



Review

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.

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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 V¹⁰; these lineages were introduced at different time-points in Goiás state. Genotype V was reported in Manaus¹¹ and Minas Gerais¹² 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 state¹¹ 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 state⁴¹; co-infection with DENV-1/-4, in São José do Rio Preto, São Paulo state⁴²; 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 state⁴⁵; co-infection with DENV-1/-2, in Barretos, São Paulo state⁴⁶; 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 “affection” 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 ($<100,000 \text{ mm}^3$), 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'-m⁷G-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 Tanzania⁷⁸; 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 etiological 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 medications¹¹⁶ 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 etiologic

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 cytoplasm¹³³; 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/mm³.^{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 Malaysia¹⁷⁰; 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}

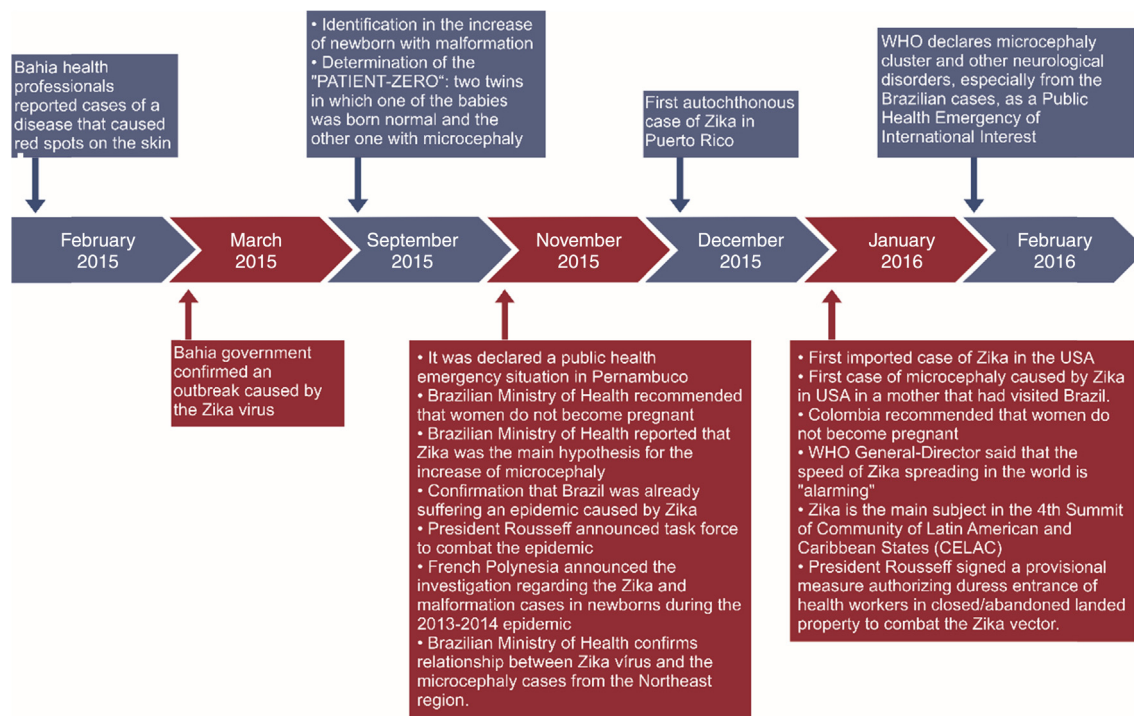


Fig. 1 – Timeline of the ZIKV introduction in the South America.

Adapted from http://www.bbc.com/portuguese/noticias/2016/02/160128_zika_virus_microcefalia_trajectoria_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.

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