FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA

MESTRADO INTEGRADO EM MEDICINA – TRABALHO FINAL

BEATRIZ HELENA DE SOUSA E CASTRO NUNES VICENTE

Vírus Zika: uma revisão

ARTIGO DE REVISÃO

ÁREA CIENTÍFICA DE INFECIOLOGIA

Trabalho realizado sob a orientação de:

PROFESSOR DOUTOR VÍTOR DUQUE

MARÇO/2017

Index

Acknowledgements

Firstly, I would like to express my sincere gratitude to my advisor Professor Vítor for his motivation and knowledge. Without his precious support it would not be possible to conduct this research.

Secondly, I would like to thank my family and friends for supporting me throughout writing this thesis.

Abstract

Unlike other mosquito-borne relatives capable of causing severe human diseases, the Zika virus has initially appeared in a discreet manner, causing asymptomatic or mild exanthematous febrile infections which were considered little or no problematic.

Even though the virus was discovered in Uganda more than 60 years ago, less than 20 human cases were identified before 2007. Epidemics in the Pacific islands associated with the Asian lineage of the virus in 2007 and 2013 emerged before the explosive outbreaks in Latin America in 2015. These events, along with the first association of the virus with severe neurological alterations and the development of congenital malformations, ended up triggering international concern.

Despite recent efforts, vector-related knowledge, modes of transmission, pathogenesis and diagnosis are still scarce, thereby demanding rapid and thorough study to be concluded in useful time.

Thus, as no specific antiviral treatment exists and the development of the vaccines is still in progress, epidemic control of Zika virus infection requires a series of protection measures, which comprise, apart from direct fight against the vector, personal protection against mosquito bites, prevention among those who travel to and from these sites, and the education of the communities where the virus has been identified, particularly in view of reducing the risk of venereal mother-to-child transmission through appropriate sexual education.

Keywords: Zika Virus; *Aedes aegypti;* Epidemiology*;* Pathogenesis*;* Zika Fever; Microcephaly; Guillain-Barré syndrome; Treatment.

Resumo

Contrariamente a outras doenças transmitidas por mosquitos e capazes de causar doenças graves no ser humano, o vírus Zika surgiu de forma discreta, causando infeções assintomáticas ou caracterizadas por uma febre exantemática ligeira considerada pouco ou nada preocupante.

Não obstante o vírus ter sido descoberto há mais de 60 anos no Uganda, menos de 20 casos foram identificados até 2007. A epidemia que ocorreu nas ilhas do Pacífico em 2007 e 2013, associadas à estirpe Asiática, surgiu antes do surto da América Latina em 2015. Estes eventos, juntamente com a primeira associação do vírus a alterações neurológicas graves e malformações congénitas, despoletaram, finalmente, a preocupação internacional.

Apesar dos esforços recentes, todo o conhecimento relacionado com o vetor, modos transmissão, patogenia e meios de diagnóstico ainda é escasso, exigindo-se estudos aprofundados e céleres em tempo útil.

Assim, face à inexistência de terapias antivirais específicas e ao facto de o desenvolvimento vacinal ainda não estar concluído, o controlo epidémico da infecção pelo vírus Zika exige uma série de medidas de proteção, que comportam não só uma luta direta contra o vetor, mas também o uso de meios de proteção individual contra a picada do mosquito, a prevenção junto daqueles que viajam de e para estes locais, e a educação das comunidades onde o vírus foi identificado, com particular enfoque na redução do risco de transmissão venérea do vírus e entre mãe e filho através de uma educação sexual adequada.

Palavras-chave: Vírus Zika; *Aedes aegypti*; Epidemiologia; Patogenia; Febre de Zika; Microcefalia, Síndrome de Guillain-Barré; Tratamento.

Index of images

List of abbreviations and acronyms

C - Capside or core;

CNCC - Cranial neural crest cells;

CNS - Central nervous system;

CSF - Cerebrospinal fluid;

CT - Computed Tomography;

E - Envelope protein;

ECDC - European Centre for Disease Prevention and Control;

ER - Endoplasmic reticulum;

GBS - Guillain-Barré syndrome;

IFN - Interferon;

Ig - Immunoglobulin;

kDa - Kilodalton;

M - Viral membrane protein;

MRI - Magnetic resonance imaging;

mRNA - Messenger ribonucleic acid;

nm - Nanometers;

NPC - Neural progenitor cells;

NS - Non-structural proteins;

OFC - Occipital-frontal circumference;

PAHO - Pan American Health Organization

PAMP - Pathogen-associated molecular patterns;

prM - Glycosylated precursor protein of protein M.

PRNT - Plaque reduction neutralization tests;

RNA - Ribonucleic acid;

RT-PCR - Reverse transcription polymerase chain reaction;

SD - Standards deviations;

WHO - World Health Organization;

ZIKV - Zika virus;

1. Introduction

The ZIKV was first found in a febrile sentinel rhesus monkey (*Macaca mulatta*) in the forest that gives it its name, the Zika Forest in Uganda, in April 1947.[1,2] Recently, the ZIKV has become a complex and urgent public health problem in many countries, being associated with cases of microcephaly in neonates and GBS in adults. The real extension of this ultimate spread of Zika is hard to predict.[1]

The major challenges in fighting Zika include halting the progression of the virus, resorting to preventive measures, such as mosquito control, and the development of diagnosis methods, antivirals and vaccines. A better understanding of the disease and its epidemiology can also help halt its spread.[3]

The purpose of the present bibliographic review is to explore the history of ZIKV, understand its virology, molecular biology, epidemiology and routes of transmission, its involvement in neonatal microcephaly and GBS and, ultimately, the prospects for stopping its progress.

2. Methods

A systematic review of the literature on ZIKV was performed using the electronic database PubMed. The research encompassed articles written in English and French, between 1947 and October 2016. The search terms were “Zika” as well as “epidemiology”,” “clinical manifestation,” “neurological symptoms,” “microcephaly, “Guillain-Barré syndrome” “cardiovascular symptoms,” “eye disease,” “vertical transmission” and “treatment”.

The bibliography mentioned in the search results was carefully reviewed. The enquiry also considered epidemiology bulletins from WHO, PAHO and ECDC.

3. History and epidemiology

The Scottish virologist Georg W. A. Dick isolated the ZIKV in April 1947 in the blood of a rhesus monkey in Uganda´s Forest[1], during a research aimed at studying the yellow fever virus and identifying additional arboviruses.[2] The virus was later found in *Aedes africanus* from the same area[1,2] in 1948.[4]

While the virus was first reported in humans in 1952[5], evidenced by the presence of neutralizing antibodies in the sera of east African residents[6], the first characterization of human disease occurred in Nigeria in 1954 (2 cases based on seroconversion and one case based on virus isolation from the serum of a febrile girl). In 1966, the virus was detected in Asia and isolated from *Aedes (Stegomyia) aegypti* mosquitoes, but it was only eleven years later, in Indonesia, that diagnosis of the first human infection has taken place, by seroconversion in seven patients that presented fever, malaise, stomach ache, anorexia and dizziness.[2]

Cases of infection remained temporarily confined to Africa[1]. No more than 20 cases were reported in the first 60 years after its discovery.[4] However, in 2007, sera of five humans presenting febrile disease and two *Aedes albopictus* - all sampled in Libreville (Gabon) - tested positive when screened by RT-PCR for ZIKV. In that same year, an outbreak of the Asian strain come about in Yap, an island of Micronesia, and *Aedes (Stegomyia) hensilli* evinced to be the principal vector. Presenting fever, rash, conjunctivitis and arthralgia, 49 confirmed and 59 suspected cases were diagnosed using RT-PCR and serological analyses.[2] Since there were no reports of deaths during this outbreak[6], ZIKV was not seen as an important emerging pathogen, thereby being envisaged as a relatively mild clinical disease.[4]

Prior to 2014, there was no evidence of ZIKV circulation in the Western Hemisphere but that was before the outbreak in French Polynesia in 2013-14 involving *Aedes (Stegomyia) polynesiensis*. The French Polynesia outbreak affected about 11% of the local population (though with no recorded deaths [6]), who manifested typical signs and symptoms of

low-grade fever, maculopapular rash, arthralgia and conjunctivitis. It is worth noting that this was the first time that GBS was associated with ZIKV. In addition, the outbreak helped to document the risk of transmissions through blood and the detection of viral RNA in semen, saliva and urine.[2] After reaching French Polynesia, the virus spread across other islands in Oceania (New Caledonia, Cook Islands and Easter Island), where *Aedes aegypti* and *Aedes albopictus* were founded.

The first cases of autochthonous transmission, that is to say, cases in which the disease was locally onset rather than being acquired from a different location or country and introduced into the community, occurred in Easter Island in February 2014.[7]

Late in 2014, patients with signs of rash, mild fever and arthralgia were noticed in Northeastern Brazil and the case numbers increased during the first months of 2015.[2] It is estimated that between 500,000 and 1,500,000 ZIKV infections occurred in Brazil by autochthonous transmission since March 2015.[4] Although there are doubts as to how the virus entered in Brazil, it was assumed that it have been brought by the participants of big events that took place in the country during 2014: the 2014 Football World Cup and the 2014 World Sprint Championships. Recent phylogenetic studies, however, suggest that the introduction occurred sooner, between May and December 2013.[2] The climate changes associated with El Nino in north and eastern South America in 2015, on the background trend of global warming, might have facilitated the rapid spread of *Aedes* mosquitoes and ZIKV.[4] Notwithstanding, by the end of 2015, autochthonous cases had been identified throughout the country. So far, the virus was diagnosed in 47 countries and territories of America[8] and travel-related cases from endemic and epidemic regions were also reported in Europe, Australia and Japan.[4,9]

As in French Polynesia, cases of GBS have increased significantly in Brazil and other Latin American countries. However, it was the abrupt increase in the number of children born with microcephaly in Brazil since the epidemic began (an increase of

more than 20 times that of the historical average[6]) that triggered global concern[2,4] and led the WHO to declare the cluster of microcephaly cases and other neurological disorders as Public Health Emergency of International Concern, on February 1, 2016.[10] From the beginning of the outbreak until February 2016, more than 5200 cases of microcephaly have been identified in Brazil, though only 37% of them were associated with congenital ZIKV infection.[2]

October 2016 data revealed that, since 2007, 73 countries have reported evidence of ZIKV (**Figure 1**). Remarkably, 67 of these countries evince cases from 2015 onwards, 7 exhibit possible endemic transmission or evidence of local mosquito-borne Zika infections in 2016, and 10 present episodes of local mosquito-borne Zika infections during or before 2015. Since February 2016, 12 countries, including European countries, have reported evidence of person-to-person transmission of ZIKV. Besides, 22 countries have reported microcephaly and other CNS malformations potentially associated with ZIKV infection or suggestive of congenital infection and 19 countries have reported an increased incidence of GBS and/or laboratory confirmation of a ZIKV infection among GBS cases.[11]

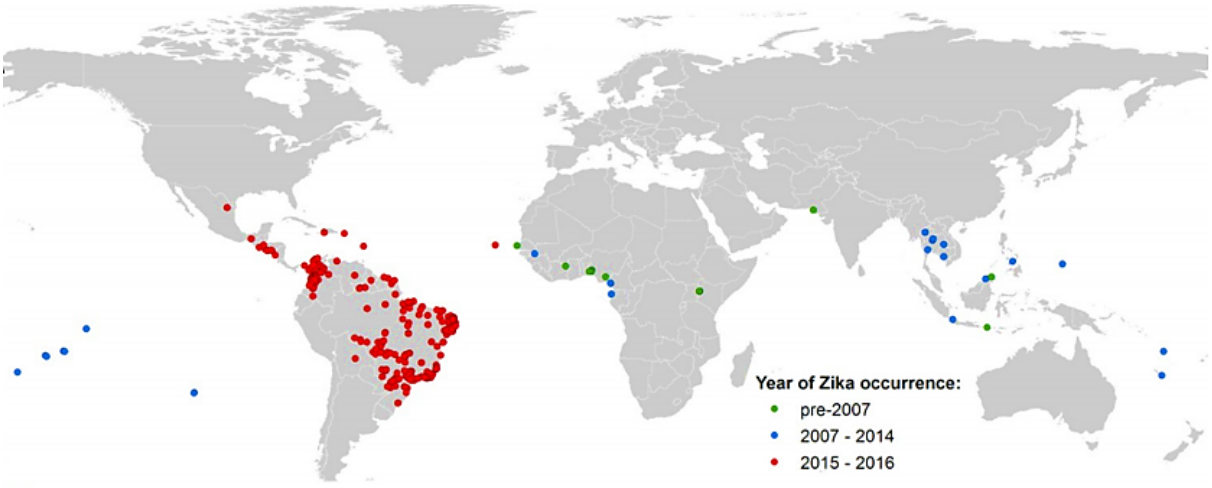

Figure 1 - Map showing the distribution of ZIKV over the years. [Adapted from 12]

4. Virology and molecular biology

4.1. The virus

Zika is an arbovirus member of the family *Flaviviridae* and genus Flavivirus[13] with an enveloped icosahedral virion of 40-50 nm in diameter that contains nonsegmented, single-stranded, positive-sense RNA genome. Its genome has 10,794 nucleotides[1] in length and early filtration studies indicated that the size of ZIKV was in the range of about 30–45 nm in diameter.[5] Being a mosquito-borne virus related to other flavivirus namely yellow fever virus, dengue virus, West Nile virus and, particularly, to Spondweni virus, it is transmitted by many *Aedes* spp. mosquitoes, including *Aedes africanus*, *Aedes aegypti*, *Aedes hensilli*, and *Aedes luteocephalus*.[1]

The virion is composed by a central core that contains the C with 13 kDa in size[1], in association with the viral RNA. This nucleocapside is involved by a lipid bilayer originated from the host cell and containing the M and E proteins that compose its smooth outer shell.[13] Whereas the E protein is the primary antigen site[9] and the major protein involved in receptor binding and fusion, the M protein is a small protein that is hidden under the E protein layer (**Figure 2**). Both proteins are organized in icosahedral symmetry consisting of 60 repeating units.[14] The M protein is expressed as a larger prM that assists the folding of E protein as a sort of chaperone and prevents premature fusion of the particles prior to their release from the infected cell. Additionally, its cleavage into M protein also promotes the maturation of the viral particles[13]. The glycosylation of the E protein is a determinant of neuroinvasion because it increases both axonal and transepithelial migration .[1,15] C, M and E proteins are classified as structural proteins, but ZIKV also express 7 NS (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[16] The function of the NS proteins remain unknown but it is believed to play an essential role in various replication stages[9], assembly, and also in antagonizing the host innate response to infection.[15]

