THE PREVALENCE OF DENGUE VIRUS IN BRUNEI DARUSSALAM DURING JANUARY - NOVEMBER 2010

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Abstract. The aim of this study was to retrospectively determine the prevalence of dengue virus from April 2010 to November 2010 in Brunei Darussalam. A total of 250 serum samples from dengue diagnosed patients were examined. All serum samples were tested for dengue IgM and IgG antibodies and dengue NS1 antigen using the PanBio dengue ELISA commercial kit. To determine the prevalence of dengue virus serotype in the country, serotyping was performed for the 14 samples that were positive for NS1 antigen. Dengue virus serotyping was carried out using the conventional reverse transcriptase-polymerase chain reaction (RT-PCR). Of the 250 serum samples included in the study, 196 were laboratory dengue confirmed cases. Dengue virus serotype 1 (DENV-1) was the predominant circulating serotype, followed by DENV-3 and DENV-2. This is the first report of DENV-3 isolation in Brunei Darussalam.

Keywords: dengue virus, serotyping, Brunei Darussalam

INTRODUCTION

Dengue is a mosquito-borne virus belonging to the genus *Flavivirus*, of the family Flaviviridae (Gurugama *et al*, 2010). It is a single-stranded enveloped ribonucleic acid (RNA) positive-strand virus coding for a polyprotein comprised of approximately 3,400 amino acids (Deubel *et al*, 1988). The entire genome size of the virus is approximately 11kb in length (Deubel, 1988). The genome is composed of three structural protein genes, a nucleocapsid, a membrane-associated protein, an envelope protein and seven non-structural protein genes: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Chambers *et al*, 1990; Bangs *et al*, 2006; Osman *et al*, 2009).

Dengue virus is comprised of four serotypes which share genetic and antigenic features: dengue virus serotypes 1 (DENV-1), 2 (DENV-2), 3 (DENV-3) and 4 (DENV-4) (Chambers *et al*, 1990; Gibson and Vaughn, 2002; Hilaire and Clarke-Greenidge, 2008). These four serotypes cause similar symptoms (Ross, 2010). Persons living in an area endemic for dengue fever maybe infected with up to three or four serotypes during their lifetime (Hilaire and Clarke-Greenidge,

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2008). Infection with a different serotype during a secondary infection is a risk factor for a more severe dengue manifestation, such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) (Gubler, 1998).

DENV-2 is the most common cause of DHF, followed by DENV-3, DENV-4 and DENV-1 (Jamaiah *et al*, 2007). Dengue infection causes a wide spectrum of clinical symptoms, ranging from asymptomatic infection to life-threatening illness; common symptoms include high fever, headache, backache, joint pains, nausea, vomiting, eye pain and rash (Lorono-Pino, 1999; Shamala, 2008, Gibbons, 2010).

Dengue viruses infect at least 50-100 million individuals worldwide annually, resulting in approximately 500,000 hospitalizations and 24,000 deaths, mostly in children (Osman *et al*, 2007).

The global prevalence of dengue infection has grown in recent decades. The most affected areas are in the tropical and subtropical areas, particularly in Southeast Asia (SEA) which includes Brunei Darussalam (Osman *et al*, 2007). Dengue infection is either endemic or epidemic in most countries in SEA (Osman *et al*, 2007). The prevalence of dengue infection in many countries had changed from hypoendemicity (one serotype) to hyperendemicity (multiple serotypes), which increases the risk for DHF (Gubler, 1998). The mean number of annual cases of DHF in SEA has increased from fewer than 10,000 in the 1950s and 1960s to more than 200,000 in the 1990s (Gibbons, 2010). Eighty-five percent of the population in SEA is at risk for dengue infection (Badaruddin, 2008).

Brunei Darussalam has a total land area of 5,765 km^2 and is located on the northwestern coast of the island of Borneo. It is made up of four districts: Brunei-Muara, Tutong, Belait and Temburong. In 2010, Brunei had an estimated total population of 395,027 (Brunei Ministry of Health, 2011).

From 1992 to 2006 there were 398 laboratory confirmed dengue cases in the country (Osman *et al*, 2007). The largest number of dengue cases occurred in 2003 (163 cases) (Osman *et al*, 2007). Badaruddin (2008) reported *Aedes aegypti* was the main vector in the transmission of dengue in this country, which is found abundantly in Water Village, located in the Brunei/Muara District. In 2006, DENV-2 was found to be the main serotype circulating in Brunei Darussalam followed by DENV-1 (Osman *et al*, 2009). In 2010, there were 304 laboratory confirmed dengue cases reported in the country.

