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Mosquito-transmitted viruses – the great Brazilian challenge

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ABSTRACT

Arboviruses pose a serious threat to public health worldwide, overloading the healthcare system and causing economic losses. These viruses form a very diverse group, and in Brazil, arboviruses belonging to the families *Flaviviridae* and *Togaviridae* are predominant. Unfortunately, the number of arboviruses increases in proportion with factors such as deforestation, poor sanitation, climate changes, and introduction of new viruses like Chikungunya virus and Zika virus.

In Brazil, dengue is endemic, along with the presence of other arboviruses. The situation is complicated by the scarcity of diagnostic infrastructure and the absence of approved vaccines for these diseases. Disease control, thus, relies solely on vector control. Therefore, enhanced clinical knowledge and improved general awareness about these arboviruses are indispensable to tackle diagnostic inadequacies.

Introduction

Arboviruses (arthropod-borne viruses) pose a serious threat to public health worldwide, especially in the tropical and subtropical countries, overloading the public healthcare system and causing economic losses. Despite these huge risks, the number of cases tends to increase because of diverse concomitant factors. Deforestation, migration, disordered occupation of urban areas, and poor sanitation as well as ongoing climate changes, which further aids the vectors of these diseases to colonize new areas, will significantly increase the strength of population at risk.

These arboviruses form a very diverse group. In Brazil, the main arbovirus causing epidemics belongs to the families *Flaviviridae* and *Togaviridae*.[1] In addition to the endemic arboviruses such as dengue virus (DENV), other neglected arboviruses also cause epidemics, such as Mayaro virus (MAYV). This situation, coupled with the introduction of Chikungunya virus (CHIKV), followed by Zika virus (ZIKV), in the Brazilian territory highlights the importance of continuous survey and research about these viruses. Improved awareness

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about these viruses among physicians, healthcare personnel, and concerned authorities as well as general public in the affected areas is indispensable for disease control. This review will focus on the endemic DENV, the neglected MAYV, and the newcomers CHIKV and ZIKV.

Dengue fever

Background

DENV are the most important human arboviruses found worldwide, transmitted by mosquitoes of the genus *Aedes*, the main vector being *Aedes aegypti*, and are responsible for morbidity and mortality. This group is the etiological agent of dengue fever (DF). DENV activity in Brazil, during its trajectory, is demonstrated by the high number of cases reported as well as the number of states involved in the epidemics. *Ae. aegypti* is observed in ~80% of the country, and the difficulties of implementing successful vector control are well known. Explosive epidemics have become a socially and politically significant public health problem, with great economic impact.[2]

The DENV species includes four genetically and antigenically different serotypes (DENV-1, -2, -3, and -4). DENV are members of the family *Flaviviridae*, genus *Flavivirus*. Like other flaviviruses, DENV have a single-stranded positive-sense RNA genome, 10,700-nucleotide-long, that is translated as a single polyprotein and post-translationally cleaved into three structural proteins: capsid, premembrane and envelope; and seven nonstructural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5.[3]

DENV-1 was the most predominant serotype in Brazil in the 1980s, and DENV-2 replaced it in the 1990s; subsequently, DENV-3 took the position in 2000, followed by DENV-4 in 2007.[4,5]

DENV-1

DENV-1 was first observed in the eighties. Phylogenetic studies classified DENV-1 into five genotypes, namely, I, II, III, IV, and V, on the basis of their genetic diversity.[6] The genotypes I, IV, and V were observed in the country, unlike II and III.[7,8]

Nucleotide sequencing subdivided the genotype V into three lineages.[9] The authors suggested that it was introduced by four different events: the first in 1984–1985, second in 1997–1999, and third and fourth in 2004–2007. Two distinct lineages were reported for viruses belonging to genotype V[10]; these lineages were introduced at different time-points in Goiás state. Genotype V was reported in Manaus[11] and Minas Gerais[12] states.

DENV-2

Co-circulation of DENV-1 and DENV-2 in Brazil began in 1990, initially in Rio de Janeiro, and subsequently in other states.[13–16] Similar to other countries in the Americas, the introduction of this strain coincided with that of the Southeast Asian genotype DENV-2 into the continent. Two additional DENV-2 epidemics occurred in 1998 and 2007–2008 in Brazil. In 2001, a large outbreak of DENV-2 occurred in Manaus.[17]

Two lineages of DENV-2 have been reported in Brazil.[18] Phylogenetic analyses of DENV-2 showed that genotype III (Southeast Asian/American) was the only one that circulated over the past 19 years in Brazil, from 1991 to 2008.[19] Sequencing of samples collected in 2011 showed the presence of DENV-2 of the Asian/American genotype in Manaus.[11] Salvador et al. later isolated an American genotype strain in Brazil.[20]

DENV-3

Phylogenetic studies have classified DENV-3 into five genotypes, namely, I, II, III, IV, and V, on the basis of their genetic diversity.[21] In Brazil, DENV-3 was first isolated from an autochthonous case in December 2000, in the state of Rio de Janeiro. A large DENV epidemic occurred in 2001–2002 and DENV-3 was assigned to genotype III.[22,23] These DENV-3 isolates appeared to arise from single introduction of GIII.[24]

Co-circulation of DENV-3 genotypes I and III was later observed in Minas Gerais, Brazil. The genotype I was identified in outbreaks occurring during 2002–2004.[25,26] Analysis of the gene sequences of mosquitoes naturally infected with DENV-3 confirmed the circulation of genotype I in Minas Gerais.[27]

DENV-3 genotype III is prevalent in Brazil and has also been observed in Manaus, Amazonas state[11] and in São José do Rio Preto, São Paulo State.[28] Phylogenetic analysis of the DENV-3 genotype III isolated from 107 samples collected between 2001 and 2009 showed that four instances of genotype introduction might have occurred in Brazil because of the detection of four phylogenetically distinct lineages. Three lineages were probably imported from the Antilles and Caribbean, while the fourth one was probably introduced through Colombia or Venezuela.[29]

A gap of eight years between two instances of introduction has been suggested.[30] Both lineages seem to be co-circulating simultaneously, although lineage II is predominant in South and Northeast Brazil, indicating that periodic DENV serotype-specific peaks in incidence coincide with the introduction of new lineages in Brazil every 7–10 years.

DENV-4

DENV-4 was first reported in Roraima State during 1981 and 1982.[31] DENV-4 reemerged in Manaus, Amazonas State in 2007.[25] The virus was subsequently identified in the northern Brazilian states of Amazonas and Pará.[32] In the Southeast region, the first episode occurred in the states of Rio de Janeiro and São Paulo in 2011.[33,34]

Partial genomic studies have confirmed that the predominant virus in Brazil is directly associated to the Caribbean strains, and belongs to genotype II. Phylogenetic analyses of different strains demonstrated the presence of two distinct genotypes I and II in Brazil.[11,32,34–40]

Co-infections

In Brazil, clinical cases of co-infection with two serotypes have been reported in some outbreaks. Co-infection with the serotypes DENV-1/-4 and DENV-2/-4 were observed in Mato Grosso state[41]; co-infection with DENV-1/-4, in São José do Rio Preto, São Paulo state[42]; co-infection with DENV-1/-4, DENV-2/-4, DENV-1/-2, and DENV-3/-4, in Manaus, Amazonas state[11,43,44]; co-infection with DENV-2/-3, in Tauá, Ceará state[45]; co-infection with DENV-1/-2, in Barretos, São Paulo state[46]; and co-infection with DENV-1/-2, in Cuiabá, Mato Grosso state.[47,48] Co-infections with more than one serotype were also detected in *Ae. aegypti*.

Clinical manifestations

In 1780, an epidemic of "breakbone fever" was reported in Philadelphia, in which patients showed some or all of the following symptoms: high fever, headache, myalgia, arthralgia, nausea, vomiting, rash, and hemorrhagic manifestations.[49] In 1801, a similar syndrome was given the name of "dengue" (meaning "affectation" in Spanish) to describe the plaintive demeanor of patients.[50] Dengue hemorrhagic fever, a severe illness form, emerged a little more than 60 years ago, when 21 cases of a severe febrile illness in children living in or near Manila were identified.[51]

All four DENV serotypes cause similar forms of illness.[52] DF is a complex illness, with a wide spectrum of clinical manifestations, which, often, are unrecognized or misdiagnosed as other fever-causing tropical illness. Among symptomatic cases of DF, a wide variety of clinical manifestations can be observed, ranging from mild febrile illness to severe DF and potentially fatal DHF.[53] After an incubation period of 3–5 days (usually 5–8), the illness begins abruptly and passes through three phases: febrile, critical, and recovery. DF (non-severe) is characterized by a combination of two or more signs and symptoms in a febrile individual in an endemic area, including nausea, vomiting, rash, aches, and pains, with a positive tourniquet test, according to the latest World Health Organization classification.[54] These symptoms occur during the early febrile stage.[55] In the critical phase, rashes are observed along with the appearance of petechial exanthem, which occurs around the time of defervescence, typically on days 3–7, and is associated with capillary leakage and hemorrhage.[56] Abdominal pain and tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, restlessness, and hepatomegaly are warning signs in potentially severe cases of DF. The severe cases are characterized by capillary leakage, which can lead to shock or fluid accumulation, causing respiratory distress, severe bleeding, and organ failure, including the liver, central nervous system, and heart. Thrombocytopenia (<100,000 mm^3), not necessarily restricted to the severe form, and "hemoconcentration" (increase in hematocrit) may occur, which may be associated to plasma leakage.[52] Only a small proportion of patients progresses to more severe form.[57] Although severe illness is historically associated with pediatric populations in the hyper-endemic regions,[58,59] current trends show that adults may also be at risk.[60–63] The risk factors for the development of severe DF include prior infection by the heterotypic serotype,[64] but, currently, the ability to predict the development of complications such as shock due to systemic vascular leak syndrome is currently poor.[53] Although the clinical definition of DF is available, clinical and laboratory presentations do vary in some cases, often overlapping with other infections; therefore, laboratory confirmation is considered plausible.

