Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (1) : 2020 : 149-157

ISSN-0972-3005

ZIKA VIRUS: AN EMERGING THREAT

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(Received 26 September, 2019; accepted 2 December, 2019)

Key words: *Zika Virus, Ugand, Nigeria*

Abstract – Zika Virus (ZIKV) is one of the most emerging severe threat to the world. Zika virus was first determined in zika forest in Uganda from the blood sample of rhesus monkey. In 2007, the outburst of zika virus was in Africa and Asia and later on it was found in the Yap State in Micronesia. Since after that, zika virus was widely spread around the world, including various countries such as Polynesia, French, Southeast Asia and on the other parts of Central and North America. Since 2007 outbreak of Zika virus with the quick transmission have made virus the international concern. The virus is an arthopode-borne virus and is a member of flaviviridae and genus *flavivirus* and transmitted through *aedes* mosquitos. The devasting effect of the disease may harm to humans also can transmit from mother and child which may lead to the microcephaly. The virus shows the symptoms such as yellow fever, rashes which is difficult to discern the viral infection. Antiviral therapeutics and the preventive strategies must be used to cure zika virus. The proper control measures should be taken.

INTRODUCTION

Zika virus disease is one of the most emerging severe threat to the world. Zika virus was first determined in Zika forest in Uganda from the blood sample of Rhesus monkey. In 2007, the outburst of Zika virus was in Africa and Asia and later on it was found in the Yap State in Micronesia (Krause *et al.*, 2017; Kindhauser *et al.*, 2016). Since after that, Zika virus was widely spread around the world including various countries such as Polynesia, French, and Southeast Asia and on the other parts of Central and North America. Since 2007 outbreak of Zika virus with the quick transmission have made virus the international concern.

Zika virus is an arbovirus which is single stranded RNA with the flavivirus species (Musso, 2016). *Aedes* mosquitoes transmit the diseases like Dengue, Yellow Fever and was mainly found in tropical and sub-tropical regions6. Zika virus was first described in 10 years- old - female in Nigeria in 1954. Zika virus is causative agent and causes fever also rash conjunctivitis are the symptoms of the Zika virus disease. Zika virus is also related to neurological complications in adults and microcephaly in fetus. World Health Organization (WHO) has announced Zika virus is considered as a long term public health concern due to its serious long term effect on children and adult.

MICROCEPHALY

The condition in which the head size, which is measured by the occipitofrontal circumference, is less than 3 standard deviation is known as microcephaly, this condition is an example of a serious form of congentital defect. The size of the brain is comparatively smaller than a normal person of same sex and age. There are two distinct form of microcephaly, one in which there is stagnant growth of the brain in the pregnancy period at approximately 32 weeks, due to continuing decrease in neuron counts. The second type is caused due to lack of dendetric connections among children after their birth, this leads to failure in growth of brain size. According to Giacomino's classification, microcephaly can be classified into 3 distinct types-

a. Microcephalia vera- here the brain size remains small, despite any sign of deformation or injury.
b. Microcephalia spuria- seen in case of any injury to the brain or any pathological changes.
c. Microcephalia combinata- this shows a reduced brain size, due to some injury.

The reason of such a disease can be both genetic and non-genetic. The genetic reason for such a disease would be, any of the following- alcohol

consumption during pregnancy, poor parental care including malnutrition, incomplete placental development, abnormal weight-gain during the gestation period, exposure to teratogens during pregnancy, metabolic disorders, non- accidental head injury or Rubenstein-Taybi syndrome, and viral infections

Epidemiology

In April 1947 the Zika virus was first observed in a Rhesus macaque monkey which has placed in a cage of Zika forest of Uganda, near Lake Victoria. Monkey developed fever, so the blood sample was tested and from the result it has been seen that Zika virus is developed in 1965. Also at the same time it was observed in human in Nigeria in 19549. There was rare infection in Africa and Asia in until 2007, but in 2007 a very high epidemic occurred in yap Island, Micronesia. Zika virus outburst in Oceania from 2013-2014. There was an epidemic in 2015 and 2016 in the America. In April 2015, there was outburst in Brazil and also spread to other countries Central, South and North America, and the Caribbean. In January 2016, WHO announced virus is likely spread through the Americas and in February 2016 WHO declared cluster of Guillain-Barre syndrome and microcephaly reported in Brazil. In Brazil, over 1.5 million people were infected by Zika, and 3,500 cases of microcephaly reported during October 2015 and January 2016. Around 455 cases of Zika virus infection were seen in Singapore during August 2016 to November 2016. Two cases of Zika fever reported in Angola in 2017.