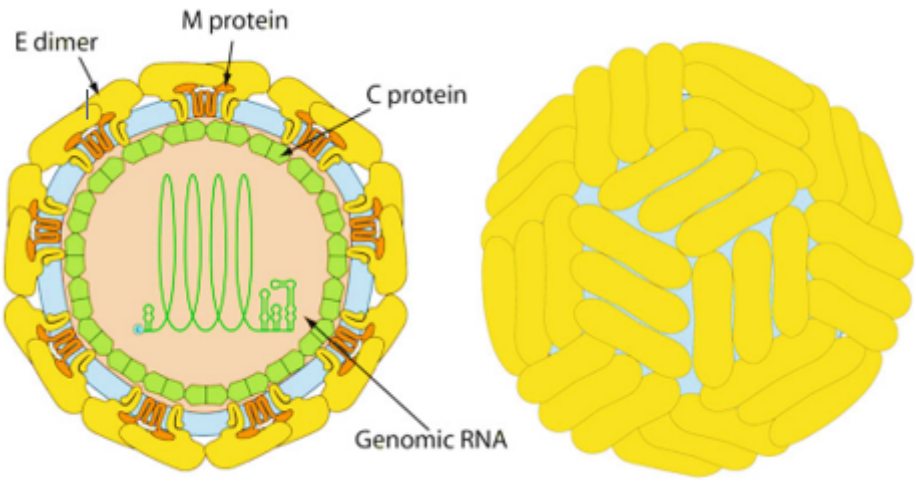

Figure 2 - Structure of a generic flavivirus. [Adapted from 17]

4.2. Genome and phylogenetic

The single-stranded RNA molecule has a positive polarity with a cap structure at its 5´end that is important to the efficiency of translation and for evasion of immune response.[13] The genome is about 11 kilobases in length[1] and encodes a polyprotein comprising about 3400 amino acids that will be cleaved into the mature viral structural and non-structural proteins.[13]

Although the ZIKV strain was isolated in 1947, genetic studies suggest that an ancestor might have manifested itself by the early 1900s in Uganda.[2] Sequencing the E, NS3 and NS5 encoded genes by means of detailed genetic analysis of the RNA sequence, it was possible to realize that the ZIKV identified in Africa has split into two major lineages: African and Asian/American.[9] Within this second lineage, a new one (American) emerged following the introduction of the virus into the Western Hemisphere, and it currently includes strains from Brazil and Latin America.[18] A major characteristic of the American ZIKV lineage is its rapid radiation, consistent with a pattern of intense diversification, as the lineage expands into new territories with immunologically naïve populations.[2]

Recent studies identified several sites within the Zika viral genome that were under strong negative selection pressure, a fact that suggests purging of deleterious polymorphisms

in important functional genes as well as the possibility of recombination, which is a rare occurrence in the flavivirus.[19] A specific change observed is the recurrent loss and gain of the N-linked glycosylation site in the E protein. This might well explain mosquito-cell infectivity[6] and the pathogenicity of ZIKV.[13] However, as recombination has not yet been experimentally achieved, these results should be interpreted with caution.[18] If confirmed, changes in ZIKV might include unpredictable replication efficiency and changes in antigenic epitopes, host tropism and virulence[1] as well as neurotropic behavior. That is probably why, despite circulating throughout Africa and Asia since the latter half of the 20th century, ZIKV infections have not been associated with significant human pathology until now.[16]

4.3 Host cell-virus interactions

ZIKV can infect a broad range of cells from different tissues within species. In mosquitoes, it replicates in the midgut and salivary glands; in mammalians, it replicates mainly in human neural progenitors[2] and brain cells, (including neurons and astroglial cells) and also in skin cells like fibroblasts, keratinocytes and immature dendritic cells.[1,13,20] The ability to replicate in a large number of hosts and cells is probably connected with its transmission cycle, which includes replication on mosquito (vector) and mammalian cells (host).[13]

Flavivirus particles bind to the surface of target cells through interactions between viral surface glycoproteins and cellular surface receptors.[1] Several entry and adhesion factors facilitate infection.[20] Then, virions that undergo a receptor-mediated endocytosis are internalized into clathrin-coated pits by the fusion of the viral envelope with the membranes of the cellular endosomes from the host cell, a process triggered by acidic pH inside cellular endosomes.[13] Once uncoated, the virus envelope releases the viral RNA into the cytoplasm thereby starting translation and replication (**Figure 3**).

To copy the viral genomic information, the virus induces a network of membranes derived from the host ER to which it associates the synthesis of a negative-strand viral RNA that will function as a template for the progeny viral RNA .

At this point, *de novo* synthesized positive strand-RNA is packed into progeny virions that bud from the ER to form enveloped immature virions, which will follow through the Golgi complex. In the acidic environment of *trans*-Golgy network, conformational changes occur, and consequently the prM is cleaved into M protein by the host protease furin, and then released from the infected cell[15] into exosomes.[16]

Although flavivirus replication is thought to occur in the cellular cytoplasm[13], it should be noted that one study unveiled that ZIKV antigens can also be found in infected cell nuclei. This finding suggests a location of replication that differs from that of other flaviviruses and thus merits further investigation.[20]

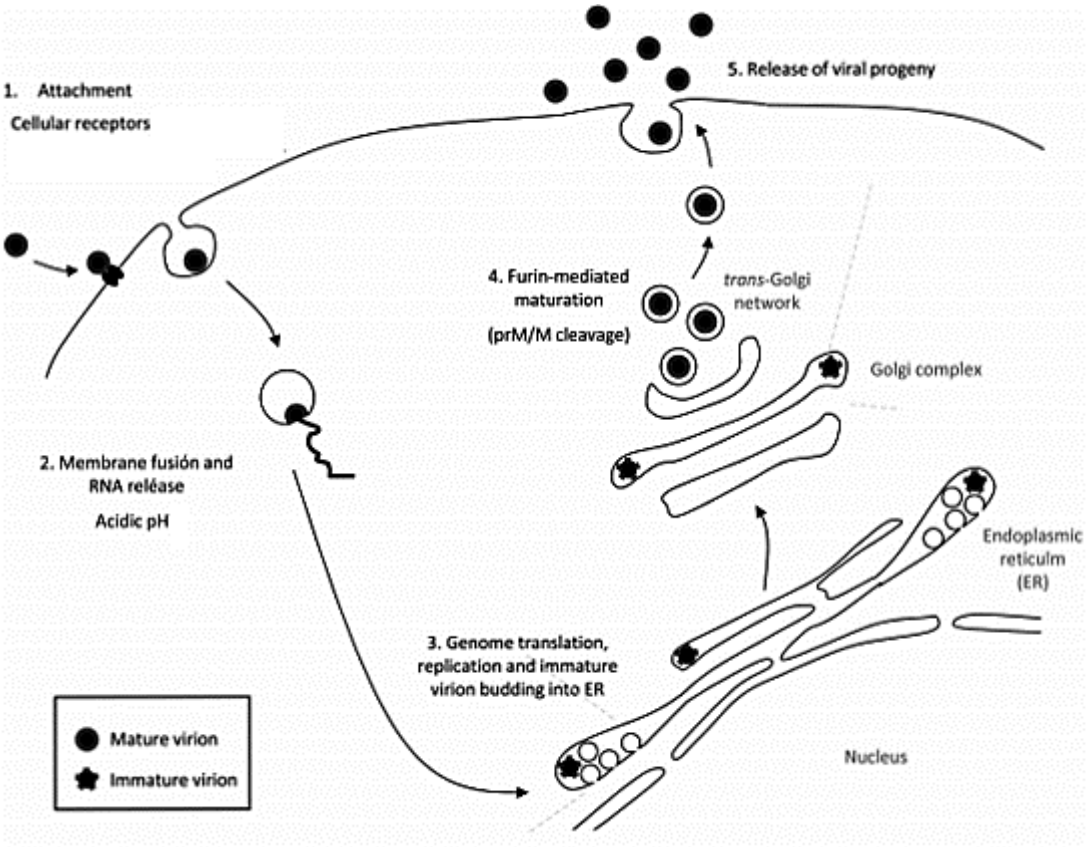

Figure 3 - Schematic view of the ZIKV cellular lifecycle. [Adapted from 13].

5. Transmission Cycle

5.1. Vector-borne transmission

5.1.1. General characteristics

For the transmission of arboviruses, the virus must be present in sufficient quantity in the blood/tissues of the vertebrate host so it can be picked up by the arthropod when it bites the natural host. Whenever viremia is absent or exists only at a low titer, infection chain is aborted. Another prerequisite is that the vertebrate host should lack neutralizing antibodies.[21] Then, the infectious virus seeks to survive and/or to replicate in a specific tissue of the arthropod. The continuity of the cycle demands sufficient infectious amounts to be delivered to another natural host. Undoubtedly, this is only possible because the virus has the potential for adapting to different host cells and body temperatures.[9]

In Africa, ZIKV exists in a sylvatic transmission cycle (**Figure 4**) involving non-human primates and local species of aedes mosquitoes; in Asia, a sylvatic transmission cycle has not yet been identified. Several mosquito species, including *Aedes africanus, Aedes luteocephalus*, *Aedes furcifer* and *Aedes taylori,* are likely vectors in Africa and Asia.[22] Similarly, seroprevalence of ZIKV has been demonstrated in various old-world non-human primates and other mammals such as elephants, zebras and rodents, but the role of these animals as virus reservoirs remains undetermined. It is possible that they act as natural ZIKV reservoirs hosts while forest-dwelling mosquitoes allow for the transmission of the virus between them.[23-24]

In urban and suburban environments, ZIKV is transmitted in a human-mosquito-human transmission cycle (**Figure 4**). Humans are amplifying hosts for ZIKV and urban cycles between humans and mosquitoes sustain and cause epidemics.[17] Overcrowding, lack of access to piped water, presence of stagnate water, household sewage give rise to the perfect conditions for the augmented transmission of ZIKV. Indeed, the recent outbreaks of ZIKV disease occurred mainly in areas evincing striking levels of poverty.[23]

Two species of *Aedes*, *Aedes aegypti* and *Aedes albopictus*, have been linked with nearly all recent ZIKV outbreaks, although two other species, *Aedes hensilli* and *Aedes polynesiensis*, were thought to be the vectors in the Yap and French Polynesia outbreaks, respectively. *Aedes aegypti* and *Aedes albopictus* are the only known aedes species in the Americas.[22] The mosquito population proliferates during heavy rainfalls, which enables ZIKV to surpass the forest boundaries and to spread into nearby villages. Consequently, the infection is transmitted to local inhabitants, who trigger the dissemination of ZIKV into larger urban centers.[24]

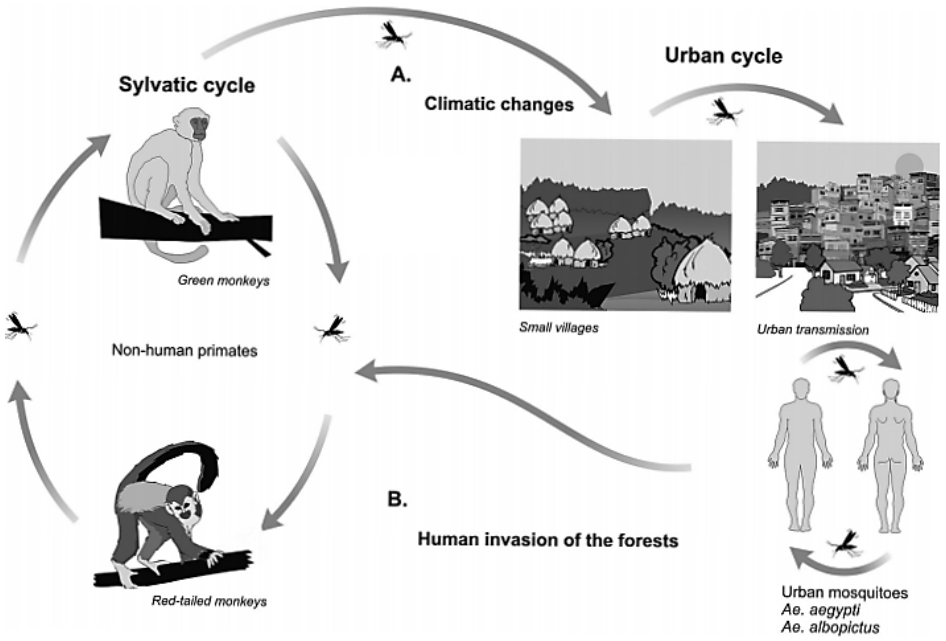

Figure 4 - Transmission of ZIKV in its sylvatic and urban cycles. [Adapted from 24]

Albeit *Aedes* mosquitos are globally distributed, warm tropical and subtropical regions are the native habitats of most species. Some species show a limited distribution (e.g., *Aedes luteocephalus* in Africa and *Aedes hensilli* in the Pacific Islands) but others have a broad geographic span (e.g., *Aedes aegypti* and *Aedes albopictus*).[20] Despite the association of *Aedes aegypti* and *Aedes albopictus* with outbreaks, both were found to have unexpectedly low but similar vector competence. However, *Aedes aegypti* exhibits high vectorial capacity because it feeds primarily on humans, often biting multiple humans in a single blood meal. Interestingly, it has an almost imperceptible bite and lives in close association with human habitation.[22] *Aedes aegypti* is currently distributed in Asia, Oceania, the Americas and in few regions of Africa and Europe; *Aedes albopictus* is also widely distributed, being currently circulating in Asia, America, northern Australia and in some areas of Africa and southern Europe. Contrary to *Aedes aegypti*, it can hibernate and survive in cool climates.[13]

The mosquitoes lay around 100 eggs at a time near stagnant water and bite people both indoors and outdoors.[25] Eggs can survive for long periods of time in a dry state, and are known to reintroduce large numbers of the mosquito after a cold, dry winter. Contrastingly, in the presence of water, it will hatch into a larva in 2 to 4 days. After 4 days, the larva develops into

a pupa and then, after another 2 days, into an adult mosquito. The life span of a mosquito is about 10 days for males and 1-2 months for females. Thus, female mosquitoes have to be transmission competent within this framework so that the life cycle of arbovirus might be completed.[9,22]

Generally, males only feed on plant nectars. Therefore, it is primarily the female mosquito that feeds on vertebrate blood, which enables the maturation of the eggs. [9,22] Following the ingestion of a bloodmeal, the virus will first infect midgut epithelial cells of the vector, where virions interact with receptors and penetrate the midgut cells. Uncoating, transcription, and translation of the virus genome inside the cell is followed by virion maturation and releasing. Then, infectious virions propagates from the midgut epithelium and infect secondary target organs. If the arbovirus is blocked at the early stages of midgut infection (e.g., receptor binding, uncoating, transcription, or translation failures), this is considered a midgut infection barrier. If infectious virions do not disseminate to hemocele (or disseminate but do not infect secondary target organs), this is called a midgut escape barrier, which can prevent transmission.[26-27] Viral infection disseminates through the mosquito body via hemolymph.

