

## SHORT REPORT

### Asian genotypes of dengue virus 4 in Brazil

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#### SUMMARY

Dengue virus, commonly transmitted by mosquitoes, causes a human disease of significant social impact and presents a serious public health problem in Brazil. This report describes the unusual emergence of DENV-4 in northern Brazil after a nearly 30-year-long absence. DENV-4 genotype I is of Asian origin and was identified in the serum of patients receiving treatment at a hospital serving the Salvador area (Brazilian state of Bahia). The identification of dengue virus serotypes through molecular and phylogenetic analysis is essential for predicting disease severity or fatal illness, principally in endemic countries such as Brazil.

**Key words:** Asian lines, Brazil, DENV-4.

Dengue fever (DF) is one of the most important arthropod-borne viral diseases and represents a serious public health problem in Brazil. It is transmitted to humans by mosquitoes of the *Aedes* genus, common to tropical and subtropical countries. Infection with dengue virus (DENV) can be asymptomatic or cause an undifferentiated fever. In some cases, however, mild DF can develop into a life-threatening dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).

DENV is an enveloped virus of the Flaviviridae family, genus *Flavivirus*, and contains a positive single-stranded RNA genome. There are four closely related antigenic serotypes: DENV-1, DENV-2, DENV-3, and DENV-4.

The first recorded DENV cases in Brazil occurred in the 19th century; however, with the eradication of the DENV-transmitting mosquitoes, there were no reported cases between 1923 and 1981. However, cases of DF reported in 1981–1982 in the State of Roraima (Northern Brazil) were identified as DENV-1 and DENV-4, probably from Central America or the northern parts of South America. Since then, DF has become endemic to Brazil and the incidence of DENV has increased in the last decade in many parts of the country. In 2010, DENV-4 re-emerged after almost 30 years' absence [1] in northern Brazil and spread to several Brazilian states.

Sequencing and analysis of the DENV-4 envelope gene (E) are used to characterize viral genotypes and geographical distribution [2]. Phylogenetic analysis reveals four distinct DENV-4 groups: genotypes I, II, and III are linked to human disease, and genotype IV is restricted to the sylvatic cycle. These genotypes can also be associated with distinct geographical

