Universitat Pompeu Fabra Barcelona

ZIKA VIRUS

The causal agent of a new congenital syndrome

Anna Palomar Cros

Juana Díez Antón

Treball de Fi de Grau

Biologia Humana

Promoció 2012-2016

Index

SUMMARY. Zika virus, a flavivirus transmitted by *Aedes* mosquitoes, was first identified in 1947 in a sentinel rhesus monkey in the Zika forest area of Uganda, and later on in humans in Nigeria. Despite having been discovered almost 70 years ago, only about a dozen human infections were reported before 2007 and were mainly confined to the African continent. The Zika virus came to global attention when it caused an explosive outbreak in the island of Yap in Micronesia in 2007 followed by a major outbreak in French Polynesia in 2013. The virus continued to spread and entered the Western hemisphere where a major outbreak was reported in May 2015 in Brazil. For many years Zika virus was considered self-limiting causing a mild disease and with no long-term consequences. However, since the end of 2015, there has been reported an increase in the incidence of Guillain-Barré syndrome (GBS) and foetal neurological complications including microcephaly, raising serious worldwide public health concerns. Believed to be only transmitted by mosquitoes, now there have been proposed other non-vector-borne transmission routes including sexual transmission, vertical transmission and blood transfusion transmission. Recently many efforts have been invested in clarifying such worrisome relationship and in developing ZIKV models either *in vitro* or *in vivo*. Here, we broadly review what is currently known about this emerging virus and focused on its consequences for brain development.

1. Background and significance of global concern about Zika virus

Infectious diseases have been raising serious worldwide public health concerns since the beginning of the XXI century, as humanity has been facing almost every year new emerging viruses such as avian flu, Dengue, Chikungunya, Ebola or now the Zika virus. The impact of these diseases is immense, not only affecting individuals health but economy and society. Moreover, infections during pregnancy have been known to have a significant impact on neonatal morbidity and mortality, as well as pregnancy outcome. The most common pathogens associated with congenital manifestations are summarized by the acronym TORCH (Toxoplasmosis, Others, Rubella, Cytomegalovirus, and Herpes simplex virus). Now, because its potential teratogenic effects resulting in central nervous system damage, Zika virus has become a new and in the future possibly a common TORCH agent (1). Zika virus was first isolated in 1947 in Uganda (2) and has remained relatively hidden, until a recent series of outbreaks in 2013 in French Polynesia and Brazil in mid-2014, where it has been associated with Guillain-Barré syndrome, microcephaly and other devastating neonatal malformations (3,4). The virus is classified within the *Flavivirus* genus of the *Flaviviridae* family, which includes other globally relevant viruses such as Dengue, Yellow fever or West Nile and is mainly transmitted by *Aedes* mosquito (5). However, during the past few years, non-vector-borne transmission routes have been proposed, such as sexual transmission, vertical transmission and blood-borne transmission (6–8). Moreover, there is no treatment or vaccine available to prevent its transmission. Previously believed as a mild illness, these unexpected neurological complications in new-borns have pointed Zika virus as the causal agent of a new congenital syndrome. During the past few years many efforts have been invested in clarifying such worrisome relationship and recent evidence either in vitro or in vivo models is available now linking Zika virus with

neurological disorders. Therefore, the present review hypothesizes a causal linkage between Zika virus infection and induced pathology in the nervous system and extensively summarizes the recent information for ZIKV in order to achieve three main objectives: (i) understand how the virus has globally spread and its newly described modes of transmission, (ii) highlight the clinical manifestations of ZIKV infection and its possible neurological complications, and (iii) evaluate the presumed linkage between ZIKV infection and microcephaly among other new-borns malformations, by reviewing the available experimental evidence.

2. Methods

Extensive daily search on PubMed, Scopus, and Web of Science during a period of time between May and July 2016, for all publications containing the word “Zika” in any field. A general filter such as the relevance of the journal, the originality of the article and its availability was applied, with restriction on date of publication to 2011 and later on. Early investigations performed when the Zika virus was first discovered were also included. Finally, the bibliography was elaborated using Mendeley.

1. Worldwide spreading of the Zika virus

Zika virus was first isolated in April 1947 from the serum of a febrile monkey, which was being employed as a yellow fever sentinel monkey, in the canopy of Zika forest, near Entebbe in Uganda (2). Therefore, the virus took his name from the geographical area where it was first isolated. In the same forest, in 1948 the virus was recovered from a lot of *Aedes africanus* caught on a tree, suggesting that the virus might be mosquito-borne (2,9). Within a couple of years, in 1952, the first human cases with serum neutralizing antibodies to ZIKV were reported in Uganda and the United Republic of Tanzania (9). Then, in 1954, the virus was successfully isolated from a young girl serum in Eastern Nigeria (10). However, until 1964 it was not confirmed that ZIKV caused human disease, when a researcher in Uganda was infected while he was working on the virus (10). In the ensuring years, ZIKV was sporadically detected in human patients, by serological methods, all in African and Asian countries, but remained in the obscurity because of the benign nature of the infection and the few reported cases (11,12). ZIKV came to global attention in 2007 when it caused the first major outbreak in humans in the Pacific Island of Yap (**Figure 1**) in the Federated states of Micronesia (13). The way through the virus was introduced is still unknown, but it has been proposed that the most likely source of this outbreak was by travel or trade involving an infected person or mosquito (14). Within four months 73% of Yap residents were infected with ZIKV, but only mild symptoms including fever, headache and skin rash were observed (13).

Along his worldwide spreading the virus underwent genomic recombinations, and on 2012 two geographically distinct strains were recognized, the African and the Asian (15). In October 2013 ZIKV caused a second major outbreak in French Polynesia (**Figure 1**) and spread to other Pacific Islands, including Easter Island, the Cook Islands, Solomon Islands, Vanuatu and New Caledonia (16,17). More than 32.000 patients were assessed for suspected Zika virus infection, with an estimated percentage of

more than 11% of the population (17,18). The identified genotype, similarly to the epidemic Yap Island, belonged to the Asiatic lineage (18). Surprisingly, when the virus reached French Polynesia there was a 20-fold increase of Guillain-Barré syndrome, an autoimmune disease that might cause temporary paralysis (3,19). In March 2015 ZIKV reached Brazil (**Figure 1**), and first autochthonous cases were reported in this country, raising a new major outbreak (20). It has been speculated that the virus entered the country during the Va'a World Sprint Championship in August 2014, where competitors from French Polynesia, New Caledonia, Cook Islands, and Easter Island, have been participating (21). The Brazilian Ministry of Health estimates that between 440.000 to 1.300.000 cases of ZIKV infections may have occurred in 2015 in Brazil (22). By October 2015, in north-eastern Brazil, there was detected a 20-fold increase in microcephaly rate from 2010, with more than 3000 cases of neonates with microcephaly including deaths (4). The outbreak was initially concentrated in north-eastern Brazil, however, the virus rapidly spread throughout Latin America and the Caribbean, and within 1 year most countries in the region reported autochthonous cases (23).

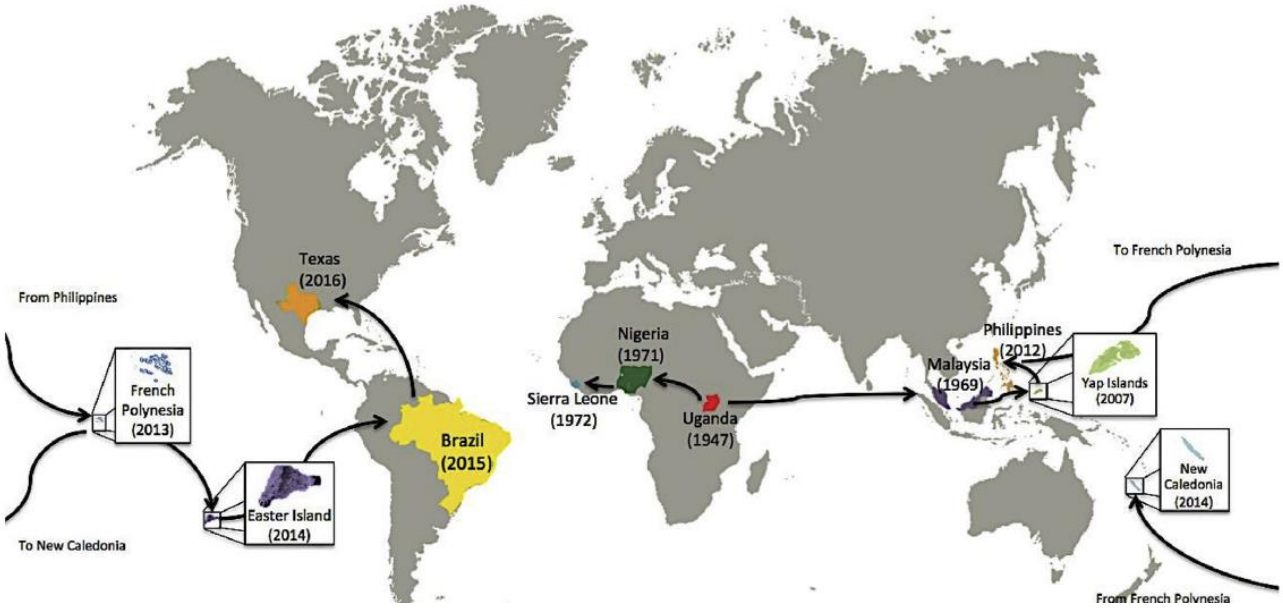

Figure 1. Global Zika virus spread and entrance in the Western hemisphere. Extracted from (144).

Therefore, the uncontrolled spread of the virus and the potentially novel "congenital Zika syndrome" lead the World Health Organisation to declare the ZIKV infection as a Public Health Emergency of International Concern on the 1st of February of 2016 (24). By April 2016, the association between ZIKV infection and microcephaly was confirmed by the United Stated Centres for Disease Control and Prevention (25). As of 13 July 2016, 65 countries and territories report continuing mosquito-borne transmission (26). Further spread of the virus is anticipated, and during the current outbreak imported cases have already been reported in the United States, Europe, and elsewhere in travellers returning from Latin America and the Caribbean (27,28). Moreover, as it has been previously speculated with the entrance of ZIKV in Brazil, during mass gatherings, people are in risk of acquiring imported and locally prevalent infectious diseases. Hence, the 2016 Olympic and Paralympic Games in Rio de Janeiro in August is a potential threat of ZIKV infection to the attendees of the event (29,30).