Viral content within the mosquito is high on the day of feeding (inoculation), decreases to undetectable levels by day 10, increases by day 15 and remains high from day 20 to day 60. These findings suggest an incubation period in mosquitos of approximately 10 days.[20] The vector-borne complete cycle and its relation with the *Aedes aegypti* lifecycle is described in **Figure 5.**

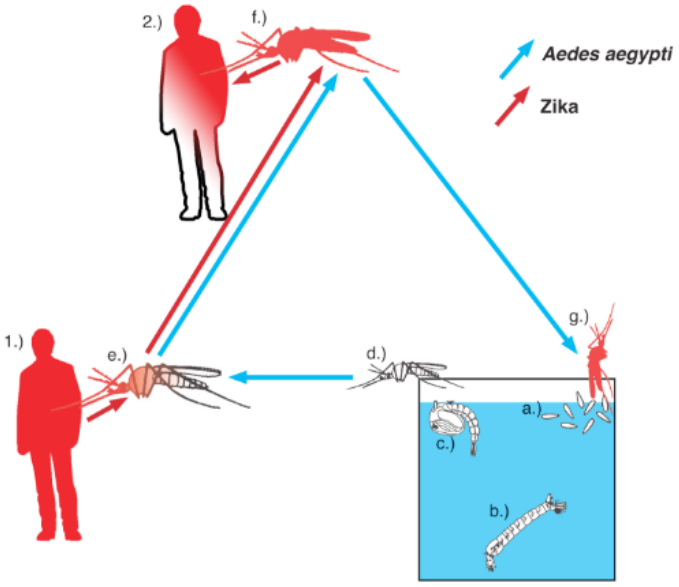

Figure 5 – Relation between the ZIKV transmission cycle and Aedes aegypti lifecycle:

ZIKV transmission: The virus passes from an infected human (**1**) through a mosquito to an uninfected human (**2**).

***Aedes aegypti* lifecycle:** A mosquito begin its life as an egg (**a**), then develops into a larva (**b**), a pupa (**c**), and an adult (**d**). Adult females feed on humans, potentially becoming infected (**e**) with the ZIKV and then infecting healthy humans with the virus (**f**). After feeding and mating, the female lays eggs of her own (**g**). [http://necsi.edu/research/social/pandemics/transmission.html, consulted on 2-10-16].

A 2014 study has allowed the identification of ZIKV in a pool of male *Aedes furcifer* without identification of infected females. This finding strongly suggests transovarially or venereal transmission of ZIKV in this species and highlights that vertical transmission may be an important mechanism of local maintenance.[28]

Whereas *Aedes* mosquitoes are the most important and common vectors of ZIKV, different species have also been purported as potential vectors.[23] Mosquitoes from the genus *Anopheles*, *Mansonia* and *Culex* have already been identified as infected with ZIKV, probably contributing to the zoonotic cycle of ZIKV transmission.[28] However, the simple detection of a virus in a mosquito sample does not assures it as a vector.[29]

4.1.3. Humans, non-humans primates and other vertebrates

During outbreaks, humans are the primary host for ZIKV. One important doubt is whether viral titers are high enough to initiate a new cycle every time an infected person is bitten by a non-infected mosquito.[13] The estimated number of genome copies circulating in ZIKV-infected patients during the 2007 outbreak in the Pacific Island of Yap was reported to be lower than those found among patients infected by other arbovirus, such as Chikungunya or Dengue virus[31]. Still, it is in line with other dead-end flavivirus infection in humans such as that of West Nile virus.[32] Further experiments are needed to clarify this issue.

Although it is known that epizootics occur in non-human primates, doubts have been cast as to whether they are an obligatory reservoir in the transmission to humans.[13] Recently, an acute symptomatic ZIKV infection was described in a traveler bitten by a monkey. While the transmission might have happened through mosquito bite, a potential transmission from primate bites should be considered as well.[33]

Information about the susceptibility of animal other than human and non-human primates is limited. Antibodies against ZIKV have already been found in several vertebrate species such as rodents, birds, reptiles, goats, sheep and cattle, both in Kenya[34] and in Pakistan.[35] These findings suggest that other vertebrates may also play a role in ZIKV circulation.[29] It should be noted that non-human primate population is non-existent in many of the Pacific islands where the virus was detected. The nature of viral reservoirs remains highly speculative. As one cannot exclude the possibility of other vertebrates performing this role[36], ZIKV is probably maintained in a human-mosquito-human cycle, suggesting that the virus has adapted to humans as a reservoir host.[18]

4.2. Non-vector transmission

Besides the transmission through the bite of *Aedes* mosquito, there is evidence that Zika can be transmitted through other routes (**Figure 6**):

5.2.1. Blood transfusions

Since it is a blood-borne pathogen, the transmission of ZIKV can also occur through blood transfusion, as happens with other arboviruses. That is to say that the blood donor could contaminate the blood supply, leading to Zika transmission through transfusions. This is important because ZIKV-infected blood travelers might be donors in the future.[23] During the epidemic in French Polynesia, the ZIKV RNA was found in 2,8% of blood donors who were asymptomatic at the time of donation[36]. Plus, Brazilian officers have recently confirmed 2 cases of transfusion-transmitted ZIKV.[3] Further studies are needed to assess the actual risk of ZIKV

transmission through blood products and the risk of generating a disease in the recipient. Still, a series of guidelines issued by the US Food and Drug Administration requires blood banks to follow strict rules so that a blood sample can be declared safe for transfusion.[37]

5.2.2. Sexual transmission

Transmission by sexual intercourse has been considered following the case of a patient who was infected with ZIKV in southeastern Senegal in 2008. After returning home, he experienced common symptoms of ZIKV associated with symptoms of prostatitis and hematospermia. Simultaneously, his wife developed symptoms of ZIKV and, since the woman had not traveled abroad, transmission by semen and vaginal sex was suggested.[38] A case of male to male transmission involving anal sex has also been reported.[39]

ZIKV RNA is isolated from semen in situations that remained undetectable in the blood collected at the same time, thereby suggesting that viral replication might have occurred in the genital tract.[18] Inasmuch as the risk of sexual transmission of ZIKV exists, men who reside in or travel to an area of active ZIKV transmission[40] should maintain sexual abstinence or use condom during sexual intercourse, especially if his partner is a pregnant woman.[6]

5.2.3. Vertical transmission

The possible routes of ZIKV vertical transmission are transplacental, during delivery, breastfeeding, and ultimately by close contact between the mother and her newborn[41]. All these hypotheses need further confirmation. During the French Polynesia outbreak, perinatal transmission from two mothers to their newborns was documented. Sera from the mothers and newborns were positive to ZIKV by RT-PCR and high ZIKV RNA load was detected in breast milk from both mothers.[13] During the same outbreak, a mother who presented a ZIKV infection-like syndrome 2 weeks before delivery, gave birth a newborn showing maculopapular rash but, unfortunately virological investigation was not performed.[41] Based on the temporal correlation of these cases with the Zika epidemic, the French Polynesia health authorities hypothesize that

ZIKV infection might be associated with these abnormalities provided that mothers are infected with the virus during the first or second trimester of pregnancy.[42]

The most surprising phenomenon of ZIKV infection is the unexpected number of infants born with microcephaly in Brazil during the ongoing viral outbreak, apparently as a result of their mothers being infected during pregnancy.[43] In fact, ZIKV RNA was detected in the amniotic fluid[44] and placental tissues[45] of some of the mothers and ZIKV has been found in fetal brain tissue of a baby with microcephaly[46] and other malformations as hidrancephaly and hydrops. Around 4000 cases of microcephaly and over 40 deaths related with ZIKV have been recorded since October 2015.[47] Even so, the cases with confirmed microcephaly and ZIKV RNA presence are very limited when compared to the total number of cases of microcephaly. An increased prevalence of microcephaly could be due to multiple causes including other viruses and parasites, irradiation, toxic substances, intrauterine growth retardation or chromosomal disorders.[24]

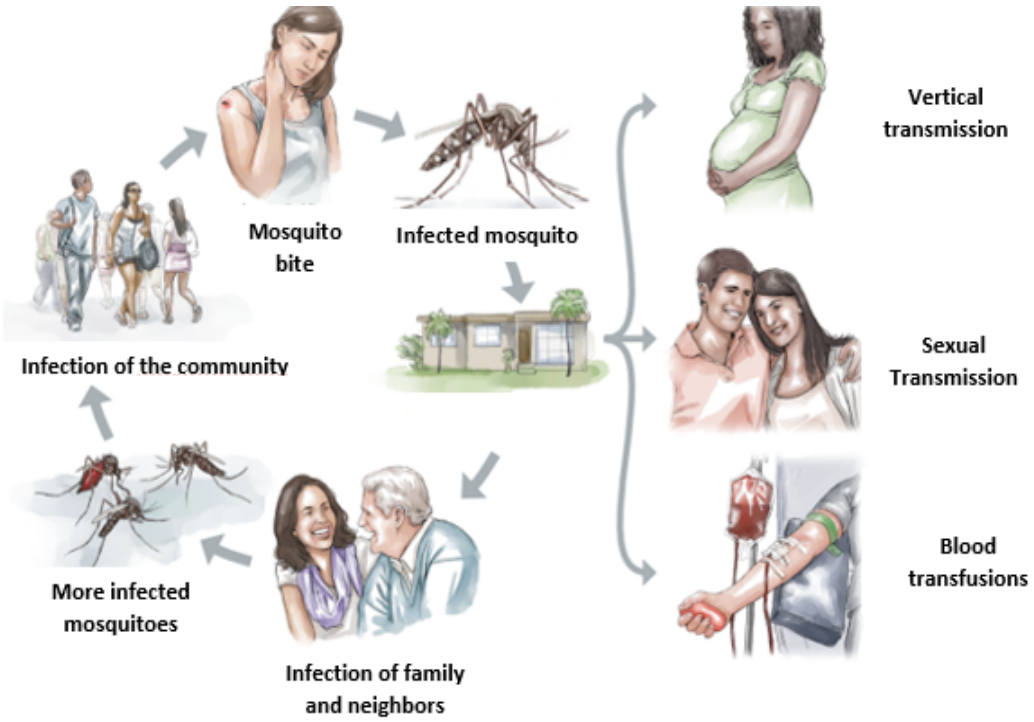

Figure 6 - Main transmission routes of ZIKV [https://www.cdc.gov/zika/transmission/ consulted on 4-10- 2016].

5.2.4. Other routes of transmission

ZIKV RNA and/or proteins have also been detected in urine[48] and saliva[49]. The detection of viral particles in saliva further suggests the possibility of other modes of transmission, such as sexual transmission (mucocutaneous transmission) and intimate contact between mother and newborn children.[6].

Two infections in laboratories have been reported[50-51] and a volunteer became infected after subcutaneous injection of infected mouse brain suspension.[52]

Other suspected routes of transmission are organ transplants, hemodialysis, through droplets respiration and needle sharing.[4,23]

6. Pathogenesis

The pathogenesis of ZIKA is still not fully understood, but experiments in mice performed 40 and 60 years ago had already suggested that ZIKV had a tropism for cells in the brain. In comparison, other animals, including cotton rats, guinea pigs, rabbits, and rhesus monkeys, did not develop CNS disease after intracerebral inoculation.[53] Usually, the symptoms appear three to eleven days after the mosquito bites[38,52] and the viremic period seems to be shorter than that of other flavivirus. Viral RNA was detected in serum from days 0-11 after onset of symptoms[6,54] and detection of the virus in saliva, urine, CSF, semen, amniotic fluid, products of conception and fetal brain may last longer than the viremia.[17]

Recent experiments have evinced the permissibility of human skin cells like dermal fibroblasts, epidermal keratinocytes and immature dendritic cells (Langerhans cells) to ZIKV infection, consistent with the skin being the initial site of ZIKV replication after mosquito inoculation.[6] It is thought that flaviviruses replicate immediately after infection in skin cells near the site of inoculation[21] and that specific interaction between the E glycoprotein of the viral particle and cell surface receptors allows the entry of the flaviviruses into the target cells.[18]

However, despite the enquiries, the key cellular receptors remain relatively unknown and their importance in viral entry have yet to be clearly established.[35]

ZIKV seems to be formed within the ER network, but as aforementioned, one study has suggested that ZIKV antigens could be found in nuclei of infected cells.[32] After the viral infection, the infected cells react in order to limit the spread of the virus, activating innate and adaptive responses: ZIKV induces an antiviral state in the infected skin cell, with greater expression of IFN type 1 (IFN-α and IFN-β) and type II genes.[23,55] Early detection of PAMP leads to the upregulation of the expression of pattern recognition receptors such as Toll-like receptor 3, retinoic acid-inducible gene-I and melanoma differentiation-associated gene-5. In addition to IFN type 1 responses, the host cell inducts apoptosis and autophagy, in order to overcome the viral infection. However, flavivirus are able to subvert the autophagy process to promote their own replication and dissemination[56] and rearrange host cell membranes to create an appropriate environment for their replication, being the ER the main source of membranes. These rearrangements result in an activation of the unfolded proteins response and simultaneous overexpress of autophagic pathways. The formed vesicles, known as autophagosomes, allow the recruitment of cytoplasmic elements, proteins and organelles as well as their degradation.In this sense, autophagy could act both positively or negatively in host immunity against ZIKV.[35,56] Infected cells of human skin explant show pyknotic nuclei, cytoplasmic vacuolation and edema in the stratum granulosum.[23]

