



## Zika Virus: Transmission and Infectivity

Emma E Taylor<sup>1</sup>, Eyad Kinkar<sup>2</sup>, Kyana St Amant<sup>2</sup> and Mazen Saleh<sup>2\*</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario, Canada

<sup>2</sup>Department of Biology, Laurentian University, Sudbury, Ontario, Canada

\*Corresponding Author: Mazen Saleh, Department of Biology, Laurentian University, Sudbury, Ontario, Canada.

Received: March 24, 2018; Published: April 25, 2018

DOI: 10.31080/ASMI.2018.01.0055

### Abstract

Since its first discovery in 1947, the Zika virus did not cause any public health concerns at a global level until 2015 in South America. The virus belongs to the family flaviviridae and it is an enveloped virus housing within its capsid a positive sense RNA genome. The primary mosquito vectors for the transmission of this virus are *Aedes aegypti* and *Aedes albopictus*, amongst others. The virus appears to infect phagocytic cells through apoptotic mimicry, utilizing receptors involved in the phagocytic uptake of apoptotic bodies. In addition to transmission through mosquitos Zika virus infection can be perinatal or congenital. In the latter, infection of the mother in the first trimester has been implicated in causing microcephaly. Not all Zika virus infections present clinical signs and symptoms but when present they may include low-grade fever, maculopapular rash, arthralgia/arthritis, edema, headaches, retro-orbital pain, conjunctivitis, maculopapular rash on face and limbs, myalgia, vomiting and other digestive disorders. Less commonly reported symptoms, as seen in other flavivirus infections, are lymphadenopathy, prostatitis, anorexia, vertigo and hematospermia. The similarities between the Zika virus and the Yellow Fever, Japanese Encephalitis, Tick-Borne Encephalitis, and Dengue viruses allowed for rapid and effective development of diagnostic methods and great progress towards vaccine development.

**Keywords:** Zika Virus; Flavivirus, Microcephaly, *Aedes aegypti*

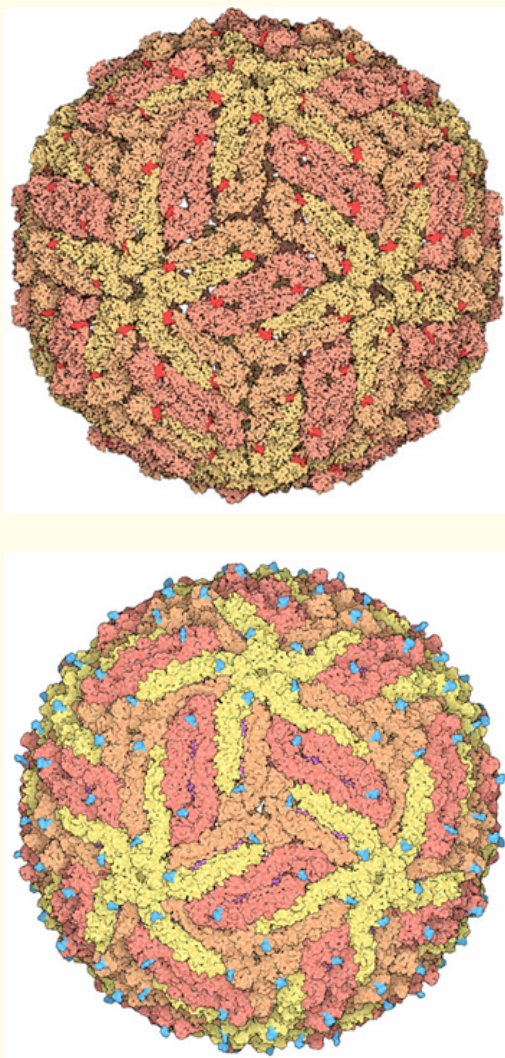
### Introduction

The Zika virus (ZIKV) is a mosquito-borne arbovirus that belongs to the family Flaviviridae, genus Flavivirus [1]. Flaviviruses have similar size, symmetry, and form under electron microscopy. The majority of the members in this genus are transmitted through the bite of an infected arthropod - classifying them as an arbovirus [2]. The most common vectors of flaviviruses are mosquitoes or ticks. Other flaviviruses that are related to ZIKV are: Yellow Fever virus (YFV), dengue virus (DENV), Hepatitis C virus, West Nile Virus (WNV), Japanese encephalitis (JE) and tick-borne encephalitis viruses [1]. ZIKV was first distinguished from these similar illnesses in 1947 by the Rockefeller Foundation whilst serosurveys of jungle Yellow Fever on rhesus monkeys were being performed in the Zika Forest, Uganda [1]. The ZIKV was subsequently identified in humans, but remained of little concern to global health organizations for the next 5 decades. Unexpectedly, Zika fever became epidemic in Yap islands, 2007, French Polynesia, 2013, followed by Brazil and parts of the Americas in 2015 - 2016 [3]. Associated with these outbreaks are neurological manifestations including Guillain-Barré syndrome (GBS) in adults and microcephaly in neonates, although the mechanism of causation is poorly understood [2]. If the molecular biology of the ZIKV - how it relates to and differs from other flaviviruses - can be defined, it follows that the appropriate steps can be taken for rapid and efficient clinical diagnoses of ZIKV and promote the development of effective therapeutic strategies.

### Molecular Biology of the Zika Virus Structure and Morphology

The structure of the ZIKV and other flaviviruses have been determined by x-ray crystallography and cryo-electron microscopy [4]. It has been shown that flaviviruses are enveloped viruses with a genome of approximately 11kb and have a spherical geometry and a capsid surrounded by a lipid membrane [4]. The membrane on flaviviruses contains two integrated surface proteins that are arranged in an icosahedral-like symmetry [5,6]. The genomes of these types of viruses are singular linear positive-sense RNA (+ss-RNA) [4]. While there are limited numbers of full viral sequences available, it is now known that ZIKV contains 10794 nt that encode for several structural and non-structural proteins [2,7]. The description of the viral sequence allows for the classification of Zika strains. Molecular data shows that ZIKV likely evolved in East Africa, then subsequently spread to West Africa and Asia [2]. The genotypes are denoted by the MR766 cluster, the Nigerian Cluster, and the Asian cluster respectively. There is a minimum of 88% nt homology between all isolated ZIKV strains [2,7]. Sun (2017) showed that Virus Pathogen Database and Analysis Resource (ViPR) completed the identification of 172 Zika genomic variants and found that 148 were Asian genotypes, 15 and 8 were East African and West African genotypes, respectively [8]. The low level of nucleotide divergence between all known strains has beneficial implications for vaccine and xenobiotic development.