It has been a huge challenge to diagnose Zika virus infection, which has enabled the geographical expansion of the virus. Because different arboviral infections can present the same clinical manifestations it is difficult to clinically distinguish ZIKV infection from other viral illness. In fact, ZIKV co-circulates and shares mosquito vectors with DENV and CHIKV, presenting similar manifestations including fever, rash, arthralgia and myalgia (16,31). In the case of ZIKV diagnosis the use of serology is limited because of the extensive cross-reactivity of antibodies against related flaviviruses (13,32,33). At present, in order to differentiate between cross-reacting antibodies it is used plaque reduction neutralization test (PRNT) that have to result in at least a fourfold increase in ZIKV neutralizing antibody titers when compared with other flaviviruses tested (34). However, the diagnosis of ZIKV is currently based on the detection of viral RNA from specimens by means of RT-PCR, which can distinguish ZIKV infections from DENV, CHIKV and other infections (18,35–37). Nonetheless, serum RT-PCR assays have low sensitivity rates, because ZIKV viremia in humans lasts for a short period of time of 3 to 5 days (32,38). Therefore, alternative source of samples has been proposed such as urine and saliva samples, as viral RNA is detected in these body fluids at a higher load and with a longer duration than in serum (36,39,40). Recently, a low-cost paper-based test has been designed to rapidly detect low levels of Zika virus, at clinically relevant concentrations, in human blood samples (41). In fact, this new diagnosis system is able to discriminate ZIKV infection of other flaviviral infections, and to differentiate between ZIKV strains (41).

Furthermore, the global health burden of ZIKV infection is partly justified because no ZIKV vaccines or therapeutics are available at present. Consequently, to control or limit ZIKV disease development of immediate measures should be a priority.

2. Modes of transmission of the Zika virus

2.1. Mosquito-borne transmission

Zika virus is an arbovirus (arthropod-borne transmission) classified into the *Flaviviridae* family. Similar to other mosquito-borne flaviviruses, ZIKV is mainly transmitted through the bite of an infected female mosquito of *Aedes* species. *Aedes spp* mosquitoes are distributed throughout the tropic and as in other arboviral infections, local overwintering could be an important aspect for maintenance and spread of ZIKV. They are also known to transmit other important arboviruses affecting humans such as Dengue Virus, Yellow Fever Virus or Chikungunya Virus (16,42). The Zika virus has been isolated from many species of Aedes mosquito competent for transmission including *Ae. aegypti*, *Ae albopictus*, *Ae hensilii* and *Ae. polynesiensis* (9,42–45). The ability of ZIKV to be efficiently transmitted by different mosquito species feeding humans further complicates their control and, thus, that of ZIKV. During the current outbreak in Latin America and the Caribbean, the principal vector spreading ZIKV is thought to be *Aedes aegypti*, probably justified by the urban abundance and the anthropophilic nature of this mosquito (46). This mosquito live and breeds in small quantities of water near people and their homes, and is known to feed on humans during daytime hours, especially during dusk or dawn in cloudy weather and indoors.

Although the primary species has not been identified, monkeys are presumed to serve as reservoir hosts (42). Humans are amplifying hosts for ZIKV, and urban cycles of transmission between humans and mosquitoes sustain and cause epidemics. While the bite of an infected mosquito is the principal route of ZIKV infection, there have been reported other non-vector-borne transmission routes.

2.2. Blood-borne transmission

There are growing concerns about the risk of transfusion transmission of Zika virus, particularly in susceptible population such as pregnant women and those in need of regular blood transfusions such as with sickle cell disease (47). This rising concern is partly justified by the persistence of ZIKV infection in blood. During the viremic phase of an ZIKV infected person high viral loads (up to 8.1×10^6 copies/mL) are detected in blood, and may last up to 14 days (32,48,49). During the French Polynesian outbreak, the potential for viral transmission through blood transfusion was demonstrated since blood donor viremia rates of up to almost 3% were documented in asymptomatic donators, suggesting that a ZIKV viremic donor could potentially contaminate the blood supply (8). In fact, there have been reported in Brazil some cases of ZIKV transmission through transfusions of donated blood (50). Although, further studies are needed to better establish ZIKV transmission through infected blood supplies, the Food and Drug Administration (FDA), and the European Centre for Disease Prevention and Control, have recently issued bulletins to alert national authorities and to reduce this possible route of transmission (51,52). These documents suggest deferral from blood donation for 28 days after travel to countries with ZIKV outbreaks or sexual contact with a man who has travelled to ZIKV risk regions.

2.3. Sexual transmission

During the past few years, there have been reported several cases of sexual transmission from infected man to their female partners. The first documented case of sexual transmission was reported on 2008, when a US scientific working in Senegal became ill with ZIKV and on his return to Colorado he infected his wife (6). In December 2013, during the ZIKV outbreak in French Polynesia, a patient of Tahiti presented high viral loads in semen ($1,1\text{-}2,9 \times 10^7$ copies/mL) and urine ($3,8 \times 10^3$ copies/mL), supporting ZIKV transmission by sexual intercourse (53). A third case was reported in early February 2016 by Dallas Country and Human Services when a patient, who had not recently travelled outside the U.S., developed Zika illness symptoms after sexual contact with her partner returning from a ZIKV risk region (54). Lately, several instances of suspected sexual transmission of Zika virus have been reported to CDC (55). Now, it has been well established that sexual transmission of Zika virus is due to semen infection, as it has been detected ZIKV RNA in this sample (53,56). Furthermore, it has been demonstrated that ZIKV RNA can persist in semen for up to 62 days after the onset of febrile symptoms (56). The viral persistence in this tissue could be allowed by the immune-privileged nature of the testes. Similarly, it has been now demonstrated in mice models that ZIKV RNA can sustain high viral loads in mice testes, which could explain male-to-female sexual transmission of ZIKV observed in humans

(57,58). Taken together these findings made the WHO to recommend an abstinence or safer sex during eight weeks after return from Zika areas (59).

2.4. Vertical transmission

As the emergence of ZIKV in Brazil has coincided with an alarming increase of the cases of microcephaly, with a 20-fold increase from prior years this new suggested route of transmission is of critical concern and significant interest (4). It has also been reported perinatal transmission of other flaviviruses including DENV, CHIKV, and YFV (60–62). Current evidence indicate that ZIKV is vertically transmitted from pregnant mother to foetuses. During the French Polynesia outbreak ZIKV perinatal transmission form two mother to their new-borns was proposed (7). ZIKV RNA could be detected in the serum of both mothers up to 5 days post-partum and in their new-borns within 4 days after birth (7). In addition, ZIKV RNA has been detected in amniotic fluid and foetal and new-born brain tissue (63–65). Similarly, the cerebrospinal fluid of 30/31 Brazilian neonates with microcephaly has been positive for ZIKV-specific IgM antibodies (66). Moreover, ZIKV antigen was noted within the chorionic villi of a human placenta from a mother who gave birth to a baby with microcephaly (63). Finally, it has been detected ZIKV antigen in placental tissue from a mother diagnosed with ZIKV disease, reflecting tropism for placental cells (67). However, the mechanism by which ZIKV is transmitted from an infected mother to her foetus is not well clarified. Recently, few studies performed in pregnant mice model have demonstrated that Zika virus infection during pregnancy can be vertically transmitted causing placental damage and neurodevelopmental defects in new-borns (68–70). In fact, in a recent study it has been suggested that ZIKV contemporary strain gain access to foetal compartment by directly infecting and replicating in human placental macrophages and to a lesser extent cytotrophoblasts and disrupting the placental barrier (71). Further studies are needed in order to better understand the mechanism of intrauterine transmission and the cell types involved.

2.5. Other possible transmission routes

Other ZIKV transmission routes has been sporadically reported, and so further studies are needed to establish their possible role on ZIKV epidemics. Similar to other flaviviruses, breastfeeding transmission has been proposed for ZIKV (72,73). ZIKV RNA has been detected in breast milk, and therefore ZIKV-infected mothers may be able to pass the virus to nursing children (7). In addition, ZIKV RNA could be detected in urine and saliva of infected women with even higher frequency than in blood samples and, thus, is another transmission source that have to be considered (40).

3. Clinical outcomes of Zika disease

Following the bite from an infected mosquito, the incubation period is 2 to 12 days, after which a mild flu-like illness occurs, but only in 20% to 25% of infected individuals, while most remain asymptomatic (13,42). This mild illness only lasts for 2 to 7 days after which there is no residuum and is associated

with restoration of normal number of peripheral immune cells and normal function of antigen-presenting cells (6,35). For many years, ZIKV infection was considered self-limiting and with no long-term consequences. However, during the experience in French Polynesia and the emergence of ZIKV in Brazil they have become apparent more severe complications.

3.1. Zika fever and complications

Symptomatic infected individuals present an acute “dengue fever-like” illness characterized by a low-grade fever (38ºC-39ºC), maculopapular rash, non-exudative conjunctivitis, retro-orbital headache, myalgia and arthralgia primarily on hands and feet (13). Digestive tract symptoms including nausea, vomiting, diarrhoea, constipation, abdominal pain, and aphthous ulcers may also be present (11,13). Dengue and Chikungunya often have similar presentation, and co-infection with these viruses has also been described (74). No death, hospitalisation, or haemorrhagic complication has been reported. However, during the recent epidemics in Oceania and South America outbreak, an association of ZIKV infection with more severe disease outcomes, such as Guillain-Barre Syndrome (GBS) has been also proposed. GBS is a post-infection autoimmune neuropathy that can result in weakness, paralysis or even death (19). During the outbreak in French Polynesia in 2013, as aforementioned, there was a 20-fold increase in GBS incidence, given the population size and the previously established incidence (1-2/100000 cases per year) (3,75). In a case-control study of the outbreak, 98% of patients diagnosed with GBS were positive for ZIKV infection, indicating a possible association. Furthermore, it has been reported an increase in incidence of GBS cases, with confirmed Zika virus infection in 15 countries including Brazil, Colombia, Dominican Republic, El Salvador, French Guiana, Honduras, Martinique, Suriname and Venezuela (26). Therefore, based on research to date, there is scientific consensus that Zika virus is a cause of GBS (26). In fact, GBS has been previously linked with other flaviviral infection (76). Other neurological complications potentially associated to ZIKV infection include meningoencephalitis and acute myelitis (77,78).

3.2. Congenital Zika syndrome

The major concern with Zika virus infection is in pregnant women as it has been proposed as the causal agent of a new congenital syndrome and the cause of thousands cases of devastating neuropathology and miscarriage, stillbirth, and intrauterine growth restriction (IUGR) due to placental insufficiency (79). The most prominent and commonly reported clinical outcome of this new congenital syndrome is microcephaly, which is defined with a head circumference ≥2 standard deviations below the mean for sex and gestational age at birth (4,80). Microcephaly is a rare paediatric condition, with potentially significant complications for the child, and which can be caused by several factors including genetic anomalies, postnatal brain injury, or perinatal brain injury such as congenital infections (81). During the outbreak in Brazil the dramatic increase in the rate of microcephaly has been linked to the newly epidemic of Zika virus (4,79,80,82,83). Furthermore, a retrospective analysis identified an increase in new-borns with microcephaly during the 2013-2014 ZIKV outbreak in French Polynesia (84). As of 13

July 2016, the Brazilian Ministry has confirmed 1.678 microcephaly cases in all the country potentially associated with Zika virus infection (26). Twelve more countries have reported cases of microcephaly and other central nervous system (CNS) malformations possibly linked with ZIKV infection, three of them from mother with a recent travel history to Brazil (26). Therefore the spatiotemporal association of microcephaly cases with Zika virus outbreak and the raising evidence from case reports and epidemiologic studies, has pointed to a robust scientific consensus that Zika virus is implicated in congenital abnormalities (84). Indeed preliminary data suggest that the greatest risk of microcephaly or congenital abnormalities in the affected neonates appears to be associated with ZIKV infection during the first trimester of pregnancy (23). Moreover, maternal infection with the Zika virus during this trimester has been associated with an estimated 1%, when assuming an 80% overall ZIKV infection rate and 100% overreporting of microcephaly cases, to 13% risk of microcephaly, when assumed a 10% ZIKV infection rate and no overreporting (85).

