Zika virus in Brazil and the danger of infestation by *Aedes (Stegomyia)* mosquitoes

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ABSTRACT

Zika virus, already widely distributed in Africa and Asia, was recently reported in two Northeastern Brazilian: State of Bahia and State of Rio Grande do Norte, and one Southeastern: State of São Paulo. This finding adds a potentially noxious virus to a list of several other viruses that are widely transmitted by *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* in Brazil. The pathology and epidemiology, including the distribution and vectors associated with Zika virus, are reviewed. This review is focused on viruses transmitted by *Aedes (Stegomyia)* mosquitoes, including dengue, Chikungunya, Zika, Mayaro, and yellow fever virus, to emphasize the risks of occurrence for these arboviruses in Brazil and neighboring countries. Other species of *Aedes (Stegomyia)* are discussed, emphasizing their involvement in arbovirus transmission and the possibility of adaptation to environments modified by human activities and introduction in Brazil.

Keywords: *Aedes aegypti*. *Aedes albopictus*. Arbovirus. *Stegomyia*. Culicidae.

INTRODUCTION

Ten arboviruses have recently been reviewed as important emergent disease agents in Brazil[1], of which *Aedes aegypti* can transmit dengue virus (DENV), yellow fever virus (YFV), Chikungunya virus (CHIKV)[2], Venezuelan equine encephalitis virus (VEEV)[3], and Mayaro virus (MAYV)[2][4]. In addition, *Aedes albopictus* can transmit several arboviruses[5], as detailed below.

The DENV, MAYV, YFV, Rocio, Saint Louis, and Oroupoche viruses are responsible for over 95% of cases of arbovirus infection in Brazil; DENV is the most prevalent, causing separate epidemics by dengue virus serotype 1 (DENV-1), dengue virus serotype 2 (DENV-2), dengue virus serotype 3 (DENV-3), and dengue virus serotype 4 (DENV-4), in the country[6]. MAYV was recently identified in sera of patients from Mato Grosso, Brazil, who had been previously diagnosed with dengue fever. MAYV was detected in humans of both sexes between 14 and 62 years of age, most of whom presented with fever, nausea, vomit, myalgia, arthralgia, and ocular and abdominal pain[7].

Zika virus (ZIKV), previously reported in Africa and Asia[8] and now in Brazil[9][10][11], causes dengue fever-like symptoms and is transmitted by *Aedes (Stegomyia)* mosquitoes, emphasizing the need for a review of the vectorial role of these mosquitoes and on diagnosis and disease control.

The division of *Aedes* into *Stegomyia* and other genera[12] has been accepted[13]. However, the more traditional and comprehensive *Aedes* definition, commonly used by public health workers, is utilized here; special abbreviations for genera and subgenera[14] are also utilized.

DENGUE AND CHIKUNGUNYA

The spread of DENV into the Americas from 1995 to 2010 resulted in greater incidences of infection in Brazil, compared to that found in Ecuador, Paraguay, Peru, Costa Rica, Bolivia, Argentina, Mexico, and Nicaragua. A total of 2,756,622 cases of DENV were reported in Brazil between 2002 and 2007, being 98.5% of total in South Cone[15]. The available data demonstrate the dissemination of *Ae. aegypti*, the circulation of 4 DENV serotypes, and difficulty in eradicating or controlling the mosquito.

Coincident with a water-supply crisis in São Paulo, which caused the general population to store water using improvised methods, the numbers of DENV cases (and related deaths) markedly increased in this state. With sequential infections with the 4 serotypes, DENV can cause symptoms ranging from mild influenza-like symptoms to hemorrhagic fever and death. The virus is usually transmitted by *Ae. aegypti*, but can also be transmitted by *Ae. albopictus*.

