

# Molecular Epidemiology of Dengue in Jamaica

## Dengue Virus Genotypes in Jamaica, 2007

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### ABSTRACT

*The genotypes of dengue viruses (DENV) isolated from patients with dengue in Jamaica during 2007 were determined using DNA sequencing and phylogenetic analysis of the C-prM gene junction. The 17 DENV analysed included strains of DENV serotypes 1 (DENV-1, n = 3), DENV-2 (n = 7) and DENV-4 (n = 7). All strains of DENV-1 were classified as genotype III, while 1 of 7 strains of DENV-2 belonged to the Asian American/Asian genotype, genotype I/ III (Jamaica genotype), 2 were genotype V, the American genotype and 4 strains clustered with reference strains belonging to genotype IV. The 6 DENV-4 strains from Jamaica and the control strain clustered together in a separate clade from Caribbean/ American reference strains, which belong to genotype II and Asian strains, classified as genotypes I and III.*

*There has been little evolution in the DENV-1 strains circulating in Jamaica over the years and this might reduce the risk of outbreaks due to this serotype. In contrast, the high genetic diversity in strains of DENV-2 viruses in circulation, the presence of more recently introduced genotypes and a new clade of DENV-4 might contribute to the epidemic potential of these DENV serotypes.*

*These preliminary data clearly indicate the need to maintain laboratory surveillance, and other control measures against hyperendemicity of dengue in Jamaica.*

**Keywords:** Caribbean, clade, epidemic, genotypes, phylogenetic sequencing, strain, surveillance

## Epidemiología del Dengue en Jamaica Genotipos del Virus del Dengue en Jamaica, 2007

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### RESUMEN

*Los genotipos de los virus del dengue (DENV) aislados de pacientes con dengue en Jamaica durante 2007, fueron determinados usando secuenciación de ADN y análisis filogenético de la unión del gen C-prM. Los 17 DENV analizados incluyeron cepas de serotipos de DENV 1 (DENV-1, n = 3), DENV-2 (n = 7) y DENV-4 (n = 7). Todas las cepas de DENV-1 fueron clasificadas como genotipo III, mientras que 1 de 7 cepas de DENV-2 pertenecían al genotipo asiático americano/asiático, genotipo I/ III (genotipo de Jamaica), 2 fueron genotipo V, el genotipo americano y 4 cepas agrupadas con cepas de referencia pertenecientes al genotipo IV. Las 6 cepas DENV-4 de Jamaica y la cepa de control se agruparon en un clado aparte de cepas de referencia caribeña/americana, que pertenecen al genotipo II, y las cepas asiáticas, clasificadas como genotipos I y III.*

*Ha habido poca evolución en las cepas DENV-1 que han estado circulando en Jamaica a través de todos estos años, y esto podría reducir el riesgo de brotes a causa de este serotipo. En contraste con ello, la alta diversidad genética de las cepas de los virus DENV-2 en circulación, la presencia de más genotipos introducidos recientemente, y un nuevo clado de DENV-4 podrían contribuir al potencial epidémico de estos serotipos de DENV.*

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*Estos datos preliminares indican claramente la necesidad de mantener la vigilancia de laboratorio, y otras medidas de control contra el carácter hyperendémico del dengue en Jamaica.*

**Palabras claves:** Caribeño, clado, epidémico, genotipos, secuenciación filogenética, cepa, vigilancia

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## INTRODUCTION

Dengue is the most important arbovirus infection that affects humans (1). There are 4 dengue virus (DENV) serotypes (DENV-1, -2, -3 and DENV-4) which are antigenically related but genetically distinct. Persons infected with any one of the 4 dengue virus serotypes may be asymptomatic or have a mild or severe disease course which renders complete immunity to that serotype but only partial immunity to infection with other serotypes. Dengue virus has spread globally; at least 2.5 billion people are at risk of mild or severe infection due to this virus. Across Asia and the Americas, dengue genotypes associated with increased virulence have been reported. There is also evidence suggesting that co-circulation of different dengue serotypes or genotypes in a geographical area increases the risk of severe forms of the disease, dengue haemorrhagic fever/dengue shock syndrome [DHF/DSS] (2, 3).