The dynamic nature of DF demands close monitoring and repeated clinical and laboratory evaluation, including periodic check of hematocrit and platelet counts.[65] The primary concern for clinicians treating such patients remains the fact that clinical diagnosis of DF is difficult during the early febrile phase. Careful clinical observation and judicious use of intravenous fluid therapy are crucial. No DF-specific drugs are available for therapy.

While the acute manifestations of DF are well known, only few studies have reported clinical manifestations during convalescence. The long-term consequences of the cross-reactivity of antibodies against DENV and the associated effects in plasmin activity,[66] which, in turn, increases the long-term risk of hemorrhagic phenomena in DENV-infected patients, too remain unknown. In two years, manifestations such as myalgia, arthralgia, asthenia, malaise, irritability, memory loss, headache, retro-orbital pain may be observed.[67] Some DENV-infected patients also presented with psychiatric symptoms such as thanatophobia and bug phobia.[68] Estimating the incidence of psychiatric disturbance is difficult because of the lack of adequate literature to base the estimation.[68–70]

Alphavirus

Background

Alphavirus, a genus of the family *Togaviridae*, is found in all continents, except Antarctica.[71] This diverse genus includes 31 recognized species that can infect birds, rodents, amphibians, reptiles, and human and nonhuman primates.[72]

The structural unit is a small, icosahedral capsid measuring 65–70 nm in diameter, surrounded by a lipid envelop of cellular origin. The genome is composed of a positive single-stranded 11.5-kb-long RNA molecule, with a 7-methylguanosine cap at the 5′-end and a polyadenylated tail at the 3′-end.[73] It has two open reading frames (ORFs), separated by an intergenic region. The first ORF encodes four non-structural proteins (nsP1-4), necessary for the replication of viral RNA. An internal subgenomic promoter that lies immediately upstream controls this ORF. The second ORF is translated into a single polyprotein precursor, which is subsequently processed to form the capsid protein (C), two envelope surface glycoproteins (E1 and E2), and two small peptides (E3 and 6k). The organization of the genome can be summarized as 5′-m^7G-nsP1-nsP2-nsP3-nsP4-(junction)-C-E3-E2-6K-E1-A_n-3′.[72,74]

The genus has three main clades: the Semliki Forest virus clade, the equine encephalitis virus/Sindbis clade, and the aquatic virus clade.[71] The most studied viruses of this genus are Sindbis and CHIKV. Thus, majority of the current knowledge about molecular biology is based on studies with these viruses.[75]

The clinically relevant alphaviruses can be roughly classified into two groups, on the basis of a combination of

phylogenetics, geographical distribution, and the clinical disease caused by them.

The viruses of the encephalitic group or New World alphaviruses cause a flu-like syndrome and have the potential to progress to neurological conditions.[76] These viruses occur in the Americas and are associated with severe and lethal encephalitis. This group includes the Venezuelan Equine Encephalitis (VEE), Eastern Equine Encephalitis (EEE), Western equine encephalitis (WEE), and Madariaga antigenic complex viruses.[76]

The viruses of the arthritogenic group or Old World alphaviruses have a broader distribution; they were initially identified in the Old World (Europe, Asia, and Africa). They cause malaise, rash, and sometimes incapacitating and long-lasting articular disease/myalgia.

They comprises the Ross River virus (RRV), CHIKV, Sindbis virus (SINV), MAYV, O'nyong-nyong virus (ONNV), and Barmah Forest virus (BFV).[77]

Chikungunya

CHIKV is the etiological agent causing Chikungunya fever (CF). The virus was first isolated in 1952 during an outbreak in Tanzania[78]; however, it probably occurred centuries ago in Africa. It has been implicated in explosive outbreaks in all continents.[79]

There is a serotype of CHIKV, which confers life-long immunity to recovered individuals. However, four genotypes have been described: the enzootic West African (WAf), the most widespread East/Central/South African (ECSA) genotype, the epidemic Asian genotype; and the WAf-derived Indian Ocean lineage (IOL), responsible for epidemics in India, Indian Ocean islands, and Europe since 2004.[80]

CHIKV first appeared in the Americas in late 2013, in the Caribbean. In Brazil, some cases were reported since June 2014. In September 2014, the Asian genotype reported in the Caribbean was detected in Amapá state, and the ECSA, which was never detected in the Americas, was confirmed in Bahia state. Since the first detection, more than 25,000 suspected cases of CHIKV infection have been registered in Brazil.[81,82]

CF was first described in Tanzania in 1955, when 115 patients were hospitalized because of acute onset of high fever, severe joint pain, and rash.[83] The term "Chikungunya" comes from the Makonde language, meaning "that which bends up", in reference to the posture acquired by the patient because of arthralgia. Arthralgia is the most important characteristic of this illness, which also includes fever, headache, nausea, and vomiting.[84]

Clinical manifestations

CF is an acute febrile illness that can occur in anyone at any age, and is usually self-limiting and rarely life-threatening.[85] CHIKV infection seems to induce long-lasting protective immunity, and epidemic peaks drop as an increasing percentage of the population improves their immunity.[86] After 4–7 (range, 2–12) days of incubation, the primary clinical features of CHIKV infection include sudden onset of fever, chills, headache, myalgia, maculopapular rash, and arthralgia, usually with a symmetric pattern, and especially in the wrist, knee, ankle, and small joints, which can often end up being debilitating.[87,88] The virus can be detected in the joint tissues for up to 90 days, leading to local inflammation.[89] Up to 60% of the patients can suffer recurrent episodes of debilitating chronic arthritis years after the infection is cleared.[90,91] The chronic disease produced by CHIKV is likely induced by deregulated inflammation during the acute phase of disease and/or convalescence.[92] Not all individuals infected with the virus develop symptoms.[85] Serosurveys indicate that 3–25% individuals with antibodies to CHIKV have asymptomatic infections.[93,94] The clinical presentation of CF is often similar to that of DF, except for the hemorrhagic or shock syndrome, which is rarely seen in CHIKV infection,[95] and the fact that febrile symptoms usually resolve in 3–4 days, but prominent and prolonged arthralgia affect multiple joints, is more common in CHIKV.[96–98] Blood test abnormalities such as leukopenia, thrombocytopenia, hypocalcemia, and a mild-to-moderate increase in liver function-determining values are seen during acute infection.[88,99] Other uncommon manifestations have also been observed, such as nephritis,[100] meningoencephalitis,[101] encephalopathy,[102] Guillain–Barré syndrome, acute flaccid paralysis, and palsies.[103–105] However, neurologic, ophthalmologic, and hemorrhagic diseases associated with CHIKV infection appear to be rare.[106]

High levels of CHIKV load typically last for 4–6 days and can persist for up to 12 days after symptom onset.[107,108] There is no gold standard method in CHIKV diagnostics. Classical virus detection methods such as virus isolation, detection of viral antigens or nucleic acid, and detection of host antibodies are commonly used.[109]

Treatment of CF is limited to supportive care: rest, fluids, antipyretics, and analgesics. Some studies suggest the use of some drugs such as chloroquine, acyclovir, ribavirin, interferon-α, and corticosteroids for treating CHIKV infection.[88,110–112] Nonsteroidal anti-inflammatory drugs are also used to treat the inflammation, but they can be used only after ruling out DENV infection.[109] Treatment with ribavirin (200 mg twice a day for seven days) has been effective in relieving the pain in the lower limbs.[113]

The clinical and epidemiological similarities between CHIKV infection and DF lead to misdiagnosis. CHIKV outbreaks in endemic DENV areas often go unnoticed in areas without diagnostic support.[114,115] In fact, both CF and DF are so similar that Halstead argued that the term "dengue" was the first descriptive for CF, but through the 19th century, the term passed across the globe to eventually designate actual DF.[79]

Mayaro

MAYV is the etiologic agent causing Mayaro fever (MF), a neglected disease of tropical Americas, where it is endemic. The virus was first isolated from a human in Trinidad in 1954, and since then, clinical cases have been reported in many countries in the tropical regions of South and Central America, including Trinidad, Bolivia, Suriname, French Guiana, Guyana, Peru, Venezuela, Colombia, Ecuador, Panama, and Brazil. Serological surveys also indicate the distribution in Costa Rica, Guatemala, and Mexico.[116]

Until now only two genotypes are known: the D genotype restricted to the Pará state in Brazil, and the L genotype with a wider distribution.[116] As in case of CHIKV infection, MAYV can go unnoticed.[117]

MAYV is thought to be restricted to the sylvatic cycle, mainly transmitted to non-human primates by canopy-dwelling *Haemagogus* mosquitoes. Clinical infections are accidental.[118] However, these viruses have great potential to emerge as a global pathogen, because urban mosquitoes such as *Ae. aegypti* can be competent vectors for MAYV transmission,[119] like the path followed by CHIKV in the Western Hemisphere.

