

# SEQUENCE ANALYSIS OF E/NS1 GENE JUNCTION OF DENGUE VIRUS TYPE 2 ISOLATED IN BRUNEI

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**Abstract.** A preliminary study of dengue infection in Brunei between 2005 and 2006 showed that dengue 2 was the predominant serotype. A total of five DEN-2 isolates were isolated and maintained in the mosquito cell-line, albopictus C6/36. The sequence spanning the envelope and non-structural protein 1 (E/NS1) junction (positions 2311 to 2550) of the isolates were determined and analysed at the amino acid and nucleotide levels. Alignment of the 240 nucleotide sequences among the five isolates showed changes occurring at 7 positions (2.9%) of the region. All but one nucleotide substitution (position 2319, amino acid 742 V→F) were found at the 3<sup>rd</sup> position of the codons and were silent mutations. Amino acid homology ranged from 98% to 100%. Sequence divergence of the Brunei isolates varied from 5% to 6.6% compared with dengue-2 prototype New Guinea C strain. Comparison of the Brunei DEN-2 isolates with sixty-five other strains placed them in a cluster containing Indonesian strains isolated in 1973, 1978 and 2004 and Malaysian strains isolated in 1996, 1998 and 1999 in genotype group IV.

## INTRODUCTION

Dengue is one of the most important tropical diseases that causes an economic burden in more than 100 countries in tropical and subtropical regions of the world, threatening some 2.5-3 billion people (Gubler, 1998, 2002). Annually, it is estimated that 50-100 million people worldwide are infected with the virus causing uncomplicated dengue fever (DF), with about 500,000 developing dengue hemorrhagic fever (DHF) resulting in 25,000 deaths (Gubler and Clark, 1995; Monath and Heinz, 1996; WHO, 1997; Gibbons and Vaughn, 2002). Several factors are believed to be responsible for this expanding geographical distribution of dengue, such as global population growth, unplanned urbanization, insufficient mosquito control and in-

creased air travel (Monath, 1994; Gubler, 1998; WHO, 2002). The infection in adult humans produce flu-like symptoms characterized by fever, headache, eye pain, myalgia, arthralgia, and rash (Nimmannitya, 1987) while in children, most are mild, asymptomatic and cannot be easily distinguished clinically from other viral infections (Henchal and Putnak, 1990) but may present as undifferentiated fever, classic DF or DHF (WHO, 1973; Race, 1979).

The causative agent responsible is dengue virus, genus *Flavivirus*, family Flaviviridae. The virus is transmitted through the bite of mosquito vectors *Aedes aegypti* and *Aedes albopictus*, which principally inhabit tropical and sub-tropical regions. Mosquito avoidance and control of larval breeding sites can prevent most transmission and infection. The dengue virus is a single-stranded (+)-sense, encapsulated RNA of approximately 11kb in length that contains a single open reading frame but lacks a poly(A) tail at the 3'-end (Chambers *et al*, 1990). The genome is com-

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posed of three structural protein genes, encoding nucleocapsid or core protein (C), membrane-associated protein (M) and an envelope protein (E), and seven non-structural (NS) protein genes (Deubel, 1988; Lindenbach and Rice, 2001). The order of proteins encoded is 5'-C-prM(M)-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3', similar to other flaviviruses (Deubel, 1988; Chambers *et al*, 1990). Initially, a polypeptide of 3,000 amino acids is synthesized followed by posttranslation processing involving viral and host proteases.

Dengue virus has four serotypes, designated DEN-1, DEN-2, DEN-3, and DEN-4, that are distinct based on their antigenic patterns and can be distinguished by serological and molecular methods (Chambers *et al*, 1990). Infection with any one serotype induces life-long protective immunity to the homologous serotype, but confers only transient or no cross-reactive immunity to subsequent infections by the other three serotypes. It has been accepted that secondary infection or multiple infections with other dengue virus serotypes poses a major risk factor for developing DHF due to antibody-dependent enhancement, ADE (Burke *et al*, 1988). Other factors that have been considered equally important in the pathogenesis of DHF include viral virulence (Gubler *et al*, 1978), viral load (Vaughan *et al*, 2000), host genetics (Gibbons and Vaughn, 2002), T-cell activation (Lei *et al*, 2001), and autoantibodies (Deubel *et al*, 1993)). Several phylogenetic (Rico-Hesse, 1990; Lewis *et al*, 1993; Messer *et al*, 2003) and epidemiologic (Rico-Hesse *et al*, 1997; Messer *et al*, 2002; Uzcategui *et al*, 2003) studies have highlighted the capabilities of specific viral genotypes to produce DHF epidemics in a population with variable immune status. For example, in the Western Hemisphere, the origin and spread of DHF can be linked to viruses of the genotypes from Southeast Asia, whereas American genotype viruses have been isolated solely from DF patients, therefore suggesting the