Dengue viruses possess a positive sense ssRNA genome, 11 Kb in length, comprising a single open reading frame (ORF) that codes for 3 structural proteins including capsid (C), pre-membrane/membrane (prM/M), envelope (E) and 7 non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). DNA sequencing and phylogenetic analysis of strains of the 4 DENV serotypes have identified different genotypes of each serotype (4). For example, phylogenetic analysis of the complete E gene sequences has revealed that strains – of DENV-1 may be classified as genotypes I–V, DENV-2 as genotypes I–V, DENV-3 as genotypes I–IV and DENV-4 as genotypes I–IV, respectively (5–8).

Other genomic regions of the dengue virus especially the structural and non-structural genes, may also be used to determine genotypes (4, 5).

Within each dengue serotype, there are certain genotypes or clades which are rarely transmitted to humans, others are associated with outbreaks of dengue fever only and other genotypes are associated with DHF/DSS (4, 9, 10). The increase in global spread and the co-circulation of multiple DENV serotypes, genotypes and clades in the same population together with the error prone nature of the viral RNA dependent RNA polymerase result in increasing genetic diversity of dengue viruses and the evolution of new genotypes within each serotype (4).

All 4 dengue serotypes have been circulating in Jamaica, the Caribbean and other dengue endemic countries of the Americas since the 1990s (11–14). In 1977, strains of DENV-1 caused an outbreak in Jamaica which later spread to

other countries in the region. Dengue-2 and DENV-3 were implicated in the 1968–69 outbreak of dengue-like illness on the island and DENV-2 was the cause of the 1995 dengue epidemic (12, 14, 15). DENV-1 and DENV-3 were also implicated in another dengue outbreak in 1998 (16). Dengue-4 emerged in Jamaica during 1981–1982, concurrent with a major outbreak of DHF/DSS in Cuba (14). According to one report from the Ministry of Health and the Environment (MOHE), DENV-2 was introduced to Jamaica during 2007–2008 and became the predominant serotype (17).

The nucleotide sequence of the Caribbean strain of DENV-1 and nucleotide sequence deduced amino acid sequence of structural proteins of DENV-2 Jamaica genotype were described during the 1980s. The genotypes of DENV-2 and DENV-4 strains circulating in the Caribbean between 1981–2000 have also been reported (18–24). This study was undertaken to determine the genotypes of strains of DENV-1, 2 and 4 that were identified in Jamaica during the 2007/2008 dengue outbreak.

## SUBJECTS AND METHODS

The strains of dengue viruses which were analysed in the study comprised 18 dengue isolates including three DENV-1, six DENV-2 and six DENV-4 strains from acute-phase sera from patients with dengue, seen at the University Hospital of the West Indies (UHWI), a tertiary care referral centre in Jamaica during 2007 and control strains of each of three dengue serotypes obtained from the Caribbean Research Epidemiology Centre (CAREC), Trinidad and Tobago. Tube cultures of the *Aedes albopictus* mosquito (C6/36) cell line were infected with acute-phase serum from patients or control strains of DENV 1–4 and serotypes were identified by reverse transcriptase-polymerase chain reaction (RT-PCR) assay as previously described (18). Cell culture supernatants were harvested by centrifugation at 2000 rpm at 4°C and stored at -70°C until required for testing.

The RNA was extracted from 140 µl aliquots of serum and cell culture supernatant using commercially prepared reagents (QIAamp Viral RNA Mini Kit, Qiagen, Germany). The manufacturer's instructions were followed. The RNA was used as template in the reverse transcriptase-polymerase chain reaction (RT-PCR) assay which amplified fragments of the C-prM gene region using primers and thermocycling conditions previously described by Lanciotti *et al* (18) with slight modifications (18, 19).