MATERIALS AND METHODS

A total of 250 serum samples were collected from April 2010 to November 2010, from patients clinical diagnosed with having dengue fever. The samples were stored at -80ºC at the Virology Laboratory, Department of Laboratory Services, Ministry of Health, Brunei Darussalam until used.

All serum samples were screened using the Dengue Duo Capture IgG/IgM ELISA kit (Panbio, Stockport, UK) for dengue IgM and IgG antibodies. Serum samples, positive controls, negative controls and calibrators were first diluted to 1:100. One hundred microliters of each sample was placed in each well of the microtiter plate with either anti-human IgM or anti-human IgG and incubated at 37ºC for 1 hour. The plate was washed six times with dilute buffer to remove residual serum prior to the addition of 100 l antigen-MAB complex solution

and again incubated for 1 hour at 37ºC. The plate was again washed six times, 100 l of TMB chromogen was added to each well, and the plate was incubated for 10 minutes in the dark at room temperature. After 10 minutes, the reaction was stopped with 100 l phosphoric acid. The absorbance of each well was read within 30 minutes at a wavelength of 450 nm using a microtiter plate reader (Opsys-Dynex Technologies, Chantilly, VA). The results were interpreted according to the manufacturer's instructions.

For dengue NS1 antigen determination, each serum sample was tested using a one step sandwich format with Platelia™ Dengue NS1 AG ELISA (Bio-Rad, Hercules, CA). Fifty microliters of diluent was dispensed into each well of the assay plate containing bound murine monoclonal antibodies (MAb). Then, 50 l of sample (along with a positive control, negative control and calibrator) and 100 l of diluted conjugate were also added. The bound MAb binds to NS1 antigen. The plate was incubated for 90 minutes at 37ºC followed by washing six times with washing buffer. After removing excess buffer, 160 l of TMB chromogen was added to each well and then incubated in the dark at room temperature for 30 minutes. The reaction was stopped with 100 l sulfuric acid solution added to each well. The plate was read with a microtiter plate reader (Opsys-Dynex Technologies, Chantilly, VA) at a wavelength of 450 nm and test results were interpreted following the manufacturer's instructions.

For dengue serotyping, viral RNA was extracted from 14 serum samples using a QIAamp viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Forward and reverse primers were prepared according to the manufacturer's instructions (Applied Biosystems, Carlsbad, CA). Table 1 shows the primer sequence for each primer used in relationship to their position in the dengue genome and size of the amplicon.

Accupower®RT-PCRPreMix (Bioneer, Seoul, Korea) was used to synthesize cDNA. A cocktail mixture of RNase free water, forward primer and reverse primer was prepared.Twenty microliters of the mixture was added to each PCR tube prior to the addition of 5 l of eluted RNA included as a positive control.

The amplification process was performed in a thermal cycler (iCyclerBio-Rad) using an in-house conventional RT-PCR protocol. Reverse transcription was conducted at 50ºC for 30 minutes followed by 15 minutes of TAQ polymerase activation at 95ºC for 10 minutes before continuing with 40 amplification cycles of denaturation at 95ºC for 30 seconds, primer annealing at 60ºC for 45 seconds and extension at 72ºC for 1 minute. The PCR products were viewed on 1.5% agarose gel, with a UV transilluminator (Uvitec, Córdoba, Argentina). The serotype was determined based on the size of the amplicon compared with control amplicons (Table 1).

Statistical analysis was conducted using SPSS version 16.0 for Windows (SPSS, Arraonk, NY). Age, gender, geographical location and month of infection were recorded for all dengue positive cases using a bar chart and frequency table. Variables were compared using a chi-square test.

RESULTS

Of the 250 serum samples used in this study, 196 samples (78.4%) were positive for dengue IgM, dengue IgG, dengue NS1 antigen. Dengue IgM were positive in 143

Table 1
Primer sequence of forward, D1, D1, D3 and D4 primers.

Primer	Primer sequence	Primer position	Size of amplicon	Virus serotype
Forward	5′- AGTTGTTAGTCTACGTGGACCGACA	1-25		
Reverse D1	5′- CCCCGTAACACTTTGATCGCTCCATT	317-342	342 bp	DEN-1
Reverse D2	5′- CGCCACAAGGCCATGAACAG	231-251	251 bp	DEN-2
Reverse D3	5′- GCACATGTTGATTCCAGAGGCTGTC	514-538	538 bp	DEN-3
Reverse D4	5′- GTTTCCAATCCCATTCCTGAATGTTGGTGT	726-754	754 bp	DEN-4

Table 2
Total number of dengue cases by district.