Additionally to microcephaly other manifestations associated with the congenital Zika syndrome have been reported including craniofacial disproportion, spasticity, seizures, irritability and brainstem dysfunction including feeding difficulties, brain calcifications, cortical disorders and ventriculomegaly (64,79,80,84). Indeed, ocular findings in infants with presumed ZIKV-associated microcephaly were described recently, including cataract, asymmetrical eye sizes, intraocular calcifications, macular atrophy, optic nerve hypoplasia, iris colomba, and lens subluxation (86–89). Nonetheless, the reported congenital malformations cases may represent only the tip of the iceberg, with less severe infection producing long-term cognitive or functional sequelae. Surprisingly, it has been recently proposed that Zika congenital syndrome may be able to develop in infants whose mothers had asymptomatic infections (90).

4. Biology of the Zika virus

4.1. Classification

Zika virus (ZIKV) is a member of the *Flavivirus* genus within the *Flaviviridae f*amily which includes other important human and animal pathogens such as yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV) (5). Flaviviruses belong to the group of arboviruses, which means arthropod borne viruses, referring to mosquitoes or ticks vectors for transmission. Within the mosquito-borne cluster of the genus *Flavivirus*, ZIKV is grouped in the Spondweni virus serogroup representing the only members of their clade (5,91).

4.2. Genome

The full genome of ZIKV (MR766 isolate), was first fully sequenced in 2007 (92). Similar to the other Flaviviruses members, ZIKV is a small enveloped single stranded positive RNA virus with a genome of 10,794 nucleotides and 3419 amino acids (5,92). The genome contains a single open reading frame (ORF) flanked by two untranslated regions, 5’ and 3’ UTRs (**Figure 2C**), containing the regulatory

elements that fold in stem-loop structures and regulate the synthesis of the viral RNA (92,93). ZIKV RNA genome includes a cap structure at its 5' end, crucial for proper translation of viral genome and evasion of immune response (94,95). However, contrary to cellular mRNAs, ZIKV RNA genome lacks a 3' poly(A) tract and instead ends with CU_{OH} similar to other flavivirus (**Figure 2C**). Further studies have also demonstrated that other ZIKV isolates show this same organisation (15,96). Furthermore, detailed

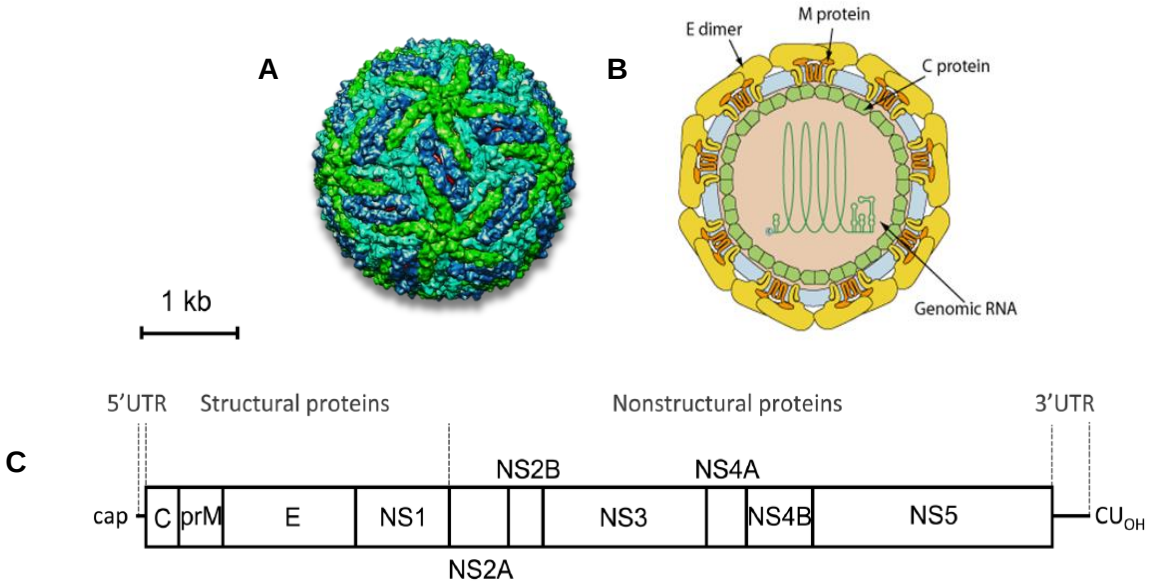

Figure 2. (**A**) Zika virus structure with surface proteins arranged in icosahedral-like symmetry. (**B**) Mature virions on the inside. (**C**) Schematic view of ZIKV genome organisation. Adapted from (145).

genetic analysis of the ZIKV RNA sequence has demonstrated that there are two distinguishable lineages of the Zika virus, the African lineage and the Asian lineage (13,15) . It has been demonstrated that there is an extra 12 nucleotide sequence in the envelope gene of ZIKV Asian genome, which is not present in the African strain (32). In fact, this 4 amino acid sequence corresponds to the 154 glycosylation motif in the envelope protein, located on a loop that is adjacent to the fusion peptide and which may be important in cell attachment and probably play a role in disease severity (97). Actually, it has been previously demonstrated the importance of glycosylation for the attachment of flaviviruses to cells (98). Interestingly, glycosylation at Asn^{154} in WNV has been linked to neurotropism (99). Taken together, these observations suggest a possible distinctive tropism and disease severity for the ZIKV Asian lineage, responsible for the recent epidemic in Brazil.

4.3. Viral proteins

The single ORF in ZIKV encodes for a viral polyprotein that is cleaved posttranslationally by host and viral proteases into three structural proteins (capsid C, premembrane prM, and membrane M) and seven non-structural proteins (**Figure 2C**). For flavivirus, these three structural proteins participate in the assembly of the virions (92). Multiples copies of C protein are complexed with the genomic RNA, conforming the core of the virions within a host-derived lipid bilayer and surrounded by an icosahedral shell composed by 180 copies each of the envelope (E) glycoprotein and the membrane (M) protein or precursor membrane (prM) protein (**Figure 2B**) (100,101). Now, the recently published cryoelectron

microscopy structures of ZIKV (**Figure 2A**) have revealed that ZIKV displays a similar structure to other well-known flaviviruses (97,102). Apart from the structural proteins the polyprotein also encodes for seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (92). NS2B acts as a cofactor for the viral serine protease NS3 that along with host proteases, co-translationally and post-translation cleaves the polyprotein into its components (103). In other flaviviruses, NS4A has been involved in membrane rearrangements associated with viral replication, NS1 and NS2A in RNA replication and viral assembly and NS2A in immunomodulation (103,104). NS5 is the most highly conserved non-structural protein of flaviviruses, and functions as RNA-dependent RNA polymerase in charge of genome replication, and also displays a methyltransferase domain necessary for capping the 5' end of ZIKV genome (105). Furthermore, it has been recently demonstrated that NS5 protein antagonizes Type I interferon signalling in humans, avoiding immune response (106).

4.4. Life cycle

During their life cycle, flaviviruses virions are presented in three major states – immature, mature and fusogenic – which are non-infectious, infectious, and host membrane-binding states (101). The immature virus particle, consisting of 60 trimeric E:prM heterodimer spikes, is assembled in the endoplasmic reticulum (107). In this immature state, the prM protein acts protecting the 12 amino acid fusion loop on the E protein (108). Subsequently, in the trans-Golgi network the low-pH environment induces conformational changes of the surface proteins and then occurs the cleavage of prM by the host protease furine, leading to the maturation of the virion into a "smooth" virus (**Figure 2B**), consisting of 90 dimeric E:M heterodimers (100,109). After the appropriate proteolytic cleavages, the fusion loop on the E protein is exposed, enabling low pH-mediated endosomal fusion (101,108). Mature virions and subviral particles are subsequently released by exocytosis (101).

4.5. Cell-virus interactions

Upon the principal mode of transmission of ZIKV, in the blood-feeding process of an infected *Aedes* mosquito, it has been recently determined that skin fibroblasts, epidermal keratinocytes and dendritic cells are highly permissive to viral infection with a strain from the French Polynesia (110). In concrete, it has been described that ZIKV entry in permissive cells is mediated by DC-SIGN, AXL, Tyro 3, and, to a lesser extent, TIM-1 (110). In ZIKV it has been suggested that TIM-1 acts as an attachment factor, concentrating viral particles in the cell surface, and transferring them to AXL, in order to assist the viral internalisation (110). The availability of a large number of receptors and/or attachment factors for ZIKV entry enables the infection of a wide range of target cells, similar to other flaviviruses, providing an evolutionary advantage for the virus (108, 109).The infection of these cells leads to an active viral replication which induces signs of apoptosis in epidermal keratinocytes, similar to observations made with DENV (111).

During viral infection, host antiviral innate immunity responses by activating a potent antiviral state in order to control the spreading of the virus. In human skin cells it has been shown that replication and dispersion of ZIKV is controlled by the activation of type I and type II interferons (IFNs). Then, ZIKV induces the transcription of Toll-like receptor 3 (TLR3), RIG-I, MDA5, and several interferon stimulated genes, such as OAS2, ISG15, and MX1 (110). Therefore, in order to supress this critical host response flaviviruses, as well as other vertebrate viruses, have developed efficient mechanisms of IFN antagonism (112). Zika virus, similar to other flaviviruses, uses the non-structural protein NS5 to supress IFN signalling (106,113). Concretely, ZIKV NS5 binds and degrades the human IFN-regulated transcriptional activator STAT2 inhibiting IFN signalling in human cells (106). Moreover infection in human skin cells with ZIKV results in the formation of an autophagosome, related with an enhanced viral replication but it remains to be determined (110).

5. Experimental evidence linking Zika virus with neurological disorders

Worldwide spreading of ZIKV and increasing evidence that point an association between ZIKV infection with more severe neurological outcomes has urged the need of understanding the pathogenic mechanism of this re-emerging virus. Within the last couple of months, several studies either in vitro or in vivo have begun to reveal mechanisms of ZIKV-induced pathology in the brain. These recent findings facilitate the development of an effective and safe vaccine to prevent a major expansion of the virus.

