Zika virus infection: epidemiology, clinical manifestations and diagnosis

Guilherme Amaral Calvet[a], *Flavia Barreto dos Santos*[b], *and Patricia Carvalho Sequeira*[b]

Purpose of review
Zika virus (ZIKV) is an arbovirus previously believed to cause only a mild and self-limiting illness. Recently, it has emerged as a new public health threat that caused a large outbreak in French Polynesia in 2013–2014 and since 2015 an explosive outbreak in Brazil, with an increase in severe congenital malformations (microcephaly) and neurological complications, mainly Guillain–Barré syndrome (GBS). Since then, it has spread through the Americas. On 1 February 2016, the WHO declared the ZIKV epidemic in Brazil a Public Health Emergency of International Concern. We reviewed the epidemiology of ZIKV infection, clinical presentations and diagnosis. We highlighted the clinical features and nonvector borne transmission of the virus.

Recent findings
Association between ZIKV infection and severe foetal outcomes, including microcephaly and other birth defects; increased rate of GBS and other neurological complications due to the ongoing ZIKV outbreak; increased evidence to date of ZIKV being the only arbovirus linked to sexual transmission; the challenge of ZIKV diagnosis; and the need for a specific point-of care test in epidemic scenarios.

Summary
The findings illustrate the emergence of a viral disease with the identification of new associated disorders, new modes of transmission, including maternal–foetal and sexual transmission.

Keywords
Guillain–Barré syndrome, microcephaly, sexual transmission, Zika virus

INTRODUCTION

Few studies related to Zika virus (ZIKV) have been published up until 2014 [1]. A literature production increase was observed following the French Polynesia outbreak in 2013–2014. But it was only after the explosive ZIKV outbreak in Brazil in 2015 where an increase in reported cases of microcephaly and neurological disorders occurred that several case reports, editorials, letters, research reports, perspectives and reviews were published, impacting the global scientific production in the field. This review describes the current epidemiology of ZIKV infections, clinical presentations and diagnosis. We highlighted the clinical features and nonvector borne transmission of the virus.

EPIDEMIOLOGY OF ZIKA VIRUS

ZIKV, a mosquito-transmitted virus in the family *Flaviviridae* and genus *Flavivirus*, was discovered in 1947 with little impact in public health systems worldwide for the following seven decades. Only 14 human cases of the disease were reported in countries in Southeast Asia and Africa [2]. In 2007, ZIKV was first detected outside of Asia and Africa, causing the first large outbreak ever reported [3]. Yap State, located at the Federated States of Micronesia in the Western Pacific, estimated that over 72% of their residents over 3 years of age were infected with ZIKV [4]. The origin of the ZIKV that caused this epidemic remains to be clarified, but it has been hypothesized

[a]Acute Febrile Illnesses Laboratory, Evandro Chagas National Institute of Infectious Diseases/Oswaldo Cruz Foundation and [b]Flavivirus Laboratory, Oswaldo Cruz Institute/Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

Correspondence to Guilherme Amaral Calvet, MD, PhD, Instituto Nacional de Infectologia Evandro Chagas, Laboratório de Pesquisa Clínica em Doenças Febris Agudas–Fundação Oswaldo Cruz, Av. Brasil, 4365 - Manguinhos, Rio de Janeiro, CEP: 21045-360, Brazil.
Tel: +55 21 38659110; e-mail: guilherme.calvet@ini.fiocruz.br

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KEY POINTS

- Emergence of ZIKV infection in Brazil in 2015 and rapid spread of ZIKV to the Americas.
- Severe congenital abnormalities and neurological complications such as Guillain–Barré syndrome are linked to ZIKV infection.
- Increasing evidence of ZIKV being the only arbovirus linked to sexual transmission to date.

that a viremic person travelling from the Philippines could have introduced it [5].