For PCR product purification, 3 µl EXOSAP-IT (Abbott Molecular, CA) was added to 45 µl aliquots of the

PCR product followed by centrifugation at 2000 rpm for 1–2 seconds. The supernatant was removed and kept at 4°C until sequenced, within two weeks. Commercially prepared reagents (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems) were used for sequencing the purified PCR products. The manufacturer's instructions were followed. In brief, the DNA strands were sequenced in both directions, each sequencing reaction contained 1.0 µl Big Dye terminator, 1.5 µl 5X buffer, 0.5 µl sense or antisense primer, 6.0 µl deionized water and 1 µl DNA. Sequencing reactions were performed in 25 cycles of denaturation at 96°C for 30 seconds, annealing at 50°C for 1 minute and extension at 60°C for 4 minutes in a Perkin Elmer model 9700 thermocycler (Applied Biosystems, Foster City, CA). The sequencing primers used were the PCR second round primers for each DENV serotype, respectively. The sequencing reaction mixtures were then column purified using DyeX 2<sup>TM</sup> (Qiagen, Valencia, CA) and DNA cycle sequencing was performed using an ABI 3130 Analyser (Applied Bio-systems, Foster City, CA).

The C-prM nucleotide sequences of each DENV serotype in FASTA format, were confirmed using Megablast software, aligned with reference sequences, downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank>), in Clustal X software and neighbour joining phylogenetic trees constructed using PAUP 4.0 beta 10 Win softwares with bootstrap analysis of 1000 replicates (20, 21).

## RESULTS

The phylogenetic relationships of nucleotide sequences of the C-prM genomic regions of DENV-1, DENV-2 and DENV-4 strains isolated from patients presenting with dengue in Jamaica during 2007 and reference strains accessed from GenBank are compared in Figs. 1–3, respectively. As shown in Fig. 1, the sequences of 3 DENV-1 strains isolated from dengue cases during 2007 and the CAREC control strain clustered with the Caribbean reference strain which was isolated in Jamaica in 1977 and DENV-1 genotype III reference strains from India. The recent DENV-1 strains showed 100% genetic homology with Caribbean and Indian reference strains isolated from 1956–2007.

Of the 7 DENV-2 strains, the control strain from CAREC and 2 strains isolated during 2007 clustered with DENV-2 American/genotype V reference strains, 3 strains isolated from Jamaican cases during 2007 were classified with Asian II/genotype II reference strains and 1 strain grouped with Jamaica genotype (American/ Asian genotype/genotype III) reference strains.

The nucleotide sequences from the 6 DENV-4 strains isolated from dengue cases in Jamaica during 2007 clustered together with the CAREC control strain with bootstrap values of 96–100% but were segregated from DENV-4 reference strains from the Caribbean, genotype II and Asian countries, genotypes I and III.

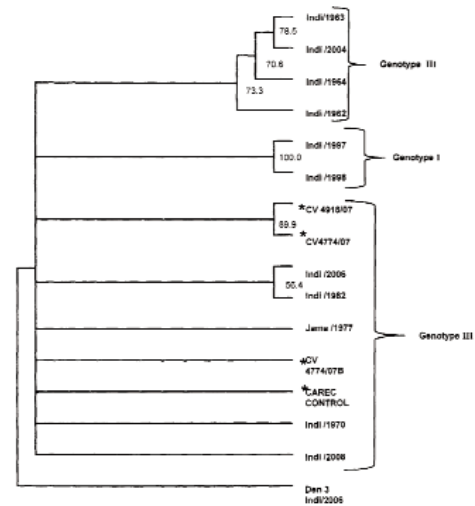


Fig. 1: Phylogenetic relationships of dengue 1 (DENV-1) viruses. Neighbour-joining, phylogenetic tree constructed from nucleotide sequences of the CprM genomic region of 3 DENV-1 strains isolated from DENV-1 dengue cases in Jamaica during 2007 and control strain (indicated by \*) and 12 GenBank reference strains from India. The reference strains are annotated by the first 4 letters of country and the year of isolation. Numbers at the nodes of the tree are bootstrap values expressed as percentages of 1000 replicates supporting each genotype. DENV-1 genotypes are indicated. The tree was rooted with a DENV-3 India 2006 reference strain.