Result of dengue laboratory testing	District				
	Brunei- Muara (*N*=208) *n* (%)	Tutong (*N* =26) *n* (%)	Belait (*N*=4) *n* (%)	Temburong (*N*=12) *n* (%)	Total
Positive	166 (79.8)	22 (84.6)	3 (75)	5 (41.7)	196
Negative	42 (20.2)	4 (15.4)	1 (25)	7 (58.3)	54

samples (73%). 89 (45.4%) were dengue IgG positive and 148 samples (75.5%) were dengue NS1 antigen positive.

Dengue infections by studied district in Brunei Darussalam

The greatest number of dengue infections were seen in the Brunei-Muara District (166 cases), followed by Tutong District (22 cases), Temburong District (5 cases) and Belait District (3 cases) (Table 2).

Age groups and gender distribution

Dengue cases were compared by gender and age group (Table 3). The prevalence of dengue infection was higher among males than females in all age groups except the 40 - 49 and 70 - 79 year old age groups. The male to female ratio was 1.3:1. The proportions of positive cases in males and females were not significantly different from each other ($p = 0.798$).

Dengue infection was the most common among patients in the 20 - 29 year old age group (27.1%) followed by the 10 - 19 years old age group (20.4%). The least number of infections was found among children in the 0 - 9 years old age group (1.5%) (Table 3).

Serotype distribution

We determine the dengue serotype in 14 NS1 antigen positive (Fig 1). Eleven (78.6%) were DENV-1 (Fig 2), two (14.3%) were DENV-3 and one (7.1%) was DENV-2 (Fig 3). Table 4 shows the serotypes by district.

DISCUSSION

The greatest number of dengue cases in Brunei Darussalam since 1992 was seen in 2010 with 304 laboratory confirmed cases (Ministry of Health Record, Brunei

Table 3
Number of dengue positive cases by age group among males and females.

Age group in years	Male	Female	Total	%
0-9	2	1	3	1.5
10-19	27	13	40	20.4
20-29	32	21	53	27.1
30-39	24	15	39	19.9
40-49	12	20	32	16.3
50-59	6	5	11	5.6
60-69	8	6	14	7.1
70-79	1	3	4	2.1
Total	112	84	196	100

Table 4
Frequency of serotypes by district.

	District				
Serotype	Brunei- Muara	Tutong	Belait	Temburong	Total
DENV-1	9	2	0	0	11
DENV-2	1	0	0	0	1
DENV-3	2	0	0	0	2
Total	12	2	0	0	14

Darussalam). Dengue infection was most prevalent in the Brunei-Muara District. This may be because more than half of the country's total population live in this district. People from other districts and outside the country migrate to the Brunei-Muara District to seek employment, goods and services. The only international airport in the country is also located in this district which serves as a transport link for travelers from outside the country which can increase the risk of dengue virus transmission to the local community (Osman *et al*, 2007).

All the serum samples used in the study were from patients clinically diagnosed as dengue with fever for less than 7 days. There was limited documentation for clinical diagnosis of the patients in the laboratory test requested form, therefore all the three tests : NS1 antigen, IgM and IgG ELISA were performed to confirm the dengue infection. We had difficulty in obtaining paired samples as most of our cases were from out-patients setting.

Only 5 cases were found in Temburong District compared to 26 cases in 2006. An increased awareness of vector control could be one of the factors for this decline. The small number of positive cases in Tutong and Belait Districts could be due to fewer breeding sites for the vector population.

The male to female ratio of 1.3:1 indicates both genders are almost at equal risk of contracting dengue infection. One

study found women are at higher risk of dengue infection (Martin *et al*, 2007). In India and Singapore men were twice as likely to contract dengue infection as women (Goh *et al*, 1987; Agarwal *et al*, 1999). In our study women had a slightly lower risk of contracting dengue than men, this could possibly be due to the fact that more men are involved in outdoors activities.

In our study, the high proportion of dengue infections was found in the 20-29 year old age group. This finding is consistent with other countries in Southeast Asia, including Singapore, Malaysia and Indonesia (Agarwal *et al*, 1999; Ooi, 2001; Tahir *et al*, 2010). A previous study showed people aged 20-29 years were more likely to be involved in outdoor activities and travelling (Rodenhuis-Zybert *et al*, 2010). In the past, dengue infection has been thought of as a childhood disease. Our study found dengue infection is common among young adults. An age shift has been seen in Southeast Asian countries during the past few decades (Guha-Sapir and Schimmer; 2005).