In October 2013, ZIKV reached the French Polynesia in the South Pacific region and until April 2014 an estimate of 30 000 people were infected, causing the largest ZIKV outbreak ever described at that time [4,6]. Severe neurological manifestations and an increase in Guillan–Barré syndrome (GBS) were reported, suggesting a possible association between ZIKV and GBS [6]. During and after this outbreak, ZIKV rapidly spread to other Pacific islands [4]. New Caledonia health authorities declared a ZIKV outbreak in February 2014, and by the end of August, 1400 cases had been reported [7]. A small outbreak of ZIKV was confirmed in the Cook Islands in March 2014, with only 905 cases reported [8]. Local health authorities reported the first autochtonous case of ZIKV infection in Chile on 28 January 2014 after the confirmation of a suspected case on Easter Island (National Travel Health Network and Centre, http://nathnac.net/). Fifty cases of Zika in Chile were confirmed by the end of the outbreak [9].

In March 2015, researchers at the Federal University of Bahia, Brazil, confirmed the introduction of ZIKV in Brazil [10]. Serum samples from 24 exhantematic patients from Camaçari, Bahia, Brazil, were tested and seven of them presented positive diagnosis for ZIKV by real-time PCR (RT-PCR). Autochthonous transmission in Brazil was later confirmed [11▪] and, by December 2015, states from all regions of the country had already reported autochthonous virus transmission [12]. It has been estimated that the number of suspected cases of ZIKV infection ranged from 440 000 to 1 300 000 by the end of 2015 [13]. Two hypotheses regarding the ZIKV introduction in Brazil had been initially raised, with the first suggesting that the virus was introduced during the soccer World Cup event held in Brazil in 2014 [14]. Another hypothesis was the virus introduction during the World Spring Canoe championship held in Rio de Janeiro in 2014, from a viremic athlete from one of the participating Pacific countries [15]. Faria *et al.* [16] have recently raised a third hypothesis for ZIKV introduction in the Americas, suggesting the viral introduction during the 2013 Confederations Cup soccer tournament, which occurred from 15 to 30 June 2013. In all potential cases, ZIKV introduction was probably initially unnoticed because ZIKV clinical manifestations can be confused with those caused by dengue (DENV) and chikungunya (CHIKV) viruses, both already endemic in Brazil. The country has notified 120 161 probable cases and 39 993 confirmations until the 16th epidemiological week of 2016 [17]. The explosive Zika outbreak in Brazil has provided data on the association between microcephaly and/or neurological disorders and ZIKV infection. As a result, on 1 February 2016, the WHO declared the ZIKV epidemic in Brazil a Public Health Emergency of International Concern.

According to the Pan American Health Organization (PAHO) epidemiological update from 2 June 2016, a total of 39 countries and territories in the Region of the Americas have confirmed local, vector-borne transmission of ZIKV since 2015 [18].

A number of imported cases of ZIKV infection have been reported from travellers from Europe, the Americas, Asia and the Pacific returning from endemic areas where ZIKV epidemics were ongoing [19–21]. The importation of cases to areas where competent mosquitoes are present poses a risk of mass dissemination of ZIKV globally.

ZIKA CLINICAL FEATURES

It is estimated that 80% of ZIKV infections are asymptomatic [3]. The majority of clinical manifestations of ZIKV infections are usually mild and self-limiting [20,22–24] after a not well established incubation period, but probably similar to other related flavivirus (3 days–2 weeks) [25]. Figures 1–4 show some clinical manifestations of ZIKV infection.

In a report during the Zika outbreak that occurred in the state of Rio de Janeiro, Brazil, based on a large number of suspected and laboratory confirmed cases, the most common signs or symptoms were macular or maculopapular rash (97%), followed by pruritus (79%), prostration (73%), headache (66%), arthralgias (63%), myalgias (61%), nonpurulent conjunctivitis (56%) and lower back pain (51%). Fever, when present, was low grade and short-term. The authors suggest that pruritus, the second most common clinical sign presented by the confirmed cases, should be added to the PAHO case definition [26▪▪]. In another study, Jimenez Corona *et al.* [27] analysed 93 autochthonous cases of Zika in Mexico. The main clinical features were fever (96.6%), rash (93.3%), nonpurulent conjunctivitis (88.8%), headache (85.4%) and myalgia (84.3%) [27].