importance of biological differences among viral genotypes.

The evolutionary and epidemiologic relationships between many isolates of the same virus become apparent only by comparing nucleotide sequences that share a common ancestry, from which data can be generated for a phylogenetic trees to be inferred quantitatively (Zuckerkindl and Pauling, 1965). Many investigators have made comparative analyses of nucleotide and amino acid sequences of short segments of specific gene regions to study molecular epidemiology and evolution of the dengue virus strains (Rico-Hesse, 1990; Deubel *et al*, 1993; Lewis *et al*, 1993). The E/NS1 gene junction is one of the most widely studied for comparative sequence analyses due to its uniform rate of random mutation with minimal selective advantages (Rico-Hesse, 1990).

Brunei is a Malay sultanate state situated on the island of Borneo in Southeast Asia flanked by the Malaysian States of Sarawak in the west and Sabah in the east. With an area of 2,226 square miles, the country is divided into four districts, Brunei-Muara, Tutong, Belait and Temburong. Statistical report published by the Public Health Department, Ministry of Health, Brunei, indicated that from the year 1992 to 2006 dengue was present in the country and due to lack of molecular epidemiological studies, the circulating serotype was not identified. In 2005, Sabah had 1,730 DF and 40 DHF cases while Sarawak had 706 DF and 13 DHF cases (WHO Collaborating Center-Malaysia, 2005). To date, no DHF had been reported in the country but with the current increase in air and land human travel, it is anticipated that outbreaks may be on the rise which may increase the risk of DHF. This study was designed to determine the relatedness of these viruses with those of neighbors in Borneo Island, countries in the Southeast Asia region and other parts of the world. The 240 nucleotides spanning the E/NS1 junction of

five Brunei DEN-2 isolates were sequenced and analysed at the amino acid and nucleotide levels.

## MATERIALS AND METHODS

### Dengue 2 virus isolates

Five dengue 2 virus isolates from Brunei were studied (Table 1). These viruses were isolated between 2005 and 2006 from patients sera suspected of dengue fever infection. Isolates DS11/221105 and DS17/130606 were from dengue cases in the Brunei-Muara district, isolates DS31/291005 and DS04/221205 from Temburong district and isolate DS09/280106 from Tutong.

### Virus propagation and viral RNA extraction

Viruses were propagated by inoculation into *Aedes albopictus* C6/36 mosquito cell line (Igarashi *et al*, 1982). C6/36 cells were grown as monolayers in a 25cm<sup>3</sup> angle-neck tissue culture flask and inoculated with 100 µl of patient's serum or infected cell culture supernatant and kept at 37°C for 1 hour to allow for virus adsorption. The infected fluid was harvested 10 days after incubating at 28°C. All isolates were cultured for up to at least three passages. Growth was monitored and identified by the indirect immunofluorescence antibody test (IFAT) using dengue-specific monoclonal antibodies DEN 1(MAB D2-1F1-3), DEN 2(MAB 3H3-1-21), DEN 3(MAB D6-8A1-12) and DEN 4(MAB 1410-6-7) (gift from CDC, Atlanta, USA).

The presence of virus in tissue culture supernatants was determined by real-time RT-PCR. Viral RNA was extracted from the infected cells using QIAamp Viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. One-step RT-PCR amplification (Kong *et al*, 2006) was carried out using the QuantiTect<sup>®</sup> SYBR<sup>®</sup> Green RT-PCR reagent kit (Qiagen, Germany). Amplification was conducted in a BioRad iCycler system. Samples were assayed in a 25 µl reaction mixture. Thermal cycling consisted of reverse transcription step at 50°C for 30 minutes, *Taq* polymerase activation at 95°C for 10 minutes, followed by 35 cycles of PCR at 94°C for 30 seconds, 60°C for 40 seconds and 72°C for 50 seconds.

