International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 6 | Issue 3| JUL-SEP | 2016 | 166-183
Original Research Article – Biological Sciences

TRENDS AND PERSPECTIVES OF THE ZIKA PANDEMIC: A SHORT REVIEW

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ABSTRACT

Zika virus infections become an alarming current global threat. In spite of the increasing morbidity and mortality being reported around the world, there is no official vaccine declared against these infections yet. Hence, this review elucidates the various recent studies conducted in different animal models and cell lines, in the context of, disease etiology, epidemiology, serological and molecular diagnosis, disease complications, viral proliferation mechanisms, and treatment strategies. This paper also attempts to give certain critique on these diverse trends.

KEY WORDS

Zika virus, Epidemiology, Neurotropism, Animal models, Anti-viral therapies, and Vaccines.

1. INTRODUCTION

ZIKA Virus (ZIKV) was first discovered among the Rhesus monkeys of the Zika forests of Uganda in 1947 [1]. The first human case was reported almost six years later in 1953 in Nigeria [2]. Now the ZIKV infections sweep the entire world with numerous cases reported in the Americas, Africa, Asia-pacific countries, and, even certain 'imported' cases by the travelers and migrants to Europe and Oceania [**FIGURE:1]** [3-4]. There were enormous concerns on the possible transmission of ZIKV infections during the Rio Olympic and Paralympic games. [5-6]

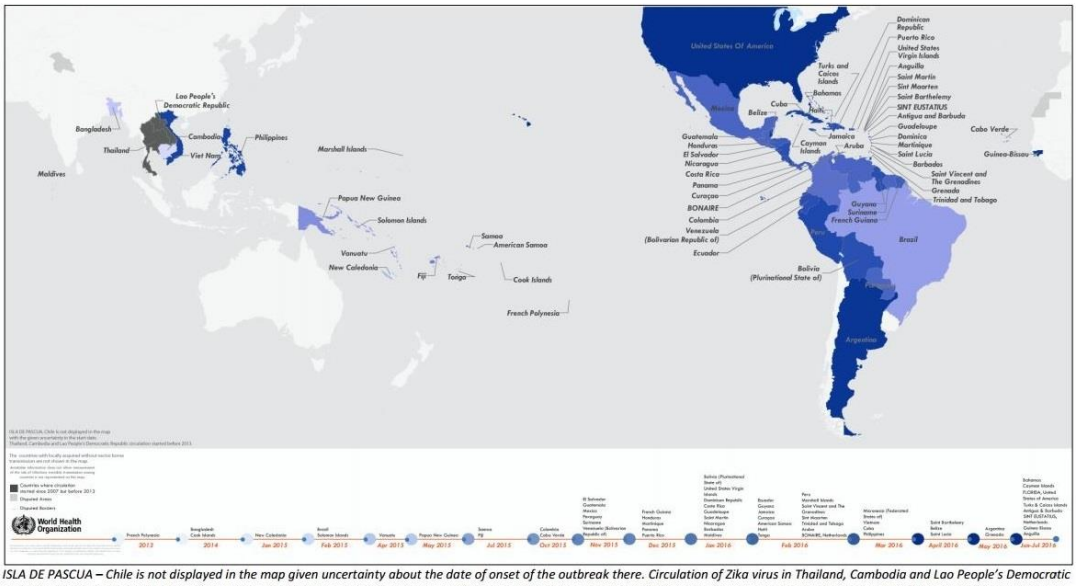
The mode of this infection is vector-borne, through the mosquito *Aedes aegypti*. The reports on vector competency of another mosquito species for ZIKV are controversial [7-10]. Sexual transmission of ZIKV is also reported [11]. An interesting case study [12] reports an asymptomatic ZIKV positive male, after the subject transmitted the infection to his female sexual partner, who developed major ZIKV symptoms. In addition, transmission via platelet transfusion [13] is also reported, resulted in monitoring of blood transfusion for ZIGV infections [14].

ZIKV along with Dengue virus (DENV) and Chikungunya virus (CHIKV) belong to the *Flavivirus* genus and the Flaviviridae family. This infection among pregnant women would result ('Vertical transmission') in Microcephaly and other brain abnormalities of the neonate [**FIGURES**:**2-3**] [15]. In adults, it causes Guillain-Barre Syndrome [16-19]. Other physiological problems such as Hearing loss in microcephalic infants [20], Abnormal heart rate and Blood pressure [21] and other cardiac problems [22] also are reported to be possible consequences.

Anthony R. Mawson-2016 [23] in their dissident hypothesis, state that higher retinoid concentration in liver could be the cause of the reported ZIKV complications and Guillain-Barré syndrome. They suggest the monitoring and comparison of retinoid levels and expression profiles among the microcephalic and control neonates. Similar view, albeit at genetic level, was expressed by Ashutosh Kumar et al. [24]

Interestingly, there are no vaccines or anti-viral therapies announced so far by the WHO and the measures available in our hands are prevention and control strategies on spread of these infections.

Reproductive planning [25-26] and Abortion [27] also are seriously considered.



ISLA DE PASCUA – Chile is not displayed in the map given uncertainty about the date of onset of the outbreak there. Circulation of Zika virus in Thailand, Cambodia and Lao People's Democratic Republic started before 2013. Countries where sexual transmission occurred are not represented in this map. Available information does not permit measurement of the risk of infection in any country; the variation in transmission intensity among countries is therefore NOT represented on this map. Zika virus is not necessarily present throughout the countries/territories shaded in this map.

FIGURE:1. Global Spread of Zika virus from 2013-2016. (Source: www.WHO.int)

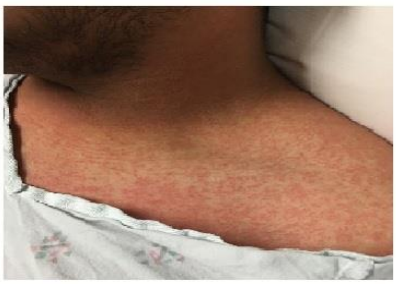


FIGURE:**2**. ***Top***. Measurement of Microcephaly in Neonates (**Source:** www.cdc.gov).***Bottom.*** **Left**. *Aedes aegypti* Mosquito (**Source:** www.cdc.gov).**Right**. Typical Maculopapular rash of ZIKV infections. (Adopted from Kristi L.Koenig et al).