In conclusion, this study found that in the period from April to November 2010, three dengue serotypes, DENV-1, DENV-2 and DENV-3 were circulating in Brunei Darussalam. Out of the 250 serum samples tested, 196 samples were positive for dengue virus. Brunei Muara District has the highest cases (84.7%) followed by Tutong District (11.2%), Temburong District (2.5%) and Belait District (1.5%). The male to female ratio was 1.3:1. The 20-29 year old age group was most affected. Of the 14 viral RNA detected and identified, 11 were DENV-1, 2 were DENV-3 and 1 was DENV-2. This study is the first reported isolation of DENV-3 serotype in the country.

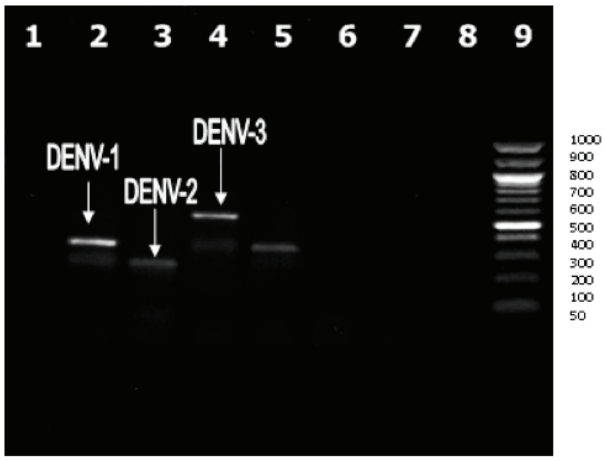

Fig 1–Gel showing positive control, DENV-1 at 342 bp, DENV-2 at 251 bp and DENV-3 at 538 bp.

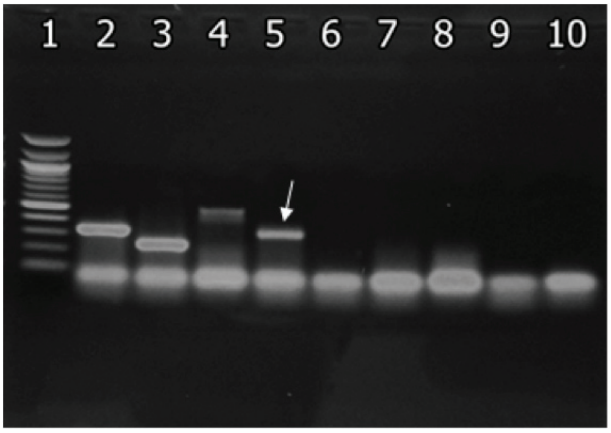

Fig 2–Agarose gel demonstrating multiplex RT-PCR product for DENV-1 serotyping. Lane 1: 100 bp DNA ladder; lane 2: DENV-1 positive control; lane 3: DENV-2 positive control; lane 4: DENV-3 positive control; lane 5: sample 114 (DENV-1 positive (+*ve*)); Lane 6: sample 115; lane 7: sample 116; lane 8: sample 117 (i); lane 9: sample 117 (ii); lane 10: sample 148.

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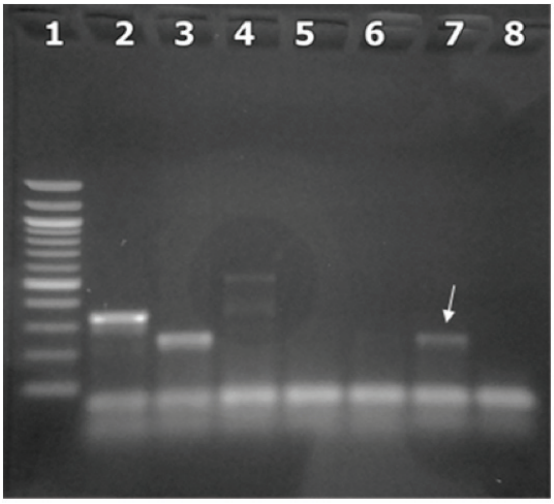

Fig 3–Agarose gel demonstrating multiplex RT-PCR product for DENV-2 serotyping. Lane 1: 100 bp DNA ladder; lane 2: DENV-1 positive control; lane 3: DENV-2 positive control; lane 4: DENV-3 positive control; lane 5: sample 155; lane 6: sample 156; lane 7: sample 157 (DENV-2 positive (+*ve*); lane 8: negative control.

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