From skin cells, the virus spreads into the lymph node and, through the bloodstream and lymphatics, into other tissues and organs as CNS, myocardium, skeletal muscles and fetus via placenta, possibly causing microangiopathy and rash.[6,23] The immune response is triggered from the moment the virus enters the host, and it involves polyfunctional T-cell activation and cytokine release.[17,56] and Ig production. IgM develops a few days after the onset and can

generally be detected up to 3 months; IgG develops within days after IgM and can be detected for months to years.[57]

Invasion of the CNS is believed to result from infection of microglial cells[6] but the pathogenesis of flavivirus invasion, its neurovirulence and neurosusceptibility of selected hosts is still unresolved. The E proteins are known to be crucial in the attachment and entering of the cells, conferring neurotropism to certain strains. The entrance into CNS may involve a direct route of paracelular passage through a leaky blood-brain barrier, associated with leucocyte diapedesis, transcytosis through leaky endothelial cells or retrograde axonal transport (**Figure 7**). The host immune response affects the course of infection, by entailing the intervention of IFN type I, endothelial adhesion molecules, E-selectin and matrix metalloproteinases.[17]

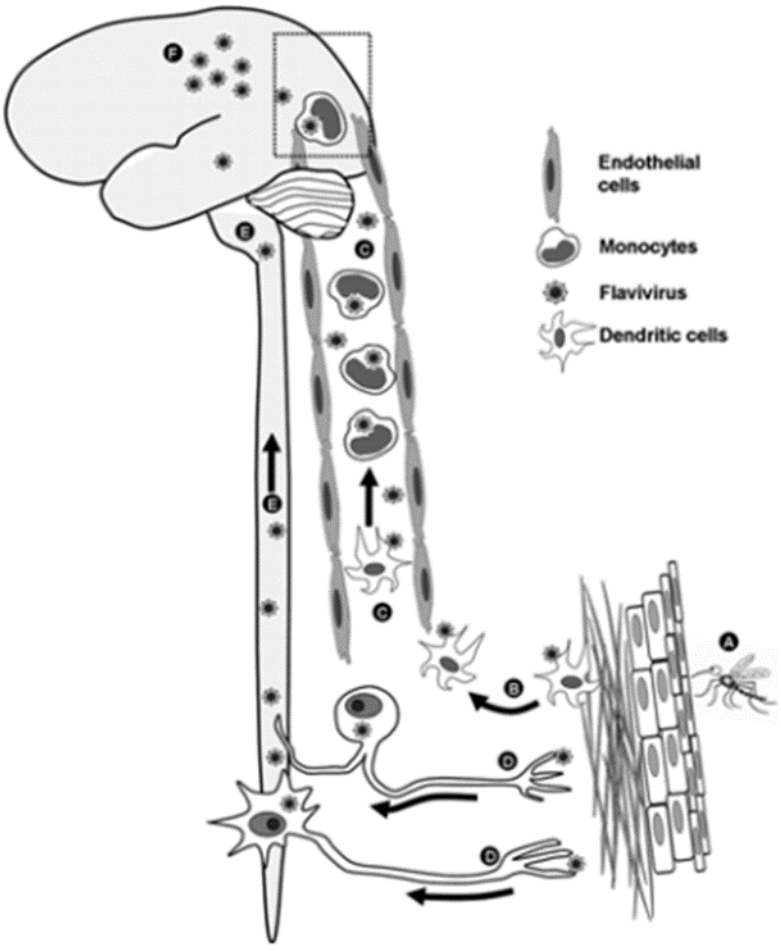

Figure 7 - Mechanisms for flavivirus entry into the CNS. **A,** Inoculation into the dermis; **B**, Uptake and transport dendritic or cells to lymph nodes; **C**, Haematogenous spread and entry into the CNS; **D**, Retrograde axonal transport into the CNS; **E**, Neuronal cell–cell spread; **F**, Associated damage to adjacent neurons. [Adapted from 17].

In a study using adult mice lacking IFN-α/β immunity, ZIKV replicated and caused disease, spreading across many tissues including CNS and testes and displaying high viral loads in the brain and spinal cord. These results are consistent with severe manifestations of ZIKV in humans and emphasize the importance of immune response in mitigating the effects of ZIKV.[53]

ZIKV virus directly and efficiently infects human cortical NPC, causing cell death, stunted growth of this cell population and transcriptional dysregulation.[58] It is believed that ZIKV leads to functional changes in the centrosomes, by altering mitotic mechanisms and increasing apoptosis. Disorientation of neural stem cells, precocious differentiation of neurons and decrease of progenitor cells are the expected consequences.[43] Early studies about ZIKA revealed that intracerebral-inoculated mice exhibited signs of encephalitis, neural degeneration (most intense in the hippocampus region), cellular infiltration in the brain and spinal cord, inclusion bodies of Cowdry type A in damaged nerve cells and widespread softening of the brain tissue with minimal inflammatory changes in the membranes of the ependymal. These lesions may be accompanied by clinical manifestations such as motor weakness and paralysis.[5] Further studies pointed to an enlargement of astroglial cells and necrosis of cells in the pyriform band cells of Ammon´s horn.[59]

Additionally, mice have evidenced skeletal myositis, myocarditis, and lung edema.[61] No other tissues, including the kidney, lung, spleen, and liver, exhibited significant ZIKV infection.[53]

While the pathogenesis of developmental retardation and organ defects remains unclear, hypoxia due to microvasculitis and thrombosis were the first hypotheses put forward.[21] Mice under 7 days of age were susceptible to lethal ZIKV infection when inoculated by an intraperitoneal route, whereas adult mice were less sensitive.[61] Studies also support the theory that the immature brain is more susceptible to infection.[17]

Recent studies concluded that ZIKV first infects the placenta and then the brain of fetus[23], a conclusion underpinned by ZIKV infection in amniotic epithelial cells and in fetal mesenchymal cells.[62] The observation of profound pathological changes in ZIKV-infected placentas, including trophoblast apoptosis, abnormal fetal capillary features and increased fetal nucleated erythrocytes, indicates malfunction of mouse placentas caused by ZIKV infection. These functional changes curtailed the fetus' blood flow and caused severe intrauterine growth restriction, ischemia, and fetal demise.[63] ZIKV infects and replicates in the primary human placental macrophages (Hofbauer cell) and cytotrophoblasts, before crossing the placental barrier to infect the fetus (**Figure 8**).[64-65] When pregnancy is not spontaneously miscarried, the virus end up probably disrupting molecular synthesis in the outer layers of the placenta, which tmight, itself, contribute to microcephaly.[66]

.

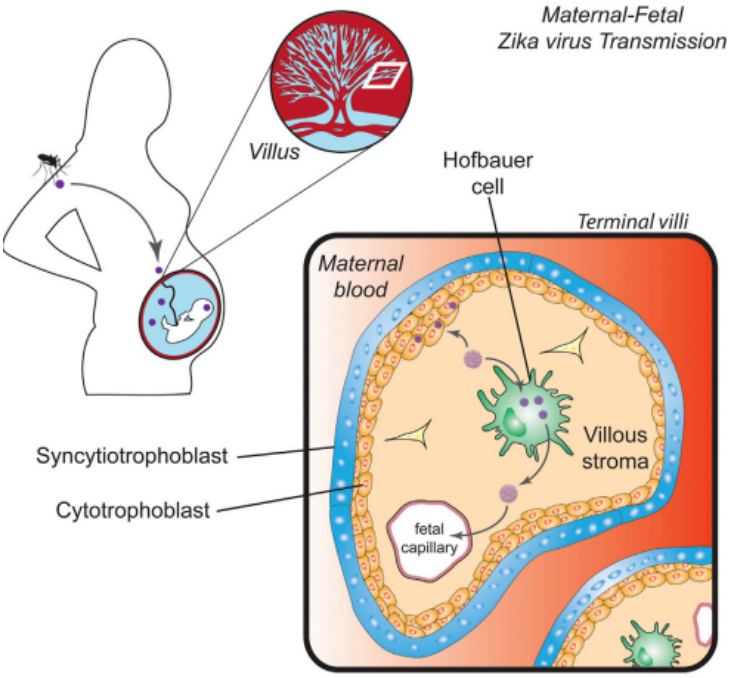

Figure 8 - Rout of ZIKV in the placenta during maternal-fetal transmission. [Adapted from 65].

ZIKV replicates preferentially in radial glia cells of dorsal ventricular zone of the fetuses[67] and in NPCs of the embryonic brain responsible for cortex development[68]. ZIKV

decreases their viability and growth as neurospheres, through down-regulation of genes involved in cell and organ development and up-regulation of those involved in immune response. This phenomenon results in inhibition of cellular proliferation and differentiation, neural apoptosis, thinning of cerebral cortex and macroscopic features observed in microcephaly[23,69] particularly smaller brains and enlarged ventricles. The cell population called CNCC gives rise, during embryonic development, to craniofacial structures, including bone, cartilage, smooth muscle, and vasculature of the head and face. The infection of CNCC by ZIKV indirectly affects neurogenesis, since it triggers cytokine secretion that leads to aberrant neural development and structural deformations of the head.[70] Human fetus infected in utero with ZIKV reveal diffuse astrogliosis and activation of microglia extending from the brain to the spinal cord, with Wallerian degeneration of the descending corticospinal tracts.[46]

Maternal antiviral immune reaction, both specific and innate, may play a role in the fetal pathophysiology as it generates indirect effects associated with high levels of inflammatory cytokines produced by infected placental tissues.[71] IFN type 1 binds to cellular receptors and mediates downregulation of the enzyme superoxide dismutase, the most powerful intracellular antioxidant.[21] Preexisting maternal non-neutralizing antibody to Zika or to other flaviviruses may enhance the probability of infection or bolster the disease in the fetus.[71]

Alternative pathogenesis had also been mentioned, namely the hypothesis that the changes observed during embryonic development and in the adult might be due to an endogenous form of hypervitaminosis A resulting from ZIKV infection-induced damage to the liver and the spillage of stored vitamin A compounds (retinoids) into the maternal, fetal and adult circulation in toxic concentrations. Such theories need further studies to be consolidated.[72]

7. Clinical Manifestations

7.1. Zika fever

Although ZIKV infection is thought to be asymptomatic in approximately 18% of infected people, the virus causes a mild, non-specific and self-limiting infection named Zika fever.[13] All ages are susceptible to infection, and a slight preponderance is observed in women.[20] The clinical course of ZIKV disease is typically mild in children infected postnatally, as it is in adults, contrary to what happens if the infection occurs during pregnancy.[73]

The classic manifestations of Zika fever include low-grade fever (37.8 to 38.5 ºC), bilateral non-purulent conjunctivitis (**Figure 9**), retro-orbital headache, myalgia, arthritis/arthralgia with periarticular edema of the small joints, edema of the extremities, a generalized, erythematous, maculopapular rash that spreads from the face to the neck, trunk, limbs (palms and soles)[21,24] (**Figure 9**) and jaundice.[13,43] Maculopapular rash presented by more than 90% of patients remains the main clinical symptom that characterizes ZIKV infection.[34] However, some patients may have more severe systemic symptoms including anorexia, high-grade fever, dizziness, chills and rigors, sore throat, hypotension, hearing disorders, subcutaneous bleeding due to thrombocytopenia, and cervical, submandibular, axillary, and/or inguinal lymphadenopathy.[3,4,21-22] Digestive complications (such as abdominal pain, vomiting, diarrhea, and constipation), mucous membrane ulcerations (aphthae) and pruritus are fairly rare[38.] Patients evicing genitourinary symptoms, including haematuria, dysuria, perineal pain, and haematospermia have also been mentioned.[49] ZIKV-related conjunctivitis may lead to an intraocular inflammation, uveitis being a potential manifestation.[74] Cardiac complications were also referred.[4]

Zika fever is usually self-limiting as symptoms resolve spontaneously after 3–7 days.[4,38] Zika fever-related rash usually resolve within the first week, but may last for up to 14 days and even become pruritic.[75] Arthralgia lasts from one week to more than a month[24] and

lymphadenopathies may be present for 2 weeks after symptom onset.[4] Non-neurological sequelae include transient hearing loss, hypotension and genitourinary symptoms.[20] A post-infection asthenia is also frequent and further investigations may be necessary to determine possible association between ZIKV infection and chronic fatigue syndrome.[4,13]

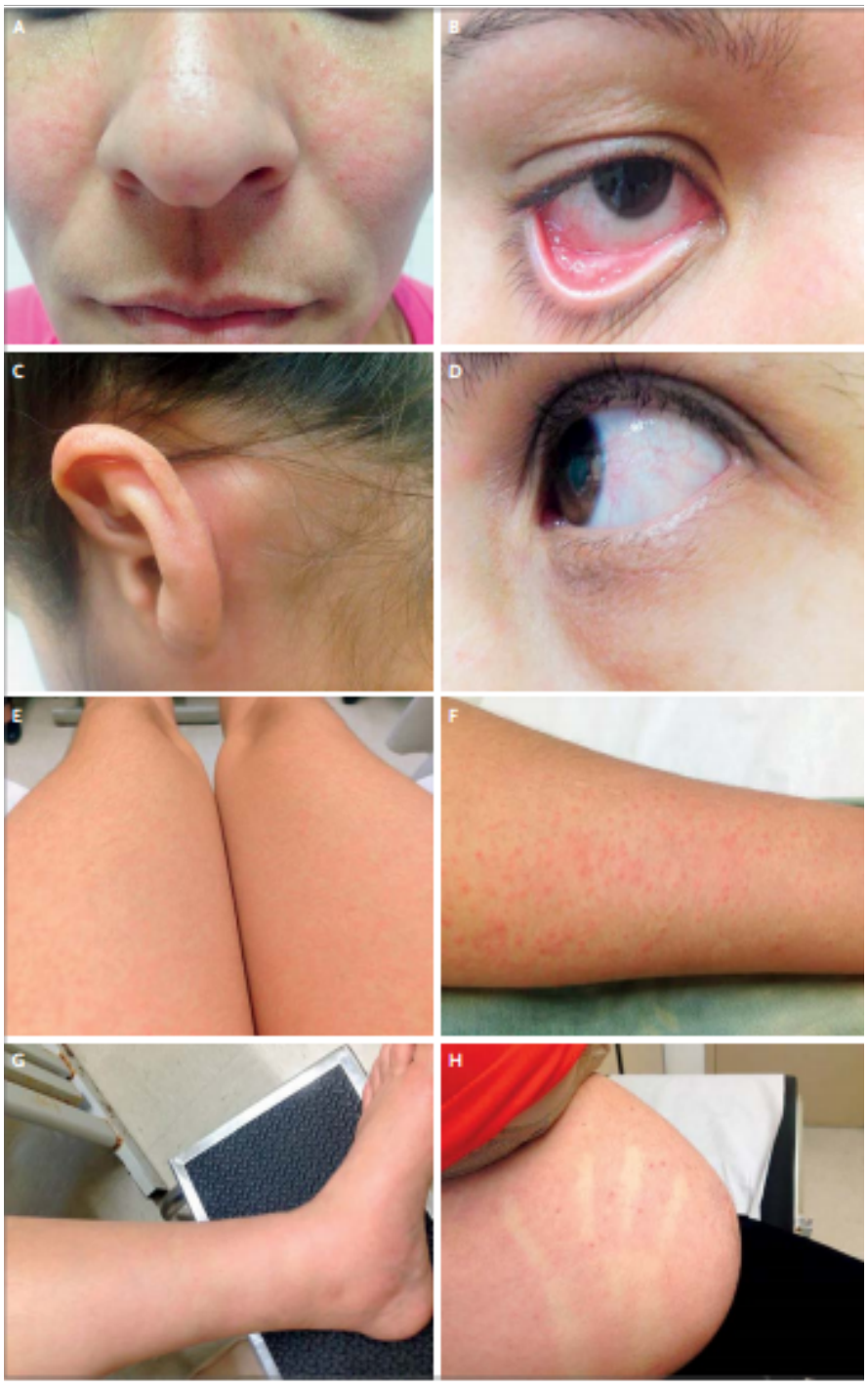

Figure 9 – Clinical features of ZIKV infection: **A**, maculopapular rash on the face; **B**, conjunctival and palpebral erythema; **C**, retroarticular lymphadenopathy; **D**, conjunctival injection with prominence of vasculature; **E**, rash on the legs; **F,** maculopapular rash on the inner arm; **G**, edema of the foot; **H**, blanching macular rash on the abdomen. [Adapted from 83].