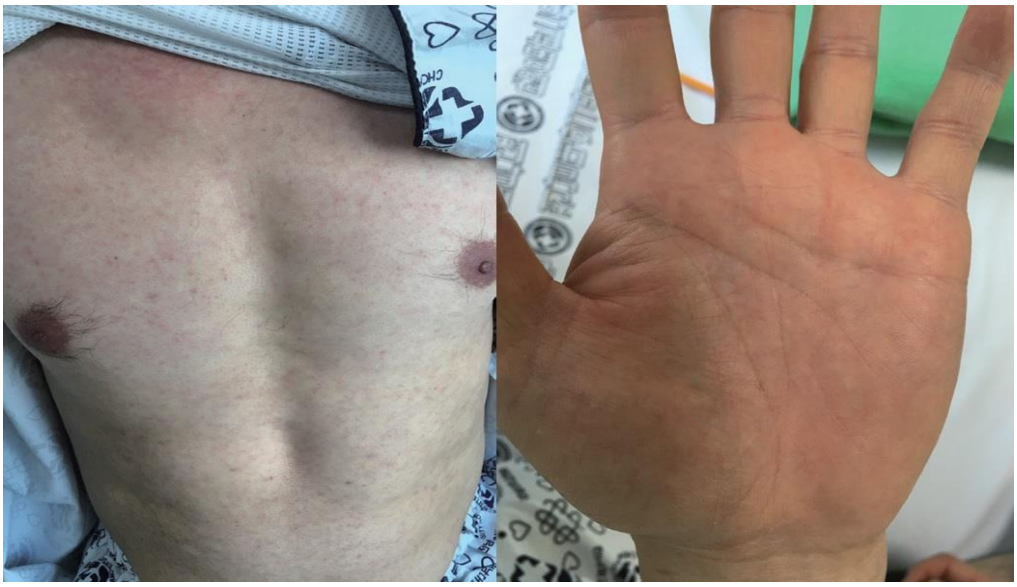


Figure:3. Clinical manifestation and virus shedding. Maculopapular rash on the trunk and palm. (Adopted from Debora Ferreira Barreto-Vieira et al., 2016).

2. TRENDS ON DETECTION AND DIAGNOSIS:

The ZIKV is reported to be diagnosable in Urine, Saliva, Serum and Semen samples. Nicastri E et al., [28] report the presence of ZIKV RNA in Cerebrospinal fluid (CSF). Presence of ZIKV RNA in tears is also reported in Rodent model [29]. Debora Ferreira Barreto-Vieira et al., [30] with their study report fine ultra-structure of ZIKV [**FIGURE**:**4**]. Rémi N Charrel et al., [31] in their extensive review describe various common clinical and molecular diagnostic parameters in different biological fluids utilized worldwide [**TABLES:1-2**]. As conventional serological diagnostic methods are limited by the cross-reactivity among the Flavi viruses, PCR- based methods are usually preferred to them. Xu MY et al., [32] developed an YBR Green based real-time RT-PCR for the detection of ZIKV RNA in cell sample. This technique analyses the replication of ZIKV in different times within the living cells. The lower limit of this method is 1PFU per ml.

Jinzhao Song *et al*., [33] developed a simple, cost-effective and accurate method for sample collection and DNA amplification which could selectively identify ZIKV with the sensitivity limit of 5 PFUs. This kit could be very useful in distant areas where no facilities are available. The underlying method combines reverse-transcription loop-mediated, isothermal amplification (RT-LAMP) assay with a Point- of -Care (POC) cassette for the detection. [**FIGURES**:**5-6**]

Another technique combining the LAMP principle and AC susceptometry for the detection of ZIKV oligonucleotides is reported [34]. This method could detect the oligonucleotides as low as 1aM (Attomolar). Calvo EP [35] et al., devised a PCR method with the second-round amplification of RNAs with specific inner primers for the specific detection of each viral RNAs during DENV-CHIKV-ZIKV co infections among febrile patients. The cross-reactivity of DENV sera with ZIKV is also studied using focus reduction neutralization tests (FRNTs) by Lalitha Priyamvada *et al*.,2016 [36]. The FRNT titres of two acute dengue sera (#33 and #39) and one naïve Flavivirus sera (#21) against ZIKV were measured [**FIGURE**:7]. With their experiment with ZIKV infecting the human FcγR-bearing monocytic cell line, U937 they suggest that DENV antibodies could also enhance the ZIKV infections.

Myrna C. Bonaldo *et al*., [37] report the isolation of ZIKA viral particles from saliva and urine of the patients from the state of Rio de Janiero, Brazil. The urine and saliva samples from patients were inoculated in Vero cell cultures. RNA isolation and quantification were performed by RT-qPCR. ZIKV was isolated from both saliva and urine samples [**FIGURE**:**8**]. Complete genomic sequence was obtained and phylogenetic analyses revealed similarities of the isolates with that of various South American epidemics [**FIGURE**:**9**]. This report recommends the utility of urine and saliva

samples to be the tools for the effective diagnosis of ZIKV.

Dhiraj Acharya et al., [38] 2016 report an Electrogenerated chemiluminescence (ECL) based method to detect ZIKV from body fluids. In this method, the virus (lower limit is 1PFU in 100µL sample) is trapped between ELC labeled -polystyrene beads (PSBs) and magnetic beads conjugated with virus specific antibodies [**FIGURE**:**10**].

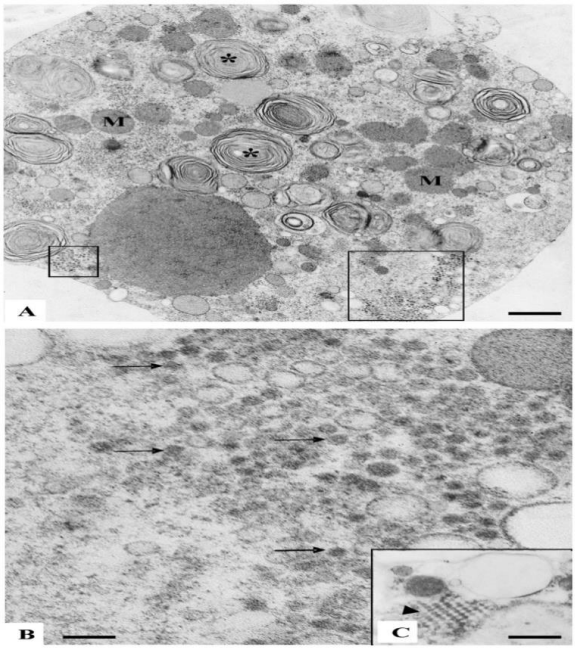


Figure:4 Vero cells six days' post inoculation with a human blood serum sample positive for ZIKV. (A) Infected Vero cell presenting numerous myelin figures (*), vacuoles, and clusters of ZIKV particles (marked areas). (B) ZIKV particles (arrows). (C) regularly arranged viral nucleocapsids (Arrow head). (Adopted from Debora Ferreira Barreto-Vieira et al., 2016).

Country imported into	Country or island imported from	No. of human cases	Zika virus RNA detection results	Positive Zika virus serology results
Australia	Cook Islands	1	Serum-positive	IgG, IgM seroconversion
Australia	Indonesia	1	Serum-positive	–
Canada	Thailand	1	Serum-, urine-positive	IgM; seroconversion in neutralization assay
Finland	Maldives	1	Urine-positive	Not tested
Germany	Thailand	1	Serum-negative	IgM, IgG
Germany	Indonesia	1	Serum-negative	IgG, IgM seroconversion; neutralization assay
Italy	Brazil	1	–	IgM; IgG seroconversion; seroconversion neutralization assay
Italy	French Polynesia	2	Serum-positive	IgG, IgM seroconversion
Japan	Thailand	1	Serum-equivocal,[a] urine-positive	IgM
Japan	French Polynesia	2	Serum-, urine-positive	IgM, seroconversion neutralization assay
Norway	French Polynesia	1	Serum-positive	IgG, IgM seroconversion
United States	French Polynesia	1	–	IgM; IgG seroconversion

Table:01 Various ZIKV diagnostic results. (Adopted from Rémi N Charrel et al.,2016).