Three laboratory-confirmed autochthonous Zika cases were reported to WHO in May 2017 after retrospective testing was performed on blood samples from Ahmedabad district, Gujarat state. The first cases one case was detected in the Gujarat state it was seen that low fever in 34-years- old women after delivering baby. Other case was found in the 22-years-old women in 37th week of gestation. And in February 2017 the third case was detected in 64-years-old man and was from acute fever.

China

In China, Zika virus was first reported in February 2016 in the traveler who returning from the Zika-indigenous countries. The guy was 34-years-old man returning from Venezuela; his blood sample was tested by RT-PCR. Around 22 cases were further reported from the Guangdong region. In 2016, another case of Zika virus infection was seen in a 38 year old female returning from the Caribbean. The same year saw another case, where a 56 year old man returning from Central America, got affected (Lim *et al.,* 2017).

Thailand

Few cases were also reported from Thailand, whose origin is known to be Cambodia. The case was first reported in 2014; the first patient with a laboratory-confirmed Zika virus infection imported into Europe in November 2013 had traveled to Thailand.

The Philippines

The Island of Philippines is located in Southeast Asia close to Yap Islands of Micronesia; Philippines saw a major outbreak of Zika virus; approximately 267 blood samples were collected during an active fever supervision in the city of Cebu from 2012 to 2013; the results were shocking as a case of Zika virus was reported by RT- PCR and virus isolation technique. This case was of a 15 year old boy who showed symptoms of mild febrile illness. By the end of February 2017, 57 cases of Zika virus infections were reported by Philippine Department of Health, this included 7 cases where pregnant women were infected. A serological study which was conducted using cross-neutralization tests showed that human population from 1953 had prominently high quantity of antibodies against Zika virus.

Japan

Only imported Zika cases have been detected in Japan. In 2013-2014 first three cases were reported in travelers returning from Thailand and French Polynesia. In September 2016 eight Zika virus cases had been reported in Japanese travelers.

America

In 2015-2016 Zika virus was reported in Americas. The outbreak began in April 2015 in Brazil, and spread to other countries in South, Central, North America and the Caribbean. In January WHO declared almost all around the America the Zika virus is spread and in February 2016, the cluster of microcephaly and Guillain–Barré syndrome cases reported and strongly suspected to be associated with the Zika outbreak – so they declared a Public Health Emergency of International Concern. Some researchers speculated that only one or two tourists might be infected during the three-week period, or

approximately 3.2 infections per 100,000 tourists.

Singapore

In Singapore the Zika virus was first reported in May 2016 and was seen in the traveler who was returning from Brazil and was detected in 47-years-old women in August 2016, which was the local zika outburst. Around 455 Zika cases have been confirmed in this outburst with the 15 disease clusters between 27th August and 30th November in 2016. 38 Zika virus cases have been reported in Singapore MOH between January and June 2017. The reproductive number decreased from 3.62 to 1.22 in the first month of the Zika virus outburst, coincident with intensive vector control and community engagement measures.

Malaysia

On September 2014, a German traveler travelling to Malaysia was found to be positive for Zika virus. Subsequently, an autochthonous Zika virus case was identified on September 2016, followed by another autochthonous case of a 67-year-old man from Petaling Jaya on December 2016. Zika Virus is prevalent in Malaysia since several decades. The first isolation of Zika virus in Asia was from a pool of *Aedes aegypti* mosquitoes collected from Malaysia on 1966. Previous serological studies had also suggested the presence of Zika in Malaysia before then. Cross-neutralization testing of human sera from Malaysian adults in 1953- 1954 showed (75%) to have Zika antibodies . Another study from the 1950's using cross- neutralization testing showed (19%) human sera from Malaysia (18%) human sera from Borneo had Zika antibodies. A seroprevalence study by plaque reduction neutralization test (PRNT) on sera collected from 71 wild or semi-captive orangutans and humans on 1996-1997 showed Zika antibodies in 8.5% and 44.1%, respectively20.

