INTRODUCTION

Zika virus infection is an emerging disease caused by Zika virus (ZIKV), a mosquito-borne arbovirus of growing public health importance. On 1st February 2016, the disease has been declared as a ‘Public Health Emergency of International Concern’ by the World Health Organization (WHO). ZIKV was first isolated in 1947 from the blood of a rhesus monkey in the Zika forest, a small isolated lake-shore forest in Uganda, from which strains of Chikungunya virus (CHIKV) had also been isolated before. The monkey was a sentinel animal in the Rockefeller Foundation’s programme for research on jungle yellow fever.

The virus is a member of the Spondweni serocomplex that belongs to the family Flaviviridae and to the genus Flavivirus. The latter comprises of approximately 70 viruses, most of which are arthropod-borne, including Yellow Fever virus, West Nile virus, St Louis encephalitis virus, Japanese encephalitis virus and Dengue virus (DENV). Flaviviruses are positive-sense, single-stranded RNA viruses, and obligate intracellular pathogens that replicate in the cytoplasm of infected cells. ZIKV sequenced in 2006, has a 10,794 nucleotide base long genome, encoding a 3419 amino acids polyprotein that is cleaved into three structural (a capsid, a precursor of membrane, and an envelope) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The NS5 protein has an RNA-dependent RNA polymerase activity.

Two major lineages from Africa and Asia have been identified through phylogenetic analyses, while two subclades from the African lineage, demonstrating ZIKV isolates from Senegal/Nigeria and Uganda, have represented West and East African lineages.

ZIKV is sensitive to ether, potassium permanganate and temperatures >60°C, but it is not effectively neutralized with 10% ethanol. Recently, researchers have demonstrated that amotosalen, combined with UV-A light, inactivates ZIKV in fresh-frozen plasma.

Transmission cycle

The main mode of transmission is probably a sylvatic cycle, involving non-human primates and mosquitoes, occurring primarily after bites by hematophagous infected mosquitoes of the Aedes (Stegomyia) genus, within the Culicidae family, such as Ae. aegypti, Ae. albopictus, Ae. africanus, Ae. apicoargenteus, Ae. luteocephalus, Ae. vitattus, Ae. furcifer, Ae. polynesiensis and Ae. hensilli. The Stegomyia subgenus includes 128 species, mostly from Africa, Asia, Oceania and recently from America (Ae. aegypti and Ae. albopictus) and Eu-

ABSTRACT

Zika virus infection is an emerging mosquito-borne disease, first identified in Uganda in 1947. It is caused by the Zika arbovirus, and transmitted by the bites of infected mosquitoes of the genus Aedes. For almost half a century, the Zika virus was reported as the causative agent of sporadic human infections. In 2007, the Zika virus emerged outside Asia and Africa causing an epidemic on the Island of Yap in Micronesia. The manifestation of the newly acquired human infection varies from asymptomatic to self-limiting acute febrile illness with symptoms and clinical features similar to those caused by the Dengue virus (‘Dengue-like syndrome’). The real-time PCR and serological methods have been successfully applied for the diagnosis of the disease. The treatment is symptomatic, since there is no specific antiviral treatment or a vaccine. During the recent outbreaks in French Polynesia and Brazil, incidents of Guillain-Barré syndrome and microcephaly were associated with Zika virus infection, giving rise to fears of further global spread of the virus. Prevention and vector control strategies have to be urgently implemented by national health authorities in order to contain future outbreaks in vulnerable populations. This review summarizes the existing information on Zika virus characteristics, pathogenesis and epidemiology, the available methods for the diagnosis of Zika virus infection and recent approaches for prevention and control.