Author (year) of published PCR assay	PCR target	PCR technique	Amplicon size (bp)	Zika virus lineage analytical	Zika virus lineage field	No. of human patients tested in studies	Sample types positive in field
Lanciotti et al. (2008)	Zika virus prM/E, target 1	Hydrolysis probe	76	Asian, African	Asian	>200 (combined set)	Serum, urine, amniotic fluid
Lanciotti et al. (2008)	Zika virus E, target 2	Hydrolysis probe	76	Asian, African	Asian	>200 (combined set)	Serum, urine, amniotic fluid
Faye et al. (2013)	Zika virus NS5	Locked nucleic acid probe	102	Asian, African	African	3 (B Rockx, personal communication, February 2016)	Serum
Tappe et al. (2014)	Zika virus NS3	Hydrolysis probe	94	Asian	Asian	5	Serum
Faye et al. (2008)	Zika virus E	Conventional	364	African	Asian	>15 (combined set)	Serum
Pyke et al. (2014)	Zika virus NS1	Hydrolysis probe	65	Asian	Asian	1	Serum
Pyke et al. (2014)	Zika virus E	Hydrolysis probe	71	Asian	Asian	1	Serum
Moureau et al. (2007)	Flavivirus NS5	SYBR®-green-based	269–272	African	Asian	2	Serum, urine
Kuno et al. (1998)	Flavivirus NS5	Conventional	1079	Asian, African	Asian	51	Serum
Scaramozzino et al. (2001)	Flavivirus NS5	Conventional (semi-nested)	220	African	Asian	1 (L Barzon, personal communication, February 2016)	Serum, urine
Maher-Sturgess et al. (2008)	Flavivirus NS5	Conventional	800	African	Asian	1	Serum
Ayers et al. (2006)	Flavivirus NS5	Conventional	863	–	Asian	1	Serum, urine, nasopharynx

Table:02. Various ZIKV lineages and diagnostic markers (Adopted from Rémi N Charrel et al.,2016)

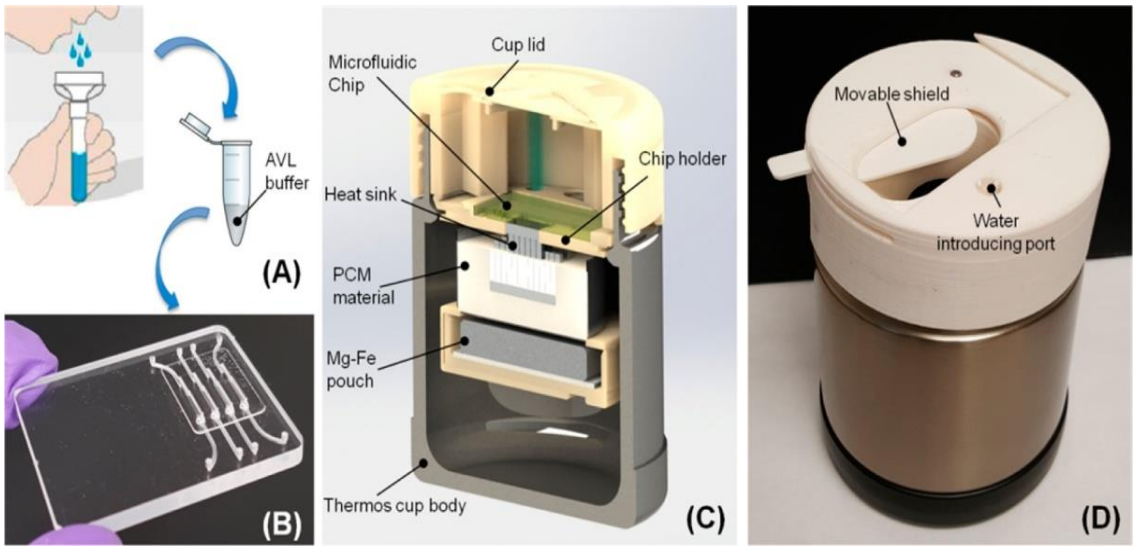


Figure:5 (A) Schematic of saliva sample preparation. Saliva samples are first collected in a saliva collection tube and then lysed in Qiagen binding/ lysis (AVL) buffer. (B) The lysed sample is filtered through the isolation membrane of our microfluidic cassette for nucleic acid extraction. (C) Exploded view of the chemically heated cup. The cup consists of a thermos cup body, a 3D-printed cup lid, a chip holder, PCM material, heat sink and single-use Mg–Fe alloy pack heat source. (D) A photograph of the chemically heated cup for point of care molecular diagnostics of ZIKV. (Adopted from: Jinzhao Song *et al*., 2016).

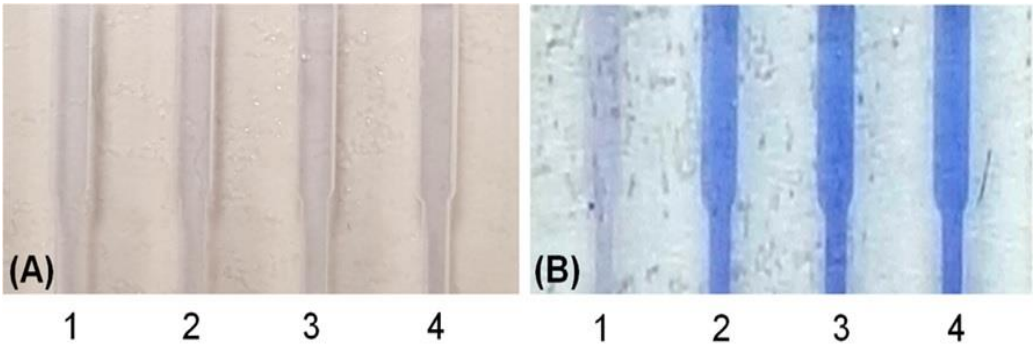


Figure :6 Photographs of the isothermal amplification reactors (A) before and (B) after 40 min incubation in the chemically heated cup. Leuco crystal violet dye is used as an amplification indicator. Amplification reactors 1, 2, 3, and 4 contain 0, 5, 50, and 500 PFU of ZIKV (Adopted from: Jinzhao Song *et al*., 2016).

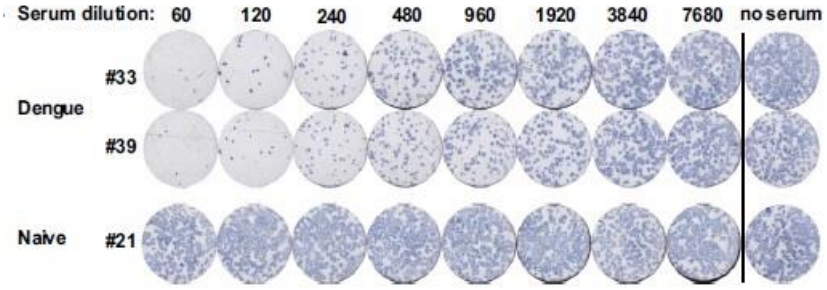


Figure:7 The Neutralization of ZIKV by DENV sera in different dilutions (Adopted by Piriyamvada et al.,2016).

