

# Dengue Virus Serotypes in Jamaica, 2003–2007

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## ABSTRACT

**Background:** Dengue virus (DENV) infection is increasing in prevalence and severity globally. The severity of dengue is influenced by several factors including the immune response, viral and host genetic factors.

**Method:** The DENV serotypes were determined in 770 serum samples from dengue immunoglobulin (Ig) M antibody positive ( $n = 469$ ), dengue IgM negative ( $n = 185$ ) and dengue antibody negative ( $n = 116$ ) patients with suspected dengue who presented during ( $n = 150$ ) or after ( $n = 620$ ) the acute phase of illness during 2003–2007. Dengue antibodies were detected by enzyme-linked immunosorbent assays and DENV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) performed on serum and cell culture supernatants of C6/36 mosquito cells inoculated with acute phase serum ( $n = 150$ ).

**Results:** Based on serological profiles, 41% of acute phase sera and 66% of post acute sera were from patients with current primary or secondary dengue, while 41% and 35% of acute and post-acute phase sera, respectively, were from patients with secondary dengue or past exposure only. Dengue virus RNA was found in 20/770 samples (2.6%). Only 1.5% (9/620) of sera collected after the acute phase of illness tested positive for DENV RNA compared with 2.6% (4/150) of sera collected during the acute phase and 7.3% of cell culture supernatants inoculated with acute phase serum (11/150,  $p = 0.001$ ). All four serotypes including DENV-1 (3/20, 15%), DENV-2 (7/20, 35%), DENV-3 (3/20, 15%) and DENV-4 (7/20, 35%) were identified over the five-year period. These results also showed that DENV-1, 2 and 4 were present during 2007 and that DENV-2 and DENV-4 were the likely causative viruses of the 2007–2008 dengue outbreak in Jamaica. The three strains of DENV-3 were isolated from infants less than three years of age with primary infection during 2006.

**Conclusion:** This study highlights the increasing threat of dengue and severe dengue disease to the Jamaican population. Preventative measures including laboratory surveillance and vector control should be strictly maintained at the highest level.

**Keywords:** Dengue virus, immunity, Jamaica, outbreak cell cultures

# Serotipos del Virus del Dengue en Jamaica, 2003–2007

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## RESUMEN

**Antecedentes:** La infección por virus del dengue (DENV) está creciendo en prevalencia y severidad a nivel global. La severidad del dengue está influida por varios factores, incluyendo la respuesta inmunológica así como factores virales y genéticos del huésped.

**Método:** Los serotipos del DENV fueron determinados en 770 muestras de suero de pacientes positivos al anticuerpo inmunoglobulina M (Ig) contra el dengue ( $n = 469$ ), negativos a la IgM contra el dengue ( $n = 185$ ), y negativos al anticuerpo contra el dengue ( $n = 116$ ). Estos pacientes, con sospecha de dengue, se presentaron durante ( $n = 150$ ) o después ( $n = 620$ ) de la fase aguda de la enfermedad en el periodo de 2003-2007. Los anticuerpos contra el dengue fueron detectados mediante ensayos inmunoabsorbentes ligados a enzimas (ELISA) y DENV ARN mediante reacción en cadena de la polimerasa con transcriptasa inversa (RT-PCR) realizados sobre suero y sobrenadantes del cultivo de células C6/36 de mosquitos, inoculadas con suero de la fase aguda ( $n = 150$ ).