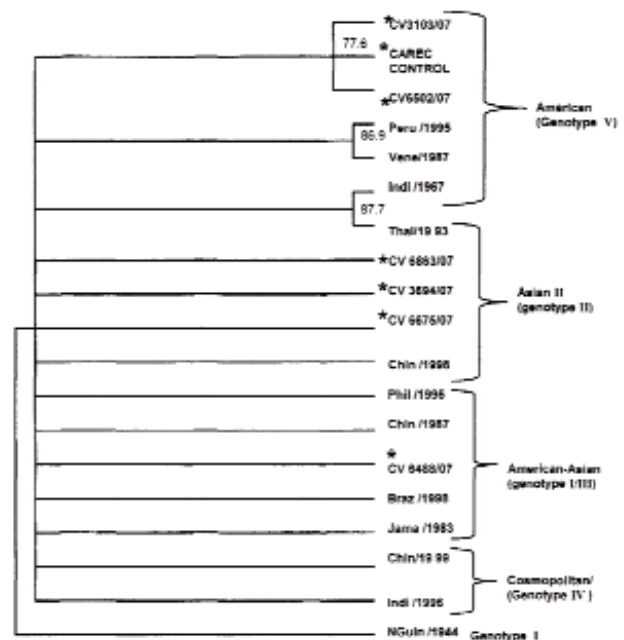


Fig. 2: Phylogenetic relationships of dengue 2 (DENV-2) viruses. Phylogenetic tree generated by neighbour-joining analysis of nucleotide sequences of the CprM genomic region of 6 DENV-2 strains isolated in Jamaica during 2007 and the control strain (indicated by \*) and 12 DENV-2 reference strains accessed from Gen Bank. The reference strains are annotated by the first 4 letters of country and the year of isolation. Number at nodes of tree and bootstrap values are expressed as percentages of 1000 replicates supporting each genotype. The DENV-2 genotypes are indicated. The tree was rooted with a DENV 1944 reference strain.



Fig. 3: Phylogenetic relationships of dengue 4 (DENV-4) viruses. Phylogenetic tree generated by neighbour-joining analysis of nucleotide sequences of the CprM genomic region of 6 DENV-4 strains isolated in Jamaica during 2007 and control strain (indicated by \*) and 13 DENV-4 reference strains accessed from Gen Bank. The reference strains are annotated by the first 4 letters of country and the year of isolation. The numbers at the nodes of the tree are bootstrap values expressed as the percentage of 1000 replicates supporting each genotype. The DENV-4 genotypes are indicated and the tree is rooted with the DENV-2 Jamaican 1981 reference strain.

## DISCUSSION

Dengue virus-1 has been circulating in Jamaica since 1977 when it caused an epidemic (14). The DENV-1 strains identified in this study belonged to genotype III and were highly genetically conserved in the C-prM region when compared to isolates from 1977. The lack of genetic evolution in DENV-1 viruses in Jamaica might reduce antigenic variation among Jamaican strains and the risk of dengue outbreaks caused by DENV-1 in the near future. It is possible that new DENV-1 strains could be introduced to Jamaica from other geographical areas increasing the outbreak potential of this serotype. A recent publication from India also reported genetic conservation in DENV-1 strains over the past 60 years (19). Pires Neto *et al* (25) also noted that there was no change in dengue-1 and dengue-2 genotypes in Brazil since their introduction in 1988 (25).

