

# Advances in Zika Virus: Searching for the Missing Link

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## Abstract

The Zika virus (ZIKAV) is an emerging arbovirus of the *Flaviviridae* family with two main lineages; the African and the Asian lineages. It is an enveloped single stranded positive sense RNA virus of approximately 11kb. Scientists recently linked ZIKAV with a rare birth defect microcephaly characterized by small head and brain development and abnormalities. The brain damage in Zika babies is worse than the researchers initially anticipated. Researchers believe that ZIKAV prevents parts of the brain not yet formed from proper development, which might in the long run, be associated with severe abnormalities. No one knows how the babies survive, how well their brain develops and functions remains a mystery. There is no effective vaccine or proven specific therapeutic treatment for ZIKAV infection to date. The development of suitable therapeutic molecules and elucidating anti-viral agents against ZIKAV viral infection is therefore imperative, and a recent near-atomic resolution of a mature ZIKAV offers an opportunity to accelerate new research in this area. Thus, this review seeks to consolidate information from various scientific work carried out worldwide, and provide a clear and comprehensive outline of research and current issues needing further ZIKAV investigation.

## Keywords

Zika Virus, Microcephaly, Symptoms, Testing, Treatments and Future Perspectives

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## 1. Introduction

The World Health Organization (WHO) declared that the recent cluster of microcephaly cases and other neurological disorders reported in Brazil, following a similar cluster in French Polynesia in 2014, constitutes a Public Health Emergency of International Concern (PHEIC). This announcement was made following a convened Emergency Committee, under the International Health Regulations, to gather advice on the severity of the health threat associated with the continuing spread of ZIKAV disease in Latin America and the Caribbean. The Emergency Committee emphasized the need for a coordinated international response to improve surveillance, the detection of infections,

congenital malformations, and neurological complications, to intensify the control of mosquito populations, and to expedite the development of diagnostic tests and vaccines to protect people at risk, especially during pregnancy [1].

With the coming up of the 2016 summer Olympic Games in Rio de Janeiro, there has been public concern, evidenced through wide media coverage, that ZIKAV may infect athletes and visitors to the games. The U.S. Olympic Committee announced that athletes who are concerned about Zika should consider skipping the games [2] and public health experts are concerned with that the virus may spread beyond Latin America. Updates from 1 January 2016 to 23<sup>rd</sup> March 2016, by the Centers for Disease Control and Prevention (CDC), reported no vector-borne cases of Zika

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infection, and 273 travel associated ZIKAV disease in the United States, in which 19 were in pregnant women and 6 were sexually transmitted. The US Territories, on the other hand, had 282 locally acquired infections, 35 in pregnant women [3]. The European Centre for Disease Prevention and Control continues to publish a rapid risk assessment report, taking into account the growing public health concerns of the risk of establishment of local vector-borne transmission during the 2016 summer, and the risk to EU travelers and citizens residing in areas with local active transmission [4].

### 1.1. The Origin of ZIKAV

ZIKAV was first isolated in 1947 from a febrile sentinel rhesus monkey (Rhesus 766) in the Zika Forest of Uganda, Africa [5]. In January, 1948 a second isolation was done at the same site from the mosquito *Aedes africanus* [6]. In 1958, three cases of human infection from ZIKAV during an epidemic of jaundice in Nigeria suspected of being yellow fever were reported [7]. Recent outbreaks of ZIKAV fever in different regions of the world demonstrate the potential for the arbovirus to spread through territories where the *Aedes* vector is found. From 1951 to 1981, there was evidence of ZIKAV infection to human from other African countries including Egypt, Tanzania, Uganda, Gabon, Central African Republic and Sierra Leone [8] as well as in parts of Asia including Malaysia [9], Thailand [10], Cambodia [11], India, Vietnam and Indonesia [8]. In 2007, a large epidemic caused by ZIKAV on Yap Island, Federated States of Micronesia infected three quarters of the local population [12]. Since October 2013, French Polynesia has experienced the largest outbreak of ZIKAV infection ever reported, with an estimate of 28,000 ZIKAV infections in early February 2014 (about 11% of the population) [13-14] with an additional health threat posed by *Ae. albopictus* mosquitoes [15]. In February 2014, the public health authorities of Chile confirmed the first case of indigenous transmission of ZIKAV infection on Easter Island. In May 2015, the public health authorities of Brazil confirmed autochthonous transmission of ZIKAV in 14 States [16].