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**Resultados:** De acuerdo con los perfiles serológicos, 41% de los sueros de la fase aguda y 66% de los sueros de la fase post-aguda, provenían de pacientes con dengue primario o secundario corriente, mientras que el 41% y el 23% de los sueros de las fases agudas y post-agudas, provenían de pacientes con dengue secundario o exposición pasada solamente. Se halló ARN del virus del dengue en 20/770 muestras (2.6%). Sólo el 1.5% (9/620) de los sueros recogidos tras la fase aguda de la enfermedad resultaron positivos al ARN del DENV, en comparación con 2.6% (4/150) de los sueros recogidos durante la fase aguda y 7.3% de los sobrenadantes del cultivo celular inoculados con suero de la fase aguda (11/150,  $p = 0.001$ ). Los cuatro serotipos incluyendo DENV-1 (3/20, 15%), DENV-2 (7/20, 35%), DENV-3 (3/20, 15%) y DENV-4 (7/20, 35%) fueron identificados en un período de cinco años. Estos resultados también mostraron que DENV-1, 2 y 4 estuvieron presentes durante 2007 y que DENV-2 y DENV-4 fueron probablemente los virus causantes del brote de dengue en 2007-2008 en Jamaica. Las tres cepas de DENV-3 fueron aisladas de infantes menores de tres años de edad con infección primaria, durante 2006.

**Conclusión:** Este estudio señala la creciente amenaza del dengue y el carácter severo de esta enfermedad para la población jamaicana. Medidas preventivas, incluyendo la vigilancia de laboratorios y el control de vectores, deben mantenerse al más alto nivel.

**Palabras claves:** Virus del dengue, inmunidad, Jamaica, brote, cultivos celulares

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## INTRODUCTION

Dengue virus (DENV), a single stranded, positive-sense RNA virus is transmitted to humans by the *Aedes aegypti* mosquito and causes an estimated 100 million cases of dengue fever, 500 000 cases of the more severe disease, dengue haemorrhagic fever (DHF), and 25 000 deaths annually (1–4). The four serotypes of DENV (DENV-1, -2, -3 and DENV-4) are antigenically related but genetically distinct. Infection with one DENV serotype is believed to provide long-term immunity to that serotype but not to other serotypes. Therefore, persons can be infected with multiple serotypes during their lifetime (5). Persons with secondary DENV infection have a greater risk of DHF than those with primary DENV infection, indicating that cross-reactive immunity to more than one DENV serotype is a risk factor for severe disease (5).

The DENV serotypes and genotypes in circulation vary between countries and within countries over time. Multiple DENV serotypes, genotypes and clades may co-circulate in the same location (6, 7). Dengue virus -1 and -2 were the most frequently reported serotypes in the Americas during the 1990s while DENV-2 and DENV-3 became the predominant serotypes during the period 2000–2007. According to reports from the region, all four dengue serotypes have been circulating in Jamaica and other countries of the Americas since the 1990s (8–11).

The occurrence of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) and severe epidemics in some regions has been associated with the introduction of certain DENV serotypes and/or genotypes (5, 6, 12, 13).

This study investigated the dengue serotypes in circulation in Jamaica during the period 2003 – 2007.

## SUBJECTS AND METHODS

Serum samples from 770 patients with dengue-like illnesses including dengue IgM positive ( $n = 469$ ), dengue IgM negative ( $n = 185$ ) and dengue antibody negative ( $n = 116$ ) patients were studied. The sera were selected consecutively from a cohort of 2248 patients with dengue-like illnesses who presented at the University Hospital of the West Indies (UHWI), a tertiary referral centre, during 2003–2007. The sera selected for the study were those from patients whose date of onset of illness were recorded. The serum was separated from 5–10 ml clotted blood samples which had been collected from each subject, submitted to the microbiology laboratory for viral investigations and stored at  $-70^{\circ}\text{C}$  until tested. Ethical approval was obtained for the study and the relevant clinical data were obtained from the patients' hospital records.

The sera ( $n = 770$ ) were tested for dengue IgM and IgG antibodies using commercially prepared enzyme linked immunosorbent assay (ELISA) kits (Focus Diagnostics, Cypress CA) according to the manufacturers' instructions.

Tube cultures of the *Aedes albopictus* high temperature (HT) mosquito (C6/36) cell line were inoculated with 50  $\mu\text{l}$  aliquots of undiluted serum from patients ( $n = 150$ ) in the acute phase of illness (onset  $\leq 5$  days) or control strains of DENV-1–4 serotypes. Following inoculation, the cultures were incubated at  $33^{\circ}\text{C}$  for seven days and maintained with Eagles' minimum essential medium (MEM), every three – four days as described (14). Cell culture supernatants were then harvested by centrifugation at 2000 rpm at  $4^{\circ}\text{C}$  and stored at  $-70^{\circ}\text{C}$  until required for testing.