Whereas haemorrhagic signs have not been observed, other severe manifestations have been reported, including neurological (GBS and meningoencephalitis), autoimmune (thrombocytopenic purpura and leukopenia)[76] and fetal (microcephaly and other malformations, such as optical) lesions.[6]

Zika fever-related death appears to be extremely rare, but a number of probable cases have been reported, especially among immunocompromised patients and neonates with suspected congenital ZIKV infection.[4]

7.2. Guillain-Barre Syndrome and other severe neurological complications

GBS is an autoimmune polyradiculoneuropathy causing acute or subacute flaccid paralysis that can culminate in death and that has been previously associated with other flaviviral and bacterial infections.[2,13] The syndrome is characterized by a damage in the peripheral nervous system with a loss of the myelin insulation,[35] which results in a rapid-onset muscle weakness consisting in progressive paralysis over 1–3 weeks due to the immune response. GBS comprises several recognizable variants with distinct clinical and pathological features (**Figure 10**).[77] The patients usually present motor dysfunction, beginning distally and progressing proximally, bilateral weakness of the arms and legs or tetraplegia, areflexia (reduction or absence of deep tendon reflexes), myalgia, sensory disturbances (paresthesia) and involvement of the cranial nerves and muscles (peripheral facial palsy), possibly affecting eye movements and swallowing reflex. These symptoms can last from few weeks to several months. The risk of GBS increases with age, being more commonly diagnosed in men than women.[21,23,78] The clinical outcome is related to a 5% death rate and 20% of the patients remain with significant disability.[4,78] So far, ZIKV-induced GBS has been described as transient in duration since the majority of patients has fully recovered.[2]

More studies are needed to understand the linkage between ZIKV infection and GBS, particularly the pathophysiological mechanisms at play. Both humoral and cellular immune responses against epitopes of antigens expressed by Schwann cells, myelin, or axons have been postulated to be responsible for this acute autoimmune neuropathy (**Figure 10**).[7] Possible alternative mechanisms include immunopathology due to viral antigen mimicry with a host protein, virus sequence changes resulting in enhanced tropism for the peripheral nervous system and an association with prior or concurrent immune responses to DENV.[9,53] Antibodies that recognize gangliosides also play a critical role in the pathogenesis of GBS.[7,53]

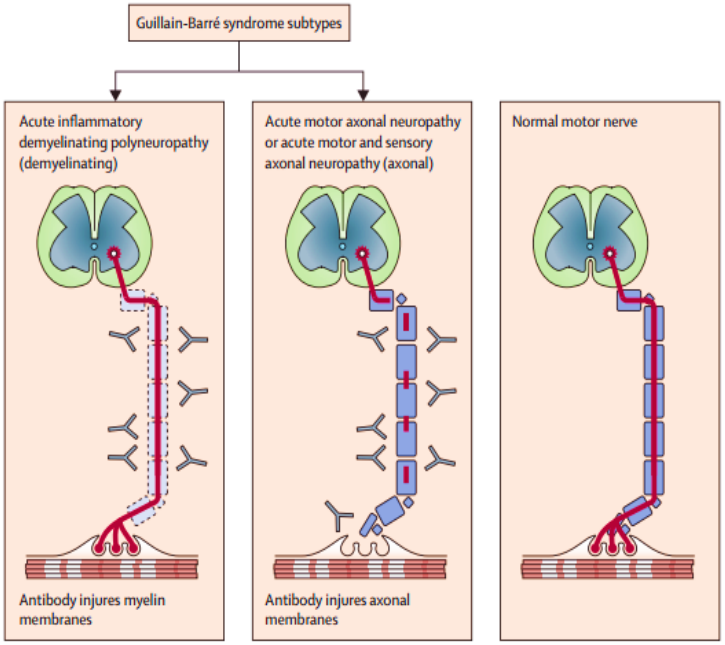

Figure 10 - Major GBS subtypes. The antibody-mediated effector pathways cause glial or axonal membrane injury with consequent conduction failure. [adapted from 77].

Another neurological commitment recently associated with ZIKV is acute myelitis causing pain, limb weakness, conjunctival hyperemia with no fever, paresthesia, but without signs of meningeal irritation nor sensory or motor deficits. Spinal Cord MRI showed lesions of the cervical and thoracic spinal cord with oedema, and a high concentration of ZIKV was detected in serum, urine and CSF by PCR.[79] No clear relationship has been established between myelitis and ZIKV but the virus can also be involved as a causing agent in inflammatory neurological disease.[24]

Other neurological complications potentially linked to ZIKV infection include encephalitis, meningoencephalitis, vertigo, myelitis, facial paralysis, ophthalmological (photophobia and hypertensive iridocyclitis) and auditory manifestations.[4,80,81] Clearly, the neurotropism of ZIKV may partially explain all these neurological and related manifestations. Nonetheless, more details and studies are required to ascertain their association.

7.3. Microcephaly and congenital Zika syndrome

Fetal abnormalities can be detected in up to 29% of ZIKV-positive pregnant women. Preliminary analyses demonstrate that the highest risk of microcephaly or congenital anomalies occurs during the first trimester of pregnancy[82], although ZIKV congenital abnormalities have been observed in fetuses of women who were infected by ZIKV at any week of gestation.[83]

Microcephaly is defined as a smaller OFC of the head for gestational age and sex, evident prior to 36 weeks of gestation (primary microcephaly) or postnatally (secondary microcephaly) (**Figure 11**).[7] Multiple causes of primary microcephaly have been identified, such as environmental factors, genetic causes, maternal diabetes, TORCH viruses [**t**oxoplasmosis, and **o**ther (syphilis, varicella-zoster, parvovirus B19), **r**ubella, **c**ytomegalovirus, and **h**erpesvirus] and different pathogens. Recently, ZIKV was also associated with development of primary microcephaly.[18,24] It can occur as a result of fetal brain disruption sequence, a process in which, after relatively normal brain development in early pregnancy, collapse of fetal skull follows the destruction of fetal brain tissue.[22] Secondary microcephaly is caused by virtually anything that alters the orderly development and functioning of the CNS, including neural migration disorder of neurons, a prenatal insult, a block to normal development and a degenerative process.[84] In any case, it is associated with intellectual disability, development delay and seizures in those newborns that manage to survive.[24]

No standard definition of microcephaly exists.[53] One proposed method claims that a newborn with OFC equal to or lower than two SD below the mean is characterized as microcephalic, and below three SD is referred as severe prognosis of microcephaly (**Figure 11**).[24] Microcephaly can be asymmetric, meaning a small head on an otherwise normally proportioned body, or symmetric, meaning that the small head is proportional to a small overall body size.[53]

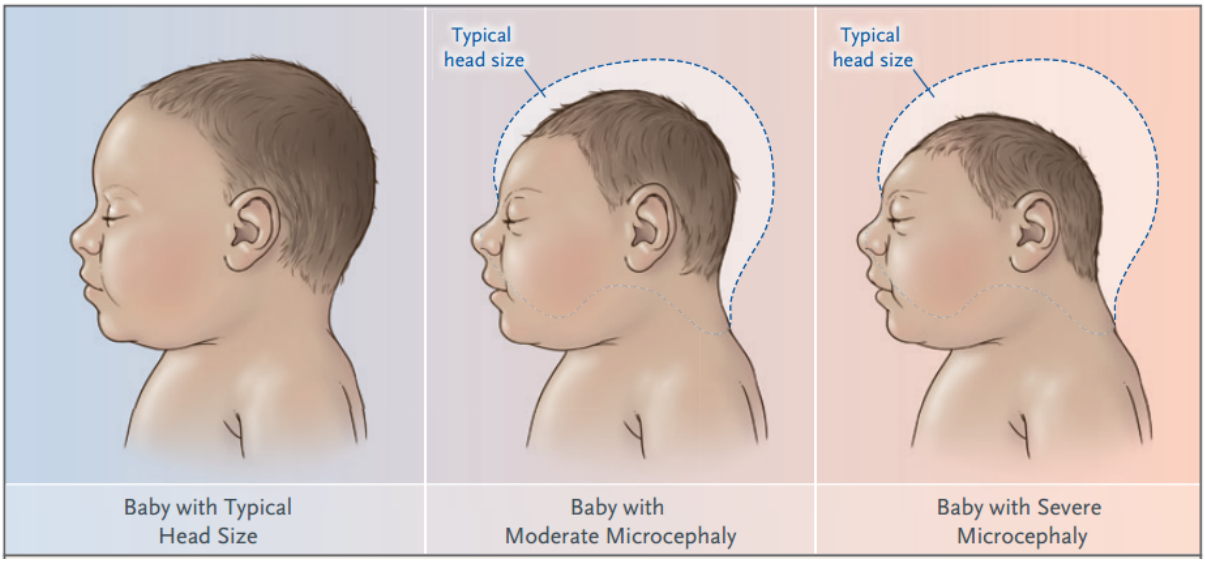

Figure 11 - Infants with moderate or severe microcephaly associated with maternal ZIKV Infection, compared with a typical newborn. [Adapted from 22].

Autopsies of fetuses with microcephaly revealed marked cerebral atrophy, ventriculomegaly, extensive intracranial dystrophic calcifications involving the cortex and subcortical white matter in the frontal, parietal, and occipital lobes, simplified gyral patterns or agyria, holoprosencephaly, cortical displacement, dysgenesis of the corpus callosum, cerebellar hypoplasia and mild focal inflammation.[46,85-87] Histopathological findings include necrosis preferentially targeting neurons, degenerative changes of the glial and neuronal cells, white matter loss and axonal rarefaction multifocal collection of filamentous, granular and neuron-shaped calcifications in the cortex, subcortical nuclei and subcortical white matter, and diffuse astrogliosis in the subarachnoidal space. Activated microglia cells, macrophages and

mononuclear inflammatory cell infiltrate and neurophagy can also be present throughout the gray and white matter, as well as perivascular infiltrates of T-cells and B-cells. The brain stem and spinal cord may also exhibit Wallerian degeneration of the lateral corticospinal tract with preservation of the ascending dorsal cords.[21,85]

Although microcephaly has received thorough attention as a pregnancy-related consequence of ZIKV infection, an expanding spectrum of fetal malformations associated with intrauterine ZIKV infection, currently referred to as congenital Zika syndrome, continues to be described. This syndrome includes not only microcephaly and fetal brain damage, but also a range of developmental ocular, musculoskeletal, craniofacial, genitourinary and pulmonary abnormalities.[85]

Congenital ocular findings concomitant with microcephaly have also been associated with ZIKV infection during pregnancy. The lesions include microphtalmia, microcornea, cataract, focal pigment motting and hemorrhages of the retina, falciform folds; chorioretinal athropy, macular neuroretinal atrophy, optic nerve abnormalities (hypoplasia and severe cupping of the optic disk), abnormal vascular development (tortuosity, early termination, absence), bilateral iris coloboma, foveal reflex loss, lens subluxation and retinal dysplasia.[22,45,88-90] These ocular abnormalities are present in 34,5% of microcephalic infants observed and affect both eyes in 70% of them.[89] Curiously, identical eye damages were found in childs with no identifiable microcephalic (**Figure 12**).[91]

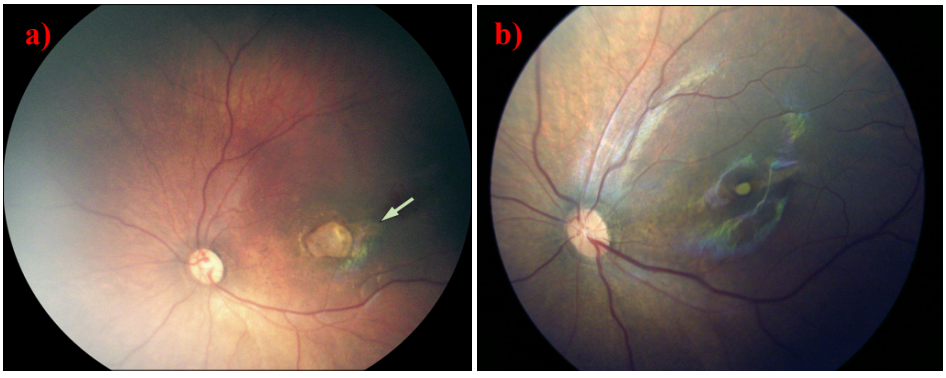

Figure 12 – Ocular findings in infants infected with ZIKV. **a)** Macular neuroretinal atrophy in an infant with microcephaly; **b)**, Chorioretinal scar on the macular region of the left eye with no associated optic disc findings in an infant without microcephaly. [Adapted from 90 and 91]