### cDNA synthesis

cDNA synthesis and PCR of the target sequence were carried out using a commercial kit (AccessQuick<sup>™</sup> RT-PCR System, Madison, WI 53711-5399, USA). Primers 5'-GAGAGGAGCGAAGAGAATGG-3' (forward) and 5'-TCAGCTCAGTGGGTTGAGG-3' (reverse) were annealed at two sites, 2154-2173 and 2737-2755 flanking the E/NS1 gene junction of dengue 2 virus genome. Amplification was conducted in PTC-200 Peltier thermal cycler using 40 cycles of 94°C for 30 seconds, 55°C for 45 seconds, 72°C for 1 minute, with a final incubation at 72°C for 5 minutes. Gel electrophoresis was used to confirm the size of the target PCR product which consisted of 602 base pair fragment encompassing the 240 nucleotide E/NS1 gene junction region. The

Table 1  
Brunei DEN-2 isolates used in the study.

Isolate	Location	Year isolated	Passage
DS31/291005	Temburong	2005	3
DS11/221105	Brunei-Muara	2005	3
DS04/221205	Temburong	2005	2
DS09/280106	Tutong	2006	2
DS17/130606	Brunei-Muara	2006	2

PCR product was purified using QIAquick PCR purification kit (Qiagen, Germany).

#### Sequencing and analysis of PCR product

DNA sequencing of the PCR product were carried out by a commercial laboratory (MACROGEN, Korea) and the nucleotide sequences have been deposited in NCBI GenBank. Sixty-three E/NS1 nucleotide sequences from different strains and countries around the region and other parts of the world obtained from NCBI GenBank (accession numbers: EU031572-EU031576, AF400004, AJ556809, AJ556811, AJ886813, AY858035, AY858036, D44542-D44548, M20558, M32932-M32971, U87321, U87339-U87342, U87350, U87358, U87366, U87376-U87377 and U87380) and used in the study are shown in Table 2. Analyses of the sequence data were performed by using CLUSTAL W (<http://www.ebi.ac.uk/clustalw/>). Phylogenetic analyses were conducted in MEGA4 (Tamura *et al*, 2007) and tree was constructed from aligned nucleotide sequences using the neighbor-joining method (Saitou and Nei, 1987). Evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei, 1993) and branch topology was verified by generating 1,000 bootstraps.

## RESULTS

Fig 1 shows the gel-electrophoresis of the amplicons encompassing E/NS1 target sequence obtained from the five dengue 2 Brunei isolates and dengue prototype 1-4. The expected 602 bp PCR product were obtained from isolates DS09/280106, DS31/291005, DS17/130606, DS11/221105, DS04/221205 and prototype DEN-2. The primers set used amplified a 200 bp PCR product from prototype DEN-3 RNA template but those from prototype DEN-1 and 4 were not obtained.

Alignments of the nucleotide and deduced amino acid sequences of the five Brunei DEN-2 isolates at the E/NS1 gene junction are shown in Fig 2. Differences occurred at 7 positions (2.9%) of the sequenced region. Isolate DS04/221205 showed 100% identity with isolate DS31/291005. Isolates DS09/280106 and DS17/130606 had 4 nucleotide substitution (A→G at position 2376 and 2385, C→U at position 2442 and G→A at position 2496), whereas 6 substitutions were found in isolate DS11/221105 (G→U at position 2320, A→G at position 2376, 2379 and 2385, C→U at position 2442 and A→U at position 2487). The substitutions were generally located at the



Fig 1—Gel-electrophoresis of PCR product of dengue virus E/NS1 sequence. Lane 1, 100bp DNA ladder; lane 2, isolate DS09/280106; lane 3, DS31/291005; lane 4, DS17/130606; lane 5, DS11/221105; lane 6, DS04/221205; lane 7, negative control; lane 8, prototype DEN-1; lane 9, prototype DEN-2; lane 10, prototype DEN-3; lane 11, prototype DEN-4. PCR amplification of the E/NS1 gene junction gave a 602 base pair fragment. The amplified products were resolved on a 2% agarose gel and stained with ethidium bromide prior to visualization under UV.