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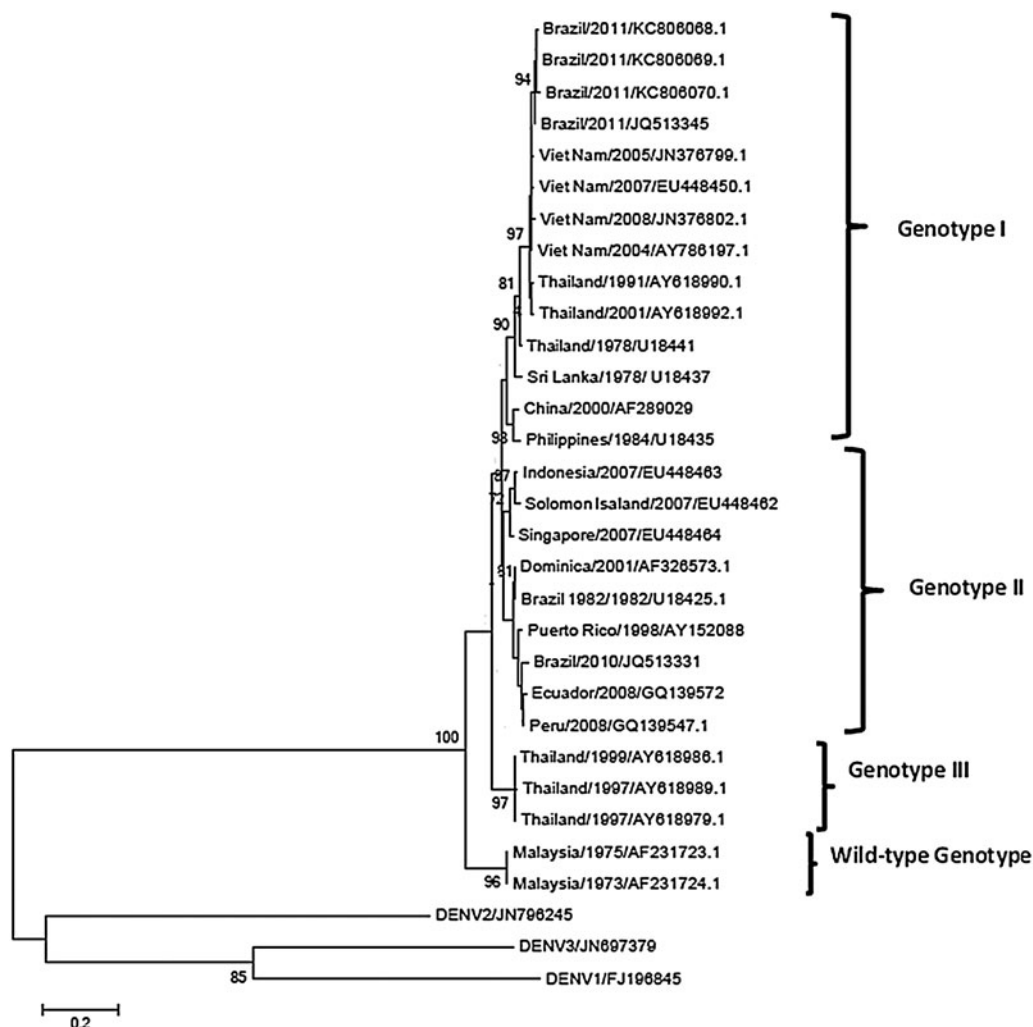
regions. Genotype I is found in the Philippines, Thailand, Sri Lanka, and Japan; genotype II is common in Indonesia, the South Pacific, and Latin America. The genotype III group consists of Thai lines that are distinct from other isolates of this region, and genotype IV consists of sylvatic isolates found in Malaysia. DENV-4 genotype II has been present in the Americas for over two decades [3]; however, incidences of genotypes I and III in Brazil have been singular events. Here, we report the emergence in Brazil of a DENV-4 genotype I strain of Asian origin.

We examined 214 serum samples from patients with clinically suspected DF who were treated at a hospital in the city of Salvador in 2011 (Hospital Aliança, Bahia, Brazil). The Ethics Committee for Human Research of Salvador University approved this study (protocol number 04-10-48). Medical records were examined for information regarding symptoms, timing of sampling, and travel history (all patients denied travelling abroad in the 6 months prior to their hospital visit). Samples were analysed with the Dengue Duo Rapid test (Bioeasy, Brazil) for IgM/IgG anti-DENV and NS1 antigen for disease confirmation. Viral RNA was extracted with the QIAamp viral RNA kit (Qiagen, Brazil), according to the manufacturer's protocols. Total RNA was reverse-transcribed with SuperScript II Reverse Transcriptase (Invitrogen, Brazil). The viral serotype was characterized as described previously [4]. Briefly, the cDNA was amplified with primers D1 and D2 in a 50- $\mu$ l reaction using the following cycling conditions: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min. Nested polymerase chain reaction (PCR) was performed with the D1 primer and TS1–TS4 type-specific internal primers for the C-prM genomic junction region [4]. Amplification products were analysed by agarose gel (2%) electrophoresis. The E gene of DENV-4 (1485 nt, position 939–2425) was amplified with serotype-specific primers D4742 (5'-TGGGATTGGAAC AAGAGCTGAGACATGGATGTC-3') and D4C P1838 (5'-CGTGTATGACATCCCTTGATTCTC AATTTCTCCA-3') for the reverse-transcription reaction, and D41236 (5'-GGGTGGGGCAATGGCT GTGGCTTGTGG-3') and D4CP2536 (5'-GGGG ACTCTGGTTGAAATTTGTTACTGTTCTGTCC-3') to amplify a 1485-bp product by PCR [2]. Samples were sequenced on an ABI-PRISM 3100 Genetic Analyzer with BigDye Terminator<sup>®</sup> v. 3.1 Cycle Sequencing RR-100 (Applied Biosystems, USA). Multiple E gene sequence alignment was performed in ClustalW and