Other tissues may also be affected by ZIKV congenital infection. In a recent report, a fetus miscarriage (thirty second week) presented, in addition to microcephaly, hydranencephaly, intracranial calcifications, destructive lesions of posterior fossa, and further abnormalities such as hydrothorax, ascites and subcutaneous edema. The mother had an asymptomatic ZIKV infection during the first trimester of gestation.[92] Arthrogryposis of the fetus legs and arms, placental calcifications, intrauterine growth restriction, redundant scalp skin, polyhydramnios, anasarca, craniosynostosis, clubfoot, acetabular dysplasia, cryptorchidism, hypospadias, pulmonary hypoplasia and single umbilical artery are amongst possible findings.[23,45-46,85]

Apart from fetal deaths and miscarriages in ZIKV-positive pregnant women, it is worth underlining cases of newborn lethality within the first twenty hours after birth[45] due to brainstem dysfunction, cerebral lesions, absence of swallowing and polymalformative syndromes.[23] The miscarriages occurred during the first trimester (between eleven and thirteen weeks) and the women who present fetal deaths were infected during the second and third trimesters, after evidencing maculopapular rash during the acute phase of infection.[45,83]

8. Diagnosis

8.1. Zika fever

The diagnosis of infection by ZIKV is based on clinical, epidemiological and laboratorial criteria. Symptoms of ZIKV disease are nonspecific and may easily be confused with other arbovirus-induced diseases, which may result in misdiagnoses; thus, the differential laboratory diagnosis is important (**Figure 13**).[6]

8.1.1. Differential diagnosis

Recently, many publications have emphasized the occurrence of dual infections, including Zika and Dengue and Zika and Chikungunya co-infections. Their clinical

manifestations are very similar and they circulate in identical conditions and territories. Molecular platforms enabling a more precise diagnosis and distinction of these viral diseases are thus needed. The list of the main differential diagnoses includes [93,94]:

- Dengue;
- Chikungunya;
- Parvovirus;
- Rubella;
- Measles Fever;
- Rickettsiosis;
- Malaria;
- Leptospirosis;

Laboratorial diagnosis of ZIKV can be performed by direct detection of the virus, viral nucleic acid, viral antigen or antibody or by a combination of these techniques. The method depends on the purpose of the performed test (clinical, epidemiological or vaccine development), type and laboratory facilities and expertise available, the sample and collection time.[6]

Culture-based methods for ZIKV detection are used in public health and research laboratories but are not generally available for clinical purposes. The reference method for the isolation of ZIKV and other arboviruses is intracerebral mouse inoculation.[95]

8.1.2.1 Molecular methods

The molecular diagnosis is based on the detection of viral RNA in different types of body fluids: whole blood, serum, EDTA plasma, saliva and urine.[57] In blood samples, as the viremic period is short, detection is possible for a period of one to five days after the onset of symptoms[6]. Negative results do not exclude the diagnosis because the sensitivity of RT-PCR is estimated to be around 40%.[94]

RNA of the virus is detectable in urine at a higher load [6] and with a longer duration (detected up to twenty days once it become undetectable in serum) than in serum. So, patients observed after the fifth day of disease should undergo RT-PCR on urine.[13,20,94]

Saliva has also been used as an alternative sample for routine ZIKV RNA detection, showing positivity more often than blood samples. Nevertheless, in contrast with urine, it did not increase the window of detection, and ZIKV RNA detection was found negative in some saliva samples while tested positive in blood.[13,49] Using a combination of samples (blood/saliva/urine) is recommended.[49]

At least 1 report suggests that the virus persists in semen for up two months and that, for unknown reasons, viral load in the semen can be roughly 100 000 times that of blood or urine. Nasopharyngeal swabs have also tested positive by RT-PCR.[3]

The RT-PCR technique must be applied for amplification of the NS5 coding regions.[21] For patients with suspected ZIKV disease, a positive RT-PCR result confirms ZIKV infection, and no antibody testing is indicated.[96] Nonetheless, reliance on the use of molecular diagnostics to rule out ZIKV infection requires careful consideration, as clinicians and diagnostic laboratories' experience on emerging diseases is unsurprisingly limited. Several noncommercial RT–PCR tests for ZIKV have been described in the literature, but few provide validation using the most recent viral strains and fully documented clinical specimens.[57]

8.1.2.2. Serological methods

The serological diagnosis of flaviviruses is complex, mainly due to extensive cross-reactivity between antibodies triggered by different flavivirus infections or by vaccination.[6] Besides that, an acute flavivirus infection might boost cross reactive antibodies owing to previous infections (such as dengue virus) or to vaccination against another flavivirus (like yellow fever or Japanese encephalitis).[57,94]

A small number of ZIKV serological tests have been described in the literature based on ELISA - using whole viral antigen or recombinant protein - or on immunofluorescence assay. Despite the risk of cross-reactivity, the most specific serological method for flaviviruses are PRNT, but even in these tests, antigenically related viruses should be included as controls.[21]

All these tests have had only limited validation, demanding from the laboratory community a better validation data for serology testing in the field.[57]

Owing to the decline of the level of viremia over time and to the possible inaccuracy of information on illness onset, a negative RT-PCR result does not exclude ZIKV infection. Therefore, serum IgM antibody testing must still be performed even if RT-PCR is negative.[96] It is generally considered that serological tests are able to detect IgM from the fourth day and IgG from the twelfth day.[94] Therefore, positive serologic test results should be confirmed through an alternative platform such as PRNT, even though flaviviral cross-reactivity can pose problems in confirmatory assays.[20] IgM antibodies for flaviviruses remain detectable for two or three months and sometimes for a longer period of time, while the specific IgG antibodies remain detectable for several months.[23]

For serum specimens collected less than seven days after the onset of symptoms, the combination of a negative RT-PCR result and negative IgM antibody testing suggests that there was no recent infection. However, a negative IgM antibody test, in the absence of RT-PCR testing, might reflect specimen collection before development of detectable antibodies, and, accordingly, does not rule out infection.[96] If that the test is performed between two and twelve weeks after exposure, negative serological results (non-reactive IgM and IgG) indicate that infection did not occur.[94]

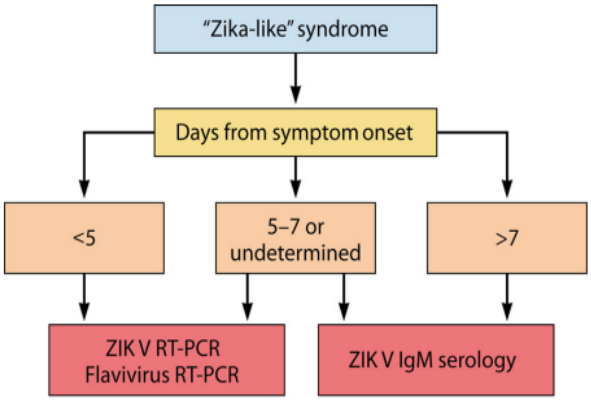

Figure 13 - Schematic flow diagram for Zika fever diagnosis [Adapted from 18].

8.1.2.3. Laboratory abnormalities

The laboratory abnormalities are nonspecific. Mild to moderate leukopenia, thrombocytopenia, albuminemia, presence of bile pigment in urine, slight elevation of assayed concentration of serum lactic dehydrogenase, gamma-glutamyl transferase and markers of inflammatory activity (proteins, fibrinogen and ferritin) are among the identified changes. In general, there are no significant abnormalities in these parameters and the findings are observed in many other viral infections.[18, 20,94]

8.1.2.4. Imagiology

In cases encompassing neurological involvement, some imaging tests may be used to identify lesions at the CNS, such as CT and MRI (**Figure 14**).[79,97]

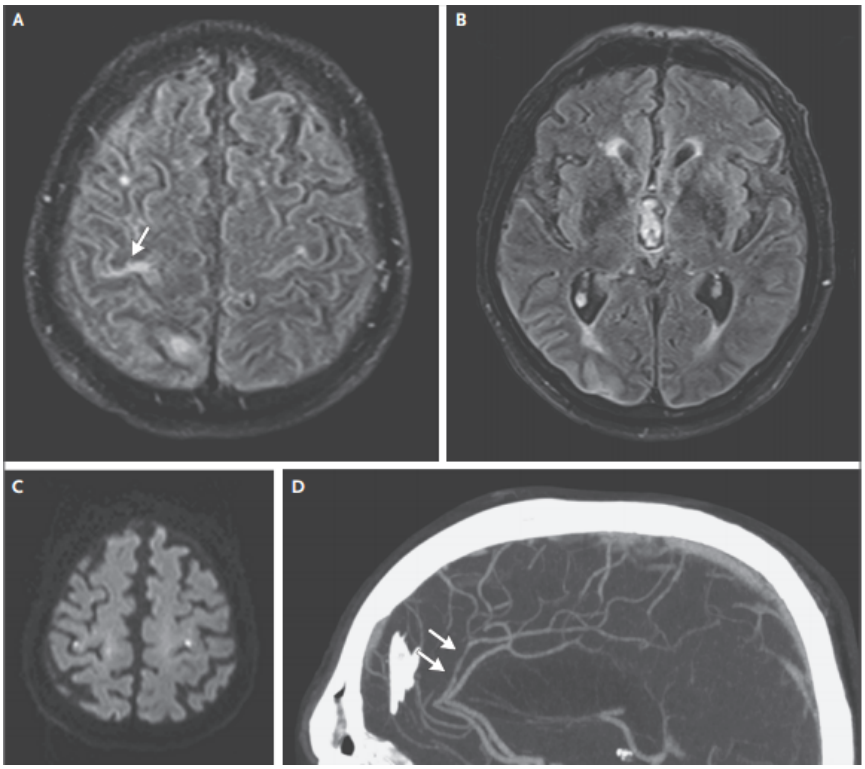

Figure 14 - MRI of a case of meningoencephalitis associated with ZIKV. Fluid-attenuated inversion recovery was used. **a**, subcortical white-matter hyperintensities in the right frontal region, the right parietal region, bilateral rolandic regions and **b**, right temporo-occipital region. **c**, multiple punctuated hyperintensities on diffusion-weighted sequences that suggest ischemic foci. **d**, angiogram shows an irregular narrowing of the right callosomarginal artery [adapted from 97].

8.2. Congenital abnormalities

After performing transfontanelle ultrasound, CT or MRI, one must rank as congenital malformations associated with ZIKV all the fetus that present at least one of the following criteria: cerebral calcification (especially periventricular, in the parenchyma, thalamic areas and basal ganglion); presence of ventricular abnormalities; lissencephaly; hypoplasia of brain stem and cerebellum; hypoplasia of the cerebellar vermis; widening of the posterior fossa greater than 10mm; agenesis or hypoplasia of the corpus callosum; abnormality of white matter attenuation. Other ultrasound findings not directly related to the fetal nervous system may include arthrogryposis, intrauterine growth retardation, arterial flow abnormalities in the cerebral or umbilical arteries, oligohydramnios or anhydramnios.[22,44,82-83,94]

8.2.1. Laboratorial diagnosis

8.2.1.1. Molecular methods

For the confirmation of congenital ZIKV infection and the relationship with fetal abnormalities, RT-PCR must be performed on amniotic fluid after amniocentesis.[22,24] ZIKV genome can be found in the amniotic fluid of the pregnant women while the virus is detected in their urine or serum.[86] In newborns suspected of being infected, or whose mother is confirmed as infected, it is advised to perform a histopathological analysis of the placenta and a RT-PCR analysis of the umbilical cord blood, placenta and urine of the two first days of life. A salivary analysis should also be performed. The sensitivity and specificity of these tests is not yet known and a specific pediatric clinical monitoring should be implemented if the RT-PCR for Zika tests positive in cord blood or in the new-born or if clinical and imaging neurological abnormalities are identified.[43]

After the birth, imaging follow-up should continue in cases of asymptomatic newborns but whose mother is infected or in cases where the newborn has obvious anomalies. The follow-

up consists of transfontaneal ultrasounds, MRI, examination of the eye and determination tests of auditory acuity.[43]

In stillbirths, histopathological analysis and RT-PCR of the placenta and fetal tissues must be accomplished.[43]

8.2.1.2. Serological methods

For asymptomatic pregnant women residing in an area with local ZIKV transmission or that traveled from areas with known cases of infection, IgM testing should be carried out upon initiation of prenatal care and mid-second trimester. The same goes for situations where fetal abnormalities are detected during ultrasound evaluation.[96] The IgM research can also be performed in the newborn although its specificity and sensitivity is unknown.[43]

8.2.1.3. Laboratory abnormalities

Infected mothers' asymptomatic children must be subject to clinical monitoring; in order to search for thrombocytopenia and elevated liver enzymes.[43]

8.2.1.4. Imagiology

Congenital abnormalities in the fetus can be identified by individual or combined methods of ultrasound (**Figure 15**), CT and MRI (**Figure 16**). In ultrasound examination, OFC must be determined at different stages of development of the fetus, always using the same biometric reference curve.[43] Ultrasound findings can be detected from the eighteenth to twentieth week of pregnancy onwards.[94] MRI or CT should be applied whenever the ultrasound raises doubts and when the mother is confirmed for ZIKA infection.[43,98]

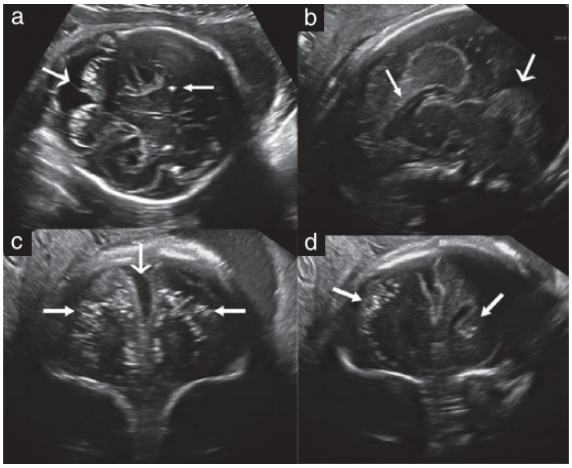

Figure 16 - Ultrasound of a confirmed case of microcephaly. **a**, transabdominal axial ultrasound image shows cerebral calcifications with failure of visualization of a normal vermis (**large arrow**) and calcifications are also present in the brain parenchyma (**small arrow**); **b**, transvaginal sagittal image shows dysgenesis of the corpus callosum (**small arrow**) and vermis (**large arrow**); **c**, coronal plane shows a wide interhemispheric fissure (**large arrow**) due to brain atrophy and bilateral parenchymatic coarse calcifications (**small arrows**); **d**, calcifications are visible in this more posterior coronal view and can be seen to involve the caudate (**arrows**). [adapted from 43]