Cambodia

Zika virus was first detected in Cambodia on August 2010, serological assay, and virus isolation on a blood sample collected during fever surveillance by the United States Naval Medical Research Unit No. 2 (NAMRU-2). The sample came from a 3-year-old boy in Kampong Speu province, with the infecting strain belonging to the Asian lineage . In 2016, Institute Pasteur du Cambodge performed retrospective testing on 2,400 acute serum samples collected from suspected dengue patients between 2007 and 2016. Only 5/2,400 (0.2%) were found to be positive by RT-PCR; one each from 2007, 2008 and 2015, and two from 2009. In November 2016, Zika was reported in a man from Kampong Cham province.

Indonesia

Zika virus infection was first reported from Indonesia in two Australian travelers; one returning from Jakarta on 2012 and the other from Bali on 2015. Retrospective testing of 103 dengue negative blood samples from a dengue outbreak in Jambi province on December 2014 to April 2015 yielded one Zika virus infection belonging to the Asian lineage. On November 2015, a Zika case in Sulawesi was confirmed by molecular and virological techniques. Previously, serologic evidence of Zika virus infection had been reported from a fever study conducted in Java on 1977-1978; with 7/219 (3.2%) inpatients showed elevated Zika antibodies by hemagglutination inhibition test (HI). In addition, a study from 1983 demonstrated the presence of Zika antibodies by HI on 9/71 (12.7%) blood samples from Lombok. These serological studies should be interpreted with caution given the potential cross-reactivity of the assays used.

Structure of Zika virus

Structure of Zika virus Structural and functional studies of Zika virus proteins will provide useful information to understand the viral pathogenesis and guide structure based drug discovery. Zika virus is enveloped by icosahedral non-segmental 10kb positive- sense RNA genome. Zika virus encodes a polyprtein that can be cleaved into three

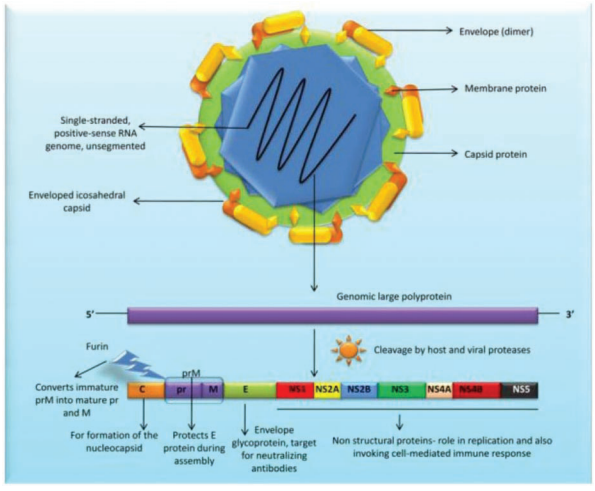

Fig. 1. Diagrammatic representation of structure of Zika virus

structural proteins- envelope (E), capsid (C), precursor of membrane (prM) and seven non-structural(NS) proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS524. Mature Zika virus is around 3.8Å resolution and structure is observed under cryoelectron microscopy. Virions in Zika virus are 60nm in size and shape is spherical. Zika virus genome is around 10,794 bases in length with

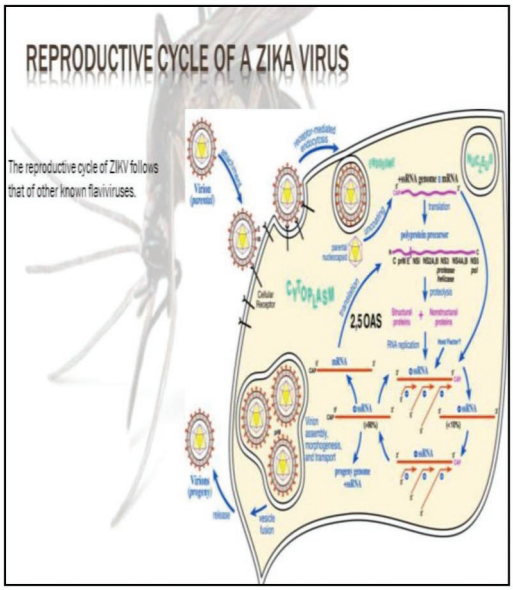

Fig. 2. Reproductive Life Cycle of Zika Virus

positive sense linear with RNA. For Zika virus cleavage both viral and host proteases are required.