Key words Aedes; Brazil; Flavivirus Polynesia; Yap, Zika virus
Europe (Ae. albopictus)\textsuperscript{16}. Ae. aegypti is widely found in tropical and subtropical regions\textsuperscript{3} and it is the primary vector of DENV and Yellow Fever virus, while it has also been implicated as vector of CHIKV\textsuperscript{16}. Ae. albopictus, also known as ‘tiger mosquito’, originated in Southeast Asia, but it has recently invaded Africa, America, Australia and Europe, through the transportation of goods and increasing international travel\textsuperscript{17}. Aedes genus mosquitoes breed in various habitats including riverbeds, rock pools and treeholes, and in moist, warm regions worldwide. They are implicated in the endemic and epidemic transmission of the aforementioned viruses, whenever they reach very high densities. Other domestic species, such as Ae. aegypti, have become well adapted to urban environments, breeding in man-made containers, and therefore, playing a significant role in the transmission of several viruses\textsuperscript{7, 16}. After the blood meal, ZIKV multiplies inside the arthropod vector where it remains until its transmission to the mammalian reservoirs by the next blood meal\textsuperscript{15}. The extrinsic incubation period in the mosquitoes has been reported to last approximately for 10 days\textsuperscript{8}. Other mechanisms of maintenance and transmission probably include the vertical or horizontal transmission of the virus, which has been detected from several natural mosquito pools\textsuperscript{1}.

Although epizootics occur in monkeys\textsuperscript{8}, the virus reservoir is not fully identified. Big mammals, such as orangutans, zebras, elephants, as well as smaller mammals such as rodents, have been reported to be potentially obligatory reservoirs in the transmission cycle in humans\textsuperscript{15}. Antibodies have been detected in several other animal species, such as water buffalos, goats, hippos, impalas, kongonis, lions, sheep, and wildebeests\textsuperscript{11}. Experimental transmission of ZIKV to mice and monkeys has also been demonstrated by researchers\textsuperscript{5}. A few days after ZIKV first isolation from a rhesus monkey in 1947, mice that were inoculated with the monkey’s serum also became sick and the virus was isolated from their brains\textsuperscript{5}. One year later, ZIKV was isolated in the same forest from a pool of Ae. africanus mosquitoes\textsuperscript{6}. In areas without non-human primates, humans probably serve as primary amplification hosts and potentially as reservoir hosts if their viremia is sufficient in duration and magnitude\textsuperscript{11}. The first evidence of human infection was reported in 1952, with the presence of neutralizing antibodies in blood sera, collected from individuals from East Africa, while eight years later, the virus was isolated from a patient in Uganda\textsuperscript{18}.

In non vector-borne ways of transmission that have been reported, the maternal-fetal transmission of ZIKV has been documented throughout pregnancy, while pregnant women can be infected in any trimester\textsuperscript{19}. Additionally, ZIKV RNA has been detected in breast milk, but transmission through breastfeeding has not been reported\textsuperscript{20}. Although, human sexual transmission of an arbovirus has not been documented, sexual intercourse has been reported as a less efficient mode of transmission\textsuperscript{21}. It is reported that a female became infected after sexual contact with her husband, who had traveled to an endemic region and after his return he presented common symptoms of the ZIKV disease and also hematospermia and prostatitis\textsuperscript{22}. Moreover, the potential for the virus’ transmission through blood transfusion has been demonstrated during the French Polynesian outbreak, since 2.8% of the blood donors, who were asymptomatic at the time of the donation, became infected by the ZIKV disease\textsuperscript{14}.

**Epidemiology**

The geographical distribution of ZIKV includes East and West Africa, South Asia (mainly the Indian subcontinent) and South East Asia, Micronesia, French Polynesia\textsuperscript{1, 9} and recently South America\textsuperscript{23}, while it is believed that ZIKV emerged in Uganda circa 1920, most probably between 1892 and 1943\textsuperscript{6} (Fig. 1). Although the geographical range of most Aedes species may be limited by cold temperatures, which are unfavourable for eggs survival, this mosquito species distribution is expanding due to global warming\textsuperscript{24}. In addition, there are species, such as Ae. albopictus that can survive and multiply in more temperate regions, due to the ability of their eggs to diapause in the winter\textsuperscript{24}. However, the increase in global temperature most likely has affected the vectors’ survival, the viral replication and infective periods, expanding the geographic distribution of the mosquitoes, increasing their biting rate and decreasing the extrinsic incubation period of the pathogen\textsuperscript{25}. Moreover, ZIKV may have been co-circulating in several regions for many years, but remained undetected, as clinical manifestations might have been attributed to other endemic arboviruses, such as DENV and CHIKV\textsuperscript{26}.