ZIKV has mature and immature forms, which have diameters of approximately 50 nm and 60 nm respectively [4]. Mature virions contain two virus-encoded membrane proteins, denoted M (membrane) and E (envelope), and have a relatively smooth morphology [4]. This is contrasted by the immature virions which are spiky and contain E and prM (precursor membrane) proteins [4,5]. There is a difference because 60 trimer projections of prM-E heterodimers make up portions of the surface of immature virions while 90 E proteins having the C-terminus end being anchored to the viral membrane contribute to the mature viral surface [4]. The Zika virus closely resembles the Dengue virus in this way (see Figure 1).



**Figure 1:** The Dengue Virus (left, [57]) and the Zika Virus (right, [58]). From visual representation of these two similar flaviviruses one can see that their morphology is quite similar. The likeness between the two viruses will hopefully allow for the development of a ZIKV vaccine, or cross-neutralizing therapeutic agents based upon the existing therapies for DENV.

A feature of ZIKV that also closely resembles other flaviviruses is the attachment of a glycan loop to the E proteins of the viral membrane [9]. The virus isolated during the Yap outbreak in 2007 and thereafter, it has been noted that the glycan loop is significantly longer than the analogous carbohydrates seen in viruses such as Dengue or Yellow Fever [4]. In older isolated strains (including

MR766 from Uganda, 1947), the E protein either has a modified glycan loop, or lacks one entirely [10]. It can therefore be hypothesized that a glycosylated E protein increases ZIKV virulence and may have contributed to its current global prevalence and to the divergence of different strains. On the other hand, other research stated that post-translational modification events have involvement more towards viral replication and maturation; due to ZIKV strains possess variations located at amino acid 154 in the E protein which is known as the glycosylation motif. The 12 nucleotides that are present in several flaviviruses at that location; majority are absent in ZIKV strains [11-14]. An additional source of Zika variability that is comparable to DENV morphology is the pre-mature or incomplete cleavage of prM proteins leading to partially mature virions [15]. These particles have the ability to become infectious and are a complication of drug development since this process is quite variable and results in alternate protein conformations [16,17]. Research has shown that DENV is pleomorphic; due to the variability in the prM content which led to the existence of different states of DENV including, protein dynamics as well as temperature dependent transition. Lui (2017) showed that the presence of NS1 (non-structural protein 1) in the blood system of infected person determines the aggressiveness of ZIKV transition to *Aedes aegypti*. Their results showed that the strains that caused the recent epidemiological events in South America are more infectious than the Cambodian strain (FSS13025). Their phylogenetic analyses indicated that NS1 possesses single amino acid substitution at location 188 (alanine to valine). Their findings showed that acquiring Valine at that location enhance the viral infectivity and its prevalence in *A. aegypti*, which could be the reason for Asian lineage stain of ZIKV viral transmission and the cause of the recent epidemiological events in South America [18]. Therefore, the question would be asked is Does post-translational modification affecting the structural heterogeneity of ZIKV?

Another striking feature of the ZIKV is the constant dynamic motion of the membrane proteins as suggested by cryo-EM [19]. Viral neutralization assays also conclude that the viral epitopes are transiently accessible due to different conformations that the E membrane protein can adopt throughout the viral life cycle [20]. The ability to undergo dynamic conformational changes is known as viral breathing [4]. The consequences of viral breathing as related to ZIKV are not well understood, however it is thought to simultaneously contribute to the high thermal stability of ZIKV and the difficulty in developing anti-viral agents that target viral epitopes [19,20]. Additionally, viral breathing may help to expose parts of the virus that interact with TIM/TAM receptors and may be a mechanism of infection, as described in the next section. Viruses belonging to the family Flaviviridae have accordingly similar morphological features, but there are some unique features of the ZIKV that warrant further investigation including, sequence variations, and glycosylation event.

### Structure and Morphology

The molecular mechanism of ZIKV infection is poorly defined at the present time but we continue to make progress in that direction. Envelope proteins on the viral membrane surface mediate attachment to the host cell and are thought to induce engulfment and direct endosomal membrane fusion [4]. Host cell-receptor

molecules may differ from species to species, and likely from tissue to tissue [4]. Some implicated host proteins that facilitate entry into dermal fibroblasts, keratinocytes and dendritic cells are TIM-1 (T-cell immunoglobulin mucin domain) and TAM (Tyro3, Axl and Mer) entry and adhesion factors [21]. These receptors normally induce phagocytic cellular activity when they bind extracellular phosphatidylserine from apoptotic remains, however, research suggested that they may interact with the lipid membrane of the virus, which is transiently exposed as a result of viral breathing [21-23]. It can be hypothesized that ZIKVs gain access to cells by way of apoptotic mimicry as viral breathing, giving access to host TIM/TAM receptors, which engulf and internalize the viral particle [22,23].

### Viral Life Cycle and Replication

Even though ZIKV life cycle is still unclear, research relate ZIKV to Flaviviruses' life cycle and replication. Flaviviruses replicate via the attachment of the viral E protein to host receptors, thus mediating internalization into the host cell [5]. This is accomplished by receptor-mediated endocytosis or by apoptotic mimicry; as recent research has concluded that TIM-1 facilitates the interaction between the viral E protein and the targeted cells which then transfers the virus particles to TAM for the endocytosis event. As their results suggested that the expression of AXL and Tyro3 showed to be up to 70% infectivity whereas the expression of TIM-1 and 4 resulted in basal infectivity [21-23]. On the other hand, TIM-1 was shown to be involved the viral infectivity and transmission during the course of pregnancy since TIM-1 was shown to be greatly expressed on many cell types including mid-gestation, late-gestation, and placental cells. As for AXL and Tyro3, they were found to be under expressed [24]. Following internalization there is fusion mechanism between the virus membrane and the host's endosomal membrane, leading to the release of the viral genomic RNA into the cytoplasm, known as 'acidic-pH-triggered' [5]. Due to ZIKV's huge similarities to the DENV type 2 and other flaviviruses, the viral assimilation structure reveals important closer similarities between ZIKV and DENV including, three domains (I, II, and III) as they are connected to the two transmembrane (TM) helices and anchored stem regions by a short linker. Therefore, the acidic environment leads to dissociate the E dimer and expose the fusion loop (FL), since it was assumed that the FL is also available at the tip of ZIKV's domain II, to facilitate the interaction with cytosolic membranes. More structural reorganization causes domain III to relocate and arranging a trimer hairpin-like structure in which the FL and the TM regions are merged creating two lipid bilayers. That allows the release of the genomic RNA. The viral RNA is to be served as mRNA and translated into a polyproteins which they code for three structural and seven non-structural proteins (Capsid 'C', prM, E, and NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 respectively) [4,5,25]. Although research on ZIKV is still on going, the questions remain to be answered are Does the structure of all Flaviviruses utilize the same route in influencing host interactions and virus tropism?

