

First report of autochthonous transmission of Zika virus in Brazil

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In the early 2015, several cases of patients presenting symptoms of mild fever, rash, conjunctivitis and arthralgia were reported in the northeastern Brazil. Although all patients lived in a dengue endemic area, molecular and serological diagnosis for dengue resulted negative. Chikungunya virus infection was also discarded. Subsequently, Zika virus (ZIKV) was detected by reverse transcription-polymerase chain reaction from the sera of eight patients and the result was confirmed by DNA sequencing. Phylogenetic analysis suggests that the ZIKV identified belongs to the Asian clade. This is the first report of ZIKV infection in Brazil.

Key words: Zika virus - “dengue-like syndrome” - Brazil

Zika virus (ZIKV) is an emerging arthropod borne virus (arbovirus) transmitted by *Aedes (Stegomyia)* mosquitoes. The virus belongs to the genus *Flavivirus*, family *Flaviviridae*, and is closely related to other flaviviruses of public health relevance including dengue (DENV), yellow fever and West Nile viruses (Pierson & Diamond 2013, Faye et al. 2014).

ZIKV was first isolated from a rhesus monkey in the Zika Forest of Uganda, in 1947 (Dick et al. 1952). For half a century, the virus was described as causing sporadic human infections in Africa and Asia, until 2007 when a Zika fever epidemics took place in Yap Island, Micronesia (Duffy et al. 2009). In 2013, a large epidemic was reported in French Polynesia, concomitant with a dengue epidemic caused by serotypes 1 and 3. ZIKV has been considered as emergent since 2007 (Cao-Lormeau et al. 2014, Ios et al. 2014).

In the early 2015, records of patients presenting a “dengue-like syndrome” appeared in the public health service in the city of Natal (05°47'42"S 35°12'32"O), state of Rio Grande do Norte, Brazil. A physician specialist in infectious disease evaluated the patients and the clinical signs and symptoms and laboratory findings indicated a non-DENV and non-Chikungunya virus (CHIKV) infection. Symptoms included arthralgia, oedema of extremities, mild fever, maculopapular rashes frequently pruritic, headaches, retroorbital pain, no purulent conjunctivitis, vertigo, myalgia and digestive disorder.

By March 2015, the Molecular Virology Laboratory of Carlos Chagas Institute, Oswaldo Cruz Institute,

state of Paraná, Brazil, received 21 acute-phase serum specimens from those patients exhibiting the “dengue-like syndrome”. Sera were transported and kept at -80°C until use. The protocol was approved by the Brazilian Ethical Committee (42481115.7.0000.5248).

After RNA extraction, reverse transcription-polymerase chain reaction (RT-PCR) was performed as a rapid molecular tool to detect viral infection in acute-phase samples. Viral RNA was extracted from 140 µL of sera by using QIAamp viral RNA mini kit (Qiagen, USA) according to the manufacturer's instructions.

The sera were first tested for DENV by RT-PCR (Lanciotti et al. 1992), ELISA for non-structural protein 1 antigen detection (Dengue Early ELISA, PanBio) and anti-DENV IgM (Dengue IgM Capture ELISA, PanBio), all yielded negative results. Moreover, all samples rendered negative results in RT-PCR assays for CHIKV infection (Lanciotti et al. 2007).

Since clinical symptoms were compatible with ZIKV infection, RT-PCR assay to specifically detect ZIKV RNA was performed, as described by Faye et al. (2008), with the modification that, instead of a one step, a two step protocol was used (cDNA synthesis followed by the PCR).

Amplicons of 364 bp encompassing the envelope protein coding region were obtained for eight out of 21 samples (Fig. 1). Two of the resultant amplicons were purified with High Pure PCR Purification kit (Roche) and sequenced in an ABI 3500 Genetic Analyzer (Applied Biosystems). Sequences were assembled using the Assembler tool (hpa-bioinformatics.org.uk/cgi-bin/assembly_tool/seq_assemble.cgi?no=2) (GenBank accessions KR815989 and KR815990) and their identity were confirmed by Basic Local Alignment Search Tool (BLAST) and compared to other ZIKV sequences. Results demonstrated a high identity with sequences from the ZIKV Asian lineage. Despite the similarity of the clinical findings, the remaining samples resulted negative in RT-PCR, probably due to the short (and low) viraemia period (between the 3rd and 5th day after the onset of clinical symptoms).