7.1 Experiments in vitro

5.1.1. Cell culture

Consistent with its transmission cycle, which includes replication in mosquito (vector) and mammalian cells (host), ZIKV can infect and replicate in a wide range of cells. Firstly, it can infect several mosquito cell lines, such as C6/36 (*Aedes albopictus*) and AP-61 (*Aedes pseudoscutellaris*) (6,37). Additionally, ZIKV is able to replicate in several mammalian cells, including PS-C1 cells (porcine kidney), LLC-MK2 cells (*Macaca mulatta*, rhesus monkey kidney) and Vero Cells (*Chlorocebus aethiops,* African green monkey kidney) (114,115). Regarding human cell lines, it has been recently demonstrated that ZIKV infects human skin cells, including skin fibroblasts, epidermal keratinocytes, and dendritic cells (110). Contemporary ZIKV strain can also infect and replicate in primary human placental macrophages and cytotrophoblasts, suggesting a possible route for ZIKV vertical transmission(71).

Furthermore, three recent studies have filled a major gap in ZIKV biology demonstrating a marked neurotropic nature of the virus and identifying *in vitro* ZIKV target in the neural lineage (68,116,117). ZIKV efficiently infects human neural progenitor cells (hNPCs), a constitutive population of the developing embryonic brain (68,116,117). It has also been demonstrated that ZIKV can also infect immature neurons, however, exhibiting lower levels of infection (68,116). *In vitro* infection of hNPCs with ZIKV leads to an efficient secretion of infectious ZIKV particles both using a viral multiplicity of infection (MOI) of 10 and 1, but in neurons only at MOI 10 (68,116). Intriguingly, an early animal study showed

ZIKV infection of neurons and astrocytes in mice and observed enlarged astrocytes (118). These results shown in immature neurons raise critical questions about ZIKV-induced pathological effects on neurons and other neural cell types in the brain, as well as potential long-term consequences (116). ZIKV-infected cells shown signs of endoplasmic reticulum membrane rearrangements, with both vesicle packets and convoluted membranes, exhibiting the characteristic intracellular “virus factory” pattern of flaviviruses (68,116,117,119,120). Besides the marked neurotropic nature of the virus, it has been demonstrated that ZIKV derives in an increased cell death and an attenuated growth of the hNPCs population (68,116,117). This cytopathic effect, characterized by cell rounding and pyknotic nuclei, is mainly due to significant increased activation of caspase-3 and cell-cycle dysregulation (116,117). Consistently, upon viral ZIKV infection there are a large number of differentially expressed genes, with a downregulation of genes involved in cell-cycle-related pathways, and an upregulation of genes enriched in transcription, protein transport, and catabolic processes (116). Although ZIKV causes cell death in hNPCs, it has been observed a limited activation of inflammatory or immune responses, suggesting a ZIKV stealthy modulation of immune pathways, or an inherent hypoimmunogenecity of isolated hNPCs with respect to ZIKV (117). After the initial cytopathic phase, the survival hNPCs subpopulation can continue to produce the virus for up to 28 days, mirroring the viral persistence observed in humans (64,80,117). Taken together, these recently established ZIKV *in vitro* models may be instrumental to better understand the molecular mechanisms underlying the pathophysiological effects of this re-emerging virus.

5.1.2. Mini organoids and neurospheres

Given the high complexity of human brain, it has been challenging to model many brain disorders *in vivo*, including ZIKV-associated microcephaly, urging the need for an *in vitro* model of brain development. Currently, embryonic stem cells (ESCs) and human-derived iPSCs can be differentiated towards different neuron subtypes or even into 3D organoids systems (121). Whereas neurospheres model initial characteristics of neurogenesis, brain organoids recapitulate the coordinated cellular and molecular early events, including gene expression and cortical layering (122). Moreover, these new established systems are complementary models and a powerful tool for studying and understanding the pathogenesis during embryonic brain development *in vitro*. For this reason, some recent approaches to understand ZIKV-associated malformations in the brain have been undertaken in neurospheres or cerebral organoids (68,122–124).

Recently, Garcez and colleagues have developed an in vitro ZIKV model by using human iPSCs cultured as: neural stem cells (NSCs), neurospheres, and brain organoids to evaluate in 3D culture models the consequences of ZIKV infection during neurogenesis and growth (122). Firstly, they exposed human iPS-derived neural stem cells (NSCs) to ZIKV, which induced a productive infection in these cells, and then cultured NSCs for three days *in vitro* (DIV) to generate neurospheres. Whereas mock-NSCs produced round neurospheres, ZIKV-infected-NSCs generated neurospheres with morphological

abnormalities, cell detachment, and an increased caspase 3/7- mediated cell death (122). After six DIV, hundreds of neurospheres grew under mock conditions, however, only few ZIKV-infected neurospheres survived, contrary to DENV2 infected NSCs, and showed viral particles bounded to the membranes and apoptotic nuclei, an indicator of cell death (122). These results suggest that ZIKV infection, triggers cell death in human neural stem cells and consequently disrupts the formation of neurospheres in vitro (122). These findings are consistent with those described by Tang and colleagues in hNPCs (116). In parallel, human iPS-derived brain organoids infected with ZIKV were cultured for 11 DIV, emulating the first trimester of development. Then, it was observed a reduction of 40% on the average growth area of ZIKV-exposed organoids compared to control and to DENV2-exposed organoids (122). Therefore, these new findings suggest that ZIKV infection during the first trimester of brain development may result in severe damage, both in neurospheres and cerebral organoids.

Similarly, Dang and colleagues have provided a new model for ZIKV infection during foetal brain development by using human ESC (hESC)-derived cerebral organoids and mice neurospheres (123). They infected with the prototype MR766 ZIK strain mice neurospheres and early day 10 human cerebral organoids, overlapping with the emergence of the neuroepithelial layer and transition from embryoid body (123). Similar to previous findings, ZIKV-infected neurospheres exhibited a reduced growth compared to control (122,123). At day 5 post-infection ZIKV-infected cerebral organoids showed a net average of 45.9% decrease in growth compared to healthy mock-treated cerebral organoids (123). Moreover, they observed strong co-localization of ZIKV envelope in NESTIN–positive cell populations, which is a marker of NPCs. Actually, ZIKV-infected NESTIN-positive cells showed unhealthy morphology and signs of apoptotic activation, indicating that ZIKV targets NPC population and leads to a neurodevelopment disruption (123). Consistent with previous findings in human skin cells, ZIKV infection in cerebral organoids and neurospheres induces the expression of TLR3 leading to a significant reduction in overall size (108,122,124). Pathway analysis has shown that ZIKV-mediated TLR3 activation leads to an enrichment of networks associated with positive regulation of neurodevelopment and regulation of synapse structure or activity (123). Surprisingly, it has been previously linked TLR3 with neurodegenerative disorders and negative regulation of axogenesis (126–128). Taken together these results suggest that ZIKV induces the expression of TLR3 disturbing neurogenesis and apoptotic pathways in NPCs possibly contributing to the ZIKV-mediated microcephaly (123). Furthermore, TLR3 is highly expressed in early neurodevelopment and decreases as the NPC population differentiates (126). This confined expression of TLR3 in the first stage of the brain development may be linked to the observed trimester-specific response of human foetal brains to ZIKV infection (123,129).

In the same line of investigation Cugola and colleagues have tested two 3D neural cell culture systems, neurospheres and cerebral organoids, with $ZIKV^{BR}$ and $ZIKV^{AF}$ (68). They have generated neurospheres by growing human NPCs in suspension, and compared ZIKV infection between both historical lineages. Interestingly, the Brazilian strain at MOI 10 displayed morphological abnormalities with signs of cell death and generate neurospheres significantly smaller than the $ZIKV^{AF}$-infected neurospheres (68).

These results confirm previous findings that ZIKV targets NPCs leading to an increased cell death and consequently an impaired growth and morphology of healthy neurospheres (68,122,123). They have also used hPSCs and hESC to generate brain organoids and to evaluate the impact of both ZIKV strains on human cortical development. Then, they have infected these organoids with both $ZIKV^{BR}$, $ZIKV^{AF}$ and YFV and compared to mock-infected organoids at 24 and 96h post-infection (68). $ZIKV^{BR}$-infected organoids shown a major reduction of the cortical plate thickness compared to others. In order to establish the potential mechanistic adaptive differences between $ZIKV^{BR}$ $ZIKV^{AF}$ brain organoids from non-human primate (chimpanzee) pluripotent stem cells were also generated (68). In these organoids, $ZIKV^{BR}$ was not able to replicate, differently to $ZIKV^{AF}$, suggesting that the Brazilian strain has undergone adaptive modifications in human cells (68). In fact, it has been recently proved that $ZIKV^{BR}$ is going through codon usage adaptation towards biases observed in highly expressed human genes (130). Therefore, it has been hypothesized that microcephaly might be a unique feature of the $ZIKV^{BR}$ strain (68).

Now, Quian and colleagues have described an improved method to generate cerebral organoids that better model human cortical development (124). Moreover, they have established a new model for ZIKV pathogenesis in more complex 3D tissue. ZIKV shows specific tropism for NPCs, including outer radial glial cells (oRGCs), and leads to a productive infection in these target cells (124). ZIKV infection of early stage forebrain organoids resulted in damaging effects simulating many features of microcephaly, such as an increase of cell death and a supressed proliferation of infected NPCs leading to decreased neuronal layer thickness, a reduced overall size and enlarged lumen/ventricles (124). Furthermore, ZIKZ-infection of 80-day-old organoids (which resemble to the second trimester of pregnancy) also resulted in a preferential infection of NPCs and oRGCs (124). All together this new information provide evidence that ZIKV preferentially infects NPCs, in a productive manner, and leads to characteristic features that resemble human microcephaly.

5.2. Experiments in vivo

5.2.1. Zika virus pathogenesis in non-pregnant mice model

When the virus was first discovered in 1947, a few initial studies were undertaken, mostly in mice, in order to characterize the virus and its tropism (2,9,44,114,118). The first isolation was made in April 1947 from the serum of a febrile sentinel rhesus monkey, and from a lot of *A.africanus* in January 1948, both in the Zika forest, in Uganda (2). Then, the virus was intracerebrally inoculated into mice in order to evaluate the pathogenicity of ZIKV (9). It was early seen that the virus was highly neurotropic in mice because infectious virus was only recovered from infected brain mice, and no other tissue (9). Mice of all ages tested were susceptible to intracerebral infection with Zika, however, only mice younger than 2 weeks were highly susceptible to intraperitoneal inoculation of the virus (9). The signs of infection in mice were detectable about 5-6 days post-infection, when the virus titer peaked (9). Those changes mainly consisted of degeneration of nerve cells, especially in the hippocampus, resulting in an

enlargement and extension of astroglial cells with affection of the pyriform cells of Ammon's horn (44,118). Moreover, it was early found that Zika virus infected and replicated in both astroglial cells and in neurones (44). Besides mice, other animal models, such as cotton-rats, guineapigs and rabbits were resistant to intracerebral inoculation with ZIKV (9).