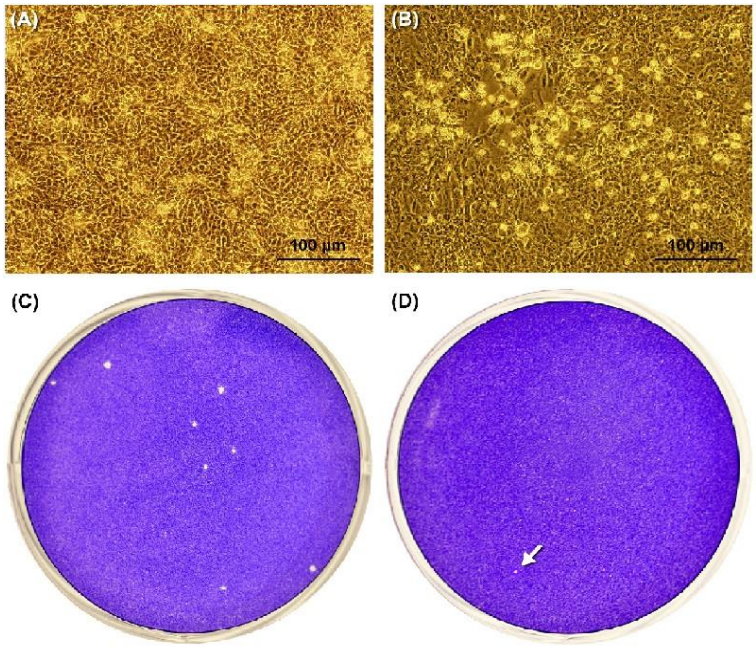


Figure:8 Isolation of Zika virus in Vero cell from the saliva of patient 6. Phase contrast optical microscopy of culture flasks containing (A) Mock-infected Vero cells and (B) saliva-infected Vero cells presenting a clear visible cytopathic effect. Viral plaque detection in saliva (C) and urine (D). The white arrow shows the unique viral plaque detected in the urine sample. (Adopted from Myrna C. Bonaldo *et al*., 2016).

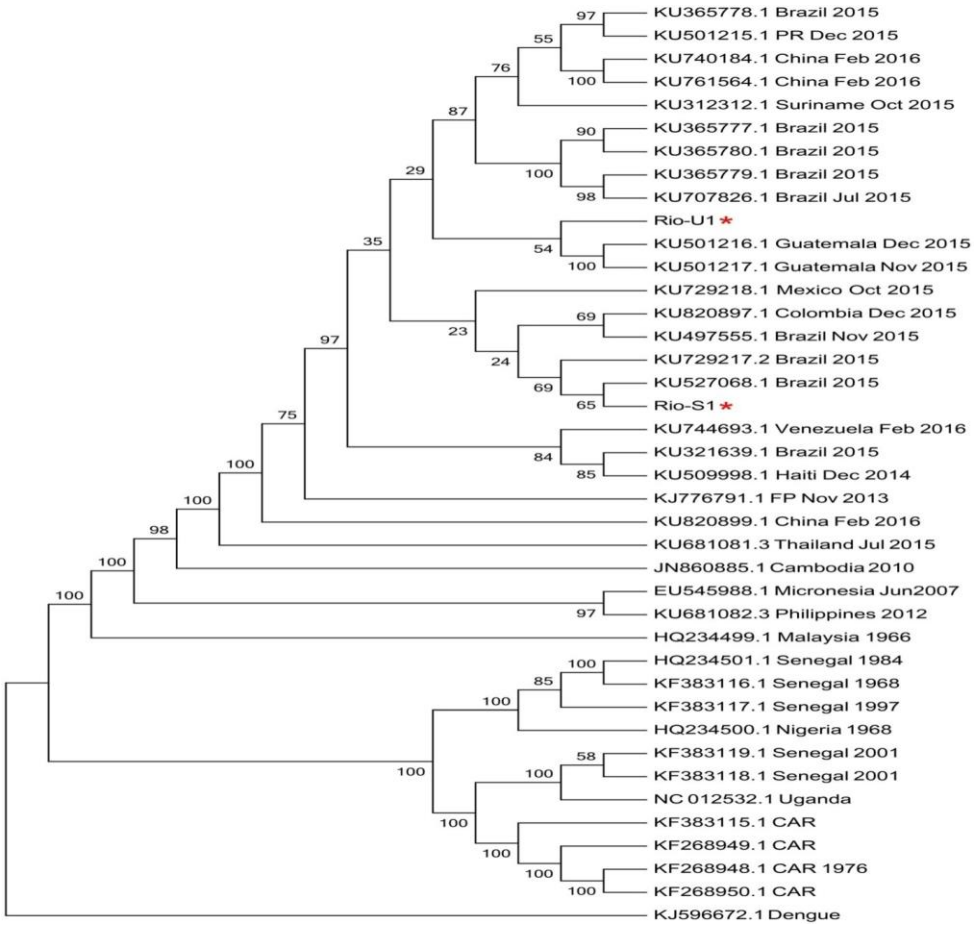


Figure: 9. Phylogenetic analyses of strains Rio-U1 and Rio-S1 marked with *(*Red*) with other strains of various epidemics. (Adopted from Myrna C. Bonaldo *et al*., 2016).

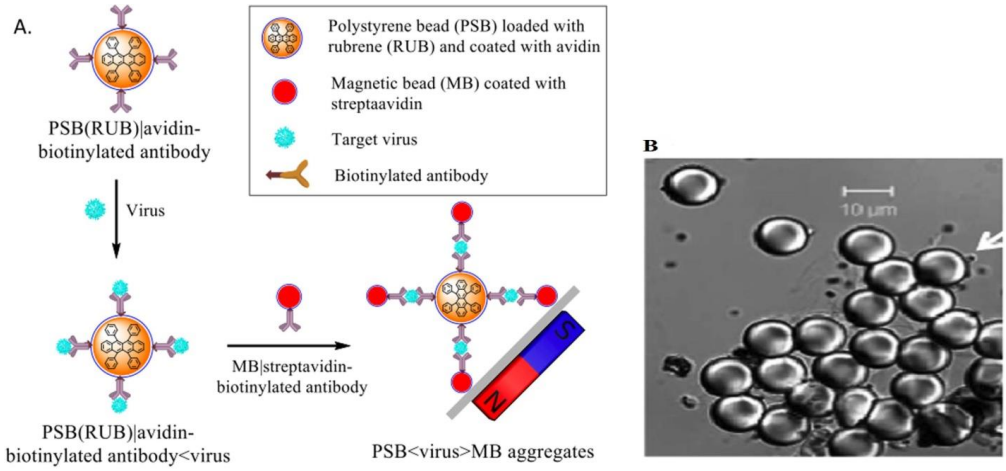


Figure: 10 A: Diagram representing the procedures for the detection of Virus. B: A phase-contrast image of PSB < ZIKV > MB aggregates showing the binding of anti-ZV2-MB (1 µ m diameter, arrow) to the surface of anti-ZV2-PSB (10 µm diameter) in the presence of ZIKV. (Adopted from Dhiraj Acharya et al., 2016)

3. EVIDENCES FROM GENETIC AND SYSTEM BIOLOGICAL APPROACHES:

The genetic diversity of ZIKV strains from various epidemics is widely reported [39-40]. Meghan May et al. [41], using system biological approach, report the specific diversity of O-linked glycome across various ZIGV species. Sang-Im Yun et al [42]. report the complete RNA genome sequence of three genetically distinct ZIKV strains MR-766 (Uganda,1947), P6-740

(Malaysia 1966) and PRVABC-59(Asian derived origin from Peurto Rico,2015).