For almost half a century, ZIKV has been reported as the causative agent of rare human infections, and mosquito and sentinel animal surveillance studies have demonstrated the endemicity of ZIKV in Africa and South-east Asia. In addition, serological studies among humans have demonstrated the widespread occurrence of the virus from Africa to Southeast Asia. In 2007, ZIKV emerged outside Asia and Africa for the first time and caused an epidemic on the Yap Island of the Federal States of Micronesia, where approximately three quarters of the residents were infected. Until then, only 14 cases of human ZIKV disease within Africa and Asia have been documented. ZIKV RNA was detected from the serum of the infected patients and it was considered that most likely ZIKV was transmitted by an infected mosquito (Ae. hensilli) or a vireaemic human with an undetected infection. Therefore, the accessibility of air travel and the plethora of the mosquitoes in this region raised big concern for the virus’ spread to other countries in Oceania or America.

In 2013 ZIKV caused a large outbreak in French Polynesia, South Pacific. According to the epidemiological evidence, probable vectors were Ae. aegypti and Ae. polynesiensis, while phylogenetic analysis showed that the viral strains were most closely related to those found on Yap Island and in Cambodia, within the Asian lineage. It is estimated that there were 29,000 suspected cases and 8,510 clinical cases, representing the largest outbreak so far. The outbreak spread to other Pacific Islands, such as New Caledonia, Cook Islands, Easter Island, Vanuatu, and Solomon Islands, probably by infected individuals coming from French Polynesia. The magnitude of the epidemic could be explained by the low levels of pre-existing immunity against ZIKV, since the proportion of IgG specific antibodies in blood donors was 0.8%.

In early 2015, several cases of patients presenting symptoms of ZIKV disease were reported in northeastern Brazil, a dengue endemic area. Large population mobility, widespread occurrence of the transmitting vectors, and favourable climatic conditions have been implicated for the disease spread, causing up to 1.3 million estimated cases by the end of 2015. ZIKV was probably introduced in Brazil during the FIFA World Cup tournament in 2014, when thousands of supporters from several countries visited Brazilian cities, possibly contributing to the infection of Ae. aegypti mosquitoes. Moreover, in August 2014, a world Championship canoe race was held in Brazil, and four Pacific countries participated in this contest. ZIKV has also begun to spread northwards at a rapid rate across several countries of South and Central America. The phylogenetic analyses demonstrated that the viral strain was related to that isolated from patients in French Polynesia and spread among the Pacific Islands, while it was placed in a clade with sequences similar to the Asian lineage. In Europe, the first case of laboratory-confirmed ZIKV infection was diagnosed in a German traveler returning from Thailand, in 2013. Although, the patient reported to have used insect repellents regularly, he had several mosquito bites and became ill a few days after returning back.

Pathogenesis and clinical manifestations

Although, there is insufficient information about the pathogenesis, it is believed that mosquito-borne flaviviruses enter host cells through inoculation and replicate initially in dendritic cells, spreading to the lymph nodes and the bloodstream. Mice infected with ZIKV have demonstrated neuronal degeneration, cellular infiltration and softening in the brain, while monkeys have presented transient pyrexia. The manifestation of the newly acquired human infection varies from asymptomatic to self-limiting acute febrile illness, like an influenza-like syndrome, with symptoms and clinical findings similar to illness resulting from infection with DENV (‘Dengue-like syndrome’) and CHIKV, including fever (from 37.8 to 38.5°C), malaise, headache, maculopapular rash, myalgia, arthralgia (especially of the small joints of hands and feet), and conjunctivitis. Also, there have been reports of chills, dizziness, anorexia, vomiting, diarrhoea, constipation, abdominal pain, mucous membrane ulcerations, pruritus, lymphadenopathy, hypotension, and post-infection asthma that may be frequent. The viremia period lasts approximately three days, between the third and fifth day after the onset of the clinical symptoms, while the mean duration of the symptoms ranges from 3–6 days up to two weeks. However, it is estimated that 80% of the individuals infected with ZIKV are asymptomatic.