According to preliminary estimates from the Brazilian ministry of health, between 440,000 to 1,300,000 cases of ZIKAV infections may have occurred in 2015 in Brazilian states with laboratory-confirmed autochthonous cases of ZIKAV [17]. As of 4 December 2015, the Brazilian ministry of health also reported 9,300 suspected cases of Chikungunya (ChikV) and approximately half a million probable cases of DENV to PAHO [18-19].

### 1.2. What Is the ZIKAV

The *Flaviviridae* family, which includes ZIKAV, DENV, West Nile, Yellow Fever, and Japanese Encephalitis viruses.

There are two main lineages of ZIKAV: the African lineage and the Asian lineage [20 - 22]. ZIKAV infects non-human primates through the bite of several infected *Aedes* species; *Ae. africanus*, *Ae. aegypti* and others as vectors [24-26] with cyclic epizootics in monkeys [27] and maintains a sylvatic cycle. Infection may also occur through the secondary sexual transmission [28], while blood transfusion [29] and perinatal transmission [30] have been indicated. ZIKAV has been isolated from several *Aedes* mosquito species, including *Ae. Albopictus* in Gabon, Africa [31]. *Ae. aegypti* is widespread in the tropical and subtropical regions of the world, including Singapore [32] and *Ae. albopictus* is now established in many parts of Europe, especially Mediterranean countries [33]. Recent reports of imported cases of ZIKAV infection from south-east Asia or the Pacific to Europe [34] or Japan [35] highlight the risk of ZIKAV emergence in parts of the world where the vector is present.

### 1.3. Common Symptoms

ZIKAV usually remains in the blood of an infected person for up to one week. Infection is reported to be symptomatic in 18% of cases only [12]. Early symptoms of infection are very similar to malaria, dengue and bacterial infection, and include low grade temperature (37.8 – 38.5°C), headache, malaise, arthralgia / myalgia, non-purulent conjunctivitis or conjunctival hyperemia [16]. The pathogenesis of ZIKAV involves in infecting dendritic cells near the site of inoculation, and then spread to lymph nodes and the bloodstream. Current control measures include isolation of the infected patient under a bed net during the viremic phase (which lasts one week), and instituting integrated vector control measures. Exposed populations and visitors to areas endemic for the vector, *Aedes aegypti*, are advised to use repellants, wear appropriate clothing that minimize skin exposure, and use repellants, insecticides or nets [16].

There is no vaccine or specific treatment for ZIKAV infection, therefore treatment is geared toward relieving symptoms [16]. Treatment is symptomatic and supportive, including rest and the use of Acetaminophen or Paracetamol to relieve fever. The use of antihistamines to control pruritus usually associated with the maculo-papular rash are recommended. Using Aspirin is not advised due to the risk of bleeding and developing Reye's syndrome in children younger than 12 years of age [16].

### 1.4. Serological Testing

The serological tests (ELISA or immunofluorescence) to detect specific IgM or IgG against Zika virus can be positive after 5 to 6 days following the onset of symptoms. In endemic areas, epidemiological studies in Nigeria and Cameroon showed a high prevalence of antibodies against

ZIKAV as exhibited [36-37]. There can be cross-reactivity with other Flaviviruses, especially DENV and yellow fever (YFV) or, less frequently, with West Nile virus (WNV) [16]. In Yap's epidemic in 2007 resulted in an attack rate of 14.6 / 1,000 inhabitants and a sero-prevalence of 75% after the epidemic. However, this prevalence could easily have been overestimated, due to cross- reaction between antibodies directed against ZIKAV and other arboviruses such as DENV [38-39].