In addition, G.Piorkowski et al [43] report the complete coding sequences of ZIKV isolated from the place Martinique and named 'ZIKAV strain MRS_OPY_-Martinique_PaRi_2015'. The open reading frame (ORF) was found to comprise three structural proteins (Capsid, Pre-membrane/ Membrane, and Envelope) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Using Maximum Likelihood analyses in MEGA6 they suggest that it is of Asian lineage and shares nucleotide similarity with strains isolated from French Polynesia and Brazil. An interesting work by Formijn van Hemert et al [44] report the unique Codon usages within ZIKV, which could translate into various key molecules of viral mechanisms. Another similar work [45] is reported in the context of Microcephaly

Meanwhile, construction of bioinformatic tools for the analyses of ZIKV RNA Sequence data [46] and epitope analyses for vaccine development [47] also are reported. Similarly, Dikhit MR et al., [48] using Immuno-Informatics identified various MHC Class 1 - restricted epitopes in Capsid, Envelope and NS proteins of ZIKV, against which, effective vaccine could be developed. Alam et al., [49] used similar approaches and found epitopes in Envelope glycoproteins. In fact, certain monoclonal antibodies (mAbs) against ZIKV Envelope proteins were reported [50] to be effective against various ZIKV strains of African, American and Asian origin. By virtue of cross-reactivity mAbs against DENV Envelope epitope which were isolated from the Dengue patients also found to be protective against ZIKV [51]. This conclusion is in contradiction with earlier [32] evidences, which suggest that DENV mAbs could aggravate ZIKV infections.

In addition, Victor Satler Pylro et al., [52] report the creation of the database 'ZIKV – CDB' for analyzing the small noncoding RNAs (sncRNAs) involved in the pathogenesis of ZIKV.

4. ON PATHOGENESIS:

Various bioinformatic and experimental studies report the link between ZIKV infections and microcephaly in neonates. Using transcriptomic analysis, Gene ontology, and pathway analyses Alyssa J. Rolfe et al., [53] report that human neural progenitor cells (hNPCs) are the high targets of ZIKV, along with the activation of numerous pro-inflammatory cascades. This result is confirmed by another report [54] with the Puerto Ricon ZIKV stain PRVABC59, suggesting the fetal primary human fetal neural progenitors (hNPs) to be the primary targets during neural infections [**FIGURE:11**]. In addition, Feiran Zhang et al., [55] with their Gene expression and Bioinformatic approaches on Asian ($ZIKV^{C)}$, African ($ZIKV^{M)}$, and DENV strains report the strain-specific influence of important hNPCs pathways. Li H et al., [56] also generated similar results from their study.

Another study [57] upon Neuroblastoma (NB) cell lines exposed to the PRVABC59 strain, report that undifferentiated are much susceptible to ZIKV infections than the differentiated neurons. This result explains the major absence of ZIKV complications in adults. In this study, cell lines of male origin such as CRL- 2267, CCL-127, CRL-2271 and SMS-KCNR, of female origin such as CRL-2266, and CRL- 2149, and terminally differentiated olfactory neuroblastoma cell lines such as T-268 and JFEN were used [**FIGURE.12**]. Altogether these studies explain the pathogenesis behind the onset and aggravation of Microcaphaly among neonates.

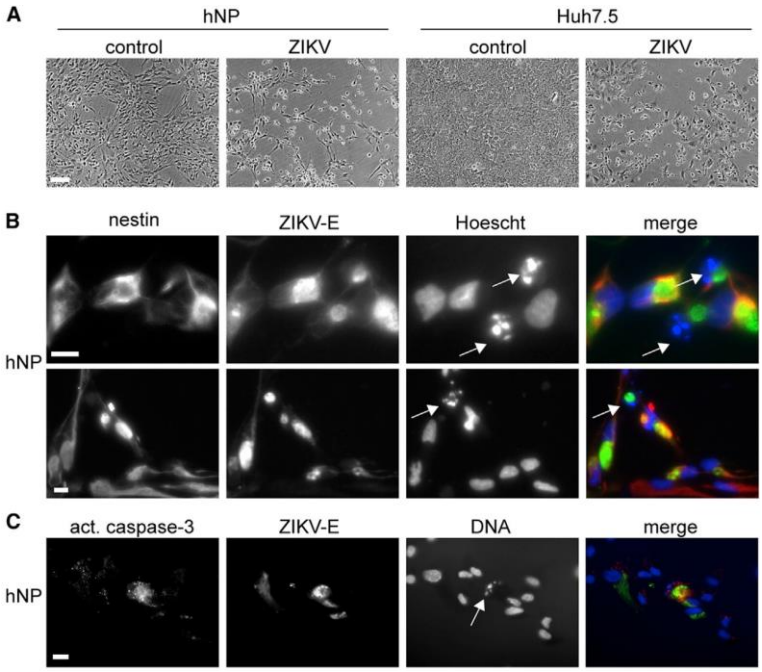


Figure:11 ZIKV Is Partially Cytopathic, but Poorly Immunogenic, in hNPs (A) Bright-field microscopy images of ZIKVinduced cytopathic effects in hNPs and Huh7.5 cells 4 days after infection. (B) Indirect immunofluorescence of ZIKV infection in hNPs. Cells were stained with antibody in order to detect ZIKV E protein and Hoechst DNA stain. (C) Indirect immunofluorescence of ZIKV infection in hNPs. Cells were stained antibodies in order to detect ZIKV E protein and activated caspase 3. (B and C) White arrows highlight pyknotic nuclei. (Adopted from Natasha W. Hanners et al).