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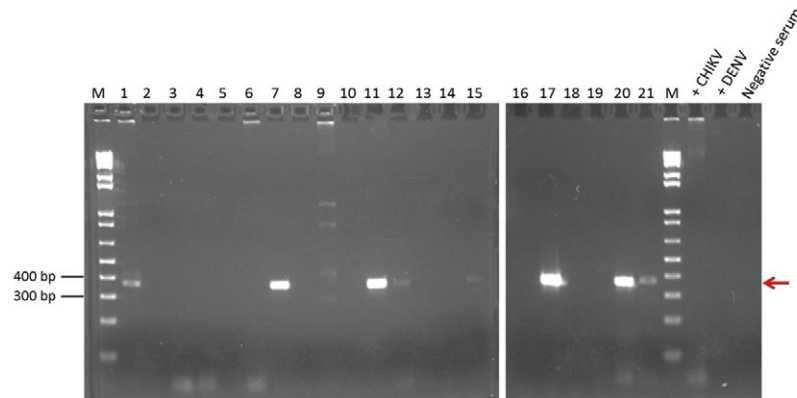


Fig. 1: agarose gel electrophoresis of reverse transcription-polymerase chain reaction assays for Zika virus (ZIKV) detection. The arrow indicates the 364 bp amplicon expected for ZIKV. RNA extracted from serum samples of patients in acute-phase of dengue fever and Chikungunya fever and a negative serum sample were included as negative controls. CHIKV: Chikungunya virus; M: molecular size marker; 1-21: serum samples.

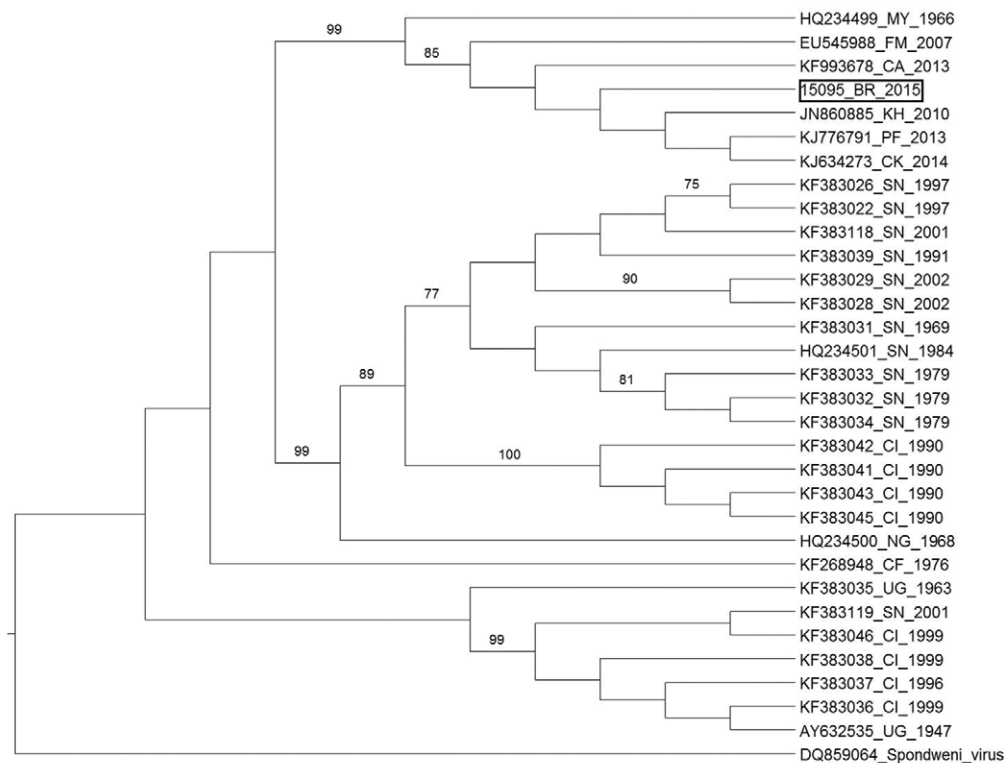


Fig. 2: phylogenetic analysis based on partial *E* gene nucleic acid sequence (nt 1655-1984 according to AY632535) of a 2015 Brazilian strain of Zika virus. The tree was inferred using the maximum likelihood algorithm based on the Kimura two-parameter model with invariant sites as implemented in MEGA 6.05. The numbers shown to the left of the nodes represent bootstrap support values > 70 (1,000 replicates). The tree was rooted with Spondweni virus and branch lengths do not represent genetic distance. Strains were labelled according to GenBank accession/country 2-letter acronym/year of isolation.