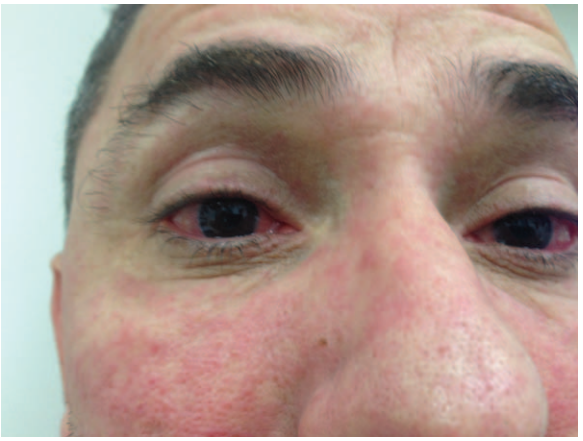

FIGURE 1. Nonpurulent conjunctivitis and facial exanthema.

Usually, ZIKV infection related fever is low grade [26▪▪], but some cases reported high fever (up to 40°C) [11▪,28,29]. Lymphadenopathy [11▪,20,26▪▪,29,30▪], severe abdominal pain [31], thrombocytopenia, enantema and haematomas have been described in patients with ZIKV infection in some reports [20,26▪▪,32–36]. Severe thrombocytopenia is uncommon, but the number of reports is growing [32,35] with some cases progressing to death [37,38]. Haematospermia has also been reported [39,40].

Clinical characteristics and outcome data on ZIKV infection in young children are scarce, but based on limited information, ZIKV infection in children is mild and similar to that in adults [34,41].

The arboviral burden of diseases caused by cocirculation of DENV, CHIKV and ZIKV in the Americas [42,43] can lead to coinfections and has been reported previously [44,45]. The clinical spectrum of manifestations and the overall epidemiological relevance of those coinfections are still unknown and should be accessed.

Fatalities attributed to ZIKV are rare, excluding foetal losses among women infected during pregnancy and newborns with severe congenital ZIKV disease. However, because the current epidemic is rapidly evolving, some deaths related to ZIKV have been reported [17,37,38,46]. In October 2015, a 15-year-old girl previously diagnosed with sickle cell disease died with vaso-occlusion, triggered by inflammation and severe splenic sequestration [46]. Another four deaths were reported in Colombia [38]. Until May 2016, three people died from complications linked to the ZIKV, according to Brazilian health officials [17]. In Puerto Rico, a 70-year-old man died of complications related to severe thrombocytopenia at the end of February 2016 [37].

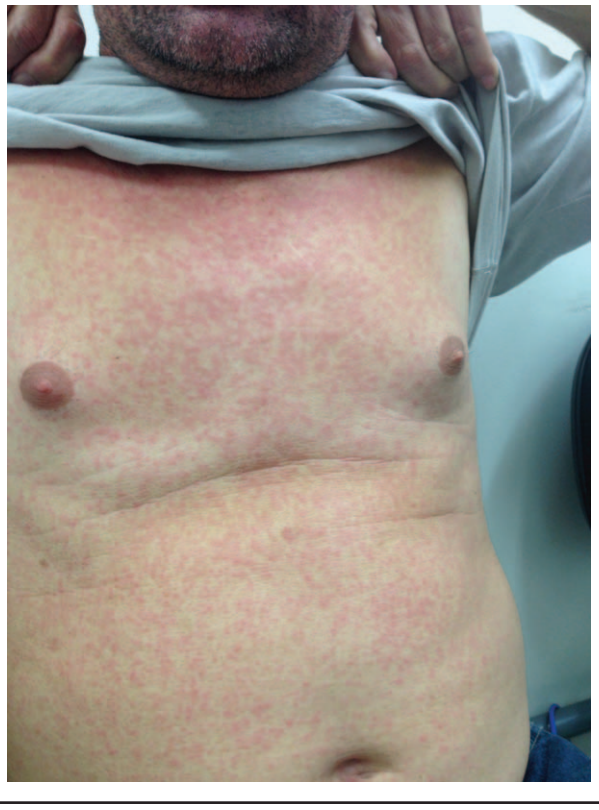

FIGURE 2. Maculopapular exanthema on the trunk.

Neurological and congenital Zika syndrome

The spectrum of the Zika clinical manifestations is increasing as the epidemic is spreading [18]. Clinical manifestations have apparently changed since the large French Polynesian outbreak in 2013–2014 [5], when severe neurological complications were reported [47▪▪], followed by an increase in severe congenital malformations in the emergence in Brazil in 2015 [48].