The sera which were taken  $\leq 5$  days after onset of illness ( $n = 150$ ) and cell culture supernatants ( $n = 150$ ) were tested for DENV RNA using previously described reverse

transcriptase polymerase chain reaction (RT-PCR) assays, which amplify the CprM gene region of the DENV genome, with modifications (15, 16). The RNA was extracted from 140  $\mu$ l aliquots of serum and cell culture supernatants using commercially prepared reagents (QIAamp Viral RNA Mini Kit Qiagen, Germany). The manufacturer's instructions were followed.

The first round dengue RT-PCR was performed in 25  $\mu$ l of a reaction mixture containing 5  $\mu$ l 5x PCR buffer, 0.5  $\mu$ l dNTP's (10 mM), 1  $\mu$ l Mg SO<sub>4</sub> (25 mM), 5  $\mu$ l nuclease free H<sub>2</sub>O, 0.5  $\mu$ l AMV reverse transcriptase (5 units/ $\mu$ l), 1.0  $\mu$ l Tlf DNA polymerase (Promega, Corp, Madison, WI) and 1  $\mu$ l (10 picomoles) of each primer (D1 and D2) and 10  $\mu$ l RNA template which yielded a 511 bp band. The thermal cycling conditions used were: 42°C for 60 minutes (min), 94°C for two minutes followed by 28 cycles of 94°C for 30 seconds (sec), 55°C for 60 sec and 72°C for 120 sec, followed by an extension period of 72°C for 10 mins and hold at 4°C. The second round dengue RT-PCR was also performed in 25  $\mu$ l of a reaction mixture containing 2.5  $\mu$ l 10x PCR buffer, 1.0  $\mu$ l dNTP's (10 mM), 0.5  $\mu$ l Mg Cl<sub>2</sub>, 5  $\mu$ l nuclease free H<sub>2</sub>O, 1.0  $\mu$ l Taq DNA polymerase (Invitrogen, Carllsbad, CA), 1  $\mu$ l (10 picomoles) of each primer (TS1, TS2, TS3 and TS4 which yielded bands of 482 bp, 119 bp, 290 bp and 392 bp for DENV-1-4, respectively) and 10  $\mu$ l DNA template (first round product diluted 1:50). The first and second round primers for the dengue RT-PCR assay have been described previously (16, 17). All PCR amplifications were performed in a Perkin Elmer model 9700 thermal cycler (Applied Biosystems, Foster City, CA). The PCR products were detected by agarose gel electrophoresis in 1x Tris-borate buffer pH 8.0 (54 g trizma base, 27.5 g boric acid, 0.5 M EDTA pH 8.0/L) using a 2% agarose gel containing ethidium bromide (0.5  $\mu$ g/ml; Sigma Chemic St Louis, Mo) at 100 Volts for one hour then visualized on a UV transilluminator and photographed.

Data were compared by chi-square analyses using Statistical Packages for Social Sciences (SPSS) Version.12.

## RESULTS

The dengue status of the 770 patients as determined by dengue ELISA results and the phase of the disease at which blood samples were taken is shown in Table 1. An overall 41% of acute phase sera and 66% of post acute phase sera were from patients with current primary or secondary dengue while 35% of acute phase sera and 41% of post acute sera were from patients with secondary dengue or past exposure to dengue.