Reproductive Life Cycle of Zika Virus:

I. Transmission through the infected mosquito bite causes microcephaly from mother to child.
II. Second image of Zika virus shows that the eruption of virus through dermal cells through infected mosquito.
III. Zika virus which crosses the placental barrier and infecting the child.
IV. Placental section (villus).
V. A part of terminal villi showing placental microphages which facilates virus entry.
VI. A child with a microcephely head.
VII. Last image shows hNPCs that proposed molecular mechanism of microcephaly.

Binding of the hNPCs with the viral particle via the AXL receptor helps in the entry of virus by the help of endosome formation. With the decrease in PH the envelope degrades which leads to the expulsion of the virion in to the cytoplasm. Few of this virion starts replicating by using the

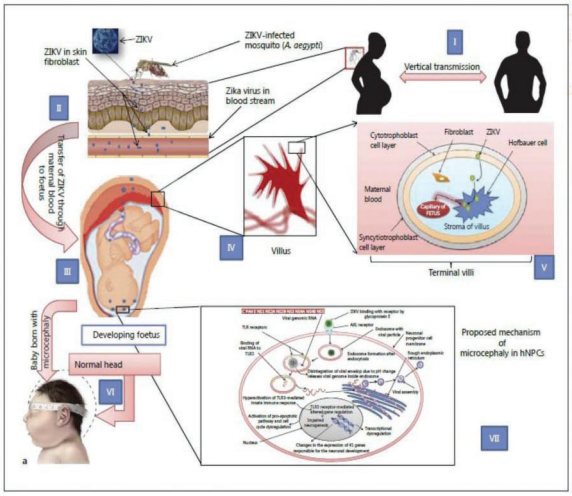

Fig.3. Zika Virus Induced Microcephaly

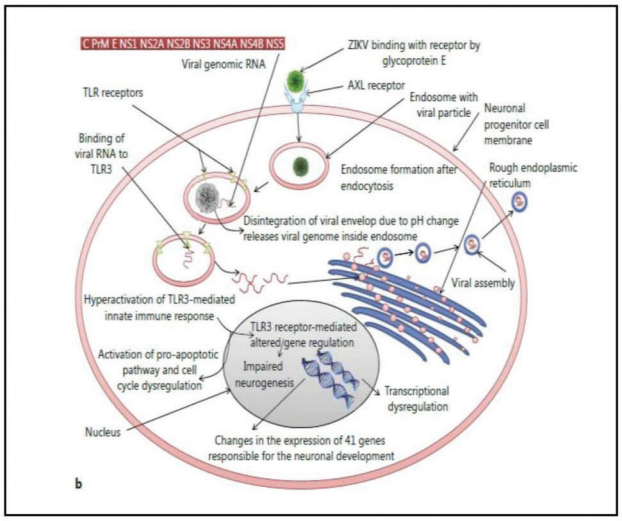

Fig. 4. Summary of the Zika virus (ZIKV) infection and proposed mechanism of microcephaly.

reproduction mechanism of the cell and this forms several viral proteins in the host. The TLR3 receptors which are present on the surface of endosome now binds to the viral RNA. Due to the down regulation of 41 specific genes, which are responsible for NPC differentiation leads to transcriptional dysregulation.

Because of this, the proapoptotic pathway is activated and rise of impared neurogenesis occurs. The down regulation of neurogensis and upregulation of apoptosis eventually leads to impaired development of the brain, or more commonly known as microcephaly.

Molecular Events Leading to Microcephaly

Epidermal keratinocytes and dermal fibroblasts

are the main target of the zika virus infection. And the infection in dermal cells circulates to different organs of the circulatory system.The infection of zika virus from mother to infants may occur by the macrophages and cytotrophoblasts. Macrophages are the main target of the zika virus infection of the placenta. The entry of the zika virus is mediated by the cell surface receptors such as AXL, heat shock protein, DC-SIGN, TYRO3. AXL the phosphatidylserine protein, which belongs to TAM receptors. In Human brain radial glia, astrocytes AXL protein is overexpressed. Entry of the zika virus through receptor mediated signaling pathway,this may lead to viral infection in neurons

MODES OF TRANSMISSION

Mosquito-Borne Transmission

Zika virus can be transmitted to humans generally through bites of an infected, female A. aegypti or Aedes albopictus mosquito. The mosquitoes are distributed through the subtropical, tropical and temperate regions and there is greater increase in the transmission. Mostly the incubation period of the Zika virus before which the mosquito is capable of transmitting the Zika virus is approximately 10 days. It has been observed that the Zika virus mosquitoes lay eggs in stored water such as vases, flower pots, bowls, tires, buckets, animal dishes. Virus carrying mosquitoes prefer to bite humans and live outdoors and indoors near humans. The female A.aegypti mosquitoes bite to the humans and infection is spread, again the other mosquitoes feed on the person carrying the virus and infection is spread to other peoples.