Oehler *et al.* [48] described the first GBS case occurring immediately after a ZIKV infection, during the French Polynesia outbreak. Cao-Lormeau *et al.* [47▪▪] provided further evidence for ZIKV infection causing GBS during the French Polynesia outbreak. As of 12 May 2016, 13 countries and territories worldwide have reported an increased incidence of GBS cases following a recent ZIKV infection [28,48–53].

Carteaux *et al.* [54] described a case of ZIKV-associated meningoencephalitis in an 81-year-old man. Mecharles *et al.* [55] reported a case of acute myelitis in a 15-year-old girl. The presence of ZIKV in the cerebrospinal fluid (CSF) of those patients suggests that the virus might be neurotropic. Roze *et al.* [56] described two cases of encephalopathy in adult patients with ZIKV infection.

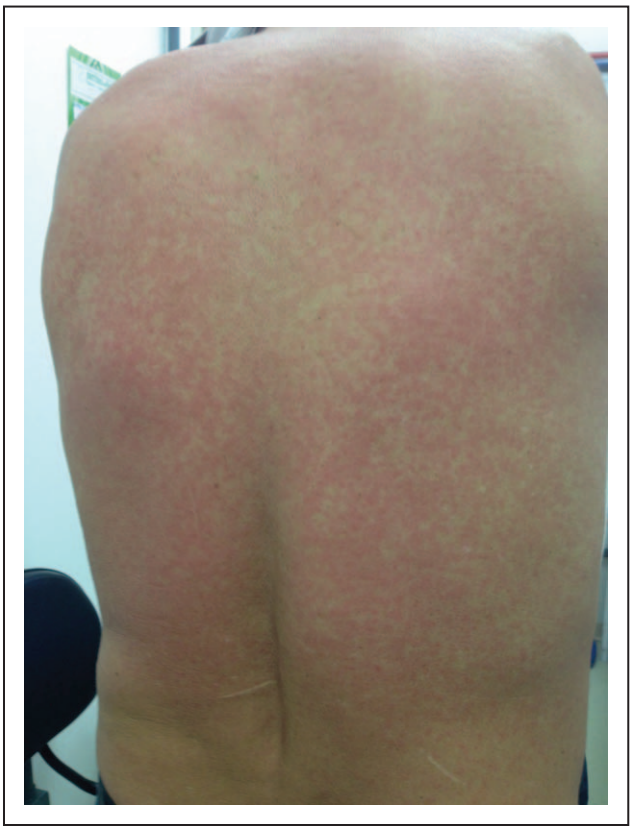

FIGURE 3. Maculopapular exanthema on the posterior trunk.

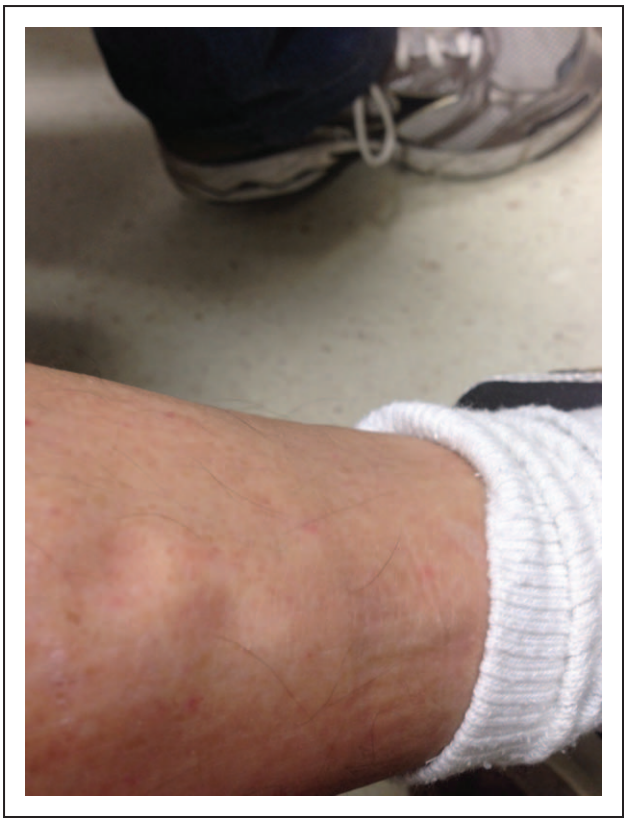

FIGURE 4. Oedema in the lower limbs.