Only 1.5% (9/620) of serum samples from suspected dengue cases taken > 5 days after onset of illness tested positive for DENV RNA compared to 2.6% (4/150) of sera taken  $\leq$  5 days after onset and 7.3% of cell culture supernatants (11/150;  $p = 0.001$ ). Of the 11 patients with DENV RNA positive cell cultures, only 4 (36.3%) also had DENV-

Table 1: Dengue status of 770 dengue suspected cases based on time of serum collection and dengue ELISA results

Category (n)	Number of cases (%)	Dengue status
<b>Post acute phase serum (620)</b>		
IgM positive only	134 (22)	primary dengue
IgM and IgG positive	273 (44)	current primary or secondary
IgG only positive	249 (41)	current secondary or past dengue infection
IgM and IgG negative	80 (13)	dengue antibody negative
<b>Acute phase serum (150)</b>		
IgM positive only	14 (9)	primary dengue
IgM and IgG positive	48 (32)	current primary or secondary
IgG only positive	52 (35)	current secondary or past exposure
IgM and IgG negative	36 (24)	dengue antibody negative

RNA positive sera by RT-PCR. During the period under study, all four serotypes were found including DENV-1 (3/20, 15%), DENV-2 (7/20, 35%), DENV-3 (3/20, 15%) and DENV-4 (7/20, 35%). Representative agarose gel electrophoresis results are shown in Fig. 1 and the distribution of

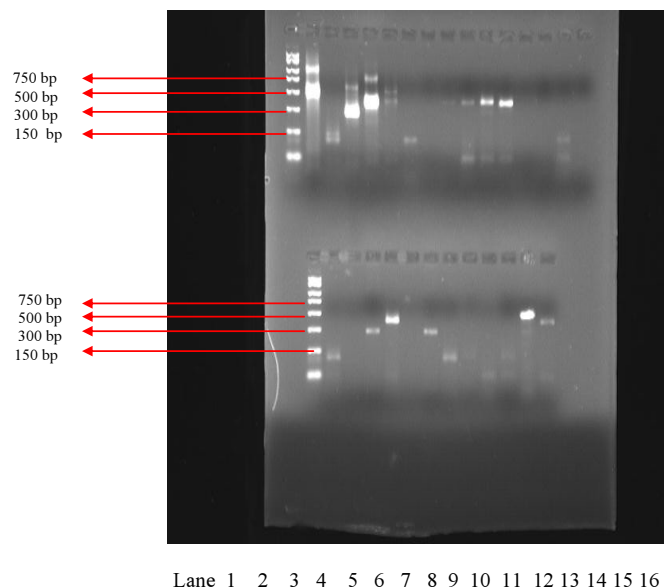


Fig. 1. Agarose gel electrophoresis of second round dengue virus reverse transcriptase-polymerase chain reaction (DENV RT-PCR) assay. Top row: lane 1, 50–2000 bp ladder; lanes 2–5, DENV 1–4 positive controls showing characteristic 482bp, 119 bp, 292 bp and 392 bp bands, respectively; lanes 6–15, samples from suspected dengue cases showing DENV-4 (lanes 6, 9–12), DENV-2 (lanes 7, 15), lane 16, negative control (molecular grade water). Bottom row: lane 1, 50–2000 bp ladder; lanes 2–13, samples from suspected dengue cases showing DENV-2 (lanes 2, 8), DENV-3 (lanes 4, 7), DENV-4 (lanes 5, 13), DENV-1 (lane 12).

DENV serotypes over the five- year period is shown in Table 2.

Table 2: Dengue virus (DENV) serotypes in circulation in Jamaica 2003–2007\*

Year	Number Tested	Positive (%)	Dengue serotype
2003	101	1 (1)	DENV-1
2004	60	1 (2)	DENV-2
2005	98	0 (0)	None identified
2006	101	3 (3)	DENV-3
2007	426	15 (4)	DENV-1, DENV-2 DENV-4

\*Dengue virus serotypes were determined by reverse transcriptase-polymerase chain reaction (RT-PCR) assay performed on serum or C6/36 cell culture supernatants.

The serology, PCR testing, demographic and clinical data on the 20 patients whose samples were DENV RNA positive are summarized in Table 3. Almost two thirds of the patients (12/20, 60%) were infants, children and young adults.