As observed in case of CHIKV, MAYV outbreaks can pass unnoticed during DENV outbreaks. It is estimated that around 1% of all DENV-like cases in the northern region of South America is caused by MAYV.[117,120]

Clinical manifestations

Similar to CHIKV, MAYV is an arthritogenic arbovirus, responsible for sporadic infections or small outbreaks in the Amazon region, usually limited to rural areas near or inside forests because of the presence of the vector.[121,122] The incubation period ranges from 7 to 12 days, with a transient short viremia period of 3–7 days.[116] MF is a non-fatal, typically DENV-like, and self-limiting acute febrile illness, characterized by headache, epigastric pain, myalgia, incapacitating arthralgia, rash, chills, nausea, photophobia and vertigo. The bilateral joint pain is the most prominent symptom; it develops during the acute phase of the disease, and can be highly incapacitating, affecting the wrists, ankles, and small joints of hands and feet, often along with edema. In more than 50% patients, it can persist for several months after the infection, and often recur.[123] The joint pain may persist for several months.[124] No mortality is associated with MAYV infection, but the illness can cause significant morbidity among rural population,[125] including intense arthralgia, temporary incapacitation to work, and hospitalization. Few cases are described in some subpopulations, especially the immunocompromised group, and are showed to be imported cases.[126] Hemorrhagic manifestations in MAYV infections have been described, although rare.[127]

Diagnosis of MAYV is based on classical viral detection methods.[116] Although high rates of antibodies are found in some rural communities residing in the Amazon basin in Brazil,[128] it is difficult to isolate MAYV, because of relatively short duration of viremia.[116] No effective vaccine or antiviral agent exists for the arthritogenic alphaviruses, and the treatment chiefly relies on supportive modalities such as non-steroidal anti-inflammatory medications[116] and chloroquine.[129] As no vaccine or specific treatment is available, vector control is the most effective approach to limit the spread of arboviruses.

Zika fever

Background

ZIKV is a member of the Spondweni serocomplex from the genus *Flavivirus* and the family *Flaviviridae*. It is the etiological agent causing Zika fever (ZF). Although it belongs to a different serocomplex group, it is similar to other flaviviruses such as Ilheus (ILHV), Rocio (ROCV), and Saint Louis Encephalitis (SLEV), which have already been isolated in Brazil.[130,131] ZIKV has a positive-sense, single-stranded, 10,794-nucleotide-long RNA genome. The genome contains 5′- and 3′-UTRs flanking a single open reading frame (ORF) that encodes a polyprotein. This polyprotein is further cleaved into three structural proteins: the capsid (C), premembrane/membrane (prM), and envelope (E), and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, 2K, NS4B, and NS5).[132]

ZIKV replication is similar to the mosquito-borne replication observed in flaviviruses, starting in the dendritic cells at the site of the bite, and then spreading to the lymph nodes and bloodstream. In general, the replicative process occurs in the cytoplasm[133]; however, it is suggested that the proteins of the ZIKV replicative complex may translocate to the nucleus of infected cells,[5] as occurs in case of the NS5 protein of DENV-2, Yellow Fever (YFV), and Japanese Encephalitis (JEV) viruses.[133,134]

At present, two known lineages are circulating in the world: African (East and West) and Asian.[135,136] In 2007, a major ZIKV epidemic was detected in the Yap island in Micronesia, caused by the Asian lineage,[137,138] and from 2013, this lineage caused epidemics in the Pacific Islands (French Polynesia, New Caledonia, Cook Islands, Tahiti, and Easter Island).[139–143] This same lineage is responsible for the epidemics in Brazil,[144–147] South and Central Americas, as well as the imported cases in North America and Europe.[148–151]

A phylogenetic study of the African and Asian lineages showed that the African lineage is more divergent than the Asian lineage, with respect to the nucleotide and amino acid sequences.[137] The most widely known African isolate is the MR766 (GenBank accession number: LC002520.1), which shows 87–90% similarity with the isolates from French Polynesia and Brazil.[152,153] However, the infections caused by the African lineage of ZIKV has never been related to congenital malformations or neurological alterations, as observed for the Asian lineage in circulation, chiefly in Brazil.[154] Thus, the genetic relationship between the lineages is not well known and needs to be studied.

What was known until now is that independent of the origin, ZIKV lineages caused minor clinical consequences, with cases of mild febrile illness. The clinical presentation of ZIKV infection is usually not specific (mild fever, rash, arthralgia, and conjunctivitis) and can be confused with other diseases – most commonly DENV and CHIKV.[155] The most frequently reported symptoms include fever, conjunctivitis, headache, myalgia, and pruritus.[156] However, it is very important to note that the clinical manifestations often described as common findings in case of ZIKV infection, such as conjunctivitis,[156] may not be noted in some cases during DENV outbreaks in endemic areas.[157] Hematological findings such as thrombocytopenia, a very common finding in DF,[158] may also be associated with ZIKV infection, but with counts generally around 100,000/mm^3.[159,160] Despite ZIKV infection being typically associated with relatively mild illness, it is also potentially associated with severe illness as well as with neurological complications such as Guillain–Barré syndrome, hearing difficulty,[161] and microcephaly, at the moment. The

association between ZIKV infection during pregnancy and microcephaly was determined after the increase in microcephaly cases in newborns in the northeastern region of Brazil in 2015. This outbreak indicated a possible association between ZIKV infection during pregnancy and fetal malformations, which can be attributed to maternal-fetal virus transmission.[162]

This relationship began to be reviewed from the cases of microcephaly and neurological abnormalities described in Brazil. The virus was isolated from the amniotic fluid of fetuses with microcephaly as well as from the blood of newborns with microcephaly, which suggested that the ZIKV is able to cross the placental barrier.[163–166] This assumption gained weightage based on the results from a study conducted in mice, which showed neurological abnormalities in newborns infected with the isolated Brazilian ZIKV.[154]

Transmission of ZIKV occurs through the bite of an infected hematophagous mosquito (thus, a vector-borne disease). Many known competent species are responsible for transmission of this virus: *Ae. africanus*, *Ae. albopictus*, *Ae. aegypti*, *Ae. apicoargenteus*, *Ae. luciocephalus*, *Ae. vitattus*, *Ae. furcifer*, and *Ae. hensilii*.[167–170] Brazilian cities are known to be infested by the anthropophilic *Ae. aegypti*, which is primarily responsible for causing epidemics in the country. However, only recently, in 2016, the virus was isolated from a pool of naturally infected *Ae. aegypti*, present in the localities of Rio de Janeiro.[171] Previous studies have identified *Ae. aegypti* naturally infected with ZIKV in Malaysia[170]; however, this virus was also detected in *Ae. albopictus* in Mexico,[172] thus making it another potential vector for the transmission of the virus into the country. Once infected, the virus incubation period in the vector may be ~10 days.[173]

Furthermore, entomological studies have recently suggested that *Culex*, a tropical domestic mosquito widely observed in Brazil, might be able to transmit ZIKV since some arboviral infections are transmitted by several species of *Culex*.[174]

The transmission of this virus occurs through the sylvatic and urban cycles and it does not require the vector. In the sylvatic environment, the cycle is maintained between mosquitoes and nonhuman primates, where rodents are also suspected of acting as hosts.[175] In the sylvatic cycle, humans are accidental hosts. In 2015, ZIKV-positive primates were identified in the state of Ceará.[176] This evidence supports the possibility that the primates act as reservoirs for ZIKV, as observed in case of YFV.[172,176]

The urban cycle occurs between mosquitoes and humans, where the *Ae. aegypti*, *Ae. albopictus*, and *Ae. africanus* mosquitoes act as the main vectors.[152] Other forms of transmission, quite a few of which are considered significant, are vertical[166,177,178] and sexual transmission.[179,180] Other routes that are still being studied include transmission via blood transfusion,[181] breastfeeding,[182] and infected host bite.[183]

Situation in Brazil

In early 2015, the Northeast region was faced with an increase in the number of cases of an unidentified disease, characterized by mild fever, conjunctivitis, rash, and joint pain, which remained for to 7 days. DF and Chikungunya, a viral fever that was first recorded in the Americas in 2013, were suspected, but not confirmed.

In late April, a preliminary test conducted by the Institute of Health Sciences of the Bahia Federal University (UFBA) identified the presence of ZIKV in biological samples collected from patients. The disease, initially noted as a mild illness, was treated as an international emergency a few months later because of the first evidence of its connection with the increase in microcephaly cases in the country (Fig. 1).[184]

According to the WHO, 39 countries have reported the virus in circulation since 2007. Of these, Brazil, French Polynesia, El Salvador, Venezuela, Colombia, and Suriname have concurrently published reports about the cases, the increase in the incidence of Guillain-Barré syndrome and microcephaly (particularly in Brazil).[185]

Gradually, ZIKV infections became a reality, with unimaginable consequences for an infection caused by arboviruses. Although the occurrence of microcephaly and Guillain-Barré syndrome is attributed to ZIKV, much remains to be investigated. On February 1, 2016, the WHO declared that the cluster of microcephaly and ZIKV, mainly observed in the Brazilian cases, as a "Public Health Emergency of International Concern" (PHEIC)[186]; this is indicative of the extent of global emergency (Fig. 1).