In view of the growing evidence linking ZIKV with more severe neurological complications, within the last couple of months, many efforts have been invested in developing a mice model of a contemporary ZIKV isolate of greater clinical relevance. However, one of the most challenging problems has been to reproduce ZIKV infection in immunocompetent mice (57,58,131). It has been recently discovered that the non-structural NS5 protein of ZIKV targets the IFN-regulated transcriptional activator human STAT2 for degradation, antagonizing IFN signalling response (106). However, mice STAT2 is refractory to ZIKV NS5 protein, suggesting that the inability of ZIKV to cause disease in mice with functional immune response is associated with the inability of ZIKV to antagonise IFN signalling in mice (106). Analogous, in DENV it was also previously demonstrated an inability to antagonize IFN signalling in mice (132,133). Consequently, several studies have been taken in the same direction by genetically depleting interferon receptors in mice (57,58,131,134).

One recent approach has established a lethal model of a contemporary ZIKV strain in mice deficient in interferon α/β and γ (AG129 mice) (134). 3- to 4-week-old or 8-week-old mixed sex mice were infected with a $ZIKV^{AS}$ strain both through intraperitoneal inoculation and foot pad inoculation. All age mice exhibited signs of illness 5 days after infection, including weight loss, lethargy and hunched posture, and finally becoming immobile and weak (134). Infection with ZIKV led to rapid viral dissemination, with higher loads in brains from young mice, and resulted in severe brain pathology potentially resembling features of human foetal ZIKV infection (134).

Another recent study, has also evaluated the effect of ZIKV injection in type-I interferon deficient mice (A129 mice) and congenic control mice (129Sv/Ev), after subcutaneous injection in the lower leg, mimicking a mosquito bite (131). Female mice aged 5-6 weeks were used for all studies, and they tested the ZIKV strain MP1751, isolated from pools of *Aedes africanus* in 1962 (131). A129 mice developed severe symptoms, including inflammatory and degenerative changes, with widespread viral RNA detection in the blood, brain, spleen, liver and ovaries (131). Whilst no histological changes were observed in the 129Sv/Ev mice injected with ZIKV, viral RNA was detected in the blood, ovary and spleen, indicating that the virus arrived the circulation and seeded into some of the organs (131). So A129 mice is a susceptible animal model for, in a future, testing of vaccines and antivirals for the treatment of ZIKV (131). In fact, this model has previously demonstrated protective effects with vaccines for CCHFV, Ross river virus and chikungunya virus (135–137).

In addition, a third study in mice has validated both immunocompromised mice (AG129 and A129) as mice models of lethal and non-lethal $ZIKV^{AS}$ infection in 3-, 5-, and 11-week-old mice (58). Both immunocompromised mice models were shown to have signs of disease and high viral loads were

detected in the spleen, brain and testes (58). The replication in the testes of male mice to surprisingly high titters, support recent reports of sexual human transmission from male to female (6,53,55). Despite both mice models exhibited signs of infection and neurological involvement, they were much more severe in the AG129 mice, characterized by “toe-walking”, tremors, and loss of balance (131). These results suggest a possible neuroprotective role of IFNγ of ZIKV infection in the brain. In fact, it has been demonstrated that IFNγ is an important signalling pathway to protection against DENV and VEEV-caused encephalitis (138,139). A review of the cytokines produced in ZIKV-infected patients showed a sustained presence of IFNγ, from early infection through the recovery phase, suggesting an important role in ZIKV CNS infection (140).

One more study carried out by Lazear and colleagues has recently evaluated the infection of a wide range of immunocompromised mice with five different ZIKV strains (57). They tested 5- to 6- week old WT C57BL/6 mice as well as congenic transgenic mice lacking key component of innate antiviral immunity (Irf3 KO, Irf5 KO, Irf7 KO, Irf3/5/7 TKO, Ifnar $^{-/-}$ (A129), AG129) for susceptibility induced by different ZIKV strains (57). They used a clinically relevant route of infection through subcutaneous injections in the foot pads and intravenous injections of recent ZIKV strains compared with the original isolate. Regarding differences observed among different virus strains, ZIKV H/PF/2013 was more pathogenic than the original Ugandan strain (57). They found that, whereas WT mice did not develop clinically apparent disease, mice lacking interferon α/β signalling succumbed to infection with different ZIKV strains (57). However the most severe form of infection was the Irf3/5/7 TKO, followed by Ifnar $^{-/-}$, suggesting a possible role for IR-3-dependent, IFN-α/β-independent restriction mechanism (141). The susceptibility to different ZIKV strains in WT mice was age dependant, since adult mice shown no signs of disease, whereas one-third of the suckling pups succumbed to infection. Contrarily, all ages A129 mice showed susceptibility to ZIKV, and morbidity and mortality was even observed in mice up to 6 months of age (57). Noteworthy, Ifnar1 $^{-/-}$ mice sustained high levels of ZIKV infection in the brain, spinal cord, and testes, which are all tissues related to relevant aspects of ZIKV disease in humans. In fact, ZIKV RNA was detected in testes and brain even after disease signs had resolved, similar to human infection where ZIKV RNA has been detected in semen up to 2 months after infection (56). Therefore, the present model in Ifnar1$^{-/-}$ is a valuable starting point to mimic and evaluate ZIKV sexual transmission. However, there are limitations to pathogenesis studies in mice lacking a critical component of innate antiviral immunity, therefore to create an even more useful animal model, infection studies with more immunologically competent mice should be taken. Actually, it has been tested whether the treatment with an IFNAR1-blocking monoclonal antibody (MAR1-5A3) could render WT mice susceptible to ZIKV infection and disease (57). Whereas MAR1-5A3-treated mice did not recapitulate the severity of ZIKV disease phenotype observed in Ifnar1$^{-/-}$, levels of ZIKV viremia were substantially higher than non-treated WT mice (57).

5.2.2. Zika virus pathogenesis in pregnant mice model

Given the urgent need to understand the basis for ZIKV vertical transmission and its neurological consequences in the newborn, a few recent studies have focused their efforts on developing an in utero transmission model of ZIKV infection. In these investigations pregnant mice have been infected with ZIKV to evaluate the effects on fecundity, neonatal infection, and brain development in order to assess the presumed association to microcephaly in humans (68–70,142). Miner and colleagues have established two mice models of Zika Virus infection in pregnancy, that support ZIKV replication and trans-placental transmission in pregnant mice (69). Using Ifnar1 $^{-/-}$ females crossed with Ifnar1 WT males they have obtained heterozygous foetuses (Ifnar1 $^{+/-}$) which exhibit a largely intact type I IFN signalling response (69). In parallel, they have developed a second model by treating pregnant WT mice females with an anti-Ifnar-blocking antibody (MAR1-5A3) 1 day prior to infection, which resulted in a less severe disease, similar to previous findings (57). Both models of pregnant pups were subcutaneously infected with an Asian ZIKV strain (H/PF/2013) at gestation day 6.5 and 7.5 (**Figure 3C**). By embryonic day 13.5 the majority of the foetuses had suffered foetal demise and reabsorption, lasting only a placental remnant (69). The foetuses who survived exhibited intrauterine growth restriction (IUGR) and growth impairment. In contrast, in pregnant WT females with prior exposure to anti-IFNAR antibody, no foetal death was observed but infection with ZIKV resulted in mild IUGR (69). Interestingly, ZIKV RNA was detected in the placenta of both models with an approximate amount of 1000-fold greater than in maternal serum, suggesting active viral replication in the placenta. Moreover, the placenta presented areas of necrosis, microscopic signs of apoptosis and vascular damage. In addition, high levels of ZIKV RNA and infectious virus were detected within the foetus head of both models, and the hindbrain and midbrain exhibited high levels of apoptosis (69). Taken together these findings suggest that ZIKV virus infection early in pregnancy results in infection of the placenta and foetal brain causing a foetal syndrome. Indeed, this study provides a valuable in utero transmission model of ZIKV infection to better understand its consequences and to evaluate antiviral therapies or vaccines.

In parallel, Cugola and colleagues intravenously infected, with a ZIKV^{BR} strain, two different pregnant mice strains at day 10-13 of gestation, to evaluate its association with birth defects (**Figure 3B**). They used a SJL mice strain, which exhibits immune system abnormalities, and a C57BL/6 strain, with functional responses of both type I and II interferon (68). The pups from the infected C57BL/6 mice showed no major body alterations and negative diagnostic qPCR assay, indicating that the virus did not cross the placenta in such strain, consistent with previous findings (58). Contrarily, the impact of ZIKV^{BR} infection in SJL pregnant females was notable leading to foetuses with either whole-body growth delay or IUGR (68). These results are consistent with the evidence supported by Miner and colleagues (69). Furthermore, ZIKV^{BR} RNA was identified in several tissues with higher loads in the brain, which confirms the neurotropic nature of the virus. In the new-borns head, similar to ZIKV^{BR}-infected human neonates, the presence of the virus produced cortical malformations with reduced number of cells and cortical layer thickness (68). Moreover these reduced cortical layer showed neurons with a ‘vacuolar nuclei’

appearance, similar to the thalamus and hypothalamus, suggesting an ongoing cellular death (68). In addition, some pups from the infected SJL mice exhibited ocular abnormalities compatible to those previously reported in humans (86,87). Interestingly, they have also demonstrated that in the mouse neural tissue ZIKV^{BR} induces the expression of genes associated with autophagy and apoptosis, consistent with formation of phagosomes reported in human skin cells (110).