The replication of viral particles takes place in the cytosol, where a double-stranded RNA (dsRNA) genome is synthesized from the genomic RNA [5]. Once the dsRNA genome is synthesized, the dsRNA genome is then transcribed and replicated. This process produces viral mRNA along with new ssRNA(+) genomes as well as the post- and co-translational modification of all viral proteins. Next, assembly of the virus occurs at the endoplasmic reticulum, the virion buds, and is then transported to the golgi apparatus via the exocytic pathway towards the trans-Golgi network (TGN). Once

in the golgi apparatus, the acidic environment leads to the rearrangement of the E proteins, the cleavage of prM protein into pr and M by the furin protease activity, maturing the virion, and allowing for the release of new virions via exocytosis [4,5]. Once released from the infected cell it disperses into the host bloodstream and lymph nodes [1].

### Modes of Transmission

The most common mode of transmission of ZIKV is via mosquito, mainly of the genus *Aedes* [1]. The main vectors associated with the spread of the ZIKV are the yellow fever mosquito, *A. aegypti*, and the Asian tiger mosquito, *A. albopictus* [27]. Recent studies show that the transmission of ZIKV could also occur via a multitude of other *Aedes* mosquito species such as: *A. africanus*, *A. luteocephalus*, *A. vittatus*, *A. furcifer*, *A. hensilli*, *A. dalzieli*, *A. polyneisensis*, *A. taylori* and *A. apicoargenteus* [1,2]. These mosquitoes live both indoors and outdoors and are found to bite humans largely during the daytime [2]. These mosquitoes are carriers of the virus once they have drawn blood from an individual who has been infected with the ZIKV – once infected the mosquitoes are able to spread the virus to others. These mosquitoes have an almost unnoticeable bite, and have the ability to bite multiple humans in a single meal [2,3]. Other modes of transmission include mother-infant transmission, sexual intercourse, and blood transfusions [2].

Even though it is still unclear where ZIKV is located in the mosquito, one may make inference to other arboviruses. Once the mosquito is infected by a virus, the virus will make its way to infect the salivary glands of the mosquito as soon as the incubation and replication periods are over at the midgut wall of the mosquito. After that mosquitoes would be ready to bite and infect other individuals as research has reported that they would live shorter lives and may lay fewer eggs. In fact, mosquito already has a short live and if viruses would kill them sooner than we would have never experienced arboviruses' diseases [26]. On the other hand, Mosquito co-infection of ZIKV with chikungunya virus (CHIKV) allows simultaneous transmission without affecting vector competence of *A. aegypti*. A recent research has shown that metabolome of *A. Aegypti* during DENV infection; as their results suggested that DENV infection precisely alters the lipid repertoire at the midgut of the mosquito (viral replication site), which could be important to highlight the metabolic pathways that may be required for successful dissemination and ultimately transmission [27]. Other research has also shown that ZIKV can be co-circulated or co-infected with other viruses including CHIKV and DENV. Their results show that *A. Aegypti* has the ability to transmit two or more viruses at the same time as their results showed that *A. Aegypti* managed to simultaneously transmit ZIKV and CHIKV through a single bite without further inference unlike other studies that showed the co-infection of CHIKV and DENV require sequential bites as well as Sindbis virus and DENV showed low rate of infectivity and infection [28]. Therefore, the question is; does DENV co-infection enhance or reduce ZIKV infectivity and pathogenicity? Does pathogenic ZIKV enhance the pathogenicity of non-pathogenic flaviviruses?

In experimental ZIKV infection models it is important to distinguish between needle-based (subcutaneous) infections of experimental animals versus mosquito bite-based infections. The latter will clearly better emulate the natural transmission kinetics in humans and other primates. There are documented differences between both routes of infections in terms of viral replication,

pathogenesis, and transmission kinetics. Differences are also seen in tissue distribution of the virus in mosquito-infected animals where the virus is detected only in hemolymphatic tissue, female reproductive tract tissue, kidney, and liver [29]. Other differences reported included delay to peak viremia and decreased genetic diversity of the virus in the infected animal population [29]. Additionally, passage of the virus from mice through the mosquitos to macaques was observed to result in alteration of the frequencies of single nucleotide polymorphism (SNP) in ZIKV populations as compared to the stock virus.

ZIKV can be transmitted from a mother to a fetus during pregnancy (otherwise referred to as congenital transmission), or transmission can occur around the time of birth (referred to as perinatal transmission) [2,30]. Congenital transmission occurs when the mother is infected with ZIKV early in the pregnancy, and before delivery the virus has passed to the fetus. On the other hand, perinatal transmission occurs when the mother is infected with ZIKV around two weeks before delivery. The virus is subsequently transferred to the fetus during or close to the time of delivery [2]. After perinatal transmission occurs, it is reported that infants can develop symptoms indicative of ZIKV, such as macropapular rash, conjunctivitis, arthralgia, and fever [30]. According to Petersen, *et al.* [2], in addition to the ZIKV symptoms, there also is evidence that ZIKV RNA is present in the amniotic fluid, further suggesting direct transmission of the virus from the mother to the fetus. Moreover, ZIKV can also be transmitted to infants and children postnatally, like adults, through the mosquito bite from infected arthropod vectors.

Recent studies are considering the possibility of ZIKV infected mothers transmitting the virus to the infants via breastfeeding practices [31]. Certain studies suggest that ZIKV can be detected in the breast milk of infected mothers, although there is not a sufficient amount of data to confidently conclude that ZIKV can be transmitted in this manner [31]. Another possible route of ZIKV transmission is through sexual intercourse between partners [2,32]. Individuals that are infected with ZIKV are able to transmit the virus through bodily fluids, even before the symptoms start to present themselves. Viral RNA can be found in the semen, vaginal fluids, and urine [32]. ZIKV is the first flavivirus that is known to be sexually transmittable between symptomatic or asymptomatic patients [32-35]. ZIKV can also be transmitted person-to-person via blood transfusions [2]. From this data, it is obvious that care must be taken to ensure individuals are aware of all the possible routes of ZIKV transmission. The knowledge may help to curb the spread of the virus across the globe.