Clinical manifestations

The current epidemic in Brazil attracted a lot of attention because of the possible association of ZIKV with the unusual increase in the occurrence of microcephaly in newborns. The current official number (confirmed until June 2016) of ZIKV infection-related microcephaly cases is 1,638 cases of microcephaly and other nervous system disorders, according to the Brazilian Ministry of Health.[187]

Some neurological symptoms such as the Guillain–Barré syndrome, characterized by weakness or paralysis caused by autoimmunity to peripheral nerves, are frequent in other viral infections too. The association with microcephaly is more complex. Microcephaly is probably a direct teratogenic effect of the virus, which affects the development of the central nervous system. There are no studies proving microcephaly to be the result of infection by other flaviviruses, and most Brazilian infants with microcephaly did not test positive for ZIKV.[188] However, the real role of ZIKV infection in the development of microcephaly remains unclear. Data obtained until date indicate a supposed causal relationship between ZIKV infection and microcephaly, which needs to be established with further detailed studies.

It was recently proposed that the antibody-dependent enhancement (ADE) phenomenon is related to ZIKV-linked complications. This effect is well established for the aggravation of infections caused by a second serotype of DENV. A strong cross-reaction is suggested between DENV and ZIKV, which is capable of stimulating the ADE effect in ZIKV-infected patients that presents antibodies against DENV. It is noted that the seroprevalence of DENV in South America population exceeds 90%, and this may contribute to future studies on pathogenesis and vaccine development against DENV and ZIKV.[189–191]

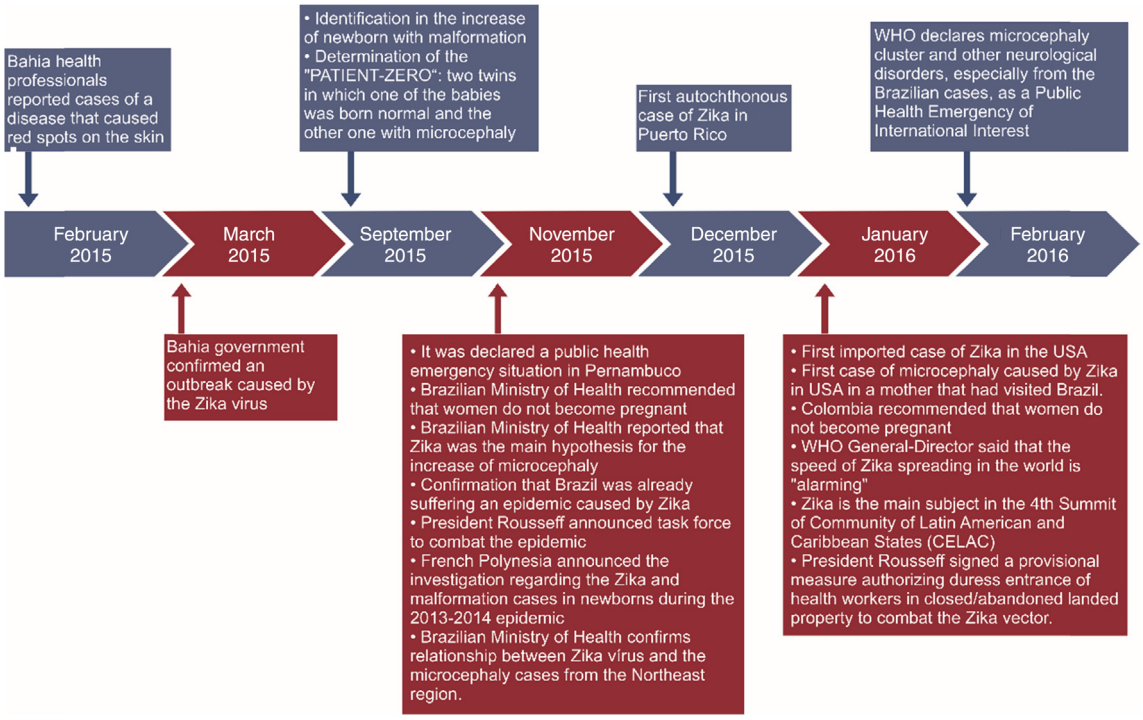

Fig. 1 – Timeline of the ZIKV introduction in the South America.
Adapted from http://www.bbc.com/portuguese/noticias/2016/02/160128_zika_virus_microcefalia_trajetoria_mdb.

Researchers believe that the relationship between microcephaly and ZIKV only emerged from the cases reported in Brazil because the country is the largest in Latin America, with a population larger and a density higher than those at previous locations affected by ZIKV epidemic, which allows the highest number of cases recorded.[192]

The Brazilian Ministry of Health is assessing changes in the microcephaly assessment protocol, emphasizing that the signs and symptoms of neurological disorders should be included as the criteria for screening newborns, irrespective of the presence of microcephaly; this, in turn, will expand the investigation and improve the Brazilian surveillance system for new cases of microcephaly.[193,194]

The fear instilled by ZIKV infections has led to a positive change in the situation; several research centers in Brazil and other countries came together to join forces in an attempt to understand the biology of this virus and to develop tools for the treatment of patients as well as for the prevention of the infection.

In São Paulo, a group of 42 laboratories, called the Zika network, and coordinated by the Institute of Biomedical Sciences (ICB) at USP, are working together to better understand the behavior of ZIKV, and thus improve the diagnostic methods and therapies and vaccine development. Regarding the development of an effective vaccine against ZIKV, the Brazilian government has partnered with the University of Texas Medical Branch – UTMB (Galveston, Texas, USA), which is a world center of arbovirus research, one of the most specialized centers in the development of vaccines, and a global reference as a center of excellence in scientific research, in an attempt to stop the consequences of infection by this arbovirus. Recently, a ZIKV vaccine was tested in mice, followed by efficiency analysis for protection against infection caused by the Brazilian strain. In this study, the researchers used a single immunization protocol with a DNA plasmid vaccine expressing the full-length domain of ZIKV pre-membrane and envelope or the purified inactivated virus vaccine; this provided complete protection in susceptible mice against the Brazilian strain.[195]

Another researcher network, coordinated by FIOCRUZ-Bahia, are involved in the development of an itinerant project called ZIBRA (Zika in Brazil Real Time Analysis), which aims to genetically map ZIKV strains collected from several locations in the Northeast regions (Natal (RN), João Pessoa (PB), Recife (PE), Maceió (AL), Salvador, and Feira de Santana (BA)), between 2015 and 2016. Once the sequences are ready, the group will be in a position to conduct epidemiological and evolutionary analyses of the virus circulating in Brazil. These data will be shared with all laboratories of the network as well as with the Brazilian Ministry of Health.[196]

Another available tool to assess the relationship between ZIKV and the host is ZIKV-CBD developed by FIOCRUZ-Minas, which gathers information on disease-associated genes, because the viral infection can interfere with gene expression by altering cellular function.[197]

Despite the advances in technology, increase in information sharing, and establishment of partnerships to better understand the behavior of ZIKV, these tools appear to be "distant" of the population, for all the work remains restricted to the laboratories. However, a quick test for the diagnosis of ZIKV, developed as a result of a collaborative project between a Brazilian laboratory and a South Korean company, has obtained the release certificate from ANVISA (National

Health Surveillance Agency). These kits will be distributed throughout the public healthcare system in Brazil once the Brazilian Ministry of Health adopts this new modality.

Final remarks

The biggest concern with arboviruses in Brazil remains the need for adequate diagnostics. Appropriate allocation of resources, development of vaccines, and therapeutic management depends on the correct assessment of the prevalence of these viruses, which in turn, depends on the diagnostic method used. However, the arboviruses discussed here share many clinical features (e.g., fever, headache, myalgia, rash). In Brazil, DENV is endemic, causing an overlap between this virus and other arboviruses. Other febrile illnesses such as measles, typhoid, leptospirosis, and influenza can also present with similar characteristics, in particular, during the early phase,[53] thereby leading to overlapping diagnosis. In many regions of Brazil, with low economic status and reduced availability of diagnostic services, diagnosis usually depends exclusively on the clinical manifestation.

These infections can be confirmed by detecting the virus, viral RNA, or antibodies. Historically, infections were diagnosed based on serology, but with the advent of molecular techniques, viral RNA can be easily detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in specimens obtained during the acute phase of infection.