A third study, carried out by Wu and colleagues, also established a vertical transmission of ZIKV virus mice model. Firstly, they infected foetal mice at embryonic day 13.5 by injecting a contemporary Asian ZIKV strain (SZ01) into the lateral ventricle (**Figure 3A**). By the day E17.5 the virus was detected in the ventricular zone (VZ) of telencephalon and in the striatum (70). In parallel, they infected intraperitoneally

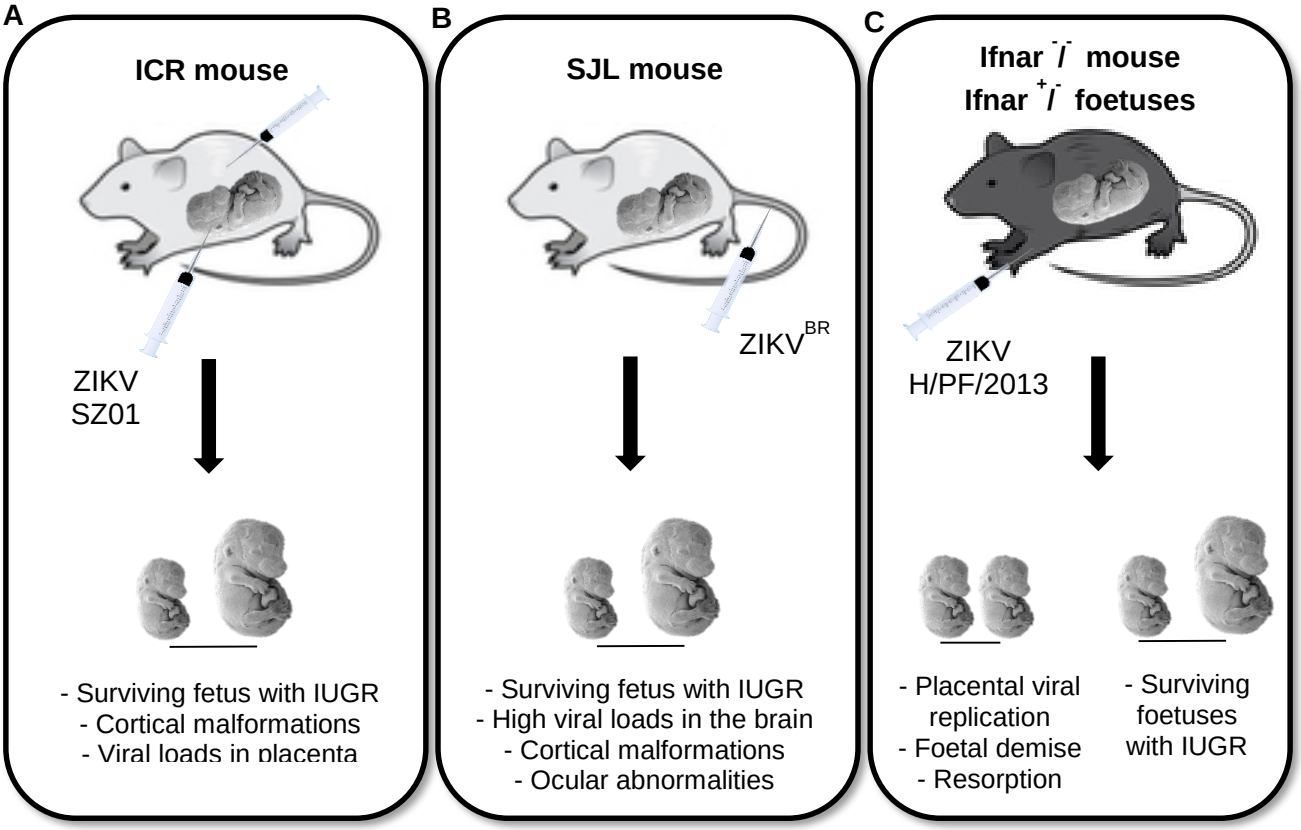

Figure 3. Pregnant mice models of ZIKV-induced pathogenesis. (**A**) ZIKV injection into the lateral ventricle of the foetal mice. (**B**) SJL mice infected with ZIKV^{BR} strain by intravenous injection. (**C**) Ifnar1 $^{+/-}$ heterozygous foetuses infected with ZIKV H/PF/2013 by subcutaneous injection in the lower leg. Adapted from (146).

C57 pregnant mice with ZIKV^{AS} at E13.5 and then evaluated the virus presence in the new-borns mice. By the third day post-infection, 5 out of 9 placentas were positive for ZIKV-specific RNA detection. Indeed it was detected a high load of ZIKV RNA in the dorsal VZ of the foetal brain, where radial glia (RG) cells, the major cortical neural progenitors, are localised (70). Their results also suggest that ZIKV vertical infection leads to an abolishment on proliferation of cortical neural progenitors which leads to a reduction of its source (70). These findings are concordant with the previous report that ZIKV induces cell death and inhibits cell cycle progression in cultured hNPC (116). Due to the reduction in the proliferative neural progenitor pool in the VZ of the foetal brain, defects in the cortical development of new-borns mice were detected. They have found a significant shorten of the cortex and a reduced cavity

of lateral ventricles with decreased surfaces in ZIKV-infected pups in comparison with control mice (70). In addition, quantitative gene expression analysis using real-time PCR has shown a downregulation of autosomal recessive primary microcephaly (MCPH)-associated genes to varying degrees in ZIKV-infected fetal brains samples. They also observed a downregulation of genes critical for cell proliferation and negative regulators of apoptotic process and an upregulation of genes involved in immune response and cell death program (70). Similar findings were stated in cultured human neural progenitors (116). Finally, they described a marked increase in the receptor of IL17a (IL17Ra) in ZIKV-infected foetal brain samples (70). Surprisingly, a recent study has demonstrated that the immune activation in pregnant mice, through IL17Ra, can give rise to alterations in the cortex development of pups.

One more investigation undertaken by Li and colleagues has brought new evidence for ZIKV infection affecting brain development. Here, the Asian lineage ZIKV has been injected directly into the cerebroventricular space / lateral ventricle of the brain in 13.5 day-old foetuses. Similar to the findings described by Wu and colleagues, in this study by 3-5 day post-infection most of the ZIKV-infected cells were located at the ventricular and subventricular zones, where many NPC are found (142). These results confirm the neural progenitor cells (NPCs) as the main target of the ZIKV (70,116,142). Moreover this report suggests that post-mitotic neurons can also be the target of ZIKV (142). ZIKV has shown to cause cell death in the infected NPC, which has been observed here and previosuly by a high presence of positive cells for the activated form of caspase 3, in the intermediate zone and cortical plate (116,142). ZIKV infection also provoke a dysregulation of NPC cell cycle and differentiation (142). Indeed this marked tropism of ZIKV for NPC in infected pups, and its cell cycle arrest and dysregulation lead to smaller brains, thinner cortical layer, and enlarged ventricles, all of them signs of microcephaly (142). Finally, through transcriptome analysis, this study confirms the previously described downregulation of cell proliferation and differentiation genes and the upregulation of immune response and apoptosis pathways by ZIKV (70,116,142).

Together, these recent studies provide important information about maternal-foetus transmission and ZIKV-related pathogenesis, building a highly valuable basis to establish a causal link between ZIKV and neurological complications observed in humans. Furthermore, these newly established mice models can be now used to evaluate possible vaccine candidates and therapies to prevent ZIKV-associated neurodevelopmental defects.

5.2.3. Zika virus pathogenesis in rhesus macaque model

However, these murine models present some limitations in extrapolating their results to human such as average gestational period or cerebral cortex size. Therefore, a model specie with larger cerebral cortex and similar gestation period like monkeys was urgently needed. There is few available data in nonhumans primates apart from the original isolation of ZIKV from a febrile rhesus monkey (2). During the first experiments carried out with ZIKV, monkeys develop an inapparent infection after subcutaneous inoculation with mouse brain virus. Only, one of five monkeys (the sentinel Rhesus 766) showed mild

pyrexia after intracerebral inoculation of the ZIKV (9). However, all monkeys evaluated exhibited viremia during the first week after inoculation and showed induction of specific antibodies about 14 days post-inoculation (9). In addition, a study has been recently initiated to daily asses ZIKV infection dynamics in rhesus macaques (143). Here, 8 monkeys, 2 of them pregnant, have been infected at gestational day 31 and 38 with the Asian ZIKV strain (H/PF/2013). Until present date, ZIKV RNA has been detected in saliva, urine and cerebrospinal fluid in all of them 1 day post-infection, in 6 of them 21 days post-infection, and up to gestational day 58, only in the pregnant monkeys (143). The daily evaluation of this new model in macaques will bring highly useful information of ZIKV pathogenesis in either adults or pregnant monkeys. Nonetheless, samples from amniocentesis at 43-36 day post-infection were negative for ZIKV RNA, in both pregnant monkeys (143). These two monkeys are expected to deliver on August-September 2016 therefore new-borns could provide relevant information about maternal-foetus ZIKV transmission.

6. Main findings of Zika virus affecting the brain

These new evidence has brought into light essential features of Zika virus which enables the understanding of its pathogenesis. Among the viral infection ZIKV in pregnant female mice targets the placenta sustaining an active viral replication and provoking severe damage in this tissue, supporting in utero transmission of the virus (69,71). In the brain, ZIKV can directly infect hNPCs with high efficiency leading to attenuated population growth through virally induced caspase-3-mediated apoptosis and cell-cycle dysregulation (116,122,124). However, the surviving neural progenitors continue to produce infectious viral particles for up to many weeks, similar to foetal neuropathological findings (116,117). Upon access to the foetal brain, ZIKV abrogates neurodevelopment by targeting the hNPCs population in all the model systems. Due to the marked tropism of ZIKV for hNPCs in infected pups, and its cell cycle arrest and dysregulation infection lead to smaller brains, thinner cortical layer, and enlarged ventricles, all of them signs of microcephaly (68–70,124,142). The provided experimental evidence demonstrates that ZIKV infection during the first trimester of brain development result in severe damage, causing a foetal syndrome that resembles the intrauterine growth restriction and spontaneous abortion observed in ZIKV-infected pregnant women. Moreover, detection of high ZIKV loads in the testes could explain male-to-female sexual transmission of ZIKV observed in humans (57,58). Finally, when evaluating the effect of various ZIKV strains in vivo it has been observed a higher lethality with the brazilian strain compared to the other strains (57). Indeed it has been suggested that $ZIKV^{BR}$ might have experienced adaptive changes in human cells (68). It has been demonstrated that the Asian lineage of ZIKV is undergoing codon usage adaptation towards biases observed in highly expressed human genes (15). These results support the hypothesis that microcephaly is a distinctive feature of recent ZIKV Asian-lineage virus, which originated in the Pacific and now is spreading in South and Central America.

7. Future challenges

The Zika virus epidemic has raised as an unexpected global threat with the explosive invasion of the Americas in 2015 and its potential linkage with thousands of cases of microcephaly in Brazil and higher rates of Guillain-Barré syndrome. The future of ZIKV epidemic is unpredictable, but as the virus rapidly spreads, the need of developing immediate measures to control or limit ZIKV disease become increasingly urgent. Previously believed to be transmitted only by mosquitos, it is now known to be transmitted during sexual intercourse, via perinatal transmission or by blood transfusion. These newly described routes of viral infection challenge the spreading control measures, and urges the need of designing gold-standard diagnosis, antivirals and vaccines which are suitable for pregnant women and foetuses. Recently, reported experimental evidence has confirmed the link between Zika virus infection during pregnancy and a new congenital syndrome. Nonetheless, it has to be well established the mechanism by which the virus infects and crosses the placental barrier reaching the developing foetal brain, and then disrupting the neural tissue. Because of the recent appearance of severe neurological complications, only time will establish the whole spectrum of Zika congenital syndrome.