Phylogenetic analysis of the sequences (Fig. 2) placed the Brazilian strains in a clade with sequences from the Asian lineage. The highest identity scores in BLAST were observed with the H/2013 (99%, GenBank accession KJ776791), CK-2014 (99%, GenBank accession KJ634273) and FSS13025 (99%, GenBank accession JN860885) isolates. The two generated sequences

were identical between themselves in the region that was analysed (Fig. 2) and was identified as 15095_BR_2015. These results corroborate previously published data about the spread of the ZIKV Asian lineage that was first reported in the outbreaks of the Pacific Islands (Musso et al. 2014). To track the introduction of ZIKV in Brazil a retrospective and more extensive analysis of additional



Fig. 3: Zika virus clinical findings in patients from Natal, state of Rio Grande do Norte, Brazil. A: lymphadenopathy; B: maculopapular rash; C: periarticular swelling.

samples from other Brazilian regions is needed, as well as a complete viral genetic characterisation. However, one plausible hypothesis is the arrival of the new emerging virus during the soccer World Cup in 2014.

Seven of the eight patients with confirmed ZIKV disease were female, the median age was 39 years (range, 18-65), all of them lived in Natal and had relatives with the same symptoms. The most commonly reported symptoms were maculopapular rash (100%) (Fig. 3) and pain. The pain scale was applied for six of them and the pain levels were high in most patients, with levels of zero (1/7), seven (2/7), nine (1/7) or 10 (2/7). The pain duration ranged from two-15 days, except for one patient who had pain for more than 21 days. Other symptoms included headache (6/8), retroorbital pain (4/8) and myalgia (6/8). Myalgia was not intense. Joint pain was reported for seven patients, including pain in the hands (5), ankle (4), elbow (4), knee and wrist (3) and foot (2); one patient reported cervical pain.

During the clinical examination, all the patients were haemodynamically normal and periarticular swelling (Fig. 3) was present in six of them, with predominance in the hands (50%). Fever was reported in six patients, but high fever (around 39°C) in only two. The duration of fever ranged from one-eight days. Submandibular or cervical lymphadenopathy (Fig. 3) occurred in three patients. No purulent conjunctivitis was observed. ZIKV can produce a clinical relevant disease, which lasts for approximately two weeks. The patients observed in Natal had intense pain resembling CHIKV infection, but the clinical course was shorter. To date, no death or complications were associated with ZIKV illness in Natal.

The blood cell count revealed that only two patients had leukopaenia, the others had normal levels of leukocytes and neutrophils and platelets were normal in all of them. The tourniquet tests were negative in seven of the patients.

We report here the first identification of ZIKV as the causative agent of an outbreak in the northeastern Brazil. It represents the first autochthonous transmission of ZIKV in the country. Although most of the patients had mild illness, clinicians and public health officials should be aware of the risk of expansion of this new emerging virus, especially given the naïve immunological status of the Brazilian population. Spreading of the disease in the country might occur by virtue of the large population mobility and the widespread occurrence of the transmit-

ting vectors. Furthermore the complex epidemiological context with the co-circulation of DENV, CHIKV and ZIKV cannot be neglected. DENV and ZIKV (Dupont-Rouzeyrol et al. 2015) or DENV and CHIKV (Caron et al. 2012) co-infections have already been reported.

The effect of the concurrent outbreaks caused by these three different arboviruses is unknown. ZIKV disease was associated with neurological and autoimmune complications in a context of concurrent circulation with DENV in French Polynesia (Roth et al. 2014). DENV also circulates in Natal. Further studies are needed to unveil whether co-infection and subsequent infection by different arboviruses can affect the course of the disease, the occurrence of severe cases and the ways of transmission (vertical, perinatal, sexual) (Foy et al. 2011, Besnard et al. 2014, Musso et al. 2015). Of interest, recent paper by Gourinat et al. (2015) shows evidence of virus secretion in urine for more than 10 days after onset of disease.

The correct clinical and laboratorial differential diagnosis between acute ZIKV, CHIKV and DENV infection will contribute to the patients' prognosis and to drive surveillance actions.

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