This was further demonstrated in November 2013, when a first case of laboratory-confirmed Zika virus infection imported into Europe was reported [40]. Serological investigations revealed anti-ZIKV-IgM and -IgG, as well as ZIKV- specific neutralizing antibodies in the patient's blood. A serum sample taken 10 days after symptom onset showed a positive result for anti-DENV-IgM but negative for anti-DENV-IgG in both the indirect immunofluorescence assay [40], and rapid test, as is also reported by others [41-43]. Testing for DENV nonstructural protein-1 (NS1) antigen were also negative. Serological tests for CHIKV, Japanese encephalitis virus (JEV), WNV, YFV, tick-borne encephalitis virus (TBEV), and ZIKAV were performed [40 - 43] and showed only positive results for anti-ZIKV-IgM and -IgG antibodies, demonstrating an acute or recent ZIKAV-infection of the patient.

During the first 5 days after the onset of clinical picture, (acute phase, viremic period) viral Ribonucleic Acid (RNA) can be detected in serum by conventional molecular techniques and the reverse transcription-polymerase chain reaction (RT-PCR), in which a negative result for dengue as the main differential diagnosis. Detection of DENV genome in urine after disappearance of the viral genome in serum samples by real- time RT-PCR has been a useful laboratory diagnostic method, suggesting that detection of ZIKAV genome in urine by real-time RT-PCR may be useful in confirming ZIKAV infection, particularly after disappearance of viraemia in serum [44].

### 1.5. Blood Transfusion

The main challenge in the prevention of arbovirus transfusion-derived transmission is the high rate of asymptomatic infections: this has been estimated at over 75% for DENV [45] and West Nile virus (WNV) [46]. For ZIKAV, there is no estimate available of the percentage of asymptomatic infections. Arbovirus transfusion-derived transmission has been reported principally for WNV [47], DENV [48] and CHIKV [49-50]. For CHIKV, the risk was evaluated as high [49-50]. From November 2013 to February 2014, tests carried out by Musso *et al*, showed that 42 (3%) of 1,505 blood donors, although asymptomatic at the time of blood donation, were found positive for ZIKAV by PCR [29].

Their results served to alert blood safety authorities about the risk of post-transfusion Zika fever, and helped set up a protocol for testing blood donated to the blood bank in French Polynesia [29], using a modified RT-PCR [49] from November 2013. The specificity of this RT-PCR assay has been previously evaluated and was confirmed by sequencing analysis conducted during the outbreak in French Polynesia [29] and its sensitivity was similar to that previously evaluated [38]. Unfortunately, the protocol used is not only time consuming, it is also expensive, and new ways for rapid and sensitive testing would be of great future value.

### 1.6. Neurological Effects

The Guillain-Barré syndrome (GBS) is a severe neurological disorder characterized by progressive muscle weakness in the arms and legs, and in some cases, weakness of muscles of the face that control eye movement or swallowing may also become weak, symptoms that can last between a few weeks to several months. In the most serious cases, this muscle weakness causes respiratory failure. Although most people fully recover from GBS, some people have permanent damage, and in 1 out of 20 cases, people have died [51]. In an outbreak in French Polynesia, Oehler *et al* reported ZIKAV infection-related neurological disorders, and the incidence of Guillain-Barré syndrome unexpectedly increased 20-fold [52]. They reported a first case of hospitalization due to GBS, where ZIKAV was indicated. A patient in her early forties, was admitted into the hospital emergency due to muscular weakness suggestive of GBS. This was 7 days after she had recovered from what was suspected to be a ZIKAV infection (influenza-like syndrome, with myalgia, febricula, cutaneous rash, and conjunctivitis). Three days after admission, she developed tetraparesis predominant in the lower limbs, with paraesthesia of the extremities, diffuse myalgia, and a bilateral but asymmetric peripheral facial palsy. She was discharged on day 13, but paraparesis and facial palsy persisted dissipating slowly. At Day 40, she was able to walk without help and had a satisfying muscular strength score of 85 / 100.

