

Science & Society

Zika Virus and
Microcephaly:
Challenges for a
Long-Term AgendaClaudia Nunes Duarte dos
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Since its introduction in Brazil in 2015, Zika virus (ZIKV) has begun to spread worldwide. One of the major infection outcomes is related to congenital malformations, but little is known about the pathogenicity of ZIKV. Here we discuss concerns about the ongoing ZIKV epidemic in the context of academic research, politics, and society.

The observation of atypical presumed cases of dengue fever by physicians in the states of Rio Grande do Norte and Bahia in Northeast Brazil in February 2014 (Figure 1) raised the possibility that they might be dealing with Zika fever. An analysis of samples by RT-PCR and sequencing confirmed the presence of ZIKV in the sera of these patients [1]. At that time, Brazil was experiencing a huge dengue epidemic that was far from controlled. Moreover, the introduction and spread of two different lineages of Chikungunya virus (CHIKV) in the north (Asian strain) and northeast (African strain) of the country since March 2014, contributed to a complex epidemiological picture, with the co-circulation of three different arboviruses, all apparently using the same urban mosquito species, *Aedes aegypti*, as a vector.

In February 2014, almost no data were available relating to ZIKV biology and pathogenesis, and only a few cases of mild, self-limited disease had been observed in humans. Epidemics caused by ZIKV were observed in the Yap Islands in Micronesia

in 2013 and in French Polynesia in 2014, but no major clinical outcomes were reported [2].

In the following months, ZIKV spread to almost all Brazilian territories. More than 2 million human cases have been estimated by the Public Health Services, whereas CHIKV infections totaled only 26 900 cases (<http://portalsaude.saude.gov.br/index.php/situacao-epidemiologica-dados-zika>). Given that CHIKV was introduced a year earlier, in 2104, and both viruses are transmitted by the widely distributed *Ae. aegypti* mosquito, the discrepancy in the number of cases could indicate additional transmission mechanisms for ZIKV infection in humans (discussed below).

By October 2015, an unusual increase in the number of birth defects, such as microcephaly and other congenital malformations, was observed in the northeast region of Brazil. There was a strong temporal relation between the beginning of the ZIKV epidemic and the birth of these babies. Indeed, the common link among these cases was that the mothers reported clinical signs compatible with ZIKV during pregnancy, mostly within the first trimester [3]. The implication of ZIKV in these clinical outcomes provoked discussion among the scientific community in Brazil because this phenomenon was unprecedented in the literature. Furthermore, the correlation between the increase in microcephaly and ZIKV infection was criticized by some members of the scientific community because notification of microcephaly to health authorities was not compulsory and, thus, the numbers from past years could have been underestimated [4]. Interestingly, a short time after the description of these clinical outcomes in Brazil, the health authorities of French Polynesia recognized a putative association between microcephaly and ZIKV. The observation of fewer of these cases in French Polynesia could be a result of abortions due to diagnosed congenital defects during the epidemics in the islands because this procedure is

not forbidden in the French territory, as opposed to Brazil.

In Brazil, 863 cases of microcephaly had been confirmed from October 2015 to March 2016, and 6480 cases are under investigation. While cohorts are being established and case-control studies are underway to definitely link ZIKV to microcephaly and other neurological manifestations, there is growing evidence to incriminate ZIKV in these congenital malformations. The first clue came from the isolation of viral RNA from the amniotic fluid of two pregnant women whose fetuses were found by ultrasound to have microcephaly [5]. Furthermore, ZIKV was detected in the placental and brain tissues of a fetus presenting with neurological birth defects, indicating vertical transmission from an expectant mother who had ZIKV symptoms at the end of the first trimester of pregnancy [6]. More recently, a study demonstrated the transplacental transmission of ZIKV through the detection of viral proteins and viral RNA in placental tissue samples from expectant mothers infected at different stages of gestation as well as in necropsy brain tissues from fetuses and newborns who died just after birth due to severe neurological disorders [7]. In this study, the possibility of a chronic placental infection was highlighted because, in two of the studied cases, both women reported ZIKV-compatible symptoms at the very start of pregnancy, and the virus persisted in their placentas until the birth of their babies. Despite the small number of cases studied, these results raise concerns about the persistence of ZIKV in some body tissues.