8. Bibliography

1. Steele RW. Zika Viurs: An Explosive Pandemic and a New TORCH Agent. *Clin Pediatr*. 2016;55(8):698-700.

2. Dick GW. Zika virus (I). Isolations and serological specificity. *Trans R Soc Trop Med Hyg*. 1952;46(5):509–20.

3. Cao-Lormeau VM. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet*. 2016;387:1531–39.

4. Schuler-Faccini L. Possible Association Between Zika Virus Infection and Microcephaly-Brazil, 2015. *Morb Mortal Wkly Rep*. 2016;65(3):59-62.

5. Cook S. A multigene analysis of the phylogenetic relationships among the flaviviruses (Family: Flaviviridae) and the evolution of vector transmission. *Arch Virol*. 2006;151(2):309-25.

6. Foy BD. Probable Non-Vector-borne Transmission of Zika Virus, Colorado, USA. *Emerg Infect Dis*. 2011;17(5):880-2.

7. Besnard M. Evidence of perinatal transmission of zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill*. 2014;19(13):8-11.

8. Musso D. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. *Euro Surveill*. 2014;19(14):14-6.

9. Dick GW. Zika virus (II).Pathogenicity and physical properties. *Trans R Soc Trop Med Hyg*. 1952;46(5):521–34.

10. Macnamara FN. Zika virus: a report on three cases of human infection during an epidemic of jaundice in Nigeria. *Trans R Soc Trop Med Hyg*. 1954;48(2):139–45.

11. Olsoni JG. Zika virus, a cause of fever in Central Java, Indonesia. *Trans R Soc Trop Med Hyg*. 1981;75(3):389-93.

12. Faye O. Molecular Evolution of Zika Virus during Its Emergence in the 20th Century. *PLoS Negl Trop Dis*. 2014;8(1):36.

13. Duffy M. Zika Virus Outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med*. 2009;360:2536-43.

14. Kay M. Zika : the origin and spread of a mosquito-borne virus. *World Heal Organ*. 2016;1-18.

15. Haddow AD. Genetic characterization of zika virus strains: Geographic expansion of the asian lineage. *PLoS Negl Trop Dis*. 2012;6(2):1-7.

16. Roth A. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. *Euro Surveill*. 2014;19(41):1-8.

17. Cao-Lormeau VM. Emerging arboviruses in the Pacific. *Lancet*. 2014;384(9954):1571-2.

18. Cao-Lormeau VM. Zika virus, French Polynesia, South Pacific, 2013. *Emerg Infect Dis*. 2014;20(11);1960.

19. Willison HJ. Guillain-Barré syndrome. *Lancet*. 2016;6736(16):1-11.

20. Zanluca C. First report of autochthonous transmission of Zika virus in Brazil. *Mem Inst Oswaldo Cruz*. 2015;110(4):569-

72.

21. Musso D. Zika virus transmission from French Polynesia to Brazil. *Emerg Infect Dis*. 2015;21(10):1887-9.

22. Heukelbach J. Zika virus outbreak in Brazil. *J Infect Dev Ctries*. 2016;10(2):116-20.

23. Pan American Health Organisation/World Health Organisation (PAHO/WHO). *Epidemiological alert - Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas*. 1 December 2015. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32405&lang=en.

24. World Health Organisation (WHO). *WHO statement on the first meeting of the International Health Regulations (2005) (IHR 2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations*. 1 February 2016. Available from: http://www.who.int/mediacentre/news/statements/2016/1st-emergency-committee-zika/en/.

25. Centers for Disease Control and Prevention (CDC). *CDC Concludes Zika Causes Microcephaly and Other Birth Defects*. 13 April 2016. Available from: http://www.cdc.gov/media/releases/2016/s0413-zika-microcephaly.html.

26. World Health Organisation (WHO). *Zika situation report – Zika virus, Microcephaly and Guillain-Barré syndrome*. 14 July 2016. Available from: http://www.who.int/emergencies/zika-virus/situation-report/14-july-2016/en/.

27. Centers for Disease Control and Prevention (CDC). *Zika virus disease in the United States, 2015–2016*. 13 July 2016. Available from: http://www.cdc.gov/zika/geo/united-states.html.

28. Massad E. Estimated Zika virus importations to Europe by travellers from Brazil. *Glob Health Action*. 2016;1:1-7.

29. Petersen E. Rapid Spread of Zika Virus in The Americas - Implications for Public Health Preparedness for Mass Gatherings at the 2016 Brazil Olympic Games. *Int J Infect Dis*. 2016;44:11-15.

30. European Centre for Disease Prevention and Control (ECDC). *RISK ASSESSMENT - Public health risks related to communicable diseases at the Rio de Janeiro Olympic and Paralympic Games , Brazil, 2016*. 13 June 2016. Available from: http://ecdc.europa.eu/en/publications/Publications/01-06-2016-RRA-Mass%20gathering-Brazil%20World.pdf.

31. Cardoso CW. Outbreak of Exanthematous Illness associated with Zika, Chikungunya, and Dengue viruses, Salvador, Brazil. *Emerg Infect Dis*. 2015;21(12):2274-6.

32. Lanciotti RS. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis*. 2008;14(8):1232-9.

33. Waggoner JJ. Zika Virus: Diagnostics for an Emerging Pandemic Threat. *J Clin Microbiol*. 2016;54(4):860-7.

34. Staples J. Interim guidelines for the evaluation and testing of infants with possible congenital Zika virus infection—United States, 2016. *Morb Mortal Wkly Rep*. 2016;65(3):63-7.

35. Zammarchi L. Zika virus infections imported to Italy: Clinical, immunological and virological findings, and public health implications. *J Clin Microbiol*. 2015;63:32-5.

36. Shinohara K. Zika fever imported from Thailand to Japan, and diagnosed by PCR in the urines. *J Travel Med*. 2016;23(1):1-3.

37. Faye O. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes.*Virol J*. 2013;10(1):311.

38. Balm M. A Diagnostic Polymerase Chain Reaction Assay for Zika Virus. *J Med Virol*. 2012;84:1501-5.

39. De M. Campos R. Prolonged detection of Zika virus RNA in urine samples during the ongoing Zika virus epidemic in Brazil. *J Clin Virol*. 2016;77:69-70.

40. Bonaldo MC. Isolation of infective Zika virus from urine and saliva of patients in Brazil. *PLoS Negl Trop Dis*. 2016:1-17.

41.Pardee K. Rapid, low-cost detection of Zika virus using programmable biomolecular components. *Cell*. 2016;165(5):1-12.

42. Hayes EB. Zika virus outside Africa. *Emerg Infect Dis*. 2009;15(9):1347-50.

43. McCrae AW. Yellow fever and Zika virus epizootics and enzootics in Uganda. *Trans R Soc Trop Med Hyg*. 1982;76(4):552-62.

44. Weinbren MP. Zika virus: further isolations in the Zika area, and some studies on the strains isolated. *Trans R Soc Trop Med Hyg*. 1958;52(3):263-8.

45. Ledermann JP. Aedes hensilli as a Potential Vector of Chikungunya and Zika Viruses. *PLoS Negl Trop Dis*. 2014;8(10):1-9.

46. Tabachnick WJ. History of domestication and spread of Aedes aegypti - A review. *Mem Inst Oswaldo Cruz*. 2013;108(1):11-17.

47. Conrad MD. Maintaining a safe blood supply in an era of emerging pathogens. *J Infect Dis*. 2016:1-8.

48. Aurby M. Seroprevalence of arboviruses among blood donors in French Polynesia, 2011-2013. *Int J Infect Dis*. 2015;41:11-2.

49. Gourinat AC. Detection of zika virus in urine. *Emerg Infect Dis*. 2015;21(1):84-6.

50. Marano G. Zika virus and the never-ending story of emerging pathogens and transfusion medicine.*Blood Transfus*. 2015;1-6.

51. European Centre for Disease Prevention and Control (ECDC). *RAPID RISK ASSESSMENT - Zika virus disease epidemic (seventh update)*. 8 July 2016. Available from: http://ecdc.europa.eu/en/publications/Publications/RRA-Zika-virus%20epidemic-seventh-update-final.pdf.

52. Food and Drug Administration (FDA). *Recommendations for Donor Screening , Deferral , and Product Management to Reduce the Risk of Transfusion- Transmission of Zika Virus*. February 2016. Available from: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM486360.pdf.

53. Musso D. Potential Sexual Transmission of Zika Virus. *Emerg Infect Dis*. 2015;21(2):359-61.

54. Dallas country department of Health and Human Services. *HEALTH ADVISORY: Sexual Transmission of Zika Virus*. 2 February 2016. Available from: https://www.dallascounty.org/department/hhs/documents/DCHHS_Zika_HealthAdvisory_20160202_final.pdf.

55. Hills SL. Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission - Continental United States, 2016. *Morb Mortal Wkly Rep*. 2016;65(5):215-6.

56. Atkinson B. Detection of zika virus in semen. *Emerg Infect Dis*. 2016;22(5):940.

57. Lazear HM. A Mouse Model of Zika Virus Pathogenesis. *Cell Host Microbe*. 2016;19(5):1-11.

58. Rossi SL. Characterization of a Novel Murine Model to Study Zika Virus. *Am J Trop Med Hyg*. 2016;94(6):1362-9.

59. McCarthy M. WHO recommends eight week abstinence or safer sex after return from Zika areas. *Bmj*. 2016;3097(May).

60. Fritel X. Chikungunya virus infection during pregnancy, Réunion, France, 2006. *Emerg Infect Dis*. 2010;16(3):418-25.

61. Center for Disease Prevention and Control (CDC). *Perinatal Transmission of Yellow Fever, Brazil, 2009*. September 2011. Available from: https://wwwnc.cdc.gov/eid/article/17/9/pdfs/11-0242.pdf.

62. Adam I. Maternal and perinatal outcomes of dengue in PortSudan, Eastern Sudan. *Virol J*. 2010;7(153):1-4.

63. Martines RB. Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses - Brazil, 2015. *Morb Mortal Wkly Rep*. 2016;65(6):159-60.

64. Driggers RW. Zika Virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities. *N Engl J Med*. 2016;374(22): 2142-51.

65. Calvet G. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: A case study. *Lancet Infect Dis*. 2016;3099(16):1-8.

66. Cordeiro MT. Positive IgM for Zika virus in the cerebrospinal fluid of 30 neonates with microcephaly in Brazil. *Lancet*. 2016;387:1811-2.

67. Noronha L. Zika virus damages the human placental barrier and presents marked fetal neurotropism. *Mem Inst Oswaldo Cruz*. 2016;111(5):287-93.

68. Cugola FR. The Brazilian Zika virus strain causes birth defects in experimental models. *Nature*. 2016;534:267-71.

69. Miner JJ. Zika Virus Infection during Pregnancy in Mice Causes Placental Damage and Fetal Demise. *Cell*. 2016;165:1-11.

70. Wu K-Y. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. *Cell Res*. 2016;26(6):1-10.

71. Quicke KM. Zika Virus Infects Human Placental Macrophages. *Cell Host Microbe*. 2016;20:1-8.

72. Barthel A. Breast milk as a possible route of vertical transmission of dengue virus? *Clin Infect Dis*. 2013;57(3):415-7.