## Epidemiology

### Early Years of ZIKV

The temporal and geographical pathway of ZIKV transmission by the arthropod vector *Aedes* is fairly well understood and can be delineated according to its time-space relationship. Firstly, the Zika virus was identified in the Zika Forest of Uganda in 1947 [3]. During a Yellow Fever serosurvey being conducted, the Rockefeller foundation distinguished and isolated ZIKV from YFV – both in a febrile rhesus monkey (Monkey 766) and in a pool of *A. africanus* mosquitoes [1,3]. The first human isolate occurred in 1952 in Uganda [3]. In the following two decades, 15 types of Arboviruses were isolated from 171 subjects [36]. In comparison to other flaviviruses such as YFV and DENV, ZIKV occurred at a low frequency, but did exhibit seasonal patterns (fewer ZIKV isolations in dry seasons and more in rainy seasons) that corresponded to the prevalence of compe-

tent mosquito vectors [37]. The virus remained relatively obscure for a number of years, though it had been reportedly isolated in mosquitoes, primates and humans in at least 14 countries, including Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone, Gabon, India, Malaysia, Philippines, Thailand, Vietnam, Indonesia, Senegal and the Ivory Coast [1,3]. In fact, it was not until 1999 that three distinct strains of ZIKV were isolated as part of a series of studies of YFV in the Ivory Coast [36,38]. In the time since then very few cases had been reported; no hospitalizations, deaths or severe hemorrhagic illness had been found [36]. This remained true until the ZIKV outbreak in Yap, of the Micronesia Pacific, in 2007.

### Emergent Years of ZIKV

The Zika virus has been considered an emergent threat to public health since the Yap epidemic in 2007 [3]. During the health crisis, no hospitalizations or deaths were recorded, but it was the first time that Zika fever was found to be hemorrhagic [3]. It was estimated that 68 - 77% of the Yap population aged 3 years or older were carriers of ZIKV antibodies, and of a population approximately 7400, there were 108 biologically confirmed ZIKV cases from April-August 2007 [3]. The generally accepted vector for the spread of the virus in this region is *A. hensilli*, although entomologists could not isolate the virus from the vector at the time of the outbreak [3]. It is thought that most infections were not reported as the majority of cases are believed to be asymptomatic. Additionally, this was the first time that ZIKV was discovered outside of the typical geographical range – Africa and Asia [1]. It was hypothesized that ZIKV could spread to other Pacific Islands or to the Americas following this emergent outbreak.

The next major Zika event to catch the attention of the general public was the French Polynesian epidemic in 2013. Iosos and colleagues indicated that approximately 10% of the French Polynesian population sought out medical attention for suspected ZIKV infections [3]. As with the Yap outbreak, no deaths caused by ZIKV infection were reported, but hospitalization and hemorrhagic fever were noted. In contrast to the inability to isolate the ZIKV from a vector in Yap (2007), entomologists were able to isolate it from two vectors in French Polynesia (2013). It was discovered that mosquito species *A. aegypti* and *A. polynesiensis* were spreading and perpetuating the viral infection [3]. Furthermore, the Zika outbreak in French Polynesia was the event that sparked investigation into the association between ZIKV infection and neurological symptoms. In fact, 72 cases of biologically confirmed ZIKV had reported neurological manifestations of the illness, and of these, 42 cases of GBS were diagnosed [3,7]. It is important to note that the country was in the midst of a Dengue viral epidemic (DENV types 1 and 3) when the Zika outbreak occurred [3]. The extreme conditions of Zika co-circulating with Dengue warrant further investigation into the effect of concurrent or serial flavivirus infections, and well as the possibility of viral cross-neutralization and viral enhancement.

Finally, March of 2015 played host to the first suspected case of ZIKV in the Americas [3]. By March of 2016, Zika is reported to have spread to 33 countries in the Americas, with the majority of cases concentrated in Brazil [3,39]. The prevalence of the ZIKV had a major global impact; not only in the eyes of the World Health Organization, but athletes, tourists and even spectators felt the negative impact of the emergent disease in Brazil. In fact, the Brazilian Ministry of Health suspected that 1.3 million cases of ZIKV infec-

tions had occurred by March 2016 [3,39]. During this time, Brazil experienced a considerable increase in the number of fetal microcephaly and congenital malformations being reported [2,3,39]. This discovery prompted French Polynesian health officials to look back at their own data. They identified an increase in the number of fetal abnormalities that coincided with the time and locations of ZIKV prevalence [3]. The mechanisms by which ZIKV can cause neurological complications are not clear as of yet. Steps must be taken to better understand the effect of ZIKV so that efficient diagnoses can be made and vaccination therapies can be developed.

## Clinical Aspects of Zika Virus Infections

### Symptoms and Diagnosis

Most of the data concerning ZIKV infections has been inferred from data collected from other flaviviruses. The similarities between these viruses that allow useful data to be extrapolated are also the cause of difficulty in the symptomatic diagnosis of Zika fever. The common symptoms of ZIKV are quite broad in nature and are characteristic of many flaviviruses, such as Dengue [2]. These symptoms can persist for 3 - 12 days and include arthralgia/arthritis, edema, fever (mild-low grade), headaches, retro-orbital pain, conjunctivitis, maculopapular rash on face and limbs, myalgia, vomiting and other digestive disorders [2,3]. Less commonly reported symptoms of flaviviruses are lymphadenopathy, prostatitis, anorexia, vertigo and hematospermia [40]. Before the outbreak in French Polynesia (2013), there was neither severe presentations of these symptoms nor hospitalization due to ZIKV infection reported [3]. Currently, there is an association between Zika fever and severe neurological complications in adults and congenital malformations in fetuses that has been the cause of some deaths (see Complications section).