Table 2  
Geographic origin and year of isolation of DEN-2 viruses used in the study.

Isolate	Country	Year	GenBank accession no.	Isolate	Country	Year	GenBank accession no.
PR159/S1	Puerto Rico	1969	M32968	JAH	Jamaica	1982	M32960
PR159	Puerto Rico	1969	M32967	NC9163	New Caledonia	1972	M32961
NGC	New Guinea	1944	M32962	PhH2172	Philippines	1983	M32963
57S	Vietnam	1987	M32948	PL-001	Taiwan	1981	M32964
1251	Tonga	1974	M32935	PL-046	Taiwan	1981	M32965
1232	Indonesia	1978	M32934	TR1751	Trinidad	1954	M32969
044	Mexico	1983	M32933	UV2039	Burkina Faso	1980	M32970
028	Philippines	1988	M32932	VEN2	Venezuela	1987	M32971
1318	Puerto Rico	1981	M32936	ThNH-7/93	Thailand	1993	D44542
1329	Jamaica	1982	M32937	ThNH-28/93	Thailand	1993	D44543
1334	Sri Lanka	1981	M32938	ThNH-52/93	Thailand	1993	D44544
1349	Burkina Faso	1982	M32939	ThNH-p11/93	Thailand	1993	D44545
1353	Sri Lanka	1982	M32940	MM-1(876/87)	Myanmar	1987	D44546
16681	Thailand	1964	M32941	MM-1(872/87)	Myanmar	1987	D44547
24H	Vietnam	1987	M32942	MM-3(1009/87)	Myanmar	1987	D44548
28741	Tahiti	1971	M32943	D80-038	Thailand	1980	U87366
348600	Colombia	1986	M32944	PUO-280	Thailand	1980	U87377
350447	Colombia	1987	M32945	D80-100	Thailand	1980	U87339
351863	Colombia	1988	M32946	D80-141	Thailand	1980	U87321
516	Thailand	1983	M32947	LF5-99	Sarawak	1999	AF40004
766635	Taiwan	1987	M32949	MY96-5176	Malaysia	1996	AJ556809
8110827	Jamaica	1981	M32950	MY98-17933	Malaysia	1998	AJ556811
8720	Indonesia	1973	M32951	MY99-19586	Malaysia	1999	AJ556813
8730	Seychelles	1977	M32952	1409	Jamaica	1983	M20558
975	Sri Lanka	1985	M32953	BA05i	Indonesia	2004	AY858035
ArA2022	Burkina Faso	1980	M32954	TB16i	Indonesia	2004	AY858036
ArA510	Ivory Coast	1980	M32955	D81-081	Thailand	1981	U87340
ArA6894	Burkina Faso	1986	M32956	KD92-201	Thailand	1992	U87358
ArD20761	Senegal	1974	M32957	KO142	Thailand	1994	U87350
DakA578	Ivory Coast	1980	M32958	CO235	Thailand	1995	U87380
HD10674	Senegal	1970	M32959	D90-206	Thailand	1990	U87376

Table 3  
Percent homology and divergence between DEN-2 Brunei isolates and prototype reference strains New Guinea C and PR159/S1.

Strains	NGC GENOTYPE II		PR159/S1 GENOTYPE I	
	Homology (%)	Divergence (%)	Homology (%)	Divergence (%)
DS31/291005	95.8	4.2	92.1	7.9
DS11/221105	95.0	5.0	90.0	10.0
DS04/221205	95.8	4.2	92.1	7.9
DS09/280106	94.2	5.8	90.4	9.6
DS17/130606	94.2	5.8	90.4	9.6