73. Kuhn S. Case report: Probable transmission of vaccine strain of yellow fever virus to an infant via breast milk. *Cmaj*. 2011;183(4):243-5.

74. Vilamil-Gómez WE. Dengue, chikungunya and Zika co-infection in a patient from Colombia. *J Infect Public Health*. 2015:1-3.

75. Oehler E. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. *Euro Surveill*. 2014;19(9):7-9.

76. Puccioni-Sohler M. Dengue: A new challenge for neurology. *Neurol Int*. 2012;4(3):65-70.

77. Carteaux G. Zika Virus Associated with Meningoencephalitis. *N Engl J Med*. 2016;374(16):1595-8.

78. Mécharles S. Acute myelitis due to Zika virus infection.

Lancet. 2016;387:1481.

79. Brasil P. Zika Virus Infection in Pregnant Women in Rio de Janeiro - Preliminary Report. *N Engl J Med*. 2016:1-11.

80. Mlakar J. Zika Virus Associated with Microcephaly. *N Engl J Med*. 2016;374(10):951-8.

81. Von der Hagen M. Diagnostic approach to microcephaly in childhood: A two-center study and review of the literature. *Dev Med Child Neuol*. 2014;56(8):732-41.

82. Sarno M. Zika Virus Infection and Stillbirths: A Case of Hydrops Fetalis, Hydranencephaly and Fetal Demise. *PLoS Negl Trop Dis*. 2016;10(2):5-9.

83. França GVA. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. *Lancet*. 2016;6736(16):1-7.

84. Cauchemez S. Association between Zika virus and microcephaly in French Polynesia, 2013-15: a retrospective study. *Lancet.* 2016;6736(16):1-8.

85. Almiron M. Zika and the Risk of Microcephaly. *N Engl J Med*. 2016:1-4.

86. Ventura CV. Zika virus in Brazil and macular atrophy in a child with microcephaly. *Lancet*. 2016;387:228.

87. McCarthy M. Severe eye damage in infants with microcephaly is presumed to be due to Zika virus. *Bmj*. 2016;352.

88. de Paula Freitas,B. Ocular findings in infants with microcephaly associated with presumed zika virus congenital infection in salvador, brazil. *JAMA Ophthalmol.* 2016;134(5):529-35.

89. Jampol LM. Zika Virus Infection and the Eye. *JAMA Ophtalmol*. 2016;134(5):535-6.

90. Pacheco O. Zika Virus Disease in Colombia - Preliminary Report. *N Engl J Med*. 2016:1-10.

91. Kuno G. Phylogeny of the genus Flavivirus. *J Virol*. 1998;72(1):73-83.

92. Kuno G. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol*. 2007;152(4):687-96.

93. Villordo SM. RNA Structure Duplications and Flavivirus Host Adaptation. *Trends Microbiol*. 2016;24(4):270-83.

94. Dong H. Flavivirus RNA methylation. *J Gen Virol*. 2014;95(4):763-78.

95. Daffis S. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature*. 2010;468(7322):452-6.

96. Baronti C. Complete Coding Sequence of Zika Virus from a French Polynesia Outbreak in 2013. *Genome A*. 2014;2(3).

97. Sirohi D. The 3.8 Å resolution cryo-EM structure of Zika virus. *Science*. 2016;5316:1-7.

98. Pokidysheva E. Cryo-EM reconstruction of dengue virus in complex with the carbohydrate recognition domain of DC-SIGN. *Cell*. 2006;124(3):485-93.

99. Beasley DWC. Envelope Protein Glycosylation Status Influences Mouse Neuroinvasion Phenotype of Genetic Lineage 1 West Nile Virus Strains. *J Virol*. 2005;79(13):8339-47.

100. Kuhn RJ. Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. *Cell*. 2002;108(5):717-25.

101. Mukhopadhyay S. A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol*. 2005;3(1):13-22.

102. Kostyuchenko VA. Structure of the thermally stable Zika virus. *Nature*. 2016;533(7603):425-8.

103. Murray CL. Architects of Assembly: roles of Flaviviridae nonstructural proteins in virion morphogenesis. *Nat Rev Microbiol*. 2009;6(9):699-708.

104. Martín-Acebes M. West Nile virus: A re-emerging pathogen revisited. *World J Virol*. 2012;1(2):51-70.

105. McMinn PC. The molecular basis of virulence of the encephalitogenic flaviviruses. *J Gen Virol*. 1997;78(11):2711-22.

106. Grant A. Zika Virus Targets Human STAT2 to Inhibit Type I Interferon Signaling. *Cell Host Microbe*. 2016;19:1-9.

107. Zhang Y. Structures of immature flavivirus particles. *EMBO J*. 2003;22(11):2604-13.

108. Yu I-M. Structure of the Immature Dengue Virus at Low pH Primes Proteolytic Maturation. *Science*. 2008;319:1834-7.

109. Zhang X. Cryo-EM structure of the mature dengue virus at 3.5-Å resolution. *Nat Struct Mol Biol*. 2013;20(1):105-10.

110. Hamel R. Biology of Zika Virus Infection in Human Skin Cells. *J Virol*. 2015;89(17):8880-96.

111. Limon-Flores AY. Dengue virus inoculation to human skin explants: An effective approach to assess in situ the early infection and the effects on cutaneous dendritic cells. *Int J Exp Pathol*. 2005;86(5):323-34.

112. Versteeg GA. Viral tricks to grid-lock the type I interferon system. *Curr Opin Microbiol*. 2010;13(4):508-16.

113. Laurent-Rolle M. The interferon signaling antagonist function of yellow fever virus NS5 protein is activated by type I interferon. *Cell Host Microbe*. 2014;16(3):314-27.

114. Way HJ. Comparative studies of some African

arboviruses in cell culture and in mice. *J Gen Virol*. 1976;30(1):123-30.

115. Buckley A. Detection of virus-specific antigen in the nuclei or nucleoli of cells infected with Zika or Langat virus. *J Gen Vriol*. 1998;69(8):1913-20.

116. Tang H. Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell*. 2016;18:587-90.

117. Hanners NW. Western Zika Virus in Human Fetal Neural Progenitors Persists Long Term with Partial Cytopathic and Limited Immunogenic Effects. *Cell Rep*. 2016;15:1-8.

118. Bell TM. Zika virus infection of the central nervous system of mice. *Arch Gesmate Virusforsch.* 1971;35(2):183-93.

119. Romero-Brey I. Membranous replication factories induced by plus-strand RNA viruses. *Viruses*. 2014;6(7):2826-57.

120. Junjhon J. Ultrastructural characterization and three-dimensional architecture of replication sites in dengue virus-infected mosquito cells. *J Virol*. 2014;88(9):4687-97.

121. Hattori N. Cerebral organoids model human brain development and microcephaly. *Mov Disord*. 2014;29(2):185.

122. Garcez PP. Zika virus impairs growth in human neurospheres and brain organoids. *Science*. 2016;352(6287):816-8.

123. Dang J. Zika Virus Depletes Neural Progenitors in Human Cerebral Organoids through Activation of the Innate Immune Receptor TLR3. *Cell Stem Cell*. 2016;19:1-8.

124. Qian X. Brain-Region-Specific Organoids Using Mini-bioreactors for Modeling ZIKV Exposure. *Cell*. 2016;165(5):1238-54.

125. Tsai YT. Human TLR3 recognizes dengue virus and modulates viral replication in vitro. *Cell Microbiol*. 2009;11(4):604-15.

126. Lathia JD. Toll-Like Receptor 3 Is a Negative Regulator of Embryonic Neural Progenitor Cell Proliferation. *J Neurosci*. 2008;28(51):13978-84.

127. Okun E. Toll-like receptor signaling in neural plasticity and disease. *Trends Neurosci*. 2011;34(5):269-81.

128. Cameron JS. Toll-like receptor 3 is a potent negative regulator of axonal growth in mammals. *J Neurosci*. 2007;27(47):13033-41.

129. Reefhuis J. Projecting month of birth for At-Risk infants after zika virus disease outbreaks. *Emerg Infect Dis*. 2016;22(5):828-32.

130. Freire CCM. Spread of the pandemic Zika virus lineage is associated with NS1 codon usage adaptation in humans. *bioRxiv*. 2015:1-8.

131. Dowall SD. A Susceptible Mouse Model for Zika Virus Infection. *PLoS Negl Trop Dis*. 2016;10(5):1-13.

132. Fernandez-Sesma A. Evasion of the human innate immune system by dengue virus. *Immunol Res*. 2012.

133. Suthar MS. Innate Immune Sensing of Flaviviruses. *PLoS Negl Trop Dis*. 2013;9(9):5-8.

134. Aliota MT. Characterization of Lethal Zika Virus Infection in AG129 Mice. *PLoS Negl Trop Dis*.2016;10(4):1-11.

135. Buttigieg KR. A novel vaccine against Crimean-Congo haemorrhagic fever protects 100% of animals against lethal challenge in a mouse model. *PLoS Negl Trop Dis*. 2014;9(3):1-14.

136. Holzer GW. Evaluation of an inactivated Ross River virus vaccine in active and passive mouse immunization models and establishment of a correlate of protection. *Vaccine*. 2011;29(24):4132-41.

137. Plante K. Novel chikungunya vaccine candidate with an ires-based attenuation and host range alteration mechanism. *PLoS Negl Trop Dis*. 2011;7(7):1-11.

138. Prestwood TR. Gamma Interferon (IFN-) Receptor Restricts Systemic Dengue Virus Replication and Prevents Paralysis in IFN- /- Receptor-Deficient Mice. *J Virol*. 2012;86(23):12561-70.

139. Paessler S. Alpha-beta T cells provide protection against lethal encephalitis in the murine model of VEEV infection. *Virology*. 2007;367(2):307-23.

140. Tappe D. Cytokine kinetics of Zika virus-infected patients from acute to reconvalescent phase. *Med Microbiol Immunol*. 2015;205(3):1-5.

141. Lazear HM. IRF-3, IRF-5, and IRF-7 Coordinately Regulate the Type I IFN Response in Myeloid Dendritic Cells Downstream of MAVS Signaling. *PLoS Pathog*. 2013;9(1).

142. Li C. Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. *Cell Stem Cell*. 2016(19):1-7.

143. Dudley DM. A rhesus macaque model of Asian-lineage Zika virus infection. *Nat Commun*. 2016;7:1-9.

144. Chang C. The Zika outbreak of the 21st century. *J Autoimmun*. 2016;68:1-13.

145. Saiz J-C. Zika Virus: the Latest Newcomer. *Front Microbiol*. 2016;7:1-19.

146. Hickman HD. Zika in the Brain: New Models Shed Light on Viral Infection. *Trends Mol Med*. 2016:1-3.