The common symptoms in the Yap and Polynesian outbreaks included rash (90–95%), fever (65–73%), arthralgia (65–70%), and conjunctivitis (55–63%), while no hospitalizations, hemorrhagic manifestations or deaths were reported. During the outbreaks in French Polynesia and Brazil, there have been reports of potential neurological and immune-mediated complications of the disease, although prior to the first epidemic, ZIKV infection was recognized as a mild disease (Table 1). Since, viral RNA has been detected in the mothers and amniotic fluid samples from the fetuses, it may have the potential to cause neurodevelopmental dysfunction in the fetus, including microcephaly, being an issue of immediate
The occurrence of such incidents in America\textsuperscript{43}. American ZIKV strains could explain the increasing occurrence of brain matter, leading to the reduced brain size of newborns with microcephaly\textsuperscript{38}. The most common findings that have been reported include widespread calcifications, cell migration abnormalities, cortical and sub-cortical atrophy, excessive and redundant scalp skin, indicating acute intrauterine brain injury\textsuperscript{40}. Ocular findings have been reported in infants as well, including fundoscopic alterations in the macular region\textsuperscript{41}.

Additionally, the infection may be associated with other severe outcomes, such as Guillain-Barré syndrome\textsuperscript{1} probably due to an unspecific immunological mechanism\textsuperscript{28} which was first reported during the large epidemic in French Polynesia. It was characterized by quadriparesis, predominantly in the lower limbs, paraesthesia of the extremities, diffuse myalgia, and a bilateral but asymmetric facial palsy, with abolition of deep tendon reflexes\textsuperscript{16, 42}. The phylogenetic similarity among the Polynesian and American ZIKV strains could explain the increasing occurrence of such incidents in America\textsuperscript{43}.

### Diagnosis
ZIKV disease in humans is usually undiagnosed due to its mild symptoms, or misdiagnosed, because more often the signs and symptoms are similar to other flaviviral diseases\textsuperscript{35}. Additionally, there are no characteristic laboratory findings, with the exception of leucopenia, while, rarely, there have been reports of cases with mild thrombocytopenia\textsuperscript{36}.

The available methods for the diagnosis of ZIKV disease involve several techniques, such as real time-PCR (RT-PCR) and serological assays. RT-PCR has been successfully applied for the diagnosis of ZIKV disease\textsuperscript{8, 15}. The diagnosis is confirmed by isolation of virus from blood samples, collected <5 days after the onset of the symptoms and viral detection using RT-PCR\textsuperscript{15}. RT-PCR may also be performed in urine samples, confirming the infection, particularly after the disappearance of viremia in the serum\textsuperscript{36}, since viral RNA is present longer in urine than in serum\textsuperscript{15} (for >10 days after the onset of the disease)\textsuperscript{23}. ZIKV RNA has also been detected from semen sample\textsuperscript{22}, and from amniotic fluid, highlighting a probable intrauterine infection\textsuperscript{19}.