The phylogenetic analysis of the DENV-2 strains which were isolated during 2007 showed high genetic diversity in this serotype. It was noted that two isolates

classified with the American genotype/genotype V which is associated with mild cases of DF while one strain grouped with the 1981 Jamaica genotype reference strain belonging to genotype III which is associated with DHF/DSS (23, 25, 26). Since DENV-2 genotype V tends to cause mild disease, any severe dengue DHF/DSS cases which were attributed to DENV-2 genotype V in the 2007/2008 outbreak might have been multifactorial including secondary infections with antibody enhancement due to infection with different dengue serotypes and the introduction of a new DENV-2 genotype.

The finding of DENV-2 strains belonging to or closely related to genotype V is of interest but not completely surprising. Previous authors have mentioned difficulties in tracking the progress of DENV-2 subtype III since its introduction into the region as strains belonging to this genotype are not serologically distinguishable from the pre-existing DENV-2 strains which belong to genotype V (23). Although it was suggested that DENV-2 genotype III might have displaced genotype V by the 1990s in the regions where both might compete, there is evidence that the American genotype, genotype V still remained in some regions of Central and South America. For example, DENV-2 genotype V was associated with a large epidemic of DF in Peru in 1995 and also circulates in Honduras (23, 24, 28). Therefore, it is difficult to assess whether or not DENV-2 genotype V was recently re-introduced to Jamaica or whether it has been circulating silently over the years, going largely unnoticed because it causes milder disease.

The finding of DENV-2 strains which clustered with genotype II/Asian II genotype is also of interest as these strains have not been reported previously from the Caribbean and this finding requires further investigation. Interestingly, two lineages of DENV-2 which grouped with the Asian-American genotype known to be circulating in the region were reported from Brazil during 2008. However, isolates from the 2007/2008 epidemics grouped separately and distinctly from 1990 and 1998 DENV-2 isolates. It was not clear to those authors whether or not a new lineage of DENV-2 had been introduced to the region (29). A recombinant strain of DENV-2 was reported in Mexico where there also is hyperendemicity of dengue (30).

Whereas DENV-4 genotype II has been reported from the Caribbean, the strains from the 2007/2008 Jamaica epidemic appear to be genetically different from those known to be circulating in the Caribbean (31). At this point, it is not clear whether or not these DENV-4 strains represent a new genotype which was introduced into Jamaica or a separate lineage of DENV-4 genotype II strains from the Caribbean. It is worth noting that DENV-4 genotype I was identified in Brazil during 2008 (31). Dengue-4 is known to be the most phylogenetically divergent of the DENV serotypes (23, 31).

The MOHE reported 25 deaths and 100 cases of DHF in the 2007/2008 outbreak. This substantial mortality rate



and the outbreak itself might partially be explained by the hyperendemicity of DENV on the island and the possible emergence/re-emergence of new strains of DENV-2 and DENV-4 in Jamaica. The co-circulation of three different genotypes of DENV-2 would be an important cause of concern.

Limitations of this study include the fact that the analysis was based on the nucleotide sequences of only the capsid-premembrane (C-prM) genomic region. Although analysis of this gene region has been reported to yield important genetic information, like other genomic regions of the virus, this precluded comparisons with strains from certain Caribbean islands for which the published analyses were based on nucleotide sequences of the envelope (E) gene region (32). It is worthy of note that the most recent publications on dengue genotypes in certain Caribbean islands pertained to strains isolated between 1981–2000 (23, 24, 28). This disparity should be addressed in future studies which provide additional genetic information on the dengue strains circulating currently in Jamaica.

In conclusion, this study provided important data on the dengue viruses which were in circulation in Jamaica most recently. By extrapolation, the results also indicate a high genetic diversity of DENV strains infecting the *Aedes aegypti* mosquito populations in Jamaica and a certain unpredictability of the virulence and antigenicity of the viruses for the human population (33). Vector control is therefore a key factor for dengue control in Jamaica as shown by Castle *et al* (12).

This report is largely preliminary and further studies are in progress to substantiate these findings. The need for more extensive laboratory surveillance and genetic analysis of representative strains of dengue viruses isolated from Jamaican cases is clear.

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