There are no approved vaccines for any of these diseases, and thus, the control of these diseases relies solely on vector control. Therefore, improved awareness about these arboviruses among physicians, healthcare personnel, and concerned authorities, in addition to a well-informed population, is indispensable to tackle, in part, the inadequacies faced in the diagnostic sector.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Figueiredo LT. The recent arbovirus disease epidemic in Brazil. *Rev Soc Bras Med Trop*. 2015;48(3):233–234.
2. Messina JP, Brady OJ, Scott TW, et al. Global spread of dengue virus types: mapping the 70 year history. *Trends Microbiol*. 2014;22(3):138–146.
3. Viruses ICoTo. Family Flaviviridae. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, eds. *Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier; 2011:1003–1020.
4. Mourao MP, Bastos MdeS, Figueiredo RM, et al. Arboviral diseases in the Western Brazilian Amazon: a perspective and analysis from a tertiary health & research center in Manaus, State of Amazonas. *Rev Soc Bras Med Trop*. 2015;48(suppl 1):20–26.
5. Nogueira RMR, Araújo JMGd, Schatzmayr HG. Dengue viruses in Brazil, 1986–2006. *Rev Panam Sal Públ*. 2007;22:358–363.
6. Allicock OM, Lemey P, Tatem AJ, et al. Phylogeography and population dynamics of dengue viruses in the Americas. *Mol Biol Evol*. 2012;29(6):1533–1543.
7. Villabona-Arenas CJ, Zanotto PM. Worldwide spread of Dengue virus type 1. *PLOS ONE*. 2013;8(5):e62649.
8. Raghwani J, Rambaut A, Holmes EC, et al. Endemic dengue associated with the co-circulation of multiple viral lineages and localized density-dependent transmission. *PLoS Pathog*. 2011;7(6):e1002064.
9. Drumond BP, Mondini A, Schmidt DJ, Bosch I, Nogueira ML. Population dynamics of DENV-1 genotype V in Brazil is characterized by co-circulation and strain/lineage replacement. *Arch Virol*. 2012;157(11):2061–2073.
10. Cunha MD, Guimaraes VN, Souza M, et al. Phylodynamics of DENV-1 reveals the spatiotemporal co-circulation of two distinct lineages in 2013 and multiple introductions of dengue virus in Goias, Brazil. *Infect Genet Evol*. 2016;43:130–134.
11. Martins VdoC, Bastos MdeS, Ramasawmy R, et al. Clinical and virological descriptive study in the 2011 outbreak of dengue in the Amazonas, Brazil. *PLOS ONE*. 2014;9(6):e100535.
12. Drumond BP, Fagundes LG, Rocha RP, et al. Phylogenetic analysis of Dengue virus 1 isolated from South Minas Gerais, Brazil. *Braz J Microbiol*. 2016;47(1):251–258.
13. Nogueira RM, Miagostovich MP, Lampe E, Souza RW, Zagne SM, Schatzmayr HG. Dengue epidemic in the stage of Rio de Janeiro, Brazil, 1990-1: co-circulation of dengue 1 and dengue 2 serotypes. *Epidemiol Infect*. 1993;111(1):163–170.
14. Zagne SM, Alves VG, Nogueira RM, Miagostovich MP, Lampe E, Tavares W. Dengue haemorrhagic fever in the state of Rio de Janeiro, Brazil: a study of 56 confirmed cases. *Trans R Soc Trop Med Hyg*. 1994;88(6):677–679.
15. de Souza RV, da Cunha RV, Miagostovich MP, et al. An outbreak of dengue in the State of Ceara, Brazil. *Mem Inst Oswaldo Cruz*. 1995;90(3):345–346.
16. Vasconcelos PFdC, Menezes DBd, Melo LP, et al. A large epidemic of dengue fever with dengue hemorragic cases in Ceará State, Brazil, 1994. *Rev Inst Med Trop Sao Paulo*. 1995;37:253–255.
17. Rocha LAd, Tauil PL. Dengue em criança: aspectos clínicos e epidemiológicos, Manaus, Estado do Amazonas, no período de 2006 e 2007. *Rev Soc Bras Med Trop*. 2009;42:18–22.
18. Oliveira MF, Galvao Araujo JM, Ferreira OC Jr, et al. Two lineages of dengue virus type 2, Brazil. *Emerg Infect Dis*. 2010;16(3):576–578.
19. Cruz ACR, Galler R, Silva EVPd, et al. Molecular epidemiology of dengue virus serotypes 2 and 3 isolated in Brazil from 1991 to 2008. *Rev Pan-Am Saúde*. 2010;1:25–34.
20. Salvador FS, Amorim JH, Alves RP, Pereira SA, Ferreira LC, Romano CM. Complete genome sequence of an atypical dengue virus serotype 2 lineage isolated in Brazil. *Genome Announ*. 2015;3(4).
21. Lanciotti RS, Lewis JG, Gubler DJ, Trent DW. Molecular evolution and epidemiology of dengue-3 viruses. *J Gen Virol*. 1994;75(Pt 1):65–75.
22. Nogueira RMR, Miagostovich MP, Filippis AMBd, Pereira MAS, Schatzmayr HG. Dengue virus type 3 in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz*. 2001;96:925–926.
23. Nogueira MB, Stella V, Bordignon J, et al. Evidence for the co-circulation of dengue virus type 3 genotypes III and V in the Northern region of Brazil during the 2002–2004 epidemics. *Mem Inst Oswaldo Cruz*. 2008;103(5):483–488.
24. Araujo JM, Bello G, Schatzmayr HG, Santos FB, Nogueira RM. Dengue virus type 3 in Brazil: a phylogenetic perspective. *Mem Inst Oswaldo Cruz*. 2009;104(3):526–529.
25. Figueiredo RM, Naveca FG, Bastos MS, et al. Dengue virus type 4, Manaus, Brazil. *Emerg Infect Dis*. 2008;14(4):667–669.