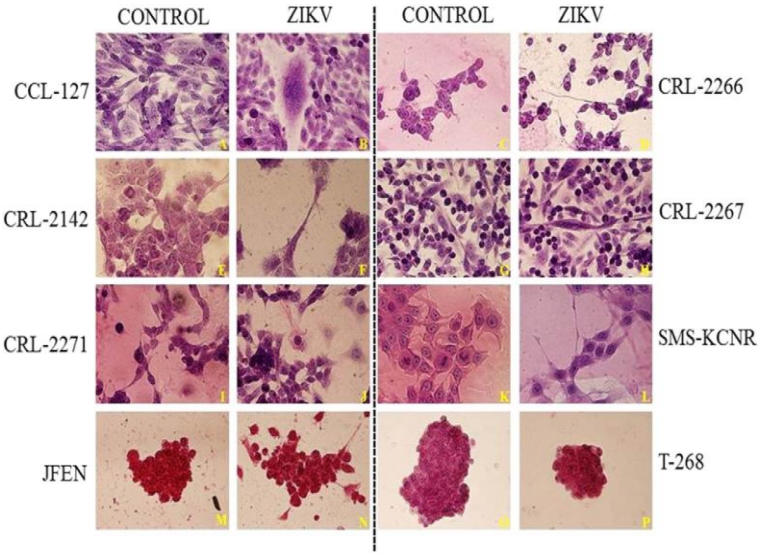


Figure:12. Morphological analyses by utilizing H&E staining. (Top row, left to right) A) CCL-127 uninfected; B) CCL-127 ZIKV infected; C) CRL-2266 uninfected; D) CRL-2266 ZIKV infected; E) CRL-2142 uninfected; F) CRL-2142 ZIKV infected; G) CRL-2267 uninfected; H) CRL-2267 ZIKV infected; I) CRL-2271 uninfected; J) CRL-2271 ZIKV infected; K) SMS-KCNR uninfected; L) SMS-KCNR ZIKV infected; M) JFEN uninfected; N) JFEN ZIKV infected; O) T-268 uninfected, P) T-268 ZIKV infected. All the infected cells, except JFEN and T-268, are showing significant ZIKV infection induced changes of the neurons and showed neuronal proliferation, central hromatolysis, enlargement of the neuronal cell body, vacuolation within the cytoplasm, shortening or abnormal increase and thinning of axonal length, syncytia formation, and neurostimulations or selective neurocytotoxicity. (Adopted from BrandonW. Hughes et al.,)

5. MODELS TO STUDY PATHOGENESIS:

Efforts to simulate the cellular manifestations of ZIKV infection during pregnancy, the subsequent microcephaly and other pathological conditions, demand the development and utilization of relevant model systems such as suitable cell lines and, importantly, animal models to study these mechanisms.

Kelli L. Barr et al [58] in their report suggest various cell lines for the effective analyses of ZIKV and Usutu viruses (USUVs). Meanwhile, Konstantin A. Tsetsarkin et al. [59] reports the development of a full length infectious cDNA clone of ZIKV, for studying viral pathogenesis and vaccine development. In addition, Marco Onorati et al., [60] report the establishment of Neuroepithelial stem cells (NES) for studying the neurotropism and microcephaly during ZIKV infections. Interestingly, Dawn M. Dudley et al., [61] from their current long term follow-up study, utilize and propose Rhesus macaques as model to study the mechanisms of ZIKV infections on human [**FIGURE** :**13**]. They suggest that this model could effectively simulate the pregnancy conditions in humans. Another group [62], in a similar work, report their study upon pregnant pigtail macaques to elucidate the fetal brain lesion followed by ZIKV infections [**FIGURES:14-15]**.

Rossi SL et al, [63] in their Review details the necessity of using *In vivo* models to understand the mechanisms of ZIKV infections during pregnancy and those of the foetus. This Review also details some studies conducted upon Mice. Similar opinions were expressed in the short communications by Na-Na Zhang et al [64]. In addition, Jonathan J. Miner et al., [29] utilized Rodent model to study the ophthalmic damages result from ZIKV infections. Using various receptor knockout mouse strains, they report that these infections could induce severe damages in the ocular systems and ZIKV RNA could be shed into the tears. In this study, mice mutant for Type 1 interferon receptor were selected and infected with ZIKV strains from French Polynesia and Brazil (termed 'Paraíba 2015') [**FIGURE:16]**. This study supports the work of Valentine G et al., [65] which analyzed various literature for the ZIKV induced neural and corneal damages. In addition, Jean-Baptiste Brault et al., [66] used mouse model to discriminate the neurotropism of various Flaviviruses.

Chick model [67] also is exploited to study the ZIKV infections during pregnancy in the first trimester, which is not possible in Rodent models. Infections in this model resulted in severe cardiac and neural damages much resembling the clinical conditions in humans. Aldo P et al., [68] from their study with human trophoblast cells, suggest that co-infection with Herpes simplex virus-2 (HSV-2), in addition to ZIKV infections, during the first trimester, could sensitize the placental cells for ZIKV entry.

Kellie Ann Jurado et al., [69] in their meticulous study with three distinct ZIKV strains, the prototype Uganda/African strain MR766 from 1947 ($ZIKV^{MR766}$), the FSS13025 Cambodian/Asian isolate from 2010 ($ZIKV^{CAM}$), and an Americas-derived virus isolated in 2016, MEX 2–81 ($ZIKV^{MEX}$), suggest that placental-specific macrophages and Hofbauer cells (HBCs) are the permissive entry sites for ZIKV to cross the maternal-foetal barrier. A similar study by Takako Tabata et al., [70] suggest that the compound Duramycin could reduce the placental infection of ZIKV.

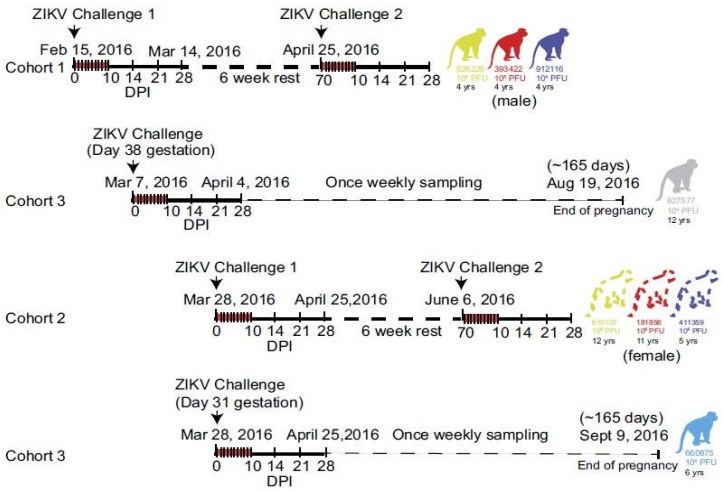


Figure :13. Schematic representation of the timeline of infection and sampling for each animal in the presented studies. Cohort 1 received the first ZIKV challenges and was then rested for 6 weeks before a rechallenge. For all studies, samples were collected daily for 10 days and then on 14, 21 and 28 days' post infection (d.p.i). as indicated by hashes in the timelines. Cohort 3 represents the two pregnant animals that were challenged on two different days. Both animals are currently in the once weekly sampling phase until the pregnancies come to term (B165 gestational days). Cohort 2 was a repeat experiment of cohort 1 that allowed for additional experiments and sample collection (for example, serum plaque infectivity) that were not feasible when we initiated cohort 1 studies. These animals are currently in a 6-week rest period and will be rechallenged on 6 June 2016. Ages of all animals are indicated under each macaque identification number. (Adopted from Dawn M. Dudley et al., 2016)

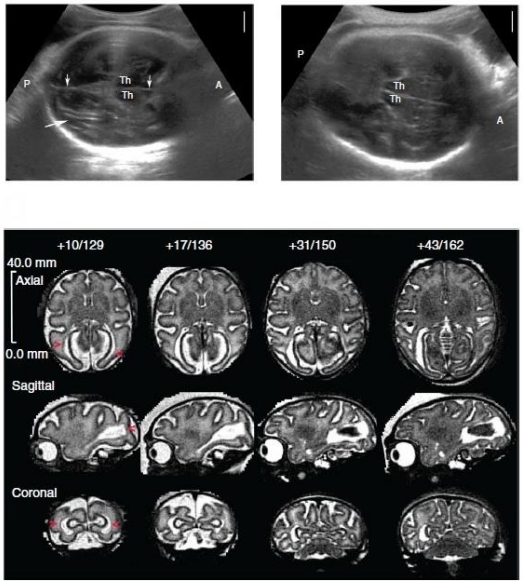


Figure:14. TOP. Right: An axial image of the fetal head obtained by ultrasound at 161 d gestation from the ZIKV-infected fetus. The large white arrow indicates a heterogeneous linear echogenic area in the posterior cerebral cortex on the right side. Other visible structures include the thalamus (Th) and falx *(small white arrows)*. Labels indicate the anterior (A) and posterior (P) orientation. Left: An analogous image is shown for a control fetus at 163 d gestation.