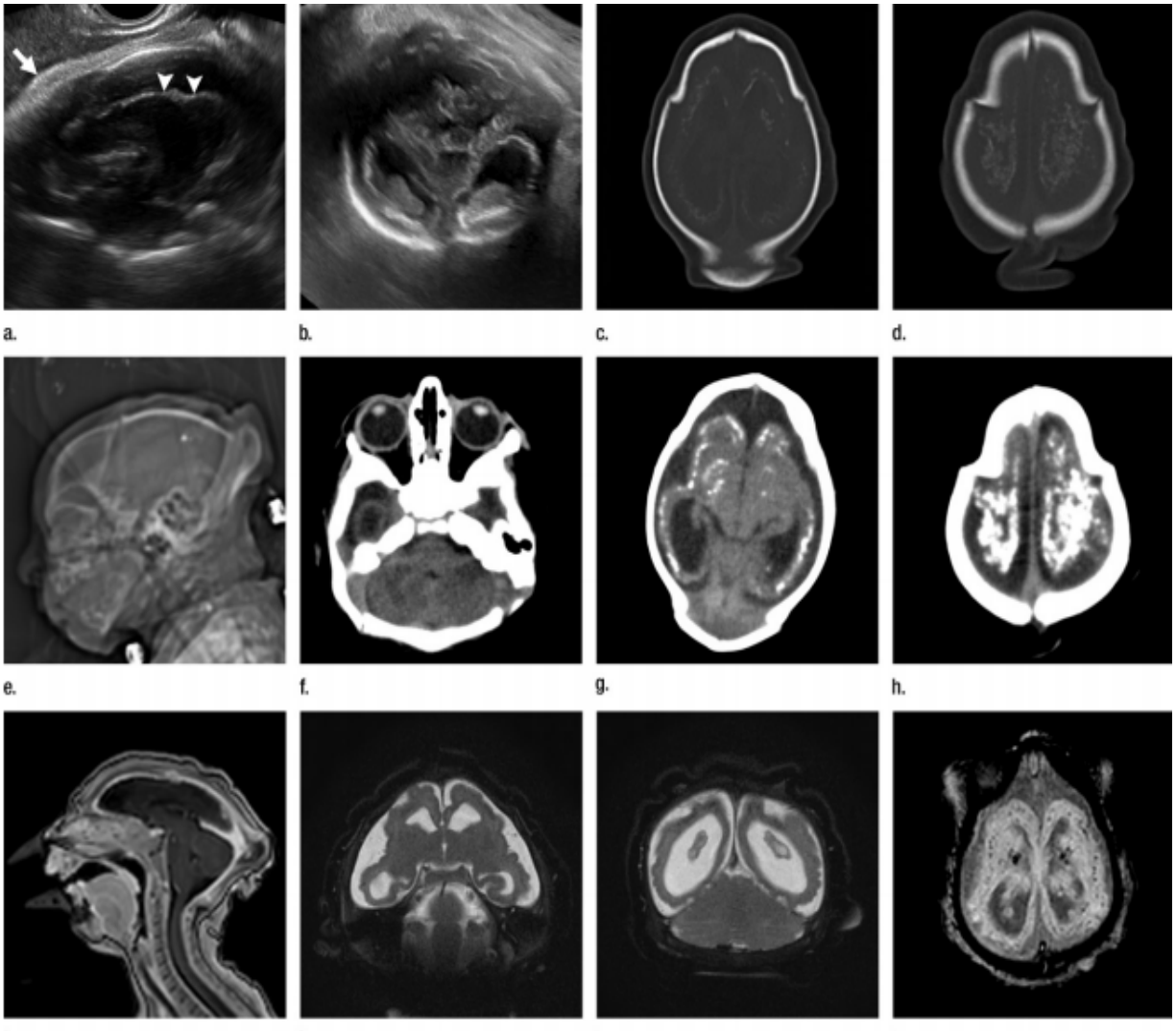

Figure 15 - Images relating to a case of an 18-year-old woman with confirmed ZIKV infection. **a**, sagittal transvaginal and **b**, coronal transabdominal ultrasound images obtained with the head upside down show a small head circumference, moderate ventriculomegaly with dense intracranial calcifications and abnormal head shape with flattened appearance and thickened skin; **c, d,** axial bone window CT images; **e**, sagittal localizer CT image; **f-h**, axial CT images show microcephaly with cerebral atrophy, and, despite ventriculomegaly, the extra-axial CSF spaces still prominent. The dense calcifications are predominantly located in the subcortical white matter at the gray matter–white matter interface. There is markedly abnormal skull shape with some eversion of the bones at the suture sites (particularly fronto-parietal sites), with redundant skin folds (particularly in the parieto-occipital region). **i**, sagittal T1-weighted, **j, k**, coronal T2-weighted and **l**, axial susceptibility-weighted MR images obtained at 1 month of age show an undersegmented midbrain, severe microcephaly, open sylvian fissures, and polymicrogyria. The dense calcifications are evident on the susceptibility-weighted image. [adapted from 98]

9. Treatment

Currently, there is still no specific treatment available for ZIKV and antiviral therapy is rarely needed, since most patients have mild courses[21] and the symptoms resolve within three to seven days.[24] The therapy focuses on relieving symptoms and includes rest and hydration with fluids, to correct loss caused by vomiting and swelling. Fever, particularly in pregnant women, may be treated with acetaminophen[6,21] that will also alleviate symptoms of headache and myalgia.[23] Aspirin and other nonsteroidal anti-inflammatory drugs can be used but are not recommended until Dengue is ruled out (due to the risk of hemorrhage) and should be avoided in pregnant women. Antihistamines may be helpful to control pruritus in patients with rash.[99] The need of hospitalization is rather uncommon and, once a patient has been infected and recovered from ZIKV, he or she are most-likely protected from future infections.[100]

In cases of ZIKV-induced GBS, the treatment is only palliative. Approaches include methylprednisolone, application of intravenous Ig and, on rare occasions, plasmapheresis. Microcephaly is a lifelong condition and there is no cure or standard treatment.[24]

Some alternative treatments are currently being developed: use of amotosalen combined with ultraviolet light A, which proved to be able to inactivate ZIKV *in vitro*;[9] the use of inhibitors of autophagosomes formation during infection to reduce viral copy numbers[35]; steroids, Ig, IFN or ribavirin.[17]

10. Prophylaxis and control measures

There is no available vaccine to prevent ZIKV infection, although several are being developed using dengue vaccine technology.[18]In addition, subunit vaccines representing ZIKV proteins, DNA vaccines expressing viral proteins and other viral vectors expressing viral antigens could be explored. It should be noted that each vaccine approach presents pros and cons, thereby requiring complementary approaches to be explored simultaneously. [2]

The mainstay of prevention consists in minimizing exposure by controlling mosquito populations and avoiding bites[6,99] particularly of pregnant women who represent the highest risk of severe disease in the form of fetal microcephaly.[2]

Historically, *Aedes aegypti* eradication strategies have proved to be inefficient, and the mosquito infestation index is very high. Several approaches could be used to reduce *Aedes aegypti* populations: elimination or protection of water containers; killing the adult female using traditional fogging with insecticide aerosols (generally not effective since these vectors tend to stay indoors where insecticides applied from trucks or airplanes are unlikely to penetrate) or indoor application of residual insecticides that may also have repellent activity;[2] release of genetically modified male mosquitoes that express a dominant, lethal gene at the larval stage, resulting in the death of all offsprings from mating with wild females, curtailing the risk of persistence of the transgene in nature;[2,6] release mosquitoes infected with endosymbiotic Wolbachia bacteria, which can spread across natural populations and suppress viral transmission by interfering with replication in the mosquito.[2-6] All these techniques are either inefficient or too complex both logistically and monetarily. An approach that might represent a near-term way is the use of lethal traps, which have been designed to be inexpensive and relatively maintenance-free. Many other designs are being tested but this sort of traps may be very useful when combined with source reduction and adulticide applications in regions where the risk of ZIKV transmission by *Aedes aegypti* is high.[2]

For personal protection against mosquito bites, residents of or travelers to Zika transmission areas are advised to wear long-sleeved shirts, pants and hats, thus minimizing the area of skin exposure. In addition, residents and visitors should sleep under mosquito nets, in air-conditioned rooms with closed windows. Apply insect repellent to exposed skin, especially during day when *Aedes* mosquito is most active and treat clothing with permethrin are other important measures.[99]

Patients with suspected ZIKV infection should be protected from further mosquito exposure during the first few days of illness, so as to avoid the infection of new mosquitoes and the risk of local transmission.[99]

Assuming that sexual transmission might be relevant in the epidemiology and to prevent fetal infection, protection by using condoms is recommended if having sex (vaginal, anal, or oral) with a male partner while traveling, or if he has just comeback from an area where the virus is actively circulating; even more, in this latter case, sexual abstinence is recommended if the partner is pregnant.[13] Application of vaginal rings shedding an antiviral substance such as Dapivirine could be useful, but it has not yet been approved for this purpose.[21]

Although the efficiency of the transmission of Zika through blood transfusions is still unknown, blood banks have to explore the travel anamnesis of their donors[21] and screenings of donated blood by RT-PCR must be performed.[1] Deferral of blood donation for one month after possible ZIKV exposure is recommended.[100] Likewise, the donated organs, particularly kidneys, from persons with a recent travel history to affected areas should be examined for ZIKV once the virus may exist in the genitourinary tract for an uncertain time duration.[23]

11. Conclusion and further directions

ZIKV infection is spreading across different parts of the world. Currently, what will happen in future regarding to ZIKV epidemic is unpredictable. The co-circulation of ZIKV with other medically important arboviruses constitutes an additional challenge, which jeopardizes the comprehension of this disease.[35]

Due to the lack of knowledge regarding this virus, its characteristics and means of transmission, nor vaccines nor effective drugs against ZIKV infection have been conceived. A thorough understanding of the molecular interactions that ZIKV establishes with the host cell during infection is also necessary to determine the targets for antiviral treatment. However,

there exists virus-specific therapeutic targets, which may lead to the development of targeted anti-ZIKV therapeutic agents.[22]

Research to develop vector control mechanisms, vaccines and antiviral therapies is essential and urgent, as well as the education of communities so that the fight against the spread of mosquito, transmission of disease and the development of congenital abnormalities is halted.[4]

12. References

1. White MK, Wollebo HS, David Beckham J, Tyler KL, Khalili K. Zika virus: An emergent neuropathological agent. Ann Neurol. 2016;80(4):479-89.
2. Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, et al. Zika virus: History, emergence, biology, and prospects for control. Antiviral Res. 2016;130:69-80.
3. Focosi D, Maggi F, Pistello M. Zika Virus: Implications for Public Health. Clin Infect Dis. 2016;63(2):227-33.
4. Chan JF, Choi GK, Yip CC, Cheng VC, Yuen KY. Zika fever and congenital Zika syndrome: An unexpected emerging arboviral disease. J Infect. 2016;72(5):507-24.
5. Dick GW. Zika virus. II. Pathogenicity and physical properties. Trans R Soc Trop Med Hyg. 1952;46(5):521-34.
6. Zanluca C, Dos Santos CN. Zika virus - an overview. Microbes Infect. 2016;18(5):295-301.
7. Anaya JM, Ramirez-Santana C, Salgado-Castaneda I, Chang C, Ansari A, Gershwin ME. Zika virus and neurologic autoimmunity: the putative role of gangliosides. BMC Med. 2016;14:49.
8. Zika Epidemiological Update, 6 October. Washington, D.C, PAHO/WHO, 2016. (http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=36436&lang=en, accessed 3 October 2016)
9. Chang C, Ortiz K, Ansari A, Gershwin ME. The Zika outbreak of the 21st century. J Autoimmun. 2016;68:1-13.
10. WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome. Geneva, WHO, 1 February 2016. (http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/, accessed 3 October 2016)

11. Zika situation report. WHO, 13 October 2016.(http://www.who.int/emergencies/zika-virus/situation-report/13-october-2016/en/, accessed 20 October 2016)

12. Messina JP, Kraemer MU, Brady OJ, Pigott DM, Shearer FM, Weiss DJ, et al. Mapping global environmental suitability for Zika virus. Elife. 2016;5.

13. Saiz JC, Vazquez-Calvo A, Blazquez AB, Merino-Ramos T, Escribano-Romero E, Martin-Acebes MA. Zika Virus: the Latest Newcomer. Front Microbiol. 2016;7:496.

14. Kostyuchenko VA, Lim EX, Zhang S, Fibriansah G, Ng TS, Ooi JS, et al. Structure of the thermally stable Zika virus. Nature. 2016;533(7603):425-8.

15. Sirohi D, Chen Z, Sun L, Klose T, Pierson TC, Rossmann MG, et al. The 3.8 A resolution cryo-EM structure of Zika virus. Science. 2016;352(6284):467-70.

16. Wang L, Valderramos SG, Wu A, Ouyang S, Li C, Brasil P, et al. From Mosquitos to Humans: Genetic Evolution of Zika Virus. Cell Host Microbe. 2016;19(5):561-5.

17. Bharucha T, Breuer J. Review: A neglected Flavivirus: an update on Zika virus in 2016 and the future direction of research. Neuropathol Appl Neurobiol. 2016;42(4):317-25.

18. Musso D, Gubler DJ. Zika Virus. Clin Microbiol Rev. 2016;29(3):487-524.

19. Faye O, Freire CC, Iamarino A, Faye O, de Oliveira JV, Diallo M, et al. Molecular evolution of Zika virus during its emergence in the 20(th) century. PLoS Negl Trop Dis. 2014;8(1):e2636

20. Plourde AR, Bloch EM. A Literature Review of Zika Virus. Emerg Infect Dis. 2016;22(7):1185-92.

21. Slenczka W. Zika Virus Disease. Microbiol Spectr. 2016;4(3).

22. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika Virus. N Engl J Med. 2016;374(16):1552-63.