SEQUENCE ANALYSIS OF E/NS1 GENE JUNCTION OF DEN-2

**A**

	<b>2311</b>	<b>2370</b>
	•	•
DS31/291005	AUAGGAGUCGUCACAUACAUGGAUAGGAAUGAAUUCACGCAGCACCCUCACUGUCUGUGUCA	
DS11/221105	.....U.....	
DS04/221205	.....	
DS09/280106	.....	
DS17/130606	.....	
	<b>2371</b>	<b>2430</b>
	•	•
DS31/291005	CUAGUAUUAGUGGGAGUCGUGACAUUUGUAUUUGGGAGUUAUGGUGCAGGCCGAUAGUGGU	
DS11/221105	.....G..G.....G.....	
DS04/221205	.....	
DS09/280106	.....G.....G.....	
DS17/130606	.....G.....G.....	
	<b>2431</b>	<b>2490</b>
	•	•
DS31/291005	UGCGUUGUGAGCUGGAAAAACAAAGAACUGAAAUGGGCAGUGGGAUUUUUAUCACAGAC	
DS11/221105	.....U.....	
DS04/221205	.....	
DS09/280106	.....U.....	
DS17/130606	.....U.....	
	<b>2491</b>	<b>2550</b>
	•	•
DS31/291005	AACGUGCACACAUGGACAGAACAUAUCAAUCCAGAAUCCCUUCAAGCUGGCU	
DS11/221105	.....	
DS04/221205	.....	
DS09/280106	.....A.....	
DS17/130606	.....A.....	

**B**

	<b>739</b>	<b>798</b>
	•	•
DS31/291005	IGVVITWIGMNSRSTSLVSLVVLVGVVTLVGLVMVQADSGCVVSWKNKELKCGSGIFITD	
DS11/221105	...F.....	
DS04/221205	.....	
DS09/280106	.....	
DS17/130606	.....	
	<b>799</b>	<b>818</b>
	•	•
DS31/291005	NVHTWTEQYKFPESPSKLA	
DS11/221105	.....	
DS04/221205	.....	
DS09/280106	.....	
DS17/130606	.....	

Fig 2–Alignment of E/NS1 junction nucleotides (A) and deduced amino acids (B) of Brunei DEN-2 isolates. Dots indicate nucleotides identical for all the isolates to the reference strain, isolate DS31/291005. Numbering of the nucleotide position are according to Hahn *et al* (1988).

third position of a particular codon causing no amino acid substitution and were of the transition (A↔G, C↔U) type. Nucleotide change which resulted in amino acid substitution was only found at position 2391 in isolate DS11/221105I, J→F at codon 742. Isolates DS31/291005, DS04/221205, DS09/280106 and DS17/130606 possessed identical amino acid sequences across the E/NS1 gene junction. Nucleic acid homology among the Brunei isolates ranged from 97.5% to 100% and the deduced amino acid sequences were highly conserved with homology ranging from 98.75-100%.

E/NS1 nucleotide sequences of Brunei isolates were compared with DEN-2 prototype New Guinea C strain (genotype II) and reference strain PR 159/S1 (genotype I) (Table 3). Nucleotide divergence of the isolates varied from 4.2% to 5.8% when compared with New Guinea C and 7.9 % to 10.0% with strain PR159/S1. The isolates showed two amino acid substitution compared with NGC strain (I→V at amino acid position 742 and P→H at amino acid position 801), whereas with strain PR159/S1, 3 amino acid changes were observed (I→V at amino acid position 742 and 764 and V→I at amino acid position 796); however isolate DS11/221105 showed the change of I→F at amino acid position 742 compared to both reference strains. The change at amino acid position 801 replacing proline (which was conserved in all DEN-2 strains compared with NGC) with histidine in the NS1 region was also observed only in all five Brunei isolates.

Alignment and comparison of the E/NS1 nucleotide and the deduced amino acid sequence between Brunei DEN-2 isolates and 63 DEN-2 strains from different geographical regions are shown in Figs 3 and 4, respectively. The strains were arranged in their genotypic grouping of Rico-Hesse based on E/NS1 nucleotide sequence analysis. Using CLUSTAL W alignment program, the phylogram tree generated five main genotypic

group (Fig 5). The results placed the five Brunei DEN-2 isolates in the cluster with Indonesian strains 1232 and 8720 isolated in 1978 and 1973, respectively of the genotypic group IV or Afro-Asia (Rico-Hesse, 1990). Other strains in the same group were West Malaysian strains isolated in 1996, 1998 and 1999, Indonesian strains isolated in 2004 and a strain from Sarawak isolated in 1999.