BOTTOM. Serial axial (top), sagittal (middle) and coronal (bottom) MR images of the fetal brain in a ZIKV-infected pregnant pigtail macaque from 10 to 43 d after inoculation (129–162 d gestation). The red arrowheads indicate T2 hyperintense foci in the bilateral periventricular regions of the occipital–parietal brain. Numbers shown at the top of the image indicate 'time after inoculation (d)/time of gestation (d)'. (Adopted from Waldorf et al.,)

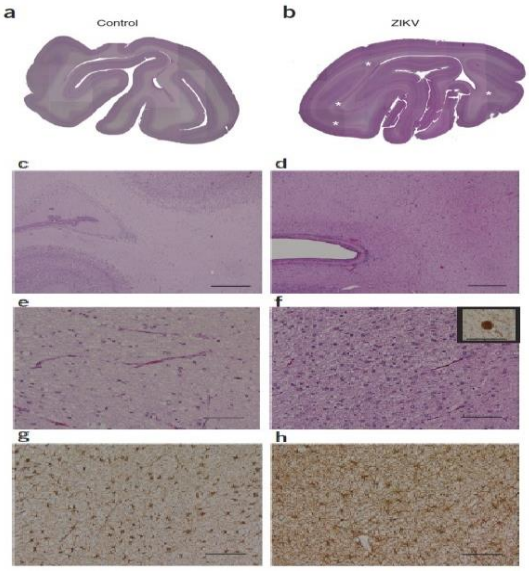


Figure:15. Neuropathology of the ZIKV-infected fetal brain and ZIKV RNA in fetal and dam tissues. (a–f) Representative H&E-stained images of the brains from one ZIKV-infected fetus (b,d,f) and a control fetus (a,c,e) are shown. White asterisks (b) indicate pink bands of white matter hypoplasia and gliosis. Neurofilament staining (f, inset) indicates an axonal spheroid, which were only seen in the ZIKV-infected fetus. (g,h) Representative image from the entire brain section showing GFAP immunostaining in the control (g) or ZIKV-infected fetus (h). (Adopted from Waldorf et al.,)

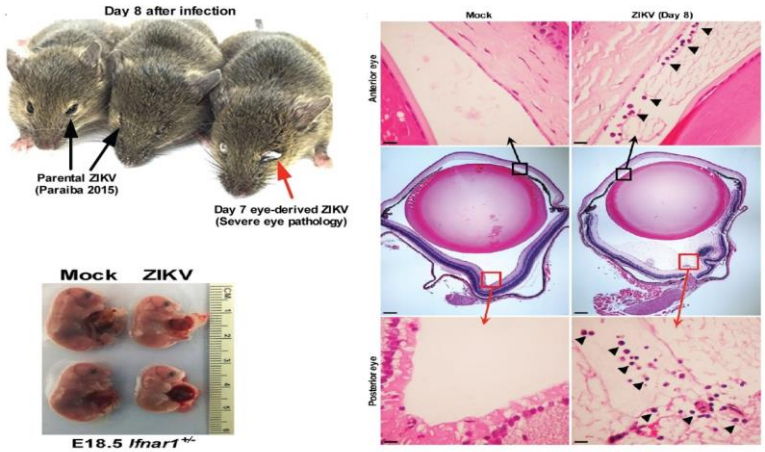


Figure:16. Left: TOP: A representative photograph demonstrating gross ocular pathology and exudate in mice inoculated with 104 FFUs of parental ZIKV Paraı´ba 2015 or eye-derived virus obtained from Ifnar 1_/_ mice. BOTTOM. A representative photograph demonstrating the average size of mock- and ZIKV-infected Ifnar1+/- fetuses on E18.5. Right. H&E-stained eye sections from mock- *(left panels)* and ZIKV-infected animals on day 8 *(right panels).* Regions shown in higher magnification are indicated by a box and displayed in the upper and lower panels. Black arrowheads indicate inflammatory cell infiltrates in the anterior *(upper panels)* and posterior (*lower panels)* chambers of the eye. (Adopted from Jonathan J. Miner et al).

6. POSSIBLE THERAPEUTIC INTERVENTIONS:

Many compounds are reported to be effective anti-Zika viral, based on recent clinical trials, pathway assays, drug screenings and various bioinformatic approaches. Byler KG et al., [71] from their *In-Silico* docking studies report the possible usage of 43 phytochemicals to be used as ZIKV NS protein binders. Various NS proteins are reported to be sharing features and mediating crucial pathogenesis and evasion mechanisms among Flaviviruses [**FIGURES**: **17-18**] [72-78].

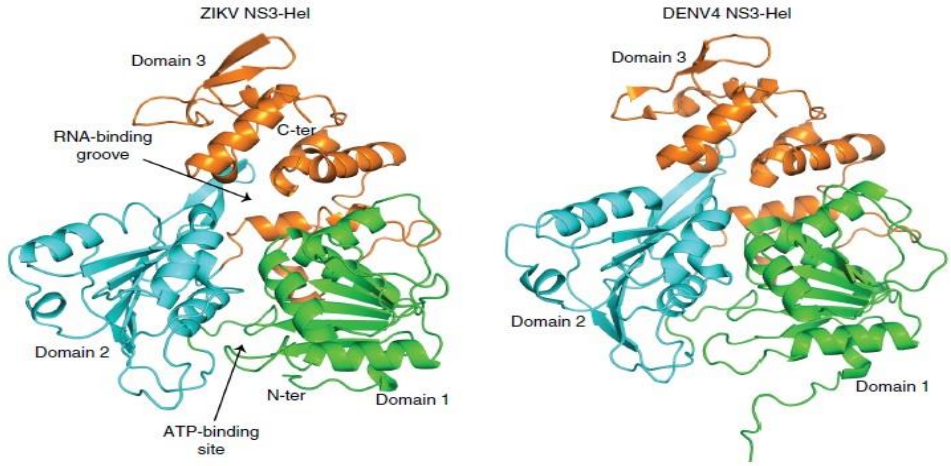


FIGURE:17. Structure comparison of ZIKV NS3-Hel with DENV4 NS3 helicase. Overall fold of ZIKV NS3-Hel residues 171–617 *(left)* and DENV4 helicase. (Adopted from Rinku Jain et al., 2016)