26. Figueiredo ML, Alfonso HL, Amarilla AA, et al. Detection of DENV-4 genotype I from mosquitoes collected in the city of Manaus, Brazil. *Virol J*. 2013;10:60.
27. Vilela AP, Figueiredo LB, dos Santos JR, et al. Dengue virus 3 genotype I in Aedes aegypti mosquitoes and eggs, Brazil, 2005–2006. *Emerg Infect Dis*. 2010;16(6):989–992.
28. Villabona-Arenas CJ, Mondini A, Bosch I, et al. Dengue virus type 3 adaptive changes during epidemics in Sao Jose de Rio Preto, Brazil, 2006–2007. *PLOS ONE*. 2013;8(5): e63496.
29. de Araujo JM, Bello G, Romero H, Nogueira RM. Origin and evolution of dengue virus type 3 in Brazil. *PLoS Negl Trop Dis*. 2012;6(9):e1784.
30. Nunes MR, Palacios G, Faria NR, et al. Air travel is associated with intracontinental spread of dengue virus serotypes 1–3 in Brazil. *PLoS Negl Trop Dis*. 2014;8(4):e2769.
31. Osanai CH, Travassos da Rosa AP, Tang AT, do Amaral RS, Passos AD, Tauil PL. Dengue outbreak in Boa Vista, Roraima. Preliminary report. *Rev Inst Med Trop Sao Paulo*. 1983;25(1):53–54.
32. Nunes MR, Faria NR, Vasconcelos HB, et al. Phylogeography of dengue virus serotype 4, Brazil, 2010–2011. *Emerg Infect Dis*. 2012;18(11):1858–1864.
33. Nogueira RM, Eppinghaus AL. Dengue virus type 4 arrives in the state of Rio de Janeiro: a challenge for epidemiological surveillance and control. *Mem Inst Oswaldo Cruz*. 2011;106(3):255–256.
34. de Souza RP, Rocco IM, Maeda AY, et al. Dengue virus type 4 phylogenetics in Brazil 2011: looking beyond the veil. *PLoS Negl Trop Dis*. 2011;5(12):e1439.
35. de Melo FL, Romano CM, de Andrade Zanotto PM. Introduction of dengue virus 4 (DENV-4) genotype I into Brazil from Asia? *PLoS Negl Trop Dis*. 2009;3(4):e390.
36. Shu PY, Su CL, Liao TL, et al. Molecular characterization of dengue viruses imported into Taiwan during 2003–2007: geographic distribution and genotype shift. *Am J Trop Med Hyg*. 2009;80(6):1039–1046.
37. Temporao JG, Penna GO, Carmo EH, et al. Dengue virus serotype 4, Roraima State, Brazil. *Emerg Infect Dis*. 2011;17(5):938–940.
38. Naveca FG, Souza VC, Silva GA, et al. Complete genome sequence of a Dengue virus serotype 4 strain isolated in Roraima, Brazil. *J Virol*. 2012;86(3):1897–1898.
39. Campos RdeM, Veiga CS, Meneses MD, et al. Emergence of Dengue virus 4 genotypes II b and I in the city of Rio de Janeiro. *J Clin Virol*. 2013;56(1):86–88.
40. Villabona-Arenas CJ, de Oliveira JL, Capra CdeS, et al. Detection of four dengue serotypes suggests rise in hyperendemicity in urban centers of Brazil. *PLoS Negl Trop Dis*. 2014;8(2):e2620.
41. Heinen LBdS, Zuchi N, Cardoso BF, Santos MAMd, Nogueira ML, Dezengrini-Slhessarenko R. Dengue outbreak in Mato rosso state, Midwestern Brazil. *Rev Inst Med Trop Sao Paulo*. 2015;57:489–496.
42. Colombo TE, Vedovello D, Mondini A, et al. Co-infection of dengue virus by serotypes 1 and 4 in patient from medium sized city from Brazil. *Rev Inst Med Trop Sao Paulo*. 2013;55:275–281.
43. Figueiredo RM, Naveca FG, Oliveira CM, et al. Co-infection of Dengue virus by serotypes 3 and 4 in patients from Amazonas, Brazil. *Rev Inst Med Trop Sao Paulo*. 2011;53(6):321–323.
44. Bastos MdS, Figueiredo RMPd, Ramasawmy R, et al. Simultaneous circulation of all four dengue serotypes in Manaus, State of Amazonas, Brazil in 2011. *Rev Soc Bras Med Trop*. 2012;45:393–394.
45. Araujo FM, Nogueira RM, de Araujo JM, et al. Concurrent infection with dengue virus type-2 and DENV-3 in a patient from Ceara, Brazil. *Mem Inst Oswaldo Cruz*. 2006;101(8):925–928.
46. dos Santos CL, Bastos MA, Sallum MA, Rocco IM. Molecular characterization of dengue viruses type 1 and 2 isolated from a concurrent human infection. *Rev Inst Med Trop Sao Paulo*. 2003;45(1):11–16.
47. Rocco IM, Barbosa ML, Kanomata EH. Simultaneous infection with dengue 1 and 2 in a Brazilian patient. *Rev Inst Med Trop Sao Paulo*. 1998;40(3):151–154.
48. Pessanha JE, Caiaffa WT, Cecilio AB, et al. Cocirculation of two dengue virus serotypes in individual and pooled samples of *Aedes aegypti* and *Aedes albopictus* larvae. *Rev Soc Bras Med Trop*. 2011;44(1):103–105.
49. Rigau-Perez JG. Severe dengue: the need for new case definitions. *Lancet Infect Dis*. 2006;6(5):297–302.
50. Rigau-Perez JG. The early use of break-bone fever (Quebranta huesos, 1771) and dengue (1801) in Spanish. *Am J Trop Med Hyg*. 1998;59(2):272–274.
51. Halstead SB, Cohen SN. Dengue hemorrhagic fever at 60 years: early evolution of concepts of causation and treatment. *Microbiol Mol Biol Rev*. 2015;79(3):281–291.
52. Back AT, Lundkvist A. Dengue viruses – an overview. *Infect Ecol Epidemiol*. 2013:3.
53. Jaenisch T, Tam DT, Kieu NT, et al. Clinical evaluation of dengue and identification of risk factors for severe disease: protocol for a multicentre study in 8 countries. *BMC Infect Dis*. 2016;16:120.
54. WHO. *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control – New Edition*. Geneva: World Health Organization (WHO) and the Special Programme for Research and Training in Tropical Diseases (TDR); 2009:160.
55. Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Dis*. 2002;2(1):33–42.
56. Whitehorn J, Simmons CP. The pathogenesis of dengue. *Vaccine*. 2011;29(42):7221–7228.
57. Simmons CP, Farrar JJ, Nguyen VV, Wills B. Dengue. *N Engl J Med*. 2012;366(15):1423–1432.
58. Kalayanarooj S, Nimmannitya S. Clinical presentations of dengue hemorrhagic fever in infants compared to children. *J Med Assoc Thailand*. 2003;86(suppl 3):S673–S680.
59. Witayathawornwong P. DHF in infants, late infants and older children: a comparative study. *Southeast Asian J Trop Med Public health*. 2005;36(4):896–900.
60. Wichmann O, Hongsiriwon S, Bowonwatanuwong C, Chotivanich K, Sukthana Y, Pukrittayakamee S. Risk factors and clinical features associated with severe dengue infection in adults and children during the 2001 epidemic in Chonburi, Thailand. *Trop Med Int Health*. 2004;9(9):1022–1029.
61. Guilarde AO, Turchi MD, Siqueira JB Jr, et al. Dengue and dengue hemorrhagic fever among adults: clinical outcomes related to viremia, serotypes, and antibody response. *J Infect Dis*. 2008;197(6):817–824.
62. Hanafusa S, Chanyasanha C, Sujirarat D, Khuankhunsathid I, Yaguchi A, Suzuki T. Clinical features and differences between child and adult dengue infections in Rayong Province, southeast Thailand. *Southeast Asian J Trop Med Public Health*. 2008;39(2):252–259.
63. Lee IK, Liu JW, Yang KD. Clinical and laboratory characteristics and risk factors for fatality in elderly patients with dengue hemorrhagic fever. *Am J Trop Med Hyg*. 2008;79(2):149–153.
64. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes, I. Infection enhancement by non-neutralizing antibody. *J Exp Med*. 1977;146(1):201–217.
65. Srikiatkhachorn A, Rothman AL, Gibbons RV, et al. Dengue – how best to classify it. *Clin Infect Dis*. 2011;53(6):563–567.
66. Huang YH, Chang BI, Lei HY, et al. Antibodies against dengue virus E protein peptide bind to human plasminogen

and inhibit plasmin activity. *Clin Exp Immunol*. 1997;110(1):35–40.
67. Garcia G, Gonzalez N, Perez AB, et al. Long-term persistence of clinical symptoms in dengue-infected persons and its association with immunological disorders. *Int J Infect Dis*. 2011;15(1):e38–e43.
68. Jhanjee A, Bhatia MS, Srivastava S. Mania in dengue fever. *Ind Psychiatry J*. 2011;20(1):56–57.
69. Rapp C, Debord T, Imbert P, Roue R. A psychiatric form of dengue after a visit to Djibouti. *Presse Med*. 2002;31(36):1704.
70. Nilsson J, Vene S, Mattsson L. Dengue encephalitis in a Swedish traveller returning from Thailand. *Scand J Infect Dis*. 2005;37(10):776–778.
71. Forrester NL, Palacios G, Tesh RB, et al. Genome-scale phylogeny of the alphavirus genus suggests a marine origin. *J Virol*. 2012;86(5):2729–2738.
72. King AMQ, Lefkowitz E, Adams MJ, Carstens EB. *Family Togaviridae. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier Science; 2011:1103–1110.
73. Lavergne A, de Thoisy B, Lacoste V, et al. Mayaro virus: complete nucleotide sequence and phylogenetic relationships with other alphaviruses. *Virus Res*. 2006;117(2):283–290.
74. Hallengard D, Kakoulidou M, Lulla A, et al. Novel attenuated Chikungunya vaccine candidates elicit protective immunity in C57BL/6 mice. *J Virol*. 2014;88(5):2858–2866.
75. Weaver SC, Reisen WK. Present and future arboviral threats. *Antiviral Res*. 2010;85(2):328–345.
76. Zacks MA, Paessler S. Encephalitic alphaviruses. *Vet Microbiol*. 2010;140(3–4):281–286.
77. Assuncao-Miranda I, Cruz-Oliveira C, Da Poian AT. Molecular mechanisms involved in the pathogenesis of alphavirus-induced arthritis. *BioMed Res Int*. 2013:973516.
78. Weaver SC, Forrester NL. Chikungunya: evolutionary history and recent epidemic spread. *Antiviral Res*. 2015;120:32–39.
79. Halstead SB. Reappearance of chikungunya, formerly called dengue, in the Americas. *Emerg Infect Dis*. 2015;21(4):557–561.
80. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol*. 2000;81(Pt 2):471–479.
81. Saúde SdVeSMd. *Boletim Epidemiológico*; 2016:2358–9450.
82. Conteville LC, Zanella L, Marin MA, et al. Phylogenetic analyses of chikungunya virus among travelers in Rio de Janeiro, Brazil, 2014–2015. *Mem Inst Oswaldo Cruz*. 2016;111(5):347–348.
83. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. *Trans R Soc Trop Med Hyg*. 1955;49(1):28–32.
84. Kucharz EJ, Cebula-Byrska I. Chikungunya fever. *Eur J Intern Med*. 2012;23(4):325–329.
85. Deller JJ Jr, Russell PK. Chikungunya disease. *Am J Trop Med Hyg*. 1968;17(1):107–111.
86. Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. *Am J Trop Med Hyg*. 2000;62(6):681–685.
87. Mohan A. Chikungunya fever: clinical manifestations & management. *Indian J Med Res*. 2006;124(5):471–474.
88. Simon F, Parola P, Grandadam M, et al. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands. Report of 47 cases. *Medicine*. 2007;86(3):123–137.
89. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Investig*. 2010;120(3):894–906.
90. Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infection. A retrospective study of 107 cases. *South Afr Med J*. 1983;63(9):313–315.
91. Schwartz O, Albert ML. Biology and pathogenesis of chikungunya virus. *Nat Rev Microbiol*. 2010;8(7):491–500.
92. Dupuis-Maguiraga L, Noret M, Brun S, Le Grand R, Gras G, Roques P. Chikungunya disease: infection-associated markers from the acute to the chronic phase of arbovirus-induced arthralgia. *PLoS Negl Trop Dis*. 2012;6(3):e1446.
93. Queyriaux B, Armengaud A, Jeannin C, Couturier E, Peloux-Petiot F. Chikungunya in Europe. *Lancet*. 2008;371(9614):723–724.
94. Sissoko D, Moendandze A, Malvy D, et al. Seroprevalence and risk factors of chikungunya virus infection in Mayotte, Indian Ocean, 2005–2006: a population-based survey. *PLOS ONE*. 2008;3(8):e3066.
95. Hasebe F, Parquet MC, Pandey BD, et al. Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. *J Med Virol*. 2002;67(3):370–374.
96. Halstead SB, Nimmannitya S, Margiotta MR. Dengue d chikungunya virus infection in man in Thailand, 1962–1964, II. Observations on disease in outpatients. *Am J Trop Med Hyg*. 1969;18(6):972–983.
97. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962–1964, I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg*. 1969;18(6):954–971.
98. Jain M, Rai S, Chakravarti A. Chikungunya: a review. *Trop Doct*. 2008;38(2):70–72.
99. Borgherini G, Poubeau P, Staikowsky F, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis*. 2007;44(11):1401–1407.
100. Solanki BS, Arya SC, Maheshwari P. Chikungunya disease with nephritic presentation. *Int J Clin Pract*. 2007;61(11): 1941.
101. Bodenmann P, Genton B. Chikungunya: an epidemic in real time. *Lancet*. 2006;368:9531–10258.
102. Lemant J, Boisson V, Winer A, et al. Serious acute chikungunya virus infection requiring intensive care during the Reunion Island outbreak in 2005–2006. *Crit Care Med*. 2008;36(9):2536–2541.
103. Rampal, Sharda M, Meena H. Neurological complications in Chikungunya fever. *J Assoc Phys India*. 2007;55:765–769.
104. Wielanek AC, Monredon JD, Amrani ME, Roger JC, Serveaux JP. Guillain–Barre syndrome complicating a Chikungunya virus infection. *Neurology*. 2007;69(22):2105–2107.
105. Bhavana K, Tyagi I, Kapila RK. Chikungunya virus induced sudden sensorineural hearing loss. *Int J Pediatr Otorhinolaryngol*. 2008;72(2):257–259.
106. Sahadeo N, Mohammed H, Allicock OM, et al. Molecular characterisation of Chikungunya virus infections in trinidad and comparison of clinical and laboratory features with dengue and other acute febrile cases. *PLoS Negl Trop Dis*. 2015;9(11):e0004199.
107. Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis*. 2007;13(5):764–767.
108. Laurent P, Le Roux K, Grivard P, et al. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. *Clin Chem*. 2007;53(8):1408–1414.
109. Mardekian SK, Roberts AL. Diagnostic options and challenges for Dengue and Chikungunya viruses. *BioMed Res Int*. 2015:834371.