BioEdit program v. 7.0.9.0. The E gene sequences from DENV-4 genotypes I–III were obtained from GenBank (<http://www.ncbi.nlm.nih.gov>) and aligned with the sequences obtained from DENV-4-positive patient samples. A maximum-likelihood phylogenetic tree was constructed using the T92+G0 model for nucleotide substitution, followed by the likelihood ratio test and bootstrap analysis with 1000 pseudo-replicates (Fig. 1). All analyses were performed in Mega v. 5.0 (<http://www.megasoftware.net/mega.php>).

The Dengue Duo Rapid test confirmed DENV infection in 93/214 samples (43.4%). Of the 93 dengue-positive samples, 77 were DENV-4, 13 were DENV-2, and three were DENV-3. The phylogenetic tree of the complete E gene sequences of the three isolates from DENV-4-positive serum samples by the Dengue Duo Rapid test (Brazil/2011/KC806068-1, Brazil/2011/KC806069-1, Brazil/2011/KC806070-1) showed that they formed a distinct, well-supported clade (bootstrap 94). The phylogenetic position of the DENV-4 isolates obtained in Salvador in 2011 demonstrated that they belonged to genotype I. Interestingly, these sequences derived from viruses that circulated in Vietnam between 2004 and 2008 (represented by Vietnam/2007/EU448450-1, Vietnam/2005/JN376799-1, Vietnam/2008/JN376802-1, and Vietnam/2004/AY786197-1), suggesting that novel segregating sites were recently acquired by the Brazilian DENV-4.

The first report of Brazilian DENV-4 descended from Asian lines described a single sample collected at Salvador, Bahia, Brazil (Brazil/2011/JQ513345) [5] and our work reinforces this finding, revealing a large number of DENV-4-positive samples in Salvador during 2011. The introduction of DENV-4 genotype I into Brazil and its detection in Salvador, Bahia, may be a consequence of tourism in the city, which receives large numbers of foreign visitors annually. The virus may have been introduced through Salvador's international harbour, which has significant commercial interactions with Asian countries. Dengue has become one of the most important emerging disease problems in international travellers, who may acquire or spread the infection to regions they visit [6]. According to the European Network on Imported Infectious Disease Surveillance, DF in Europeans travellers is mostly associated with travel to Asia [7]. In addition, GeoSentinel, a global network of medicine providers for travellers, reported that dengue was the most frequent cause of febrile illness affecting foreign travellers to Asia [8]. Travellers are routinely advised to avoid or prevent mosquito bites by



**Fig. 1.** Phylogenetic tree constructed from the DENV-4 E gene sequences. The nucleotide sequences (472 nt, position 1953–2425 of the DENV-4 viral genome) were analysed using the maximum-likelihood method. The virus names correspond to the country of origin/year collected/GenBank accession number. The strains in this study were named Brazil/2011/D4\_11, Brazil/2011/D4\_30, Brazil/2011/D4\_23, and Brazil/2011/D4\_16. The scale bar represents a genetic distance of 0.01 or 1% divergence in the nucleotide sequence.

using insect repellent and/or wearing long-sleeved shirts and trousers during the morning hours, when the mosquito *Aedes aegypti* is more active in urban areas.

The identification of DENV-4 in Salvador is of significant public concern, as serotypes 1–3 have already caused epidemics in this area. If DENV-4 infects people that have already been exposed to one of the other DENV serotypes, we may see an increased incidence of clinically severe DHF, which may be fatal [9]. These findings reinforce the need to study the molecular epidemiology of DENV infections in endemic countries such as Brazil. Our future studies will address the dynamics of the viral population when four serotypes of DENV are circulating in the same human population.

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## DECLARATION OF INTEREST

None.

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