Maternal Transmission

Zika virus has been detected in microcephaly infant born to mothers with an infection during pregnancy. It has been seen that Zika virus has the ability to cross the placenta and infect fetal nervous tissues. Placenta acts as an effective immunological barrier between the mother and the child, which protect the child from microorganisms in the mother's circulation. The potential of Zika virus to undergo utero transmission has increased global concern as regions positive for Zika virus infection. In nursing mothers Zika virus RNA has been detected in the breast milk of the infected mothers. This introduces a novel transmission mechanism in which Zika virus transmission occurs from the mother to the child. The study of Mother-Child, Zika virus RNA was detected in serum of two mothers and in the serum and the breast milk.

Blood Transfusion Transmission

Zika virus can be transmitted through blood transfusion it is observed that around 3% of the infection is transmitted through the blood donors; this makes the novel potential to the mode of transmission of Zika virus. Zika virus transmission with blood transfusion is possible as Zika virus infections are primarily acute and blood transfusion-related transmission.

Sexual Transmission

Zika virus can be transmitted through sex from one person to his or her partners. Zika virus symptoms are not seen at the early stage even though the person is infected. Zika virus is spread from one person to another, Zika virus symptoms starts after the symptoms end. Sometime the symptoms are not been detected in person.Zika virus is first arbovirus which is observed in human semen.Mosty infection load in saliva and urine of the infected individual in the detection of Zika virus RNA is reported. Sexual and salivary/urinary transmission of Zika virus is very difficult to distinguish because due to the correlated nature of behavior associated with sexual activity. Conformation of Zika virus transmission with sexual interaction could lead to change the epidemiology of Zika virus as Zika virus RNA was found to be detectable in semen over a longer period of time, as compared to blood serum.

Zika Virology and Immunology

The transmission of this disease is due to the bite of female mosquitoes, leading to infection of skin, blood from insect saliva, during feeding of blood in the host, which in this case are humans. The likelihood of the viral transmission depends on the volume of fluid present in the proboscis and other factors which includes- the volume of the salivary gland in the insect, viral replication level, viral infectious titer of the preceding host. The process of infection in host requires the following- viral envelope protein binding and particle uptake into susceptible cells, this in turn is mediated by specific receptors like- AXL, DC-SIGN, Tyro3, and TIM-1, these trigger transcriptional activation of Toll-like receptor 3 (TLR3), RIG-I, MDA5 and also interferon stimulated genes including OAS2, ISG15, and MX1, and beta interferon. Examples of cells which get

infected includes- epidermal keratinocytes, skin dendritic cells and skin fibroblasts. One of the al target for the virus is immature dendritic cells. Autophagy is explored by the virus to enhance its-replication, and pharmacologic manipulation of Zika-infected cells with 3-Methyladenine (3-MA), an inhibitor of autophagosome formation this in turn strongly reduces viral copy numbers in infected fibroblasts. The time period of the infection and persistence of the virus in the host is not known in both post-partum or intrauterine infection. Route of fetal infection, or the degree of neurotropism is also unknown. In spite of the presence of serum antibodies, related flavivirus may still persist. In certain cases the virus has been found in dead foetus and amniotic fluid. The reason of fetal infection of central nervous tissue maybe due to the infection of amniotic fluid which causes uptake of the virus via the FcRn receptors in placenta. Epitopes integrated with dengue, may result in preexistence of antibodies for these viruses leading to enhancement of initial viral replication or infection in placental cells or transfer of viruses through the placenta .

