The first system was developed by using Cambodian ZIKV FSS13025 strain. Recovered viruses were shown to be highly contagious for *A. aegypti* mosquitoes and virulent to both A129 and AG129 mice, despite the fact that, it was more attenuated than the wild-type virus [61].

The Brazilian ZIKV Paraiba 01/2015 strain and the MR766 prototype of ZIKV were lately used to generate infectious clones. Their genetic stability was further upgraded by implanting intron sequences into the NS1 and NS5 genes [62, 63]. ZIKV conveying reporter genes such as luciferase and GFP was proved to be a precious tool for virus development and

reproduction analysis, as well as antiviral tests [64, 65]. The introduction of an NS1 K265E mutation significantly increased virus production on Vero cells, which has an influence on vaccine management [66]. Employing reverse genetics, it was observed that a single serine-to-asparagine replacement (S139N) in the prM protein of ZIKV accords to foetal microcephaly exemplifying the power of the system to recognize genetic determinants of virulence [67].

A bacteria-free approach that does not require cloning, termed ‘Infectious Sub genomic Amplicons (ISA), is used to recuperate infectious viruses from PCR products in both mammalian and insect cells [68, 69]. The perception of ISA depends on the production by PCR of three to six overlapping DNA fragments that encloses the entire viral genome. The ZIKV genome is bounded by the CMV promoter and the hepatitis delta (HDV) ribozyme followed by the simian virus 40 (SV40) polyadenylation signal in the 5’ and 3’ ends, subsequently. The amplicons are then blended and transduced directly into prone cells to sanction virus recovery through as yet unspecified in cellule recombination events. The ISA method does not compel any further steps, such as cloning, propagation of cDNA into bacteria, or even RNA synthesis, unlike other bacteria free approaches [70].

Setoh *et al.*, used an altered circular polymerase extension response protocol to

produce de novo a completely functional ZIKV directly from deep progression data. This technique has the advantage of producing infectious virus in absence of prior virus isolation and passaging in cell culture and/or suckling mice, which may result in the build-up of adaptive mutations that may alter viral phenotypes [71].

Lately, infectious clones were engaged in *in vivo* and *in vitro* research on growing mutations. This system was used to assess the effect of the V2634M mutation in NS5, a mutation linked with conversion in viral reproduction efficiency besides incidence of microcephaly in Latin America. Nevertheless, the mutant infectious clone manifested no remarkable change in cell culture reproduction, and nor did it modify the pathogenesis characteristic and virulence of ZIKV in AG6 mice [72].

Besides infectious clones, the use of replicons has also been an applicable tool to study viral replication, to explore the responsibility of specific mutations and to discover novel antiviral drugs. They have the advantage of being non-contagious, which makes holding them in the laboratory safer [73, 74]. Altogether, these systems are valuable tools for exploring new antiviral compounds and for studying the physiopathology of ZIKV infection.

#### **IN VIVO MODELS:**

##### **Murine models:**

Murine models have put up noticeably to the procurement of new insights into the biology of ZIKV infection [75-78]. Mice have contributed to exemplify various aspects of ZIKV pathogenesis, including the association between ZIKV infection in pregnant women and congenital defects [79-81]. For the assessment of vaccine and antiviral candidates, mice have also been a valuable model. Due to their small size, fast reproductive rate and low cost make these animals alluring models for ZIKV studies.

#### **Non-human primates:**

NHPs summarize various aspects of the illness in humans and these are the natural hosts for ZIKV. In actual fact, the first reported ZIKV-susceptible animal model was the rhesus monkey, which had ancient significance for the invention of ZIKV in 1947. Mice when intracerebrally inoculated with viraemic rhesus monkey sera developed clinical illness, but whereas when same virus inoculated intraperitoneally in mice and monkeys had no apparent disease [82]. Since the disclosure of ZIKV in the Americas, various batches have carried out experiments in NHPs to develop countermeasures and to understand infectiveness and transmissibility [83-88]. NHP models are crucial for progressing ZIKV vaccine and drug candidates into clinics.

#### **Rhesus macaque (*Macaca mulatta*):**

This is the most commonly used NHP model for ZIKV. In order to mimic mosquito bites,

most studies have used needle infection by the subcutaneous route. ZIKV infection in infertile animals resulted in either subclinical infection or mild clinical signs of disease, such as rash, fever and conjunctivitis through these examinations. Viraemia and virus secretion in several body fluids, such as urine, saliva, tears, semen, vaginal discharge and cerebrospinal fluid, have been reported. ZIKV has also been spotted in several organs, where it resulted in gross and histopathological modifications, with a noticeable tropism to the CNS [89-93]. Even after the clearance of virus from peripheral blood, urine and mucosal secretions, ZIKV can still endure in the CNS, lymph nodes and other immune favoured sites of rhesus monkeys for weeks [94]. ZIKV infection resulted in powerful inherent and humoral and cellular adaptive reaction in these animals, which were secured from successive challenge with homologous or heterologous strains [95-99]. This monkey species are useful in distinguishing the inherent and adaptive immune response after ZIKV infection. Hirsch and co-workers, conducted one study by opting pregnant rhesus macaques by infecting them and found that ZIKV causes placental dysfunction and immunopathology. All infected dams showed inherent immune cell enabling within 5 days p.i, as demonstrated by the existence of CD169+ staining inside monocytes/macrophages, myeloid DCs and NK subsets. Infected dams disclosed marked

enabling of DCs and NK cells which elevated at 70 and 85 days p.i. Multiplication of CD8 T-cell and B-cell were also detected, with maximum reactions seen in the first two weeks after infection. The antibody titres raised through 28 days p.i. and the ZIKV selective maternal antibodies were detected as early as 6 days p.i. Neutralizing activity has been shown by collecting serum from dams and foetuses at 85 days p.i. [100]. The assessment of three vaccine platforms (inactivated vaccine, plasmid DNA vaccine or a rhesus adenovirus serotype 52 vector-based vaccine) led to the growth of nullifying antibodies and full shielding of the rhesus macaques against protest [101]. The pregnant animals that got infected culminated in longlasting viraemia, vertical transmission and microcephaly in foetuses [102]. Viraemia in non-pregnant animals is lesser than that of the viraemia in pregnant animals [103, 104]. A modern study determined that the infection of pregnant rhesus monkeys early in pregnancy summarizes many lesions that are characteristic of congenital Zika syndrome (CZS), including foetal loss, smaller brain size, and microscopic brain pathology distinguished by microcalcifications, necrosis, vasculitis, haemorrhage, gliosis and apoptosis of neuroprogenitor cells [105]. Inoculation of ZIKV intravenously reported no clinical illness, but the animals developed impermanent viraemia, viruria and virus secretion in saliva.

In the absence of major histopathological modifications, ZIKV was present in the lymph nodes and spleen in cardiopulmonary, gastrointestinal, integument and genitourinary tissues [106]. Saliva from the infected monkeys when inoculated in the palatine tonsils or conjunctiva or nasally developed infection, whereas direct inoculation of high virus ZIKV directly to the tonsils resulted in viraemia [107]. Animals inoculated through the vaginal or the rectal route had symptomatic clinical illness, but viraemia was detected in 50 and 100 % of the macaques respectively when inoculated through these routes [108].

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