The habitat of *Aedes aegypti* influences the potential areas of YFV and DENV transmission. Dengue fever occurs predominantly in urban and densely populated areas, where it is transmitted by *Ae. aegypti*, a mosquito also occasionally found in rural areas. A precarious water supply may result in the

maintenance and transportation of water containers containing mosquito eggs, and patients infected by DENV and YFV may then migrate from urban and sylvatic areas, promoting viral dissemination. Although DENV control has been promoted in Brazil, its easy transmission, due to widespread dispersion of *Ae. aegypti* and its good adaptation to urban areas, has made it very difficult. Garbage dispersed in urban areas is the most important breeding site for this mosquito[16].

Migration from rural to urban areas and living under a precarious infrastructure, including the water supply, has contributed to mosquito breeding. In the northeast region of Brazil, over 75% of breeding sites arise due to precarious water storage, while in the southeast region most breeding sites occur in other domiciliary vessels (jars, plant pots, and roof gutters)[16].

In equatorial West Africa, CHIKV has a zoonotic cycle, involving mostly non-human primates and aedine mosquitoes. Although viral antigens have been found in birds and rodents, these animals do not seem to serve an important role in the CHIKV replication cycle, as the principal vectors rarely feed on them[17]. Between epidemic periods, CHIKV is maintained in Africa among *Aedes (Stegomyia) africanus*, *Aedes (Stg.) luteocephalus*, *Aedes (Stg.) furcifer*, and sylvatic primates. Urban outbreaks in east Africa and Asia involve *Ae. aegypti*, *Ae. albopictus*, and humans[18].

Recently, CHIKV has been reported from the United States (Florida), El Salvador, Costa Rica, Panama, Caribbean islands, Venezuela, and the Guyanas[19], and caused a great number of infections in the Brazilian States of Amapá, Bahia, and Minas Gerais in 2014[2]. Because the lineage of CHIKV isolated from the Americas is different from that of the Indian Ocean Lineage (IOL), which is adapted to *Ae. albopictus*, the possibility of transmission by the latter lineage was previously considered remote[20]. However, tests of 22 populations of *Ae. aegypti* and 13 of *Ae. albopictus* from Brazil and 9 other countries showed a high susceptibility and capacity for transmission of both lineages. In fact, 96.7% of an *Ae. albopictus* subpopulation from Rio de Janeiro were capable of transmitting CHIKV, and 1 subpopulation from this city transmitted the virus as soon as 2 days after infection[21].

Large international competitions such as the World Soccer Cup in 2014 and the Olympic games in 2016 may promote the introduction of new viruses into Brazil. The occurrence of CHIKV indicated the possibility of introducing pathogens from regions with different climates, if adequate vectors are available.

Molecular methods used in Korea permitted the diagnosis of CHIKV infection in patients originally suspected as being infected by DENV[22]. Thus, these diseases will need to be adequately diagnosed in Brazil, where both have been reported.

ZIKA VIRUS

Zika virus is a flavivirus, originally isolated in 1947 from a rhesus monkey utilized as bait for a study of YFV in the Zika forest, near Kampala, Uganda[23]. It has been isolated in several African countries (Uganda, Tanzania, Egypt, Central African Republic, Sierra Leone, and Gabon), Asian countries (India, Malaysia, the Philippines, Thailand, Vietnam and Indonesia), and in Micronesia[8] [24].

Diagnosis of ZIKV infection in Brazilian patients has been done by reverse transcription-polymerase chain reactions[11]. A generic test (the Generic reverse transcription (RT)-nested-polymerase chain reaction (PCR) test for flaviviruses), followed by sequencing for specific viruses was proposed[25], and specific diagnosis of ZIKV in field-collected mosquitoes can be accomplished by quantitative real-time PCR[26].

Infection by ZIKV causes symptoms similar to those of DENV (malaise, chills, fever, headache, muscle pain, arthralgia, and periorbital pain). Similarly, no cases of permanent damage to articulations (like those caused by CHIKV) and no deaths have been observed. However, during an investigation of yellow fever in Eastern Nigeria, ZIKV was isolated from 2 icteric patients not infected by malaria or YFV[27]. In mice, ZIKV is highly neurotropic; the virus was not isolated from tissues other than brain, and pathology showed neuronal degeneration, cellular infiltration, and softening in the brain in infected, young mice. Infected Rhesus monkeys presented with transient pyrexia[23]. Seven patients from Indonesia that were serologically reactive to ZIKV showed high fever, malaise, chills, anorexia, vomiting, diarrhea, stomach aches, dizziness, leg pain, lymphadenopathy and, more worrying, hypotension[28].