Several serological assays have also been used for the detection of specific anti-Zika IgM antibodies. Enzyme-linked immunosorbent assay (ELISA) has been used as a diagnostic method at the Arboviral Diagnostic and Reference Laboratory of the Centers for Disease Control and Prevention (CDC) in Atlanta, USA, for the detection of specific IgM antibodies\textsuperscript{5}. However, in the early stages of the infection, the amount of antibodies may be very low, resulting in difficult confirmation of the diagnosis\textsuperscript{15}. Furthermore, cross-reactivity among patients with evidence of previous flaviviruses infections has been reported, especially with dengue virus\textsuperscript{5}. High diagnostic specificity has been observed by the plaque reduction neutralization test (PRNT), in which titers of specific neutralizing antibodies to ZIKV may be determined with a cut-off value of 90% (PRNT\textsubscript{90}), although cross-reactive results have been demonstrated in secondary flaviviruses infections\textsuperscript{5, 27}. According to the case classification scheme of the CDC during the epidemic in Yap, a case was considered confirmed if ZIKV RNA was detected in the tested serum or if all of the following were present: IgM antibodies detected by ELISA, PRNT\textsubscript{90} titer of at least 20, and a ratio ZIKV PRNT\textsubscript{90} titer to dengue or heterologous flaviviruses PRNT\textsubscript{90} titer of at least 4\textsuperscript{12, 27}. Recombinant NS1 microsphere immunoassay (MIA) has been successfully used, demonstrating seroconversions of IgG and IgM specific antibodies in cases which have exhibited cross-reactive results with DENV using other serological assays\textsuperscript{13}. ZIKV has also been serologically confirmed by in-house IgM antibody capture (MAC), indirect IgG ELISA using inactivated antigen\textsuperscript{28}, hemagglutination-in-

### Table 1. Epidemiology and clinical manifestations during Yap, French Polynesia and America ZIKV epidemics

<table>
<thead>
<tr>
<th>Place</th>
<th>Vector</th>
<th>Linage</th>
<th>Estimated cases</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yap Island</td>
<td>\textit{Ae. hensilli}\textsuperscript{27}</td>
<td>Asian\textsuperscript{30}</td>
<td>5,005\textsuperscript{27}</td>
<td>Mild disease\textsuperscript{27}</td>
</tr>
<tr>
<td>French Polynesia</td>
<td>\textit{Ae. aegypti} and \textit{Ae. polynesiensis}\textsuperscript{28}</td>
<td>Asian\textsuperscript{8}</td>
<td>29,000\textsuperscript{15}</td>
<td>Mild disease; potential neurological and autoimmune complications; and Guillain-Barré syndrome\textsuperscript{37}</td>
</tr>
<tr>
<td>America</td>
<td>\textit{Ae. aegypti} and \textit{Ae. albopictus}\textsuperscript{20}</td>
<td>Asian\textsuperscript{23}</td>
<td>1.3 million\textsuperscript{23-33}</td>
<td>Mild disease; potential neurological and autoimmune complications; and Guillain-Barré syndrome; and microcephaly\textsuperscript{16}</td>
</tr>
</tbody>
</table>
hibition test on serum samples\textsuperscript{44} and indirect immunofluorescence assay (IIFA)\textsuperscript{34}. Moreover, on indication of an intrauterine infection, histopathologic examination including immunohistochemical staining, along with RT-PCR, should be performed on the placenta and umbilical cord\textsuperscript{19}.

**Prevention strategies and treatment**

The low pre-existing immunity of a population is one of the factors that should be taken into account when evaluating the potential emergence of arboviruses\textsuperscript{32}. Moreover, mosquitoes of the *Aedes* genus and their breeding sites present a significant risk factor for future ZIKV outbreaks\textsuperscript{8}, along side globalization and environmental changes, resulting in the establishment of these vectors outside their native geographical regions\textsuperscript{45}. Therefore, the presence of tiger mosquitoes in Europe, together with pathogens introduced by international travelers, represent a latent threat for arboviruses transmission\textsuperscript{17}.

Some preventive measures, such as mosquito bite prevention and integrated vector management, are common for all arboviruses\textsuperscript{32}. Prevention against the infection includes individual protection against bites and eradication of vector mosquito breeding sites\textsuperscript{15}. Individual protection includes wearing permethrin-treated, light-colored clothes, using skin repellents, mosquito bed nets, air conditioning and screens\textsuperscript{15, 40}. Pregnant women have been advised by several national health authorities to avoid traveling to affected countries, while in at least five of the affected countries in America, there are instructions to avoid pregnancy\textsuperscript{39}. Regarding the safety of mosquito repellents during pregnancy and lactation, the CDC guidelines consider that all products containing *N, N*-Diethyl-meta-toluamide (DEET), picardin, and IR3535, which are approved by the USEPA (U.S. Environmental Protection Agency) are safe\textsuperscript{19, 40}.