## DISCUSSION

Researchers have successfully showed substantial genetic differences within all four dengue serotypes using comparative gene sequence analysis (Rico-Hesse, 1990; Lewis *et al*, 1993; Lanciotti *et al*, 1994,1997; Chungue *et al*, 1995; Twiddy *et al*, 2002). Analysis of a limited sequence of the viral RNA is sufficient to determine the epidemiology and classification of dengue virus strains. At present, there is no published DNA sequencing work on any of dengue strains of the Borneo Island as reflected in the limited availability of the virus sequences in the GenBank. Our study is the first to report the molecular analysis of dengue virus in Brunei. The main objectives of the study were to establish the virus genotype, localize the source of transmission and analyse the development of any variation in the nucleotide and the amino acid sequences of the virus genome. The rationale of choosing the E/NS1 gene to compare variations among strains of the same serotype was due to the high variation that exists in this particular region which has also been the focus of other phylogeny studies (Rico-Hesse, 1990).

The 240 nucleotide and deduced 80 amino acid sequences of the E/NS1 gene junction of five Brunei DEN-2 isolates were determined. Sequences from 63 DEN-2 strains were used in the comparison and Rico-Hesse DEN-2 E/NS1 classification method was used to genotype the Brunei strains. From



SEQUENCE ANALYSIS OF E/NS1 GENE JUNCTION OF DEN-2

		2311	2370
PR159	PUER R	1969	AUAGGAGUUAUCAUCACAUGGAUAGGAAUGAACUCACGUAGCACAUCACUGUCUGUGUCA
PR159/S1	PUER R	1969	.....
1251	TONGA	1974	.....
28741	TAHITI	1971	.....
348600	COLOMBIA	1986	.....
NC9163 N	CALEDONIA	1972	.....
044	MEXICO	1983	.....C.....
VEN2	VENEZUELA	1987	.....C.....
350447	COLOMBIA	1987	.....
351863	COLOMBIA	1988	.....
1318	PUER R	1981	.....A.....
TR1751	TRINIDAD	1954	.....U.....C.....
766635	TAIWAN	1987	.....C.U.....U.....C.....
PL-046	TAIWAN	1981	.....C.....U.....C.....
028	PHILIPPINES	1988	.....C.U.....U.....C.....A.....
PL-001	TAIWAN	1981	.....C.U.U.....U.....C.....
PhH2172	PHILIPPINES	1983	.....U.U.....U.....C.....
D80-038	THAI	1980	.....C.....C.U.....U.....C.....
NGC	N GUINEA	1944	.....C.U.....U.....C.....C.....
16681	THAI	1964	.....C.U.....U.....C.....C.....A.....
ThNH-7/93	THAI	1993	.....C.U.....U.....C.....C.....
ThNH-28/93	THAI	1993	.....C.U.....U.....C.....C.....
ThNH-52/93	THAI	1993	.....C.U.....U.....C.....C.....
ThNH-p11/93	THAI	1993	.....C.U.....U.....C.....C.....
PUO-280	THAI	1980	.....C.U.....U.....C.....C.....C.....
D80-100	THAI	1980	.....C.U.....A.U.....C.....C.....
D81-081	THAI	1981	.....C.U.....G.....U.....C.....C.....
MM-1	MYANMAR	1987	.....C.U.....U.....C.....C.....
MM-2	MYANMAR	1987	.....C.U.....U.....C.....C.....
MM-3	MYANMAR	1987	.....C.U.....G.....U.....C.....C.....
KD92-021	THAI	1992	.....C.U.....U.....C.....C.....
KO142	THAI	1994	.....C.U.....U.....C.....C.....
CO235	THAI	1995	.....C.U.....U.....C.....C.....
D90-206	THAI	1990	.....C.U.....U.....C.....C.....
57S	VIETNAM	1987	.....C.....U.....C.....
24H	VIETNAM	1987	.....C.....U.....C.....
8110827	JAMAICA	1981	.....C.....U.....C.....
JAM1409	JAMAICA	1983	.....C.....U.....C.....
516	THAI	1983	.....C.....U.....C.....
D80-141	THAI	1980	.....C.....A.U.....C.....
1329	JAMAICA	1982	.....C.....U.....C.....
JAH	JAMAICA	1982	.....C.....U.....C.....
1232	INDONESIA	1978	.....CG.....U.....C.....
8720	INDONESIA	1973	.....CG.....U.....C.....C.....
8730	SEYCHELLES	1977	.....U.....C.....C.....
1349	B FASO	1982	.....U.....C.....C.....
ArA6894	B FASO	1986	.....U.....U.....C.....U.....
975	SRI LANKA	1985	.....G.....U.....C.....C.....
1334	SRI LANKA	1981	.....G.....U.G.....C.....C.....
1353	SRI LANKA	1982	.....G.....U.....C.....C.....
DS31/291005	BRUNEI	2005	.....CG.....U.....C.....C.....
DS11/221105	BRUNEI	2005	.....CU.....U.....C.....C.....
DS04/221205	BRUNEI	2005	.....CG.....U.....C.....C.....
DS09/280106	BRUNEI	2006	.....CG.....U.....C.....C.....
DS17/130606	BRUNEI	2006	.....CG.....U.....C.....C.....
BA05i	INDONESIA	2004	.....CG.....U.....C.....C.....
TB16i	INDONESIA	2004	.....CG.....U.....C.....C.....
MY96-5176	MALAYSIA	1996	.....CG.....U.....C.....C.....A.....
MY98-17933	MALAYSIA	1998	.....CG.....U.....C.....C.....
MY99-19586	MALAYSIA	1999	.....CG.....U.....C.....C.....
LF5-99	SARAWAK	1999	.....CG.....C.U.....C.....C.....
PM33974	GUINEA	1981	.....G...G.U.....U.C.C.....C.....A...A.C
ArA510	IVORY COAST	1980	.....C...G.U.....U.C.C.....U.C.....A...A.C
UV2039	B FASO	1980	.....G...G.U.....U.C.C.....U.C.....A...A.C
ArA2022	B FASO	1980	.....G...G.U.....U.C.C.....U.C.....A...A.C
DakA578	IVORY COAST	1980	.....G.CG.U.....U.C.C.....U.C.....A...A.C
ArD20761	SENEGAL	1974	.....G...G.U.....C.C.....U.C.....A...A.C
HD10674	SENEGAL	1970	.....G.GG.U.....C.C.....U.CU.....A...A.C