After the description of a ZIKV-associated case of Guillain-Barré syndrome (tetraparesis predominantly in the lower limbs, paresthesia of the extremities, diffuse myalgia, a bilateral but asymmetric facial palsy, with abolition of deep tendon reflexes) in French Polynesia[29], another 40 cases of this syndrome were described in the same country[30]. Therefore, the qualification of ZIKV as a *mild cousin of dengue*[31] may not be adequate, due to the possibility of more serious disease symptoms, especially in non-immunocompetent patients.

In Italy, 2 cases of ZIKV infection in individuals arriving from French Polynesia were diagnosed, who presented with low-grade fever, malaise, conjunctivitis, myalgia, arthralgia, ankle edema, and axillary and inguinal lymphadenopathy. One patient, returning to Italy from a 12-day stay in Salvador, Bahia, in March 2015, showed leukopenia, with monocytosis and thrombocytopenia[32].

Since February 2015, the Secretary of Health Vigilance [*Secretaria de Vigilância em Saúde* (SVS)] has monitored all patients presenting with exanthematic diseases. All individuals suspected of having ZIKV infection reported by the State secretaries of Bahia, Sergipe, Paraíba, Rio Grande do Norte, and Maranhão have presented with self-limiting symptoms, mostly exanthema, pruritus, occasional fevers, headaches, arthralgia, and muscular pain, and clinical intervention was not required. Most patients were 20 to 40 years old, but their ages varied from 4 months to 98 years old. Eight patients from the Brazilian State of Rio Grande do Norte were diagnosed with ZIKV infection by the Laboratory of Molecular Virology of Carlos Chagas Institute of the Oswaldo Cruz Foundation [*Laboratório de Virologia Molecular* (LVM) *do Instituto Carlos Chagas* (ICC) *da Fundação Oswaldo Cruz* (FIOCRUZ) State of Paraná], utilizing RT-PCR, confirmed by viral genome sequencing,

which indicated that the strains were Asiatic[11]. These findings confirmed ZIKV circulation in Brazil and indicated the need for epidemiological vigilance. ZIKV was recently reported in 7 Brazilian States (the above mentioned Cities, as well as Pará and São Paulo), and a case of transmission by blood transfusion was reported[33]. The isolation of ZIKV from 3% of blood donors in French Polynesia had already raised the potential for this form of transmission[34].

The recent rise in microcephaly incidences in several northeastern states, with 1,248 cases reported in 2015 up through November 30th[35], has been strongly suspected of being associated with ZIKV, with the virus being found in the amniotic fluid of 2 pregnant women whose fetuses presented a reduction in the circumference of the head[36]. This serious effect of ZIKV infection on fetuses, not previously reported, is unsurprising considering the perinatal transmission reported for 2 women from French Polynesia[37] and the strong neurotropism of the virus[23].

Zika virus was probably introduced in Brazil during the World Soccer Cup, in 2014, when many tourists visited the Natal and other Brazilian capitals, possibly contributing to the infection of *Aedes (Stegomyia)* mosquitoes. Because dengue fever occurred in several cities where the games were played, tourists could also have acquired the viruses, possibly carrying them when going back to their respective homes.

Rapid travel and commerce link urban centers allowing for the rapid movement of both vector mosquitoes and infected humans. Commonly, humans have facilitated the widespread establishment of the vectors, with enhanced viral dissemination following shortly thereafter. Established vectors can rapidly move within cars, trucks, planes, or other forms are transportation, allowing a *hop scotch* type of viral transmission, resulting in rapidly and randomly spread outbreaks. From 2011 to 2014, concomitant infections with DENV, CHIKV, and ZIKV (Asian strain) were observed in the Pacific region[38], as could be expected given that these viruses use the same vectors (*Ae. aegypti* and *Ae. albopictus*).