Another diagnostic difficulty is the apparent lack of symptoms in a large percentage of infected people. It is estimated that only 18% of human infections result in clinical manifestation [41]. In fact, only 26% of French Polynesian blood donors that tested positive for ZIKV reported symptoms, and during a serosurvey in Yap only 19% of confirmed infected residents in reported illness associated with ZIKV [2]. While the exact Zika incubation period is unknown, it is thought to average less than one week based upon other members of the flaviviruses [2]. The virus can be considered self-limiting, as the viremic period in humans is approximately 3-5 days after the initial onset of symptoms [3,7]. Due to the non-specific manifestation of flaviviruses, biological and chemical tests should be used to confirm a diagnosis. The primary and most accurate method of detection for acute ZIKV infection is isolation and polymerase chain reaction (PCR) of the viral nucleic acid [7,22]. There is however a multitude of diagnostic tests and specific methodologies that are in practice. For example, viral nucleic acid can be detected by PCR techniques in sera and other fluids, and the presence of ZIKV antigens can be detected by immunohistochemistry analysis with monoclonal antibodies [1,42]. Diagnosis relies on reverse-transcription PCR (RT-PCR) of viral RNA from blood samples of patients believed to be infected with the virus [3]. These samples must be collected no more than 5 days after the onset of symptoms or detection of the nucleic acid in the sera may not be possible [3]. Enzyme-linked immunosorbent assays (ELISAs) are another serological test used to detect anti-Zika IgM and IgG [3,7]. However, cross-reactions with other flaviviruses can yield positive results;

notably if the patient has anti-Dengue and anti-West Nile virus antibodies [7]. IgM-capture (MAC-) ELISA tests can be corroborated with more specific plaque reduction neutralization tests (PRNTs) to reduce the possibility of a misdiagnosis due to cross-reactivity [2]. This method is labour intensive, costly and involves handling of the live virus – many labs are not equipped to run this type of tests [2].

Alternatively, RT-PCR of urine, saliva and semen samples may be used with varying degrees of success to diagnose ZIKV infection by detecting viral RNA well after the onset and resolution of symptoms [3,32,43]. In a comparative study conducted by the Centers for Disease Control and Prevention (CDC) on 53 subjects with Zika fever, infections were biologically confirmed using RT-PCR in 92% of their urine samples, 81% of saliva samples and only 51% of serum samples [44]. Furthermore, when comparing the results of sera and urine samples from 66 infected subjects, viral RNA was found in 61 urine samples; almost double the number of positive serum specimens [44]. No positive results from RT-PCR testing were found for ZIKV in sera after 5 days or longer from the onset of symptoms [44]. This is because the viremic period is relatively short, making the viral load higher and infection more detectable in these alternative fluids than when compared to blood [32]. In particular, urine and semen show high viral loads [32,43]. The infectious viral load of semen can persist for an unknown duration and potentially contribute to sexual transmissions of this disease [32]. It is also unknown how long infectious viral loads can persist in the blood and amniotic fluid of pregnant women [2]. There is currently no standard procedure for testing in prenatal and antenatal infections, although RT-PCR of cord blood and amniotic fluids has been used to confirm the presence of viral particles while ultrasonography has been used to look for congenital malformations in the fetus [2].

### Treatment and Prevention

As of the time of this review, there is no specific antiviral drug for the ZIKV infection [3,45]. The treatment is purely based on symptomatic relief – a combination of antihistamines for rashes, antipyretics such as acetaminophen for pain and fever, and fluids for hydration are usually prescribed [3,7,45]. It has been found that the infection can be complicated by administering acetylsalicylic acid which increases the risk of bleeding [41,45]. The risk of hemorrhaging is also thought to be increased by the use of non-steroidal anti-inflammatory drugs (NSAIDs) [7]. This is largely based upon the observation of hemorrhagic syndrome onset with other flaviviruses brought upon by treatment with NSAIDs [45]. In the viremic phase, individuals with suspected ZIKV infections should be isolated from other individuals and from mosquitoes to prevent further transmission of the disease [46]. Other preventative measures include protecting one's self against mosquito bites, wearing long clothes, using insect repellent containing DEET, eliminating or isolating stagnant water (egg-laying sites), making use of screened-in outdoor areas, mosquito nets, and avoiding travel to afflicted areas [3,39]. Extra precautions are to be taken for pregnant women residing in ZIKV endemic areas as they should always wear protective clothing, and should always sleep inside screened-in rooms or under mosquito nets. These means of prevention are largely based upon personal protection, as no vaccine currently exists [3].

Vaccinations for Yellow Fever (YF), Japanese Encephalitis (JE), Tick-Borne Encephalitis (TBE) and Dengue (DEN) currently exist, so it seems likely that a vaccination for the ZIKV is entirely possible [4]. In fact, there are currently a number of vaccine and drug therapies for the ZIKV infection being developed with promising results. Methodologies being tested include the use of an inactivated virus, live recombinant virus, subunit vaccines, virus-like particles, live-vectored vaccines, nucleic acid-based vaccines and repurposing existing drugs [4,47]. For example, the similarities between the epitope structure for DENV 1-4 and ZIKV may result in the development of universal structure-based vaccine that will provide cross-neutralization protection against homologous infectious particles [4]. Just as with other flaviviruses, the target of the trial-vaccine forerunners is to alter the functionality of the precursor-membrane protein (prM) or the envelope protein (E) with a nucleic acid vaccine [47]. These proteins have become the primary target of antibodies as their neutralization would interfere with host membrane receptors and membrane fusion [16,47]. This is because E is essential for viral attachment to and entry into host cells, while prM function is vital for virus maturation [17,47]. Their alteration leads to the formation of subviral particles with E glycoprotein conformations that produce high antibody titers correlating to the viral-neutralizing activity of the serum [17]. For example, Pardi and colleagues developed an mRNA-modified vaccine with lipid nanoparticles that is structurally based upon the French Polynesian (2013) ZIKV strain (H/PF/2013) [48]. A vaccinated group of test mice were found to have E protein-specific binding IgG antibodies and neutralizing antibodies, and when they were subsequently challenged against the Puerto Rican 2015 ZIKV strain, all mice blood tested negative for ZIKV RNA [48]. Additionally, Pardi and colleagues vaccinated rhesus monkeys in one of three different vaccine doses (50 µg – 600 µg). Just as with the mice, no illness or negative effects were observed due to the vaccination and E-specific antibodies and neutralizing antibodies were once again produced [48]. Six control monkeys all became infected when challenged with Puerto Rican ZIKV, while 4 out of 5 vaccinated monkeys were completely protected from viremia; one vaccinated monkey experienced low levels of transient viremia [48].