23. Atif M, Azeem M, Sarwar MR, Bashir A. Zika virus disease: a current review of the literature. Infection. 2016;44(6):695-705.

24. Slavov SN, Otaguiri KK, Kashima S, Covas DT. Overview of Zika virus (ZIKV) infection in regards to the Brazilian epidemic. Braz J Med Biol Res. 2016;49(5):e5420.

25. Lupton K. Zika virus disease: a public health emergency of international concern. Br J Nurs. 2016;25(4):198-202.

26. Black WCt, Bennett KE, Gorrochotegui-Escalante N, Barillas-Mury CV, Fernandez-Salas I, de Lourdes Munoz M, et al. Flavivirus susceptibility in Aedes aegypti. Arch Med Res. 2002;33(4):379-88.

27. Hall-Mendelin S, Pyke AT, Moore PR, Mackay IM, McMahon JL, Ritchie SA, et al. Assessment of Local Mosquito Species Incriminates Aedes aegypti as the Potential Vector of Zika Virus in Australia. PLoS Negl Trop Dis. 2016;10(9):e0004959.

28. Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y, et al. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One. 2014;9(10):e109442.

29. Ayres CF. Identification of Zika virus vectors and implications for control. Lancet Infect Dis. 2016;16(3):278-9.

30. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 2008;14(8):1232-9.

31. Pupella S, Pisani G, Cristiano K, Catalano L, Grazzini G. West Nile virus in the transfusion setting with a special focus on Italian preventive measures adopted in 2008-2012 and their impact on blood safety. Blood Transfus. 2013;11(4):563-74.

32. Leung GH, Baird RW, Druce J, Anstey NM. Zika Virus Infection in Australia Following a Monkey Bite in Indonesia. Southeast Asian J Trop Med Public Health. 2015;46(3):460-4.

33. Johnson BK, Chanas AC, Shockley P, Squires EJ, Gardner P, Wallace C, et al. Arbovirus isolations from, and serological studies on, wild and domestic vertebrates from Kano Plain, Kenya. Trans R Soc Trop Med Hyg. 1977;71(6):512-7.

34. Darwish MA, Hoogstraal H, Roberts TJ, Ahmed IP, Omar F. A sero-epidemiological survey for certain arboviruses (Togaviridae) in Pakistan. Trans R Soc Trop Med Hyg. 1983;77(4):442-5.

35. Hamel R, Liegeois F, Wichit S, Pompon J, Diop F, Talignani L, et al. Zika virus: epidemiology, clinical features and host-virus interactions. Microbes Infect. 2016;18(7-8):441-9.

36. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. 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):20761.

37. Recommendations for donor screening, deferral, and product management to reduce the risk of transfusion transmission of Zika virus, recommendations for industry. US Food and Drug Administration, 16 February 2016. (http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM486360.pdf , accessed 3 October 2016)

38. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. Emerg Infect Dis. 2011;17(5):880-2.

39. Deckard DT, Chung WM, Brooks JT, Smith JC, Woldai S, Hennessey M, et al. Male-to-Male Sexual Transmission of Zika Virus--Texas, January 2016. MMWR Morb Mortal Wkly Rep. 2016;65(14):372-4.

40. Hills SL, Russell K, Hennessey M, Williams C, Oster AM, Fischer M, et al. Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission - Continental United States, 2016. MMWR Morb Mortal Wkly Rep. 2016;65(8):215-6.

41. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. Euro Surveill. 2014;19(13):20751

42. Neurological syndrome, congenital malformations, and Zika virus infection. Implications for public health in the Americas. PAHO /WHO, 1 December 2015. (http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32405&lang=en ,accessed 25 October 2016).

43. Picone O, Vauloup-Fellous C, D'Ortenzio E, Huissoud C, Carles G, Benachi A, et al. [Zika virus infection during pregnancy]. J Gynecol Obstet Biol Reprod (Paris). 2016;45(5):415-23.

44. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? Ultrasound Obstet Gynecol. 2016;47(1):6-7.

45. Martines RB, Bhatnagar J, Keating MK, Silva-Flannery L, Muehlenbachs A, Gary J, et al. Notes from the Field: Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses--Brazil, 2015. MMWR Morb Mortal Wkly Rep. 2016;65(6):159-60.

46. Mlakar J, Korva M, Tul N, Popovic M, Poljsak-Prijatelj M, Mraz J, et al. Zika Virus Associated with Microcephaly. N Engl J Med. 2016;374(10):951-8.

47. Higgs S. Zika Virus: Emergence and Emergency. Vector Borne Zoonotic Dis. 2016;16(2):75-6.

48. Gourinat AC, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. Emerg Infect Dis. 2015;21(1):84-6.

49. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. J Clin Virol. 2015;68:53-5.

50. Simpson DI. Zika Virus Infection in Man. Trans R Soc Trop Med Hyg. 1964;58:335-8.

51. Filipe AR, Martins CM, Rocha H. Laboratory infection with Zika virus after vaccination against yellow fever. Arch Gesamte Virusforsch. 1973;43(4):315-9.

52. Bearcroft WG. Zika virus infection experimentally induced in a human volunteer. Trans R Soc Trop Med Hyg. 1956;50(5):442-8.

53. Lazear HM, Govero J, Smith AM, Platt DJ, Fernandez E, Miner JJ, et al. A Mouse Model of Zika Virus Pathogenesis. Cell Host Microbe. 2016;19(5):720-30.

54. Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, et al. First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg. 2014;91(5):1035-8.

55. Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, et al. Biology of Zika Virus Infection in Human Skin Cells. J Virol. 2015;89(17):8880-96.

56. Olagnier D, Amatore D, Castiello L, Ferrari M, Palermo E, Diamond MS, et al. Dengue Virus Immunopathogenesis: Lessons Applicable to the Emergence of Zika Virus. J Mol Biol. 2016;428(17):3429-48.

57. Charrel RN, Leparc-Goffart I, Pas S, de Lamballerie X, Koopmans M, Reusken C. Background review for diagnostic test development for Zika virus infection. Bull World Health Organ. 2016;94(8):574-84D.

58. Tang H, Hammack C, Ogden SC, Wen Z, Qian X, Li Y, et al. Zika Virus Infects Human Cortical Neural Progenitors and Attenuates Their Growth. Cell Stem Cell. 2016;18(5):587-90.

59. Bell TM, Field EJ, Narang HK. Zika virus infection of the central nervous system of mice. Arch Gesamte Virusforsch. 1971;35(2):183-93.

60. Weinbren MP, Williams MC. 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.

61. Way JH, Bowen ET, Platt GS. Comparative studies of some African arboviruses in cell culture and in mice. J Gen Virol. 1976;30(1):123-30.

62. van der Eijk AA, van Genderen PJ, Verdijk RM, Reusken CB, Mogling R, van Kampen JJ, et al. Miscarriage Associated with Zika Virus Infection. N Engl J Med. 2016;375(10):1002-4.

63. Miner JJ, Cao B, Govero J, Smith AM, Fernandez E, Cabrera OH, et al. Zika Virus Infection during Pregnancy in Mice Causes Placental Damage and Fetal Demise. Cell. 2016;165(5):1081-91.

64. Jurado KA, Simoni MK, Tang Z, Uraki R, Hwang J, Householder S, et al. Zika virus productively infects primary human placenta-specific macrophages. JCI Insight. 2016;1(13).

65. Quicke KM, Bowen JR, Johnson EL, McDonald CE, Ma H, O'Neal JT, et al. Zika Virus Infects Human Placental Macrophages. Cell Host Microbe. 2016;20(1):83-90.

66. Adibi JJ, Marques ET, Jr., Cartus A, Beigi RH. Teratogenic effects of the Zika virus and the role of the placenta. Lancet. 2016;387(10027):1587-90.

67. Wu KY, Zuo GL, Li XF, Ye Q, Deng YQ, Huang XY, et al. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. Cell Res. 2016;26(6):645-54.

68. Li C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, et al. Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. Cell Stem Cell. 2016;19(1):120-6.

69. Garcez PP, Loiola EC, Madeiro da Costa R, Higa LM, Trindade P, Delvecchio R, et al. Zika virus impairs growth in human neurospheres and brain organoids. Science. 2016;352(6287):816-8.

70. Bayless NL, Greenberg RS, Swigut T, Wysocka J, Blish CA. Zika Virus Infection Induces Cranial Neural Crest Cells to Produce Cytokines at Levels Detrimental for Neurogenesis. Cell Host Microbe. 2016;20(4):423-8.

71. Klase ZA, Khakhina S, Schneider Ade B, Callahan MV, Glasspool-Malone J, Malone R. Zika Fetal Neuropathogenesis: Etiology of a Viral Syndrome. PLoS Negl Trop Dis. 2016;10(8):e0004877.

72. Mawson AR. Pathogenesis of Zika Virus-Associated Embryopathy. Biores Open Access. 2016;5(1):171-6.

73. Goodman AB, Dziuban EJ, Powell K, Bitsko RH, Langley G, Lindsey N, et al. Characteristics of Children Aged <18 Years with Zika Virus Disease Acquired Postnatally - U.S. States, January 2015-July 2016. MMWR Morb Mortal Wkly Rep. 2016;65(39):1082-5.

74. Furtado JM, Esposito DL, Klein TM, Teixeira-Pinto T, da Fonseca BA. Uveitis Associated with Zika Virus Infection. N Engl J Med. 2016;375(4):394-6.

75. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536-43.

76. Zea-Vera AF, Parra B. Zika virus (ZIKV) infection related with immune thrombocytopenic purpura (ITP) exacerbation and antinuclear antibody positivity. Lupus. 2016.

77. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barre syndrome. Lancet. 2016;388(10045):717-27.

78. Yuki N, Hartung HP. Guillain-Barre syndrome. N Engl J Med. 2012;366(24):2294-304.

79. Mecharles S, Herrmann C, Poullain P, Tran TH, Deschamps N, Mathon G, et al. Acute myelitis due to Zika virus infection. Lancet. 2016;387(10026):1481.

80. Munoz LS, Barreras P, Pardo CA. Zika Virus-Associated Neurological Disease in the Adult: Guillain-Barre Syndrome, Encephalitis, and Myelitis. Semin Reprod Med. 2016;34(5):273-9.

81. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome--case report, French Polynesia, December 2013. Euro Surveill. 2014;19(9):20720.

82. Schuler-Faccini L, Ribeiro EM, Feitosa IM, Horovitz DD, Cavalcanti DP, Pessoa A, et al. Possible Association Between Zika Virus Infection and Microcephaly - Brazil, 2015. MMWR Morb Mortal Wkly Rep. 2016;65(3):59-62.

83. Brasil P, Pereira JP, Jr., Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika Virus Infection in Pregnant Women in Rio de Janeiro. N Engl J Med. 2016;375(24):2321-34.

84. Woods CG, Parker A. Investigating microcephaly. Arch Dis Child. 2013;98(9):707-13.

85. Alvarado MG, Schwartz DA. Zika Virus Infection in Pregnancy, Microcephaly, and Maternal and Fetal Health: What We Think, What We Know, and What We Think We Know. Arch Pathol Lab Med. 2017;141(1):26-32.

86. Calvet G, Aguiar RS, Melo AS, Sampaio SA, de Filippis I, Fabri A, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis. 2016;16(6):653-60.

87. Costa F, Sarno M, Khouri R, de Paula Freitas B, Siqueira I, Ribeiro GS, et al. Emergence of Congenital Zika Syndrome: Viewpoint From the Front Lines. Ann Intern Med. 2016;164(10):689-91.

88. Miranda HA 2nd, Costa MC, Frazao MA, Simao N, Franchischini S, Moshfeghi DM. Expanded Spectrum of Congenital Ocular Findings in Microcephaly with Presumed Zika Infection. Ophthalmology. 2016;123(8):1788-94.

89. de Paula Freitas B, de Oliveira Dias JR, Prazeres J, Sacramento GA, Ko AI, Maia M, et al. Ocular Findings in Infants With Microcephaly Associated With Presumed Zika Virus Congenital Infection in Salvador, Brazil. JAMA Ophthalmol. 2016;134(5):529

90. Ventura CV, Maia M, Dias N, Ventura LO, Belfort R, Jr. Zika: neurological and ocular findings in infant without microcephaly. Lancet. 2016;387(10037):2502.

91. Ventura CV, Maia M, Bravo-Filho V, Gois AL, Belfort R, Jr. Zika virus in Brazil and macular atrophy in a child with microcephaly. Lancet. 2016;387(10015):228.

92. Sarno M, Sacramento GA, Khouri R, do Rosario MS, Costa F, Archanjo G, et al. Zika Virus Infection and Stillbirths: A Case of Hydrops Fetalis, Hydranencephaly and Fetal Demise. PLoS Negl Trop Dis. 2016;10(2):e0004517.

93. Paniz-Mondolfi AE, Rodriguez-Morales AJ, Blohm G, Marquez M, Villamil-Gomez WE. ChikDenMaZika Syndrome: the challenge of diagnosing arboviral infections in the midst of concurrent epidemics. Ann Clin Microbiol Antimicrob. 2016;15(1):42.

94. Falcao MB, Cimerman S, Luz KG, Chebabo A, Brigido HA, Lobo IM, et al. Management of infection by the Zika virus. Ann Clin Microbiol Antimicrob. 2016;15(1):57.

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

96. Rabe IB, Staples JE, Villanueva J, Hummel KB, Johnson JA, Rose L, et al. Interim Guidance for Interpretation of Zika Virus Antibody Test Results. MMWR Morb Mortal Wkly Rep. 2016;65(21):543-6.

97. Carteaux G, Maquart M, Bedet A, Contou D, Brugieres P, Fourati S, et al. Zika Virus Associated with Meningoencephalitis. N Engl J Med. 2016;374(16):1595-6.

98. Soares de Oliveira-Szejnfeld P, Levine D, Melo AS, Amorim MM, Batista AG, Chimelli L, et al. Congenital Brain Abnormalities and Zika Virus: What the Radiologist Can Expect to See Prenatally and Postnatally. Radiology. 2016;281(1):203-18.

99. Sampathkumar P, Sanchez JL. Zika Virus in the Americas: A Review for Clinicians. Mayo Clin Proc. 2016;91(4):514-21.

100. Long D, Long B, Koyfman A. Zika Virus: What Do Emergency Physicians Need to Know? J Emerg Med. 2016;50(6):832-8.