Zika virus has been isolated from *Aedes aegypti*, *Aedes africanus*[23] [39], *Aedes luteocephalus*, *Aedes (Fredwardsius) vittatus*, *Aedes (Stg.) apicoargenteus*, *Aedes (Diceromyia) furcifer* in a village in Senegal[40], and *Aedes albopictus* in Gabon[41], representing the first report of ZIKV transmission by this mosquito in urban areas. ZIKV was also isolated from 31 pools of several species of aedine mosquitoes; from *Mansonia uniformis*, *Culex perfuscus*, and *Anopheles coustani* (one pool each); and *Aedes vittatus* and *Aedes furcifer*[40]; their vectorial roles need to be clarified. Transmission by *Aedes aegypti* was observed[42]. *Aedes (Stg.) hensilli* mosquitoes were not found to be infected by ZIKV in Yap Island, Micronesia, where an outbreak occurred in 2007[24]. However, its predominance in the collections and high susceptibility to experimental infection made it very suspect as a vector of the virus[43]. ZIKV was isolated from semen in a patient from French Polynesia[44], and non-vector borne, probably sexual, transmission was observed in humans in USA[45].

Yellos fever virus was introduced from Africa to the Americas and afterwards adapted to local sylvatic vectors[46]. ZIKV and other arboviruses currently transmitted by urban mosquitoes can also adapt to Neotropical mosquitoes and primates, jeopardizing any future control.

VECTORIAL ROLE OF *AEDES (STEGOMYIA)* MOSQUITOES

Owing to their wide distribution in the American and other continents and their involvement as vectors of several arboviruses, *Aedes (Stegomyia)* mosquitoes are of great medical importance. In Latin America, *Ae. aegypti* and *Ae. albopictus* are widely distributed and very numerous in most small and large cities. As in other continents, rapid human population growth and uncontrolled urbanization, leading to slums with inadequate infrastructure and piped water, have made it very difficult to reduce the populations of *Ae. aegypti* mosquitoes to safe levels.

Zika virus is only 1 additional virus to the list of arbovirus transmitted by the above species of this subgenus, whose vectorial role is briefly discussed below.

The *Stegomyia* subgenus includes 128 species[47], mostly from Africa, Asia, and Oceania. Some of them have been found in manmade containers[48] and/or have adapted to urban conditions. In recent years, these species have been introduced in new areas, like the Americas (*Ae. aegypti* and *Ae. albopictus*) and Europe (*Aedes albopictus*), being implicated as vectors of several arboviruses. The most important species are analyzed below.

Breeding of the sylvatic forms of *Aedes aegypti* such as *Aedes aegypti formosus* in Africa occurs mostly in riverbeds, tree holes, and rock pools, but these aedine mosquitoes have become well adapted to urban environments. Females typically oviposit and immature forms can develop in clean water or water containing a moderate amount of organic matter in various types of vessels, including barrels, tires, plant pots, roof gutters, and more rarely in leaf axils and bamboo stumps[48], stream pools, tree holes, nuts, and coconut shells[49]. *Aedes aegypti* appears to rarely invade sylvatic environments[50], but it has occasionally been identified in 2 localities in Rio Grande do Norte, namely (*Floresta Nacional* (FLONA) – *Nísia Floresta*, Natal), constituted by Atlantic forest, and Ecological Station Seridó, an area with xerophilous vegetation (*caatinga*) (Maria de Fátima M Ximenes, unpublished observation).