Blood samples taken at eight and 28 days after the beginning of the influenza-like syndrome were both positive for ZIKAV-specific IgM and ZIKAV- and DENV-specific IgG, assessed by in-house IgM antibody capture MAC - enzyme-linked immunosorbent assay (ELISA) and indirect IgG ELISA using inactivated antigen). On the last serum specimen sampled 28 days after the onset of influenza like syndrome, antibody specificity was determined by plaque reduction neutralization test (PRNT) against Dengue serotype (DENV1-4) and ZIKA, confirming neutralizing antibodies against ZIKAV and the four DENV serotypes were present in the sera of the patient [52]. These serological analyses

indicated a recent infection by ZIKA, and possible DENV infection, and indicating a stronger predisposition to GBS during ZIKAV infection, where DENV co-infection exists.

A more recent case for meningo-encephalitis (as diagnosed by Magnetic Resonance Imaging) of an 81-year old patient, associated with ZIKAV, was reported in March 2016 [53]. The patient was, on medical examination, febrile (39.1°C) and comatose, with hemiplegia of the left side, paresis of the right upper limb, a normal response to tendon reflexes, and a Babinski sign on the left side. Tests using reverse-transcriptase–polymerase-chain-reaction assay (R-PCR) of the cerebrospinal fluid indicated a positive result for ZIKAV. The patient’s neurological condition appeared to improve without specific treatment, and his cognitive function was fully recovered by day 38.

On 17 March 2016, World Health Organization (WHO) reported that, between October 2013 and April 2014, 42 patients were admitted to hospital for a median of 11 days with Guillain-Barré syndrome (GBS) during the ZIKAV outbreak, representing a 20-fold increase in incidence of GBS in French Polynesia compared with the previous four years [54]. No deaths were reported. As indicated by Oehler *et al.*, majority of these cases (88%) had reported symptomatic ZIKAV infection in the days about 6 days preceding the onset of neurological symptoms. 41 of the 42 cases of GBS (98%) had IgM or IgG antibodies against ZIKAV; furthermore, all GBS cases (100%) had positive sero-neutralization against ZIKAV. 95% of the patients has pre-existing dengue immunity, however analysis of dengue serology through immunofluorescent assay, microsphere immunoassay, and sero-neutralization, did not indicate recent dengue infection.

In May 2016, WHO also announced an outbreak of ZIKAV, infecting patients in Cape Verde, a chain of islands about 350 miles off the coast of Senegal, with strong links to Brazil. The virus was found to be of the same strain causing birth abnormalities in Brazil. This is the first report of a South American strain infection in Africa, where the local strain results in mild symptoms with no link to nervous of birth defects. The impact of the South American strain on populations in Africa, who are already exposed to low-grade immunity to the local strain of ZIKAV is yet to be elucidated [78].

## 2. Microcephaly

Babies have shown high rates of microcephaly, a neurological disorder in which infants are born with a head circumference lower than normal and usually exceeding 33 cm and an abnormally small brain, which does not develop properly. This defect can be as a result of a number of factors from different sources, such as chemicals, biological agents

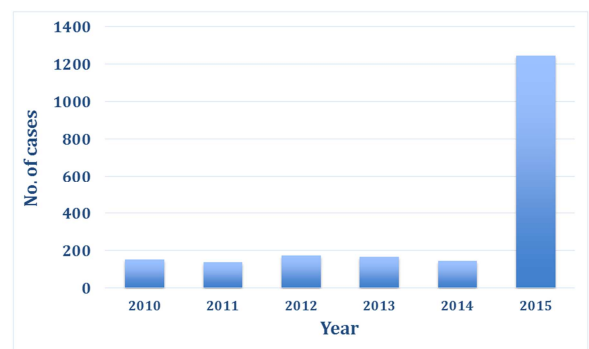
(infectious) such as bacteria, viruses, and radiation [55]. The condition usually results in death or cognitive impairment.