Control strategies include several environmental, chemical, mechanical, genetic and biological methods\textsuperscript{45}. Prevention and vector control measures rely primarily on the eradication of the mosquito-breeding sites (potted plant saucers, used tyres, moats, water reservoirs) by drying or treating them with insecticides, such as deltamethrin, which is currently considered an effective insecticide\textsuperscript{15}. While controlling adult mosquito populations with chemicals is usually ineffective in containment of mosquito-borne outbreaks; several pyrethroids, serving as chemical adulticides, insect growth regulators and larvicides, have been used, showing different modes of effectiveness\textsuperscript{45}. Traps have been suggested for the survey and monitoring of mosquito populations, the use of which is often associated with a decrease in the human biting rates\textsuperscript{45}. The sterile insect technique (SIT), is a species-specific method of insect control, which is based on the release of sterile insects to the environment, from which the males exert competitive action against the ‘wild’ ones, leading to a decrease in the females’ reproductive potential\textsuperscript{46}. Another strategy which has been previously introduced in the DENV and CHIKV endemic regions includes the incompatible insect technique (IIT), which uses the *Wolbachia* bacteria, especially *Wolbachia*-caused cytoplasmic incompatibility, replacing existing populations of male mosquitoes from the genus of *Aedes* with *Wolbachia*-infected ones, which are unable to reproduce with uninfected females, or females infected with another strain of the bacterium\textsuperscript{24, 47}. Moreover, entomopathogenic fungi and bacteria, copepods and plant essential oils have been proposed as alternatives in vector control\textsuperscript{45}.

Since vector control has been proven the most effective countermeasure against vector-borne diseases, it is imperative to implement more efficient prevention strategies; however, difficulties in the implementation of vector control measures has led to an alarming prevalence of these diseases\textsuperscript{46}. Additionally, the current spread of ZIKV poses a challenge for national health systems, due to the risk of the concurrent transmission of DENV or CHIKV by vectors of the *Aedes* genus\textsuperscript{48}. The failure of the national vector control methods is usually attributed to several factors, such as the numerous, cryptic and inaccessible breeding sites of the mosquitoes in specific geographical regions\textsuperscript{47}, the insecticide resistance that has contributed to high densities of *Ae. aegypti* populations, field operational issues related to vector control activities resulting in low entomological effectiveness and poor health education which failed to achieve a sustained community-based control of *Ae. aegypti*\textsuperscript{24}. The ineffectiveness of the vector control strategies in combination with the increasing risk of ZIKV infection, signify the urgent need for the development of an effective and safe vaccine. So far the treatment of the disease is symptomatic; combining acetaminophen and antihistaminic drugs, given that there is no specific antiviral treatment or a vaccine\textsuperscript{15}.

**CONCLUSION**

Zika virus infection has recently been demonstrated as a global threat, changing its manifestations from an endemic mild disease to an epidemic severe one, probably associated with neurological complications, such as microcephaly and Guillian-Barré syndrome. Taking into consideration the climate change and its consequences on global ecology, the fact that the majority of the human population lives in areas infested by mosquitoes of the
Aedes genus, as well as the effects of globalization and increased air travel in the spread of infectious diseases, the potential of a ZIKV pandemic is of immense concern. Molecular and serological methods have been successfully established for the laboratory diagnosis of the disease; however, since in most cases there are no typical clinical symptoms or laboratory findings, ZIKV disease may be misdiagnosed or confused with other flaviviral diseases. Therefore, more effective vector control and surveillance measures are required along with the development of effective drugs and vaccines for controlling the infection.

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