At the same time, an increase was observed in the number of reported cases of Guillain–Barre syndrome associated with previous clinical ZIKV infection. During the ZIKV epidemics in French Polynesia, an increase in Guillain–Barre cases was noticed, but only recently has a case-control study unequivocally implicated ZIKV infection in triggering this neurological syndrome [8]. The microcephaly

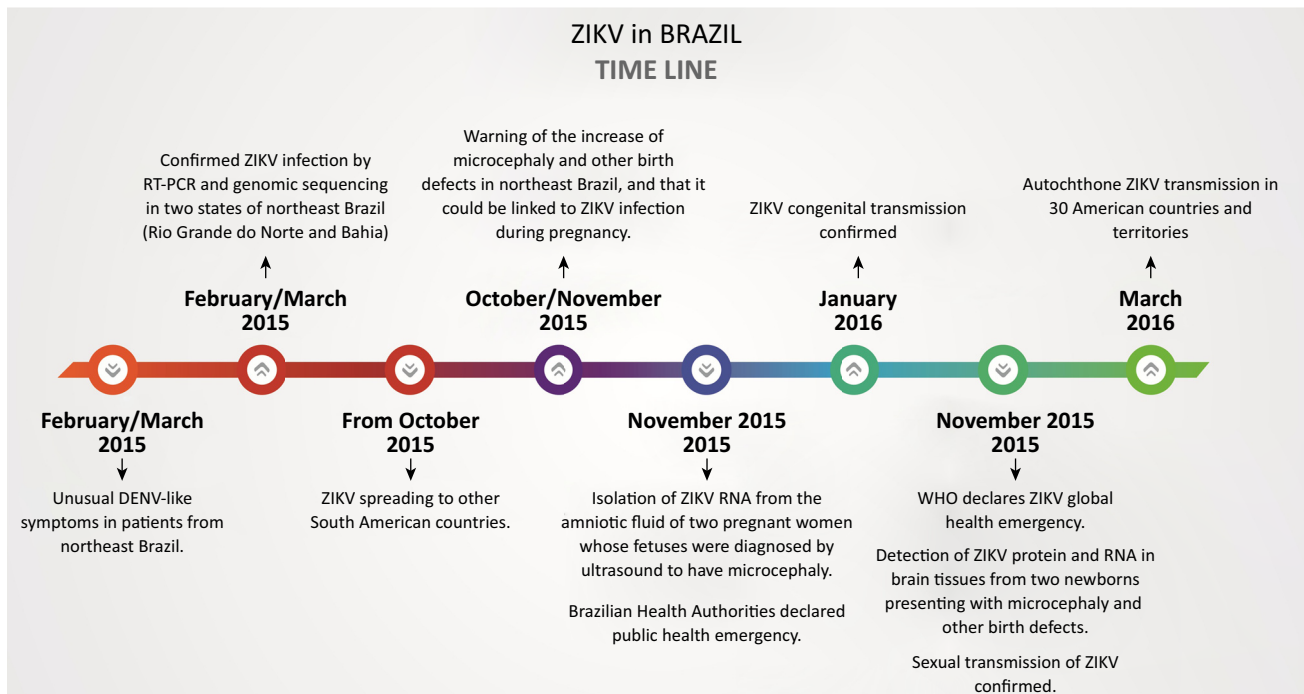


Figure 1. Timeline of Zika Virus (ZIKA) Outbreak in Brazil. Abbreviation: DENV, dengue virus.

and malformation cases are a major burden not only for the families, but also for the social security system, because these children will require assistance for their entire lives. Furthermore, because Brazilian law prohibits abortion, many expectant mothers are practicing illegal abortions and putting their lives at risk.

By the end of 2015, ZIKV had spread to other American countries, and it continues to move to other continents; the number of infections is increasing daily (www.paho.org/hq/index.php?option=com_content&view=article&id=11585&Itemid=41688&lang=en), and the first cases of birth defects linked to the ZIKV have been reported in Colombia [9].