Signs and Symptoms

Zika virus symptoms last for several days to a week after being infected usually it is mild.

i. Fever: Fever is condition in which the body temperature increases to 98.6 degree which can usually cause disease.
ii. Rash: Rash is condition in which it is like skin infection changed in the texture of the body, color of skin changes, itching, warm, sometimes it swells, blistered or cracked, dry, warm and also may bumpy and painful.
iii. Conjunctivitis (Pink eye): Conjunctivitis is condition in which the conjunctiva membranes lining in which it is inflamed or gets infected the eyelids and covering the white part of the eye. Also called pinkeye.
iv. Arthralgia: Pain in the joint. v. Headache
vi. Muscle Pain vii. Vomiting

Diagnostic Tests

In case of Zika virus the diagnostics test is very important way for the detection of the virus. Zika virus are directed by two ways i.e direct way and indirect way, in direct way it is done by identifying virus RNA in tissue or the body fluid and in indirect way the Zika virus is detected by testing for specific antibodies. The fluids may include serum, blood, urine or saliva. The serological assays results which may be cofounded by cross- reactivity between the virus genus and by the geographical overlap. The various tests are done such as virus neutralizing, immunoflurescence assay and enzyme-linked immunosorbent assay.

Control Measures

Vaccines

Various organizations are trying to develop zika virus vaccines, but most vaccines are under preclinical trial, considering that they are eligible for marketing approval. Examples of Zika Virus

Developer	Technology
CaroGen Corp.	VLV-based nano-particle
CDC	VLP expressed by DNA plasmid; live recombinant adenovirus
GeoVax Labs, Inc.	MVA-VLP technology elicits antibodies and T cells
Hawaii Biotech	Recombinant proteins produced from insect cell line, plus Alhydrogel or proprietary adjuvant
Inovio/Gene One Life Science	DNA (electroporation)
Kansas State University	DNA
NewLink Genetics Corp.	Purified inactivated virus
NIH	Zika-targeted mutation, live attenuated (longer-term); DNA; live VSV recombinant
Novavax	E protein (nanoparticles)
Pharos Biologicals, Inc.	Nanosphere delivery
Protein Sciences Corp.	Recombinant variations of Zika virus E protein
Replikins, Ltd.	Synthetic replilink peptides
Vaxart, Inc.	Recombinant Zika vaccine in room temperature-stable tablets
VaxInnate Corp.	TLR technology; vaccine antigens are genetically fused to bacterial protein flagellin
Xenetic Biosciences	Combined Zika/dengue vaccine

MVA = Modified Vacinnia Ankara, CDC = Centers for Disease Control and Prevention, TLR = Toll-Like Receptor, NIH = National Institutes of Health, VLP = virus-like particle, VSV = Vesicular Stomatitis Virus, VLV = Virus-Like Vesicles.

Vaccines under Investigation in the U.S [58].

Antiviral therapeutics

Antiviral therapeutics is important for the Zika virus, since the research is going on and the more efforts have been taken in antiviral therapeutics for the control of the virus. Zika virus is among the flavivirus and till now there is no any established antiviral treatment (Petersen *et al.,* 2016). Drugs containing the inhibitory activity are choosing to target the viral life cycle. Since the dengue and Zika virus are similar the anti dengue drugs can be preferred to be anti-zika drugs.

Preventive strategies

Preventive strategies should be taken mostly vector management, by the use of insecticides and the removal of the stagnant water, the insecticides such as (diethyltoluamide/ethylbutylacetylaminopropionate)/ larvicides, is greatly emphasized due to the lack of antiviral therapeutics and vaccines against Zika virus. For current Zika virus outbreak A. aegypti and A. albopictus are primarily responsible. Vector-pathogen and vector control strategies interaction of all possible mosquito species are advised to be considered owing to Zika virus ability to evolve. It is also recommended to wear long-sleeved shirts and long pants, even potentially insecticide-impregnated clothing, in order to minimize vector contact (Weaver *et al.,* 2016). It is recommended that public health authorities in Zika virus endemic regions provide access to contraceptives, safe abortion services and prenatal care. Efforts toward educating the population, particularly in Zika virus endemic regions and travelers, regarding the potential routes of Zika virus transmission and preventative measures should be greatly emphasized (Lazear *et al.,* 2016).

Conclusion

Zika virus is an emerging virus it is rapidly spreading all over the world, and it is important to keep up with this outburst by control threat analysis, case reporting, outburst tracking and vector. The *Aedes* mosquito is the main vector for the infection it is important to take the better prevention. Zika vrius symptoms last for many days. Various approaches have been used to produce the vaccines to flavivirus. Neither drug nor any vaccines is available for the control of zika virus infection, since it is the public health emergency.

ACKNOWLEDGEMENT

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