In 2014, Brazil had fewer than 200 cases of microcephaly; in 2015, as the ZIKAV spread, approximately 3,000 cases were recorded. Several areas in Brazil have declared a state of emergency. This is the first time the condition has been linked to ZIKAV [56].

In 2015, as of 28 November, 1,248 suspected cases of microcephaly were identified in 311 municipalities across 14 of the 26 Brazilian states and one federal district of Brazil, of which 509 cases were reported between 21 and 28 November 2015 [57]. This was a dramatic increase compared to between 150 and 200 children per year were born with microcephaly in Brazil between 2010 and 2014 (Table 1 and Figure 1), with Pernambuco state [56], the highest number of cases (646). The Ministry of Health continues to monitor and investigate the increased number of cases of microcephaly in the country, while advising pregnant women not to consume alcohol or use drugs without medical advice and avoid contact with people with fever or infection. Pregnant women are also advised to reduce the presence of disease-transmitting mosquitoes by eliminating breeding sites, and protect themselves from mosquito exposure, keeping doors and windows closed or screened, wear pants and long-sleeved shirt and use repellents allowed for pregnant women, all day, as *Aedes* is active daytime, unlike *Anopheles sp.*

**Table 1.** Cases of Reported Microcephaly Annually in 14 States in Brazil [57].

State	2010	2011	2012	2013	2014	2015
• Pernambuco	7	5	9	10	12	646
• Paraíba	6	2	3	5	5	248
• Rio Grande do Norte	2	2	4	0	1	79
• Sergipe	3	1	2	0	2	77
• Alagoas	3	7	2	3	2	59
• Bahia	12	13	7	14	7	37
• Piauí	1	0	4	4	6	36
• Ceará	8	4	9	5	7	25
• Maranhão	3	2	6	2	2	12
• Tocantins	1	0	1	4	0	12
• Rio de Janeiro	10	15	8	19	10	12
• Goiás	3	4	3	2	3	2
• Distrito Federal	3	3	1	2	2	1
• Mato Grosso do Sul	0	0	1	3	0	1



**Figure 1.** Reported cases of Microcephaly in Brazil from 2010-2015.

Human induced pluripotent stem cells (hiPSCs) was used as an *in vitro* model to investigate whether ZIKAV directly infects human neural cells and the nature of its impact [58]. The researchers compared ZIKAV's effect on cells known as cortical neural progenitor cells to two other cell types: induced pluripotent stem cells and immature neurons. Results showed that human neural progenitor cells (hNPCs) were readily infected by ZIKAV *in vitro*, with the infection spreading to 65%–90% of the cells within 3 days of inoculation. Quantitative analysis showed similar results for hNPCs derived from hiPSC lines of two different subjects. As a control, they also exposed human embryonic stem cells (hESCs), hiPSCs, and immature cortical neurons to ZIKAV under the same conditions. hESCs and hiPSCs could also be infected by ZIKAV, but the infection was limited to a few cells at the colony edge with reduced expression of the pluripotent marker NANOG. Immature neurons differentiated from hNPCs also exhibited lower levels of infection. The studies not only showed that ZIKAV directly targets hNPCs and attenuate their growth, through cell-cycle dysregulation and caspase-3-mediated apoptosis (revealed by upregulation of genes on RNA-sequence analysis), but they also established a novel platform for investigating ZIKAV mechanisms on brain development.