110. Briolant S, Garin D, Scaramozzino N, Jouan A, Crance JM. In vitro inhibition of Chikungunya and Semliki Forest viruses replication by antiviral compounds: synergistic effect of interferon-alpha and ribavirin combination. *Antiviral Res*. 2004;61(2):111–117. Feb.
111. De Lamballerie X, Boisson V, Reynier JC, et al. On chikungunya acute infection and chloroquine treatment. *Vector Borne Zoonot Dis*. 2008;8(6):837–839.
112. Mahendradas P, Ranganna SK, Shetty R, et al. Ocular manifestations associated with chikungunya. *Ophthalmology*. 2008;115(2):287–291.
113. Ravichandran R, Manian M. Ribavirin therapy for Chikungunya arthritis. *J Infect Dev Ctries*. 2008;2(2):140–142.
114. Pessoa R, Patriota JV, Lourdes de Souza M, Felix AC, Mamede N, Sanabani SS. Investigation into an outbreak of dengue-like illness in Pernambuco, Brazil, revealed a cocirculation of Zika, Chikungunya, and Dengue virus type 1. *Medicine*. 2016;95(12):e3201.
115. Furuya-Kanamori L, Liang S, Milinovich G, et al. Co-distribution and co-infection of chikungunya and dengue viruses. *BMC Infect Dis*. 2016;16:84.
116. Mota MTdO, Ribeiro MR, Vedovello D, Nogueira ML. Mayaro virus: a neglected arbovirus of the Americas. *Fut Virol*. 2015;10(9):1109–1122.
117. Vieira CJ, Silva DJ, Barreto ES, et al. Detection of Mayaro virus infections during a dengue outbreak in Mato Grosso, Brazil. *Acta Trop*. 2015;147:12–16.
118. Hoch AL, Peterson NE, LeDuc JW, Pinheiro FP. An outbreak of Mayaro virus disease in Belterra, Brazil. III. Entomological and ecological studies. *Am J Trop Med Hyg*. 1981;30(3):689–698.
119. Long KC, Ziegler SA, Thangamani S, et al. Experimental transmission of Mayaro virus by Aedes aegypti. *Am J Trop Med Hyg*. 2011;85(4):750–757.
120. Forshey BM, Guevara C, Laguna-Torres VA, et al. Arboviral etiologies of acute febrile illnesses in Western South America, 2000–2007. *PLoS Negl Trop Dis*. 2010;4(8):e787.
121. Causey OR, Maroja OM. Mayaro virus: a new human disease agent. III. Investigation of an epidemic of acute febrile illness on the river Guama in Para, Brazil, and isolation of Mayaro virus as causative agent. *Am J Trop Med Hyg*. 1957;6(6):1017–1023.
122. Pinheiro FP, Freitas RB, Travassos da Rosa JF, Gabbay YB, Mello WA, LeDuc JW. An outbreak of Mayaro virus disease in Belterra, Brazil. I. Clinical and virological findings. *Am J Trop Med Hyg*. 1981;30(3):674–681.
123. Vasconcelos PF, Calisher CH. Emergence of human arboviral diseases in the Americas, 2000–2016. *Vector Borne Zoonot Dis*. 2016;16(5):295–301.
124. Neumayr A, Gabriel M, Fritz J, et al. Mayaro virus infection in traveler returning from Amazon Basin, northern Peru. *Emerg Infect Dis*. 2012;18(4):695–696.
125. Pinheiro FP, Leduc JW. Mayaro virus disease. In: Monath TP, ed. *The Arboviruses: Epidemiology and Ecology*. vol 3. 1986:137–150.
126. Mota MT, Vedovello D, Estofolete C, Malossi CD, Araujo JP Jr, Nogueira ML. Complete genome sequence of mayaro virus imported from the Amazon Basin to Sao Paulo State, Brazil. *Genome Announ*. 2015;3(6).
127. Mourao MP, Bastos Mde S, de Figueiredo RP, et al. Mayaro fever in the city of Manaus Brazil, 2007–2008. *Vector Borne Zoonot Dis*. 2012;12(1):42–46.
128. Pinheiro FP. Arboviral zoonoses in South America, Mayaro fever. In: Steele JH, ed. *CRC Handbook Series in Zoonoses*. vol 1. 1981:159–164.
129. Taubitz W, Cramer JP, Kapaun A, et al. Chikungunya fever in travelers: clinical presentation and course. *Clin Infect Dis*. 2007;45(1):e1–e4.
130. Hayes EB. Zika virus outside Africa. *Emerg Infect Dis*. 2009;15(9):1347–1350.
131. Vasconcelos PFC, Travassos da Rosa APA, Pinheiro FP, et al. *Arboviruses Pathogenic for Man in Brazil TRAVASSOS da ROSA, APA; VASCONCELOS, PFC; TRAVASSOS da ROSA, JFS. An Overview of Arbovirology in Brazil and Neighbouring Countries*. Belém: Evandro Chagas Institute; 1998:72–99.
132. Kuno G, Chang GJ. Full-length sequencing and genomic characterization of Bagaza, Kedougou, and Zika viruses. *Arch Virol*. 2007;152(4):687–696.
133. Roby JA, Funk A, Khromykh AA. Flavivirus replication and assembly. In: Shi P-Y, ed. *Flaviviruses*. Singapore: Novartis Institute for Tropical Diseases; 2012.
134. Uchil PD, Kumar AV, Satchidanandam V. Nuclear localization of flavivirus RNA synthesis in infected cells. *J Virol*. 2006;80(11):5451–5464.
135. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis*. 2008;14(8):1232–1239.
136. Faye O, Faye O, Diallo D, Diallo M, Weidmann M, Sall AA. Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. *Virol J*. 2013;10:311.
137. Haddow AD, Schuh AJ, Yasuda CY, et al. Genetic characterization of Zika virus strains: geographic expansion of the Asian lineage. *PLoS Negl Trop Dis*. 2012;6(2):e1477.
138. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med*. 2009;360(24):2536–2543.
139. Cao-Lormeau VM, Roche C, Teissier A, et al. Zika virus, French polynesia, South Pacific, 2013. *Emerg Infect Dis*. 2014;20(6):1085–1086.
140. Dupont-Rouzeyrol M, O'Connor O, Calvez E, et al. Co-infection with Zika and dengue viruses in 2 patients, New Caledonia, 2014. *Emerg Infect Dis*. 2015;21(2):381–382.
141. Pyke AT, Daly MT, Cameron JN, et al. Imported zika virus infection from the Cook Islands into Australia, 2014. *PLoS Curr*. 2014:6.
142. Waehre T, Maagard A, Tappe D, Cadar D, Schmidt-Chanasit J. Zika virus infection after travel to Tahiti, December 2013. *Emerg Infect Dis*. 2014;20(8):1412–1414.
143. Tognarelli J, Ulloa S, Villagra E, et al. A report on the outbreak of Zika virus on Easter Island, South Pacific, 2014. *Arch Virol*. 2016;161(3):665–668.
144. Hennessey MJ, Fischer M, Panella AJ, et al. Zika virus disease in travelers returning to the United States, 2010–2014. *Am J Trop Med Hyg*. 2016;95(1):212–215.
145. Brasil P, Calvet GA, Siqueira AM, et al. Zika virus outbreak in Rio de Janeiro, Brazil: clinical characterization, epidemiological and virological aspects. *PLoS Negl Trop Dis*. 2016;10(4):e0004636.
146. Cardoso CW, Paploski IA, Kikuti M, et al. Outbreak of exanthematous illness associated with Zika, Chikungunya, and Dengue viruses, Salvador, Brazil. *Emerg Infect Dis*. 2015;21(12):2274–2276.
147. Campos GS, Bandeira AC, Sardi SI. Zika virus outbreak, Bahia, Brazil. *Emerg Infect Dis*. 2015;21(10):1885–1886.
148. Nishiura H, Mizumoto K, Villamil-Gomez WE, Rodriguez-Morales AJ. Preliminary estimation of the basic reproduction number of Zika virus infection during Colombia epidemic, 2015–2016. *Travel Med Infect Dis*. 2016;7.
149. Lednicky J, Beau De Rochars VM, El Badry M, et al. Zika virus outbreak in Haiti in 2014: molecular and clinical data. *PLoS Negl Trop Dis*. 2016;10(4):e0004687.
150. Jimenez Corona ME, De la Garza Barroso AL, Rodriguez Martinez JC, et al. Clinical and epidemiological