Of the 20 DENV RNA positive patients, 16 (80%) were dengue antibody positive and 4 (20%) were dengue antibody negative. Of the 16 patients who were both dengue RNA and

antibody positive, 4 (25%) were dengue IgM positive only, 8 (50%) were dengue IgG positive only and 4 (25%) were both dengue IgM and IgG positive. The four DENV RNA positive patients who were dengue-IgM positive only, were five years of age and under while the 4 DENV RNA positive patients who were both dengue-IgM and -IgG negative comprised two adults and two children (Table 3).

The DENV-1, -2 and -4 serotypes were found in sera from all age groups while DENV-3 was identified only in sera from three infants, less than three years of age, with primary dengue infection during 2006. Among the 8 (40%) patients whose illness onset dates and serological results were consistent with secondary dengue infection (dengue IgG positive only) DENV-4 (5/8, 62.5%) and DENV-2 (3/8, 37.5%) were the serotypes identified. Two of three cases of DENV-1, an eight-year old child and one adult, were both dengue IgM and IgG negative, suggestive of primary DENV-1 infection (Table 3).

## DISCUSSION

The DENV RT-PCR results presented here are the first such results to be reported from testing carried out in Jamaica. Previously, clinical samples for DENV RT-PCR testing were sent to reference laboratories overseas (unpublished data). There was a heavy bias in the samples towards sera from patients in the post acute phase of illness. Also the majority

Table 3: Laboratory and clinical data in 20 patients with positive dengue virus (DENV) RNA tests.

Patient ID	Age	Sex	Suspected Clinical Diagnosis	DENV-IgM	DENV-IgG	DENV-Serotype	Year
1	63 years	Female	? Dengue	Positive	Positive	DENV-1	2003
2	5 years	Male	? Dengue	Positive	Negative	DENV-2	2004
3	9 months	Female	Meningoencephalitis	Positive	Negative	DENV-3	2006
4	8 months	Male	Kawasaki, Viral Exanthema	Positive	Negative	DENV-3	2006
5	27 months	Male	Pyrexia of unknown origin	Positive	Negative	DENV-3	2006
6	27 years	Male	Dengue	Negative	Positive	DENV-2	2007
7	44 years	Male	Dengue	Negative	Negative	DENV-2	2007
8	9 years	Female	Dengue	Positive	Positive	DENV-4	2007
9	44 years	Female	Dengue, measles, rubella, leptospirosis	Negative	Negative	DENV-1	2007
10	8 years	Female	Dengue	Negative	Negative	DENV-1	2007
11	64 years	Male	Dengue	Negative	Positive	DENV-4	2007
12	5 years	Male	Dengue	Negative	Positive	DENV-4	2007
13	9 years	Male	Dengue	Negative	Negative	DENV-4	2007
14	16 years	Female	Acute viral illness	Negative	Positive	DENV-2	2007
15	N/A	Female	Dengue	Negative	Positive	DENV-2	2007
16	N/A	Female	Dengue	Negative	Positive	DENV-4	2007
17	18 years	Female	Dengue	Negative	Positive	DENV-4	2007
18	16 years	Female	Dengue	Negative	Positive	DENV-4	2007
19	54 years	Male	Viral illness	Positive	Positive	DENV-2	2007
20	19 months	Male	Sepsis, DHF	Positive	Positive	DENV-2	2007

\*N/A = Not available. DENV-IgM and -IgG were detected by enzyme-linked immunosorbent assay (ELISA). Dengue serotype was determined by reverse transcriptase polymerase chain reaction (RT-PCR) assay on serum or C6/36 culture cell supernatants inoculated with serum from patients with < 5 days onset of illness. DHF = dengue haemorrhagic fever.



of dengue cases were classified as secondary infections which is consistent with the dengue endemic/hyperendemic status of the Jamaican population (8–11).