The microcephaly prevalence in foetuses and newborns increased remarkably during 2015–2016 in Brazil. The largest increase occurred in the Northeast region, where ZIKV was first reported [57]. Oliveira Melo *et al.* [58▪▪] showed the evidence of two cases of foetal microcephaly in women reporting ZIKV-like symptoms during pregnancy by ultrasound imaging, with further ZIKV detection by RT-PCR in their amniotic fluid. The whole ZIKV genome was characterized and published [59]. A case of confirmed ZIKV infection in a foetal demise with hydrops fetalis and microcephaly was reported by Sarno *et al.* [60]. Several other ZIKV-related cases of microcephaly have also been reported [61–64]. Noronha *et al.* [65] provided additional evidence of the transplacental transmission and neurotropism of ZIKV.

Polynesia estimated that the risk of microcephaly due to ZIKV infection in the first trimester of pregnancy was 0.95% based on eight microcephaly cases identified retrospectively in a population of approximately 270 000 people with an estimated ZIKV infection rate of 66% [66▪▪].

Some pregnant cohorts were already established to study infant outcomes exposed to ZIKV [30▪,67]. Brasil *et al.* [30▪] published preliminary results of 88 pregnant women. Among the 72 women with confirmed ZIKV infection, 42 underwent prenatal ultrasonography, and foetal abnormalities were observed in 12 (29%); none of the 16 women with negative tests had foetal abnormalities. The abnormalities observed on ultrasonography varied widely [30▪].

Since 2015, several reports were published describing a wide range of congenital abnormalities probably associated with ZIKV infection *in utero*. The WHO [68] has started a process for defining the spectrum of this syndrome to map and analyse the clinical manifestations including visual abnormalities [69–71] and neuroimaging findings [72–75].

Nonvector-borne modes of transmission

Apart from perinatal transmission, there is increased evidence of nonvector-borne forms of ZIKV transmission, including sexual transmission [76]. Foy *et al.* [39] described clinical and serologic evidence indicating that one scientist transmitted ZIKV to his wife probably by sexual contact after his return home. Venturi *et al.* [77] retrospectively diagnosed a case of ZIKV infection leading to a secondary autochthonous case, probably by sexual transmission. D'Ortenzio *et al.* [78] presented a case with

more evidence supporting the hypothesis for ZIKV sexual transmission (either oral or vaginal). Deckard *et al.* [79] described the first report of ZIKV transmission from an infected man to a sex partner through anal sex.

Musso *et al.* [40] detected a high ZIKV replicative RNA load in semen samples. Furthermore, high viral load in the semen (100 000 times more) compared with urine and blood was described in a 32-year-old man infected by ZIKV [80▪]. Atkinson *et al.* [81] described the detection of ZIKV RNA in semen 62 days after the onset of illness, but infectious virus was not cultured. The prolonged presence of virus in semen could indicate a prolonged potential for ZIKV sexual transmission [81]. The CDC and WHO have issued interim guidance for the prevention of ZIKV sexual transmission [82,83].

ZIKV is potentially transmissible via blood products and organ or tissue transplantation [84,85]. So far, there is no scientific evidence supporting that ZIKV could be transmitted through human saliva, breast milk and urine, although some studies have reported infectivity viral particles confirmed by the presence of a cytopathic effect onto Vero cells [86–88].

LABORATORIAL DIAGNOSIS OF ZIKA INFECTIONS

The diagnosis of Zika infections can be performed on clinical-epidemiological and laboratorial bases. Currently, ZIKV can be detected in distinct clinical specimens such as blood (plasma, serum), CSF, urine, saliva, breast milk, semen, vaginal secretion, amniotic fluid and tissues [28,39,59,78,89–96].