### 3. Perinatal Transmission

The RT-PCR was carried out in the first reported case of perinatal transmission of the ZIKAV in 2013 [30] in French Polynesia, in a case of two mothers and their newborns. All available samples collected from Mother 1 and Newborn 1 until day 3 and from Mother 2 and Newborn 2 until day 13 were tested for ZIKAV and DENV. No other pathogens were tested for, given the co-circulation of ZIKAV and DENV (serotypes 1 and 3) [59-60]. The test for ZIKAV was RT-PCR using two primers/probe amplification sets specific for ZIKAV [38]. Results were positive when the two amplifications occurred (threshold cycle less than 38.5). A standard curve using serial dilutions of known concentrations of a ZIKAV RNA synthetic transcript was included within the RT-PCR run to estimate the RNA loads. Both mothers and both newborns had ZIKAV infection confirmed by positive RT-PCR result on at least one serum sample. All samples tested by ZIKAV RT-PCR were also tested for DENV and found negative, using a multiplex RT-PCR [30 and 60].

### 4. Breast Milk

Breast milk samples from two mothers were inoculated on Vero cells in order to detect replicative ZIKAV and were also tested by RT-PCR. The samples gave positive RT-PCR

results, but no replicative ZIKAV particles were detected in cell culture. Total protein and C-reactive protein levels were within the normal range [30]. Given the severe neonatal diseases reported with other arbovirus infections, such as CHIKV [61] and DENV [62-63], monitoring of perinatal ZIKAV infections remains important. Although no replicative ZIKAV particles were detected, ZIKAV transmission by breastfeeding must be considered [30], due to the high ZIKAV RNA load detected in breast milk. Patients living in or returning from ZIKAV-endemic or epidemic areas presenting with a 'dengue-like' syndrome but testing negative for DENV should be tested for ZIKAV, with attention paid to infected pregnant women and their newborns, as data on the impact of the infection on them are limited [29].

## 5. Viral Replication

Research on autophagy pathway in mammalian cells has shown that ZIKAV behaves like other flavivirus members (except WNV [64]), in which stimulation of autophagosome formation further enhances the replication of ZIKAV in permissive cells, whereas the presence of 3-MA, an inhibitor of autophagosome formation, strongly reduces viral copy numbers in the infected fibroblasts, indicating that, at the cellular level, ZIKAV induces autophagosome formation to and trigger apoptosis to promote replication [65-66].

Studies demonstrating the co localization of ZIKAV [65] and DENV [66] with LC3 strongly suggest that autophagocytic vacuoles are the site of viral replication. It has been speculated that autophagy may promote replication of ZIKAV infection through restriction of the antiviral innate immune response [67], by enhancement of translation of the viral genome that has entered the mammalian cells [68], or by providing additional energy and relevant membrane structures for viral replication [69]. However, the exact molecular mechanism(s) by which ZIKAV hijacks components of the autophagosome pathways remains to be determined, however, this research shows that human skin cells: immature dendritic cells, keratinocytes and fibroblasts have been shown to be readily infected by ZIKAV.

Antoine Enfissi *et. al* published the first complete genome of the American strain of ZIKAV. Phylogenetic analyses were conducted for the NS5 protein coding region, the envelope protein coding region, and the complete coding region, against the sequences available in databases, indicated similarity between South and Central America to the Surinam and French Polynesian strains, raising public concern about the possibility of increase of cases of Guillain-Barré syndrome and congenital neurological anomalies [79].

## 6. Vaccine and Treatment

Though no remedies are currently available against ZIKAV fever/ clinical ZIKAV infection, recent computational approach suggest that Zika viral envelope glycoproteins are most immunogenic and can often be considered as a good candidate for vaccine development [70]. Seeking hairpin sequences of the ZIKAV genome for potential antiviral therapeutics remains a viable target for further research. NAI is referred to as an evolutionary conserved gene silencing process that needs double stranded RNA processed into siRNA and human miRNA sequences to suppress the expression of homologous RNA (mRNA) targets in a sequence specific manner and thus considered as antiviral therapeutics [71-72]. This therapy has already been successful in repressing the expression of genes involved in pathogenic infections as well as genetic disorders.