Aedes aegypti is the primary vector of DENV and YFV, and its susceptibility to infection is genetically influenced[51]. This mosquito has also been implicated as a vector of CHIKV, and if Mayaro virus becomes adapted to urban environments, *Ae. aegypti* may also serve as a vector for this virus[2]. It has occasionally been found infected with West Nile virus, recently reported in Brazil[52], whose major vectors are *Culex* mosquitoes[53]. *Aedes aegypti* has served as the urban vector of YFV, although YFV has not been transmitted in urban settings in Brazil since 1942. However, because urban YFV infections were recently observed in Bolivia[54], they can also potentially occur in Brazil. The basic reproduction number (R_0) of *Ae. aegypti* for urban YFV transmission was 43% lower than that for the initial phase of DENV epidemics, and several cities in the State of São Paulo with R_0s>1 for DENV are high-risk areas for YFV[55]. The R_0 for CHIKV is 64.4% of that for DENV[56].

Aedes albopictus was previously distributed only in Asia, but was transported to other continents via commerce, and it is now considered an important invasive mosquito[57]. In Brazil, *Ae. albopictus* was present in 59% of municipalities in 2014[58] and in 24 of 27 states[59]. It is adapted to both urban and sylvatic habitats, including bromeliads[60], tree holes (also with *Ae. aegypti* and *Ae. vittatus*)[61], and perforated bamboo internodes[62], and is a suspected link for YFV between preserved and modified environments in the south and southeast regions of Brazil[63]. This species is both endophagic and exophagic, feeding on a wide range of hosts, in comparison to *Ae. aegypti* that feeds mostly indoors on humans.

Experimentally, *Aedes albopictus* may transmit 22 different arboviruses[64]. As a good experimental vector of YFV[65], *Ae. albopictus* could potentially transmit YFV if an overlap of their distribution occurs[63]. *Haemagogus janthinomys*, *Haemagogus leucocelaenus*, and *Ae. serratus* were found to be infected with YFV in Brazil[66][67]. In the Federal District, *Ae. albopictus* was found in São Sebastião, near the locality where YFV was isolated[66], while the northwest region of Rio Grande do Sul currently harbors several cases of DENV infection[68], with the affected areas certainly being infested by *Ae. aegypti* and possibly by *Ae. albopictus*. In central Africa, the presence of *Ae. albopictus* was more frequent than *Ae. aegypti* in suburban environments, and in Libreville (Gabon), CHIKV and DENV were isolated only from *Ae. albopictus*[69]. In Brazil, *Ae. albopictus* does not seem to be an important vector of DENV[70], but its role as vector of CHIKV and other arboviruses in Brazil needs to be more thoroughly studied.

Aedes africanus, abundant and found infected by ZIKV in the Zika forest, was initially suspected as the primary vector for this virus[23]. ZIKV has been repeatedly isolated from *Aedes africanus* in the Zika forest, and only pools from this species (among 115 pools representing several species) were positive[39]. CHIKV also was isolated from *Ae. africanus* mosquitoes and from collectors in this forest, and vertical distribution at night and during the day was different[39], emphasizing the role of this *Aedes (Stegomyia)* mosquito in the zoonotic transmission of both arboviruses.

The introduction of Aedine species of 10 genera (all included here in genus *Aedes*) was reviewed[71], and only *Aedes aegypti* and *Aedes albopictus* of *Stegomyia* were found to be dispersed from their original areas. Other species of this subgenus are normally restricted to the Pacific and Indian oceans or African localities. However, although improbable, it is possible that species such as *Aedes simpsoni*, which is associated with plant axils[72] and artificial containers[73] in Africa and whose role in YFV replication was reproduced by *Aedes albopictus*, *Aedes gardnerii*, and *Aedes desmotes* from bamboos in Philippines[74], could invade and colonize Brazil. *Aedes flavopictus* and *Aedes scutellaris* are also good candidates for invading the American continents, and the last species was found in a California port facility just after World War II and quickly eliminated (Bruce Harrison, personal communication). Several species of the Scutellaris group of *Stegomyia*, which includes *Aedes hensilli* and *Aedes albopictus*, should be considered as potential vectors

of ZIKV in Southeast Asia and Oceania[43]. The finding of *Wyeomyia mitchellii*, a sylvatic bromeliad-associated American species, in Hawaii[75] and French Polynesia[76] indicates the possibility of invasion of new regions by mosquitoes not associated to human modified habitats.