The realization that we were facing a novel epidemiological problem in Brazil resulted in the mobilization of groups from research institutes and universities. Several of them instituted 'crisis management task forces' to prioritize actions to be taken and different research groups have sought to

establish international collaborations with the National Institutes of Health, Center for Disease Control, Pasteur Institute, London School of Hygiene and Tropical Medicine, and so on. In parallel, the Brazilian Ministries of Health and of Science, Technology, and Innovation established working groups, as did the Brazilian funding agencies. However, the epidemic coincided with serious economic constraints and a political crisis in Brazil, limiting the availability of funds. In addition, the lack of clear Government policies to tackle the epidemic and establish priorities, as well as the dearth of knowledge about the virus and the disease, delayed the official recognition of an epidemiological crisis in Brazil.

Since early 2015, ZIKV has been a dominant subject in all newspapers. This was important to make society aware of the risks of the disease and how each citizen could contribute to combat a presumptive villain: the *Ae. aegypti* mosquito vector. However, as mentioned above, one

challenging observation was the increasing speed of the epidemic when compared with the rate of spread of CHIKV, which had entered Brazil 1 year before ZIKV. Given that these viruses share the same potential vector, it was hypothesized that additional mechanisms or vectors might be involved in ZIKV transmission. Accordingly, recent observations indicated that active ZIKV can be found in the saliva [10], urine [11], and breast milk [12] of patients, but whether these fluids could represent alternative routes for the transmission of ZIKV remains to be determined. Sexual transmission of ZIKV is well documented, and the number of cases worldwide is increasing [13]. The most recent WHO alert advises 'pregnant women whose sexual partners live in or travel to areas with ZIKV outbreaks to use safe sexual practices' (www.who.int/mediacentre/factsheets/zika/en/). However, similar to the other alternative routes of transmission, the epidemiological impact of this mechanism of transmission has not yet been determined.

Box 1. Outstanding Questions

Why are ZIKV clinical symptoms more severe in Brazil? Are there molecular markers of virulence (mutation or recombination) in Brazilian ZIKV strains? Is there more than one strain circulating?

Could host factors or host response be involved in the clinical outcome?

What are the target cells and tissues for viral replication?

What are the ZIKV transmission mechanisms in humans: sexual, perinatal, transplacental, blood transfusion, saliva, urine, or breast milk?

What is the role (if any) of previous infections by (or immune responses to) other flaviviruses, such as dengue virus and yellow fever, in ZIKV pathogenesis? What about co-infections with different arboviruses?

Is there a possibility of re-infection or persistence? What is the time course of the immune response? Is humoral immunity protective and long lasting?

Which mosquito vector species are implicated in viral transmission in the urban cycle?

Could mosquito vectors be co-infected? Would this impact ZIKV virulence?

Another issue of concern is that other mosquito species, such as *Culex*, which have been shown to be highly susceptible to laboratory infections by ZIKV [14], could be involved in the urban transmission cycle. ZIKV is a potential encephalitic virus, similar to West Nile virus, Saint Louis encephalitis virus, and Japanese encephalitis virus, all of which are transmitted by *Culex* mosquito species. Thus, it is reasonable that ZIKV could use the same vector. Moreover, the work of Chouin-Carneiro *et al.* [15] suggests that, although they are susceptible to infection, *Ae. aegypti* and *Ae. albopictus* are low-competence vectors for ZIKV transmission, which further reinforces the hypothesis of an alternative mosquito vector. Indeed, a literature search incriminates *Aedes* species in the outbreak in the Yap Islands in 2007 (*Ae. hensilli*) as well as the French Polynesia epidemics in 2014 (*Ae. aegypti* and *Ae. polynesiensis*) because it was the predominant genus identified, although ZIKV has never been isolated from these mosquito species [2].

Further studies are needed to address this issue.

It is important to note that the epidemiological situation regarding ZIKV in Brazil, a country with a territorial area of 8 515 767.049 km², may be underreported. Fortunately, there is a centralized health system (SUS), and all notifications are shared with the Ministry of Health and the regional health units.

Despite the multiple initiatives aiming to tackle the epidemiological emergency and the efforts to advance our knowledge of ZIKV biology, pathogenesis, susceptible animal models, transmission, and host response to infection, there are urgent needs, including cohort establishment, the development of serological diagnostic assays, and the identification of potential vectors for the implementation of blocking measures.

Several questions remain that should be answered as quickly as possible (Box 1)

and each day we are surprised by new aspects of ZIKV, which is undoubtedly becoming a worldwide health threat. Our hope is that what we are seeing is not just the tip of the iceberg.

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