As indicated in this study, there may be a limiting protective threshold against ZIKV that is dose-dependent based upon the antibody affinity for the virus, the accessibility of the epitope, and the overall effectiveness of the vaccine [48]. This is very important to consider during human trials. Antibodies that are not able to meet or exceed the protective threshold may increase viral production in monocytes, macrophage, and dendritic cells because viral-antibody complexes are facilitated in their cellular transport by Fcγ receptors. This is called antibody-dependent enhancement (ADE). The ADE phenomenon has been implicated in the pathogenesis of severe infections causing hemorrhagic fever and shock syndromes [48]. Additionally, when considering vaccination as a method of protecting both a pregnant woman and her child from ZIKV infection, a real danger is that passive immunization (maternal antibodies being shared with the fetus) will result in sub-neutralizing levels of antibodies for the fetus [48]. This may contribute to severe infantile ZIKV infections and even congenital malformations. Due to the relationship between severe ZIKV infections and fetal microcephaly and GBS in adults, vaccination and treatments must take care not to exacerbate the infection because of possible ADE effects. Other research has shown that potentially PKA inhibitor (PKI) has the ability to inhibit the replication of ZIKV at the post-entry stage

by disturbing negative-sense RNA synthesis and protein translation. Screening several cellular pathways for inhibitors led to the identification of the PKI 14-22 inhibitor as to be considered ZIKV's potential inhibitor. The study used human endothelial cells and astrocytes, since they have shown to be ideal modules for ZIKV infection, to show that PKI effectively suppresses the replication of several ZIKV strains that have African and Asian/American lineages with minimal cytotoxicity to both cells. This study has shown a potential in further exploring the therapeutic strategies in tackling ZIKV replication [49].

### Complications

As alluded to earlier in the review the ZIKV was of little concern to the global health organizations before the outbreaks in Yap (2007) and French Polynesia (2013). This was due to Zika's typically mild, uncomplicated and relatively asymptomatic presentation [50]. Since the emergence of the ZIKV as a global threat however, the incidence of infection has proportionally increased to the prevalence of severe neurological complications such as GBS in adults and microcephaly in neonates.

### Neurological Complications in Adults

Before the French Polynesian Zika event, flaviviruses such as Japanese Encephalitis, West Nile Virus and Dengue had already been suspected to cause GBS [36]. GBS is an autoimmune disorder that is characterized by rapid onset of muscle weakness, motor function disturbance and ascending paralysis following a minor viral or bacterial infection; there was an unusually high number of reported cases during the Zika outbreak in French Polynesia [51]. Sejvar and colleagues describe the average number of incidents of this syndrome per year in the country as 1-3 out of a population of 100,000 [52]. During the French Polynesian Zika outbreak, after experiencing ZIKV symptoms, 42 patients in one year had additional neurological symptoms develop, and were subsequently diagnosed with the syndrome [52]. There is clearly a spatial and time-dependant correlation between ZIKV and the GBS, however, during this time it was reported that Dengue types 1 and 3 were co-circulating with ZIKV in French Polynesia [51]. Concurrent flavivirus infections may contribute to the high incidence rate of this syndrome.

More recently in the Americas, Venezuela reported a 2-3 times increase of GBS diagnoses from the national average and associated this increase to ZIKV infections [46]. Additionally, Brazil and El Salvador reported that of all cases of GBS, more than half of the patients had Zika-like symptoms preceding their diagnosis [39,46]. The link between ZIKV infections and GBS is becoming more apparent over time. In fact, Cao-Lormeau and associates discovered that there are clinical characteristics of ZIKV-induced GBS that distinguish the disorder from other types of GBS [51]. They discovered that most of the 42 French Polynesian patients described above had electrophysiological markers (using electromyography assessment techniques) showing acute motor axonal neuropathy (AMAN) [51]. The onset of this type of syndrome was extremely rapid, but further investigation showed that the prognosis for the ZIKV-GBS patients was generally favourable. This type of GBS caused 'reversible conduction failure' within nerve segments, which is typical of the AMAN type of GBS [51]. Following these neurological findings, health organizations in countries where Zika is endemic or countries that are prime candidates to play host

to the ZIKV should be prepared to care for ZIKV patients in both physiological and neurological capacities.

### Neurological Complications in Neonates

A second source of neurological complications that have been associated with ZIKV infections are those having to do with pregnant women and their afflicted fetuses. Parallel to the unusually high number of GBS diagnoses occurring during ZIKV outbreaks, the frequency of reported cases of neonatal congenital malformations also follows the temporal and geographical path of the ZIKV. It is hypothesized that a maternal ZIKV infection results in an increase in the likelihood of microcephaly for neonates characterized by newborns having a below average size of head (regardless of sex or age) resulting in slow brain growth and brain abnormalities [53]. These nervous system complications have resulted in the death of some microcephalic neonates [46]. Though the relationship between microcephaly and ZIKV was initially not as apparent as ZIKV and GBS, etiologic and epidemiologic data encouraged countries such as French Polynesia to retrospectively consider the concurrent diagnoses of Zika fever and microcephaly. Indeed, health organizations in that country found – contrary to the average annual rate of fetal congenital malformations – 17 cases had been reported in newborns to accompany the French Polynesian Zika outbreak [3,46]. In Brazil, there was also a considerable inflation in the number of neonatal congenital malformations, nervous system defects and microcephaly that were found to coincide with Zika events [39,46].

The mechanisms of neurological damage to fetuses caused by Zika viral particles is even less understood than those of GBS. It is known that Zika RNA can be detected in both the brains of microcephalic neonates, and the amniotic fluid of the mother [46,53]. Interestingly, the only tissue of newborns to have quantifiable levels of ZIKV RNA was the brain [54]. This infers that there is a link between a maternal infection and fetal brain damage. Ultrasonography can be used to detect fetal abnormalities; caution must be taken, however, not to assume that all fetal microcephaly is the result of Zika infection. Differentiation between genetic microcephaly and acquired microcephaly that is the result of infection with the Zika virus must be made. This distinction is important so that mechanisms of neonatal neurological defects caused by a virus can be studied competently and efficiently. Additionally, Rather and colleagues discuss that pregnant women who gave birth to afflicted newborns likely contracted the virus during the first trimester of their pregnancy [53]. Petersen and colleagues corroborate this finding by suggesting that maternal infection often took hold between 7 and 13 weeks [2]. This is contradictory to the onset of fetal microcephaly not caused by the Zika virus – which usually occurs in the late second or early third trimester. This being said, it is not advisable for pregnant women to travel to areas where ZIKV is circulating during any stage of pregnancy as ZIKV can be transmitted by newly infected mothers to their infants at the time of birth, resulting in sick infants with typical ZIKV infections [2,30,53].