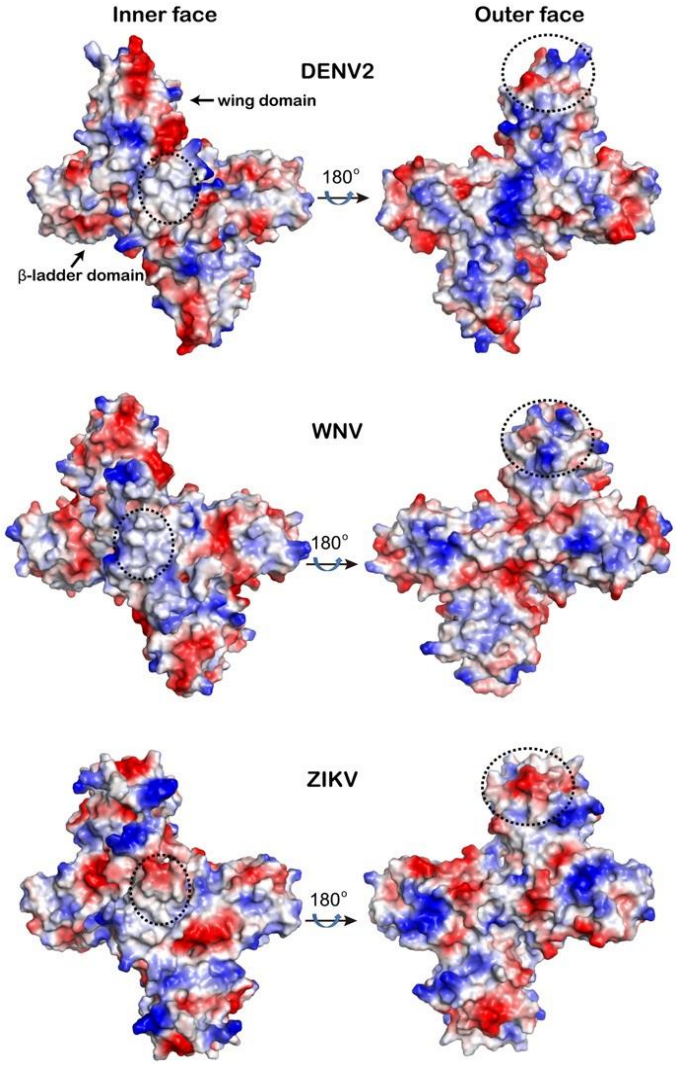


Figure:18. Variation in electrostatic surface potential of Flavivirus NS1proteins. Electrostatic surface views of NS1 from DENV2, WNV and ZIKV showing diverse characteristics on certain regions. Surfaces are colored by electrostatic potential at neutral pH from -2 kT (red) to +2 kT (blue) using PyMOL. (Adopted from Xiaoying Xu et al., 2016)

In addition, Xu M et al. [79], from their drug screenings, report various FDA approved drugs such as Emricasan, Niclosamide, to be used as antiviral and Caspase-3 inhibitor compounds. Other FDA approved drugs such as Bortezomib, Mycophenolic acid Daptomycin also reported to be anti-ZIKV compounds [80]. The two-approved anti –HCV drugs, IDX-184 and MK0608, also are reported to be optimally inhibiting the ZIKV-polymerase [81].

George Savidis et al., [82] reports that Interferon-inducible transmembrane proteins (IFITMs) could block ZIKV replication in earlier stages [**FIGURE:19**]. IFN-β along with IFN-γ are also reported [83] to be effective ZIKV inhibitors in cell cultures. The nucleoside analogs such as 2'-C-methyladenosine and Sofosbuvir also could effectively block ZIKV induced cell death and relocate pTBK1(phosphorylated TANK binding kinase-1), a mechanism which could significantly abrogate the viral pathogenesis [60].

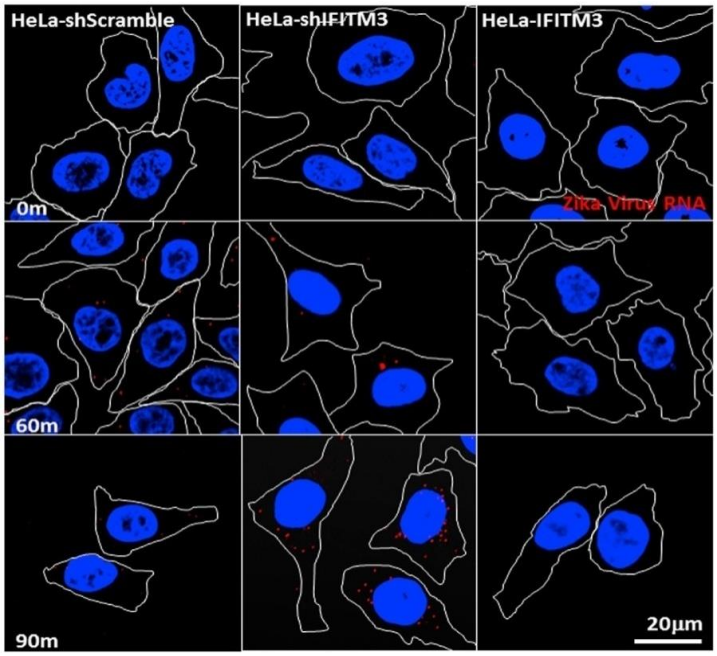


Figure:19 IFITM3 Inhibits the Early Replication of Zika Virus. HeLa cell lines were incubated on ice with ZIKV MR766 (Uganda, 1947) at an MOI of 100. At time zero, warm media was added and the cells were fixed, permeabilized, and stained for ZIKV RNA (red) and confocally imaged so as to capture a centrally located area within the cells. The nuclei of the cells were also stained for DNA with Hoechst 33342 dye (blue). White lines outline the cell boundaries based on DIC imaging. (Adopted from George Savidis et al.,2016)

Matthew T. Aliota et al., [84] reports a unique method to control the ZIKV infections via vectors. In their study, the *Ifnar* $^{-/-}$ C57BL/6 were mice infected with ZIKV. These mice have abrogated type 1- interferon signaling and are much vulnerable to infections and develop high viremia. They used *Wolbachia*, a maternally inherited intracellular bacterium and infected it upon the Colombian *Ae.aegypti* mosquito species (termed '(COL)wMel'). The mosquitoes were then fed on the *Ifnar* $^{-/-}$ C57BL/6 mice for blood meal. Infection, Vector competency, Dissemination, and Transmission rates were compared between wild type (WT) and Colombian (COL) wMel vectors. The COLwMel subjects exhibited much less competence than the WT subjects. In addition, Govindarajan M et al., [85] reports the usage of colloidal silver nanoparticles (AgNPs) to be used as possible larvicidal agents specifically against the ZIKV vector mosquitoes.

7. CONCLUSION:

One of the common features among all the countries with ZIKV epidemic, is that they are, for the most part, tropical with high humidity and rain fall. Almost no cases are reported in Europe, unless they are transmitted from travelers. So, the virulent ZIKV mutations are almost climate-dependent. We do have

the clue that ZIKV pathogenesis are not unique either, as it mostly resembles that of DENV and its structural biology shares much similarity with other Flavi viruses. So, we are not in a complete blind-zone in this area.

And most of the research ventures of developed countries of Europe and North America are focused upon Cancers, Heart diseases, Diabetes and Neurodegenerative diseases. Hence, in addition to CDC and WHO, more immediate international efforts are needed to recognize and work on these rapid epidemics such as that of SARS, DENV, Ebola Virus and, currently, ZIKV, to contain and reduce the global mortality rates.

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