Aedes koreicus and *Aedes japonicus* are 2 examples of invaders of potential health importance. Well adapted to rock pools and other containers[77][78][79][80], the former species is now widely distributed in Europe[77], and the latter is distributed in the USA and Europe[78][79][80]. The ecological and health implications of 5 invaders of Europe (*Aedes aegypti*, *Aedes albopictus*, *Aedes japonicus*, *Aedes koreicus* and *Aedes atropalpus*) were thoroughly reviewed, with the first 2 species already involved in disease transmission in Europe and *Aedes albopictus* and *Aedes japonicus* already showing widespread distribution[80].

Controlling the dissemination of vector insects to prevent the transmission of pathogens requires an understanding of several aspects of their bioecologies. Studies of *Ae. japonicus* have demonstrated that the interaction of this invasive species with other local species influences its population dynamics and the transmission of pathogens. *Aedes japonicus* coexists with other immature mosquito forms and, although not yet completely cleared, at least 2 native species (*Aedes triseriatus* and *Aedes atropalpus*) are being dislodged. Interactions with *Ae. albopictus* also can limit its area of dispersion. Stress in larval environments resulting from competition for food and abiotic factors can enhance the virus transmission efficiency of adults emerging from such habitats. *Aedes albopictus* transmits Japanese encephalitis virus and has shown vectorial competence for CHIKV and DENV, being dispersed in Europe and the United States.

The observation of sexual interference of *Aedes albopictus* male mosquitoes with females of *Ae. aegypti*[81], referred to as satyrism[82], emphasizes the need for additional studies on the ecology of *Aedes (Stegomyia)* mosquitoes and their mutual interactions.

These data illustrate the dangers of invasive species and the needs for vigilance and a good comprehension of mosquito biology and their interactions with other species. While the usual vectors of CHIKV and DENV are known, the possibility that other species may become established as efficient transmitters of arboviruses cannot be discounted. Like *Ae. aegypti*, *Ae. japonicus* utilizes several types of water containers, can be transported in tires, is anthropophilic and peridomestic[83].

The efficient adaptation of *Aedes aegypti* to urban areas makes their control difficult. Only the improvement of sanitation and municipal infrastructure of cities, an almost impossible task in areas with progressive urbanization, budgetary restrictions and low collaboration of populations, can lead to effective control. *Aedes albopictus* is less adapted to domestic environments than is *Ae. aegypti*, but is widely distributed in peridomestic habitats within cities. For example, it is easy to collect eggs laid by *Aedes albopictus* mosquitoes in small ovitraps in the neighborhood of the first author department, located near the busiest avenue of Florianópolis. In the last 10 years, ovitraps installed near State Park Dunas de Natal, the second biggest urban park in Brazil,

have been used to obtain eggs of *Ae. albopictus*, which were similarly obtained near the Federal University of Rio Grande do Norte buildings.

Studies on the feeding preferences of *Aedes albopictus* have indicated a preference for mammals in suburban areas of the USA[84]. Eighty-three percent of female *Ae. albopictus* mosquitoes in suburban areas of Singapore had fed on humans; but some were reactive to shrews, swine, dogs, cats, turtles, and other hosts in rural settings. In urban areas, all positive blood meals were from humans[85].

The wide distribution of DENV and the growing menace of CHIKV, ZIKV, and other viruses in Brazil constitute a serious problem that needs to be addressed. Although some candidate vaccines for DENV will soon be launched in Brazil, vaccines for the other 2 viruses have not been developed.

Thus, at present, the only possible intervention for controlling these arboviruses is an extreme reduction in the populations of *Stegomyia* mosquitoes. Such intervention is less difficult for *Ae. aegypti*, which rarely migrates to forest areas, in contrast to *Ae. albopictus*[84]. Alternative tools for controlling mosquito-borne diseases, like the utilization of *Wolbachia* bacteria[86] and of sterile males associated to insecticides[87] must be investigated further, but the careful reduction of potential breeding places is probably the most important method for their control.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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