### Canadian Case Study

In Canada there have been a few documented cases of travelers returning home with ZIKV. The first case of a ZIKV infection in a Canadian was in 2013, when a woman who had travelled to Thailand returned to her home in Alberta, Canada. She experienced a number of mosquito bites during her stay. Upon her return and over a 7 day period, the patient experienced intermittent periods

of fever and chills, backaches, a popular rash on her extremities, mild conjunctivitis, joint and muscle tenderness as well as retro-orbital headaches [55]. She then sought out medical attention and it was initially thought that she had contracted Dengue Fever (DENV) [55]. From the patient, Fonseca and colleagues report that blood, a nasopharyngeal swab and urine samples were collected and proceeded to be subjected to a differential diagnosis of causative pathogens.

The patient was determined to be negative for bacterial pathogens and malaria, but the blood samples resulted in a positive reaction to IgM antibodies for Dengue virus [55]. An inconsistency was discovered with the diagnosis of Dengue fever when the patient's serum samples did not show a corroborating positive result to Dengue IgG antibodies [55]. This encouraged the medical staff treating her to look at alternate Flaviviruses that might have a cross-neutralization effect with the DENV IgM antibodies. RT-PCR was subsequently performed on the patient's serum, nasopharyngeal and urine samples [55]. Fonseca and colleagues reported that the resultant nucleic acid sequences were found to be nearly identical with the Asian Zika virus strain. Following this discovery, the hypothesis of a ZIKV infection was corroborated by Zika-specific serum IgM tests that were performed using ELISA and PRNT techniques. Additionally, the incubation period from her exposure to mosquitos until the onset of her symptoms is consistent with the current model of Zika incubation periods [55]. Finally, the complete Zika viral RNA genome was isolated from the urine samples and nasopharyngeal swabs at the National Microbiology Laboratory in Winnipeg, Manitoba, Canada. It was confirmed that the patient was the first host of the Zika virus in Canada, having contracted the ZIKV infection from a mosquito-vector from her visit to Thailand [55-57].

### Conclusions

Since the isolation of the ZIKV from rhesus monkey 766 in a forest of Uganda in 1947, to its recent emergence as a global health risk, health organizations have accomplished many advancements. We are now closer to a complete understanding of the molecular biology of ZIKV and the molecular mechanisms by which it acts. Although there are not currently vaccinations or drug therapies available for the Zika fever specifically, the fact that ZIKV is very similar to other members of the Flaviviridae has allowed for a rapid progression in the development of the aforementioned viral-combatant techniques. ZIKV is still spreading across the globe by way of competent mosquito-vectors and domestic transmission from person-person via a multitude of possible routes. With further research and investigation, however, the threat of the ZIKV may diminish and with the development of clinical therapies, ZIKV may return to its mild, nonthreatening status.

### Bibliography

1. Hayes Edward B. "Zika virus outside Africa". *Emerging Infectious Diseases* 15.9 (2009): 1347-1350.
2. Petersen Lyle R., et al. "Zika virus". *New England Journal of Medicine* 374.16 (2016): 1552-1563.
3. Ios Sophie., et al. "Current Zika virus epidemiology and recent epidemics". *Medecine et Maladies Infectieuses* 44.7 (2014): 302-307.