characterization of laboratory-confirmed autochthonous cases of Zika virus disease in Mexico. *PLoS Curr*. 2016:8.
151. Rodriguez-Morales AJ. Zika: the new arbovirus threat for Latin America. *J Infect Dev Ctries*. 2015;9(6):684–685.
152. Faria NR, Azevedo RdoS, Kraemer MU, et al. Zika virus in the Americas: early epidemiological and genetic findings. *Science*. 2016;352(6283):345–349.
153. Faye O, Freire CC, Iamarino A, et al. Molecular evolution of Zika virus during its emergence in the 20(th) century. *PLoS Negl Trop Dis*. 2014;8(1):e2636.
154. Cugola FR, Fernandes IR, Russo FB, et al. The Brazilian Zika virus strain causes birth defects in experimental models. *Nature*. 2016;534(7606):267–271.
155. Musso D, Cao-Lormeau VM, Gubler DJ. Zika virus: following the path of dengue and chikungunya? *Lancet*. 2015;386(9990):243–244.
156. Heukelbach J, Alencar CH, Kelvin AA, De Oliveira WK, Pamplona de Goes Cavalcanti L. Zika virus outbreak in Brazil. *J Infect Dev Ctries*. 2016;10(2):116–120.
157. Fernanda Estofolete C, Terzian AC, Parreira R, et al. Clinical and laboratory profile of Zika virus infection in dengue suspected patients: a case series. *J Clin Virol*. 2016;81: 25–30.
158. Gubler DJ. Dengue and dengue haemorrhagic fever, Malaysia. *Releve epidemiologique hebdomadaire/Section d'hygiene du Secretariat de la Societe des Nations*. 1998;73(24):182–183.
159. Kwong JC, Druce JD, Leder K. Zika virus infection acquired during brief travel to Indonesia. *Am J Trop Med Hyg*. 2013;89(3):516–517.
160. Zammarchi L, Stella G, Mantella A, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. *J Clin Virol*. 2015;63:32–35.
161. Tappe D, Nachtigall S, Kapaun A, Schnitzler P, Gunther S, Schmidt-Chanasit J. Acute Zika virus infection after travel to Malaysian Borneo, September 2014. *Emerg Infect Dis*. 2015;21(5):911–913.
162. Control ECfDPa. *Rapid Risk Assessment: Zika Virus Epidemic in the Americas: Potential Association with Microcephaly and Guillain–Barré Dyndrome*; 2015. http://ecdc.europa.eu/en/publications/Publications/zika-virus-americas-association-with-microcephaly- rapid-risk-assessment.pdf 2016.
163. Sarno M, Sacramento GA, Khouri R, et al. Zika virus infection and stillbirths: a case of hydrops fetalis, hydranencephaly and fetal demise. *PLoS Negl Trop Dis*. 2016;10(2):e0004517.
164. Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. *N Engl J Med*. 2016;374(10):951–958.
165. Martines RB, Bhatnagar J, Keating MK, 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):159–160.
166. Calvet G, Aguiar RS, Melo AS, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. *Lancet Infect Dis*. 2016.
167. Akoua-Koffi C, Diarrassouba S, Benie VB, et al. Investigation surrounding a fatal case of yellow fever in Cote d'Ivoire in 1999. *Bull Soc Pathol Exot*. 2001;3:227–230.
168. McCrae AW, Kirya BG. Yellow fever and Zika virus epizootics and enzootics in Uganda. *Trans R Soc Trop Med Hyg*. 1982;76(4):552–562.
169. Fagbami AH. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. *J Hyg*. 1979;83(2):213–219.
170. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from Aedes aegypti mosquitoes in Malaysia. *Am J Trop Med Hyg*. 1969;18(3):411–415.
171. *Top Mosquito Suspect Found Infected with Zika*; 2016. http://www.sciencemag.org/news/2016/05/top-mosquito-suspect-found-infected-zika Accessed 28.06.16.
172. Zika. *Epidemiological Update*; 2016. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=34243&lang=en Accessed 28.06.16.
173. Boorman JP, Porterfield JS. A simple technique for infection of mosquitoes with viruses; transmission of Zika virus. *Trans R Soc Trop Med Hyg*. 1956;50(3):238–242.
174. Ayres CF. Identification of Zika virus vectors and implications for control. *Lancet Infect Dis*. 2016;16(3):278–279.
175. 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):442–445.
176. Favoretto S, Araujo D, Oliveira D, et al. First detection of Zika virus in neotropical primates in Brazil: a possible new reservoir. *bioRxiv*. 2016:049395.
177. Picone O, Vauloup-Fellous C, D'Ortenzio E, et al. Zika virus infection during pregnancy. *J Gynecol Obstet Biol Reprod (Paris)*. 2016;11.
178. Brasil P, Pereira JP Jr, Raja Gabaglia C, et al. Zika virus infection in pregnant women in Rio de Janeiro – preliminary report. *N Engl J Med*. 2016;4.
179. Venturi G, Zammarchi L, Fortuna C, et al. An autochthonous case of Zika due to possible sexual transmission, Florence, Italy, 2014. *Euro Surveill*. 2016;21(8).
180. D'Ortenzio E, Matheron S, de Lamballerie X, et al. Evidence of sexual transmission of zika virus. *N Engl J Med*. 2016;13.
181. Marano G, Pupella S, Vaglio S, Liumbruno GM, Grazzini G. Zika virus and the never-ending story of emerging pathogens and Transfusion Medicine. *Blood Transf*. 2016;14(2):95–100.
182. Dupont-Rouzeyrol M, Biron A, O'Connor O, Huguon E, Descloux E. Infectious Zika viral particles in breastmilk. *Lancet*. 2016;387(10023):1051.
183. 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):460–464.
184. Saúde Md. *Situação Epidemiológica/Dados*; 2016. http://portalsaude.saude.gov.br/index.php/situacao-epidemiologica-dados-zika Accessed 01.07.16.
185. WHO. *Zika and Potential Complications, Zika Situation Report*; 2016.
186. Heymann DL, Hodgson A, Sall AA, et al. Zika virus and microcephaly: why is this situation a PHEIC? *Lancet*. 2016;11.
187. *Saúde investiga 4.222 casos suspeitos de microcefalia no país*; 2016. http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/24350-microcefalia-1-638-casos-confirmados-pelo-ministerio-da-saude Accessed 30.06.16.
188. Vogel G. Emerging diseases. A race to explain Brazil's spike in birth defects. *Science*. 2016;351(6269):110–111.
189. *Zika Infection May Be Worse in People Already Exposed to a Common Virus*; 2016. http://www.sciencemag.org/news/2016/06/zika-infection-may-be-worse-people-already-exposed-common-virus?utm_campaign=news_weekly_2016-06-24&et_rid=33529441&et_cid=584788 Accessed 28.06.16.
190. Paul LM, Carlin ER, Jenkins MM, et al. Dengue Virus Antibodies Enhance Zika Virus Infection. *bioRxiv*. 2016.
191. Dejnirattisai W, Supasa P, Wongwiwat W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with zika virus. *Nat Immunol*. 2016;23.
192. Reuters. *Experts Question Assumption that Zika Sickens Just 1 Out of 5*; 2016.

193. *Brasil avalia mudanças no protocolo de microcefalia*; 2016. http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/24346-brasil-avalia-mudancas-no-protocolo-de-microcefalia Accessed 30.06.16.
194. França GVA, Schuler-Faccini L, Oliveira WK, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation. *Lancet*. 2016.
195. Larocca RA, Abbink P, Peron JP, et al. Vaccine protection against Zika virus from Brazil. *Nature*. 2016;28.
196. Fiocru Z. *Fiocruz Bahia mostra resultados de mapeamento genômico do zika*; 2016.
197. Fiocru Z. *Fiocruz Minas cria banco de dados para pesquisas sobre zika*; 2016.