Overall, the laboratorial diagnosis of ZIKV infection relies on the same usual strategies used for other arboviruses, with viral genome detection by RT-PCR tests on acute-phase samples and serology (ELISA and immunofluorescence) for detection of specific antibody against the virus. The virus isolation may also be used in acute samples, but it is a more laborious and time-consuming approach, also requiring a more robust infrastructure, not available in most laboratories. However, as many other flaviviruses, results based on more routinely used serological tests may be compromised by a cross-reactivity in convalescent samples due to previous flaviviruses infections. For a more reliable result, flaviviruses diagnosis should test paired acute and convalescent samples, collected 2–3 weeks after the onset of symptoms. Usually, the choice of the laboratorial approach used will depend on the goal of the analysis, laboratory infrastructure, technical expertise and sampling availability.

During the acute phase of the disease, virus or viral nucleic acid detection can be performed. The virus isolation, despite not being performed on a routine basis, can be accomplished using mosquitoes cells (such as AP-61, *Aedes pseudoscutellaris;* C6/36, *Aedes albopictus*) or mammalian cell lines (such as BHK, VERO), directly from infected mosquitoes or by innoculation into newborn mice [97–100]. Even though the virus characterization is important, the isolation may be difficult due to the low viremia during the acute phase of ZIKV infection [94].

The most commonly and widely used diagnostic technique for ZIKV diagnosis is based on the virus molecular detection by conventional or real-time RT-PCR, and some protocols have been described for detecting ZIKV within the group [101–104] or specific to the virus [94,105–107]. The viral genome detection by molecular techniques provides a definitive diagnostic result, but as viremia is transient, this approach is most reliable if performed within the first week of the disease [94]. By using quantitive RT-PCR, the RNA viral load in blood (7.28×10^6 to 9.3×10^8 copies RNA/ml), urine (2.5×10^3 to 8×10^6 copies RNA/ml), semen (1.1×10^7 to 2.9×10^7 copies RNA/ml) and breast milk (2.9×10^4 to 2×10^6 copies RNA/ml) could be determined [5,20,89,90,94,108]. Although the ZIKV genome has been detected on amniotic fluid, cord blood, CSF and placenta by RT-PCR, the method's sensitivity on those specimens is unknown [109].

Anti-ZIKV IgM and IgG antibodies can be detected by serological assays. However, the results should be carefully analysed due to false-positive results from possible cross-reactivity with other flaviviruses. The virus-specific IgM may be detectable more than 5 days after onset of symptoms. In some case, these biomarkers, indicative of an active infection, may rise as early as day 3 of illness and last over 2 months [91,94,110]. Seroconversion represented by at least a four-fold increase in paired sera, acute and convalescent sera, is desirable for a more reliable result. In a flavivirus-naive patient, a minimal cross-reactivity is obtained, but a significant cross-reactivity in patients exposed to a previous flavivirus infection is observed, even on yellow fever vaccinated patients.

Despite the gold standard for the specific detection of antiflavivirus anitbodies, the plaque-reduction test (PRNT) [111], useful for cross-reactive results on ZIKV infection where other flavivirus circulate, the assay is arduous and time-consuming, and specialized infrastructure and lab personnel are needed due to the live virus manipulation.

For the diagnosis of Zika congenital infection, CDC recommends the performance of both molecular and serological tests, as it is not known which one would be more reliable for the condition investigation [112]. With tissue availability,

immunohistochemistry assays may also be performed for viral antigen detection [62]. Furthermore, aiming to diagnose congenital ZIKV infections, CSF, placenta, blood cord and/or umbilical cord tissues from neonates or unborn foetuses shall be analysed.

CONCLUSION

Our current understanding of the pathophysiology of ZIKV infection needs to be strengthened and epidemiological studies and animal models of the disease need to be developed. Public policies need to be implemented, effective preventive measures such as vaccines created and improving efforts to reduce transmission through vectors and more studies on sexual transmission are necessary. Accurate, portable and inexpensive point-of-care tests are in need to better identify cases, especially where other arboviruses cocirculate. Due to the difficulties in propagating the virus, antigen availability in many laboratories poses a burden for the diagnosis, and the development and use of recombinant antigens are desirable.

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Conflicts of interest

There are no conflicts of interest.

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