4. Heinz Franz X and Karin Stiasny. "The antigenic structure of Zika virus and its relation to other flaviviruses: implications for infection and immunoprophylaxis". *Microbiology and Molecular Biology Reviews* 81.1 (2017): e00055-e00016.
5. Bollati Michela., *et al.* "Structure and functionality in flavivirus NS-proteins: perspectives for drug design". *Antiviral Research* 87.2 (2010): 125-148.
6. Plevka Pavel., *et al.* "Maturation of flaviviruses starts from one or more icosahedrally independent nucleation centres". *EMBO Reports* 12.6 (2011): 602-606.
7. Araujo Lucas Masiêro., *et al.* "Guillain-Barré syndrome associated with the Zika virus outbreak in Brazil". *Arquivos de Neuro-Psiquiatria* 74.3 (2016): 253-255.
8. Sun Guangyu., *et al.* "Comprehensive annotation of mature peptides and genotypes for Zika virus". *PLoS one* 12.1 (2017): e0170462.
9. Sirohi Devika., *et al.* "The 3.8 Å resolution cryo-EM structure of Zika virus". *Science* 352.6284 (2016): 467-470.
10. Faye Oumar., *et al.* "Molecular evolution of Zika virus during its emergence in the 20<sup>th</sup> century". *PLoS Neglected Tropical Diseases* 8.1 (2014): e2636.
11. Li J., *et al.* "The glycosylation site in the envelope protein of West Nile virus (Sarafenid) plays an important role in replication and maturation processes". *Journal of General Virology* 87.3 (2006): 613-622.
12. Lanciotti Robert S., *et al.* "Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007". *Emerging Infectious Diseases* 14.8 (2008): 1232-1239.
13. Haddow Andrew D., *et al.* "Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage". *PLoS Neglected Tropical Diseases* 6.2 (2012): e1477.
14. Baronti Cécile., *et al.* "Complete coding sequence of zika virus from a French polynesia outbreak in 2013". *Genome Announcements* 2.3 (2014): e00500-e00514.
15. Pierson Theodore C and Michael S Diamond. "Degrees of maturity: the complex structure and biology of flaviviruses". *Current Opinion in Virology* 2.2 (2012): 168-175.
16. Rodenhuis-Zybert Izabela A., *et al.* "Immature dengue virus: a veiled pathogen?" *PLoS Pathogens* 6.1 (2010): e1000718.
17. Dowd Kimberly A., *et al.* "Combined effects of the structural heterogeneity and dynamics of flaviviruses on antibody recognition". *Journal of Virology* 88.20 (2014): 11726-11737.
18. Liu Yang., *et al.* "Evolutionary enhancement of Zika virus infectivity in *Aedes aegypti* mosquitoes". *Nature* 545.7655 (2017): 482-486.
19. Kuhn Richard J., *et al.* "Shake, rattle, and roll: Impact of the dynamics of flavivirus particles on their interactions with the host". *Virology* 479-480 (2015): 508-517.
20. Dowd Kimberly A., *et al.* "A dynamic landscape for antibody binding modulates antibody-mediated neutralization of West Nile virus". *PLoS Pathogens* 7.6 (2011): e1002111.
21. Hamel Rodolphe., *et al.* "Biology of Zika virus infection in human skin cells". *Journal of Virology* 89.17 (2015): 8880-8896.
22. Mercer Jason and Ari Helenius. "Apoptotic mimicry: phosphatidylserine-mediated macropinocytosis of vaccinia virus". *Annals of the New York Academy of Sciences* 1209.1 (2010): 49-55.
23. Amara Ali and Jason Mercer. "Viral apoptotic mimicry". *Nature Reviews Microbiology* 13.8 (2015): 461-469.
24. Tabata Takako., *et al.* "Zika virus targets different primary human placental cells, suggesting two routes for vertical transmission". *Cell Host and Microbe* 20.2 (2016): 155-166.
25. Heinz Franz X and Karin Stiasny. "The antigenic structure of Zika virus and its relation to other flaviviruses: implications for infection and immunoprophylaxis". *Microbiology and Molecular Biology Reviews* 81.1 (2017): e00055-e00016.
26. Public Health Ontario. "Zika Virus" (2017).
27. Chotiwan Nunya., *et al.* "Dynamic remodeling of lipids coincides with dengue virus replication in the midgut of *Aedes aegypti* mosquitoes". *PLoS Pathogens* 14.2 (2018): e1006853.
28. Göertz Giel P., *et al.* "Mosquito co-infection with Zika and chikungunya virus allows simultaneous transmission without affecting vector competence of *Aedes aegypti*". *PLoS Neglected Tropical Diseases* 11.6 (2017): e0005654.
29. Dudley Dawn M., *et al.* "Infection via mosquito bite alters Zika virus tissue tropism and replication kinetics in rhesus macaques". *Nature Communications* 8.1 (2017): 2096.
30. Centers for Disease Control and Prevention. "Zika in Infants and Children – Congenital and Perinatal Transmission" (2017).
31. Colt Susannah., *et al.* "Transmission of Zika virus through breast milk and other breastfeeding-related bodily-fluids: A systematic review". *PLoS Neglected Tropical Diseases* 11.4 (2017): e0005528.
32. Musso Didier., *et al.* "Potential sexual transmission of Zika virus". *Emerging Infectious Diseases* 21.2 (2015): 359-361.
33. Fréour Thomas., *et al.* "Sexual transmission of Zika virus in an entirely asymptomatic couple returning from a Zika epidemic area, France, April 2016". *Eurosurveillance* 21.23 (2016).
34. Szabo Robert and Roger V Short. "How does male circumcision protect against HIV infection?" *BMJ: British Medical Journal* 320.7249 (2000): 1592-1594.
35. D'Ortenzio Eric., *et al.* "Evidence of sexual transmission of Zika virus". *New England Journal of Medicine* 374.22 (2016): 2195-2198.
36. Shapshak P., *et al.* "Chapter 18. Zika virus". In *Global Virology I-Identifying and Investigating Viral Diseases*. Springer New York (2015): 477-500.
37. Moore D., *et al.* "Arthropod-borne viral infections of man in Nigeria, 1964–1970". *Annals of Tropical Medicine and Parasitology* 69.1 (1975): 49-64.
38. Akoua-Koffi C., *et al.* "Investigation surrounding a fatal case of yellow fever in Cote d'Ivoire in 1999". *Bulletin de la Societe de Pathologie Exotique* 94.3 (2001): 227-230.
39. Petersen, Eskild, *et al.* "Rapid spread of Zika virus in the Americas-implications for public health preparedness for mass gatherings at the 2016 Brazil Olympic Games". *International Journal of Infectious Diseases* 44 (2016): 11-15.



40. Olson JG and TG Ksiazek. "Zika virus, a cause of fever in Central Java, Indonesia". *Transactions of the Royal Society of Tropical Medicine and Hygiene* 75.3 (1981): 389-393.
41. Zammarchi Lorenzo., *et al.* "Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications". *Journal of Clinical Virology* 63 (2015): 32-35.
42. Buckley A and EA Gould. "Detection of virus-specific antigen in the nuclei or nucleoli of cells infected with Zika or Langkat virus". *Journal of General Virology* 69.8 (1988): 1913-1920.
43. Gourinat Ann-Claire., *et al.* "Detection of Zika virus in urine". *Emerging Infectious Diseases* 21.1 (2015): 84-86.
44. Bingham Andrea M. "Comparison of test results for Zika virus RNA in urine, serum, and saliva specimens from persons with travel-associated Zika virus disease—Florida, 2016". *MMWR. Morbidity and Mortality Weekly Report* 65.18 (2016).
45. Brasil Patrícia., *et al.* "Zika virus infection in pregnant women in Rio de Janeiro". *New England Journal of Medicine* 375.24 (2016): 2321-2334.
46. Hennessey Morgan., *et al.* "Zika virus spreads to new areas-region of the Americas, May 2015–January 2016". *American Journal of Transplantation* 16.3 (2016): 1031-1034.
47. Thomas Stephen J. "Zika virus vaccines-a full field and looking for the closers". *New England Journal of Medicine* 376.19 (2017): 1883-1886.
48. Pardi Norbert., *et al.* "Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination". *Nature* 543.7644 (2017): 248-251.
49. Cheng Fan., *et al.* "Suppression of Zika virus infection and replication in endothelial cells and astrocytes by PKA inhibitor PKI 14-22". *Journal of Virology* 92.4 (2018): e02019-e02017.
50. Musso Didier., *et al.* "Detection of Zika virus in saliva". *Journal of Clinical Virology* 68 (2015): 53-55.
51. Cao-Lormeau Van-Mai., *et al.* "Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study". *The Lancet* 387.10027 (2016): 1531-1539.
52. Sejvar James J., *et al.* "Population incidence of Guillain-Barré syndrome: a systematic review and meta-analysis". *Neuroepidemiology* 36.2 (2011): 123-133.
53. Rather Irfan A., *et al.* "Zika virus infection during pregnancy and congenital abnormalities". *Frontiers in Microbiology* 8 (2017): 581.
54. McNeil DG. "Zika: The Emerging Epidemic". WW Norton & Company (2016).
55. Fonseca Kevin., *et al.* "First case of Zika virus infection in a returning Canadian traveler". *The American Journal of Tropical Medicine and Hygiene* 91.5 (2014): 1035-1038.
56. Goodsell D. "Protein Data Bank. "Molecule of the Month – Dengue Virus".
57. Goodsell D. Protein Data Bank. "Molecule of the Month – Zika Virus".

**Volume 1 Issue 5 May 2018**

**© All rights are reserved by Mazen Saleh., et al.**