Computational studies have shown that the 3'UTR of ZIKAV is crucial for viral replication, translation and pathogenicity, and it is therefore an obligatory target for RNAi [73], a post transcriptional gene silencing technique, which has also been successfully used against various viral infections such as hepatitis B [74] and C [75] infection.

Of particular interest is a recent study by Sirohi *et al.* [80], who studied a strain of Zika virus isolated from a patient infected during the French Polynesia epidemic and determined the structure to 3.8Å, using cryoelectron microscopy to create a three-dimensional picture of Zika. The structure of Zika virus is similar to other known flavivirus structures except for the ~10 amino acids that surround the Asn154 glycosylation site found in each of the 180 envelope glycoproteins that make up the icosahedral shell. The shell is made up of 180 copies of two different proteins. These, like all proteins, are long chains of amino acids folded into particular structures to create a protein molecule. The glycosylation site and surrounding residues on Zika virus may also be involved in attachment to human cells, and the differences in the amino acids between different flaviviruses could signify differences in the kinds of molecules to which the virus can attach and the different human cells it can infect. In many other viruses it has been shown that as the virus projects a glycosylation site outward, an attachment receptor molecule on the surface of a human cell recognizes the sugars and binds to them. This site could be a good spot to target an antiviral compound.

## 7. Conclusion and Future Perspectives

ZIKAV infection is primarily a vector-borne and, with the increase of transportation of goods through shipping

networks forming the largest possible route for transporting the *Aedes* mosquito across continents, it would only take suitable environments for the disseminated vector to replicate in new areas. This is further complicated by globalized air transportation. For example, over 100 million air passengers alone enter Europe annually from international regions that include regions that are endemic regions for ZIKAV. Although infection on air transportation are unlikely, growth of suitable vector populations in warm conducive climate (especially during summer), to accelerate disease transmission, remains a public health concern. The real incidence of ZIKAV fever is unknown, due to clinical manifestations mimicking dengue virus infection, and to lack of simple reliable laboratory diagnostic tests. Most ZIKAV patients are asymptomatic, even when they are high infective within the first week of infection and therefore testing is important. Care must be taken in interpreting results of serological tests, due to cross-reactivity amongst Flavivirus serology [25] and use of the RT-PCR adds great value to differentiation. However, this test is relatively expensive and time consuming, making it difficult to use as a screen test for screening in certain situations, such as at boarder entry points or during blood transfusion.

Clinical symptoms for ZIKAV have been shown to be very similar to Dengue and Chikunguya, and this can confuse the treatment regime, as non-steroidal anti-inflammatory drugs are contraindicated [16]. However, this similarity can be looked at as an opportunity to develop vaccine and vector control strategies, especially as many endemic areas such as, Brazil and Mexico are making efforts towards a dengue vaccine (Sanofi Pasteur), and considering that the pathways targeted and involved in infection appear to be the same the same in both ZIKAV and Dengue, one may postulate that this could be useful in general therapy against Flavivirus. Inhibition of dengue virus has previously been *in vitro* using artificial miRNAs, efficiently targeting highly conserved regions of the dengue viral genome [76]. It has further been shown that silencing putative cysteine rich venom protein through siRNA highly reduced infection of *Aedes Aegypti* cells by the dengue virus [77]. Due to their close relationship, similar strategies could be applied to ZIKAV. Current published studies on RNAi therapy on Zika do not exist, it is still a pending area for further research. More research is needed to understand the increase in cases of microcephaly in areas with an outbreak ZIKAV infection, and attention needs to be paid to infected women and their newborns, while also elucidating how early during pregnancy could there be likelihood of microcephaly occur. New and novel systems that allow for deeper investigations into ZIKAV effect on the brain remain paramount.

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