Compared with the dengue IgM and IgG ELISA, the sensitivity of the DENV RT-PCR was substantially reduced. This could be caused by the fact that the samples were mostly collected from patients who presented for medical attention after the viraemic phase of illness. While the RT-PCR is highly sensitive, specific and successfully identifies the dengue virus serotype in clinical samples, it usually yields positive results in viraemic sera collected within two to five days of onset of fever. Sera collected after the period of viraemia are more likely to yield positive serological tests such as IgM capture ELISA (18). The results also show that the sensitivity of the DENV RT-PCR for detection of DENV in serum samples may be substantially increased, several folds, by first inoculating the sera into C6/36 cell cultures and testing the culture supernatants by DENV RT-PCR. This finding is in agreement with previous reports (19).

The results also confirm DENV-2 and DENV-4 as the causative viruses of the 2007 – 2008 dengue outbreak in Jamaica. The Ministry of Health and Environment (MOHE) reported that 1585 of 5461 suspected cases were confirmed in the outbreak, including 101 cases of DHF and 25 deaths due to dengue over the period (20). Dengue-2 and DENV-3 were also implicated in the 1968 – 69 outbreak of dengue-like illness in Jamaica and DENV-2 was the cause of the 1995 dengue epidemic (9, 10, 21). According to one MOHE report, DENV-2 was introduced to Jamaica during 2007 – 2008 and became the predominant serotype. However, the results suggest DENV-4 as the more likely predominant genotype during 2007 and that DENV-2 and DENV-1 were in circulation as early as 2003 – 2004.

Dengue-4, which was also listed as a causative virus of the 2007 – 2008 epidemic, emerged in Jamaica during 1981 – 1982 concurrent with a major outbreak of DHF/DSS in Cuba which was caused by DENV-4 (8).

Perhaps because of the deficits in surveillance of DENV serotypes in Jamaica over the years, there is insufficient information on tracking the activity of DENV-3 on the island. However, subsequent to the 1968 – 69 epidemic DENV-3 was again implicated in an outbreak in Jamaica, the Caribbean and the Americas during 1998 (22). During the five-year period covered by this study, 2003 – 2007, DENV-3 serotype was identified only in 2006 and the affected patients were infants less than three-years of age. The DENV-3 serotype re-emerged in Latin America in 1994 after an absence of 17 years and was first isolated in Cuba, where it caused a small outbreak, in 2002 (23). It is important that the presence of DENV-3 in Jamaica be monitored as the strain of DENV-3 genotype III currently circulating in the Americas is related to the strain which caused DHF epidemics in India and Sri Lanka (23–26). The current level of DENV-3 activity in Jamaica is not clear and it is uncertain whether or not this serotype eventually will supersede

DENV-1, DENV-2 or DENV-4 in the near or distant future. Nonetheless, even sporadic appearances of DENV-3 should be considered an important threat to the non-immune susceptible persons including infants who are less likely to have adequate protective cross-reactive antibodies and older persons who might have cross-reactive enhancing dengue antibodies.

All 4 dengue serotypes were in circulation in Jamaica during 2003 – 2007. Our results clearly illustrate the varying susceptibility of persons in the population to DENV serotypes. Adequate, sustained vector control is therefore essential to avert outbreaks of DF and DHF/DSS. In addition to providing valuable information on the dengue serotypes, the results of this study also support recommendations made by the present and previous authors that in dengue endemic countries like Jamaica it is important that a panel of tests for dengue including serology, nucleic acid testing for early diagnosis, cell culture and rapid protein/antigen tests be available to compensate for limitations in each test. The advantages of the various methods of diagnosis of primary and secondary dengue and their specific applications in the Jamaican setting have been discussed (27–29).

The limitations of this study include collection of the majority of serum samples after the acute phase of illness, delays in transport to the laboratory, incomplete or missing biodata and deficiencies in clinical data submitted with the samples.

In conclusion, the major objectives of this study including the establishment of nucleic acid tests for early diagnosis of dengue and serotype determination to strengthen surveillance in Jamaica were achieved. This study highlights the vulnerability of the Jamaican population to the increasing threat of severe dengue disease and recommends that the necessary preventive measures including laboratory surveillance and vector control be maintained at the highest level.

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