2371

2430

PR159 PUER R 1969 CUGGUUUUAGUGGGAAUCGUGACACUGUACUUGGGAGUUUUGGUGCAGGCCGAUAGUGGU  
 PR159/S1 PUER R 1969 .....  
 1251 TONGA 1974 .....  
 28741 TAHITI 1971 .....  
 348600 COLOMBIA 1986 ..... A .....  
 NC9163 N CALEDONIA 1972 .....  
 044 MEXICO 1983 ..... U . A .....  
 VEN2 VENEZUELA 1987 ..... U . A .....  
 350447 COLOMBIA 1987 ..... C . . . . . A .....  
 351863 COLOMBIA 1988 ..... C . . . . . A .....  
 1318 PUER R 1981 ..... C .....  
 TR1751 TRINIDAD 1954 ..... A ..... C ..... C ..... C .....

766635 TAIWAN 1987 . . A . . . . . G . . . . . G . . . . . CG .....  
 PL-046 TAIWAN 1981 . . A . . . . . G . . . . . G . . . . . CG .....  
 028 PHILIPPINES 1988 . . A . . . . . G . . . . . G . . . . . CG .....  
 PL-001 TAIWAN 1981 . . . . . G . . . . . G . . . . . CG .....  
 PhH2172PHILIPPINES 1983 . . . . . G . . . . . G . . . . . CG .....  
 D80-038 THAI 1980 . . A . . . . . G . . . . . G . . . . . A . . . . . CU . . . . . U . . . . . U .....  
 NGC N GUINEA 1944 . . A . . . . . G . . . . . G . . . . . G . . . . . U .....  
 16681 THAI 1964 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 ThNH-7/93 THAI 1993 . . A . . . . . G . . . . . U . . . . . U . . . . . C . . . . . A .....  
 ThNH-28/93 THAI 1993 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 ThNH-52/93 THAI 1993 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 ThNH-p11/93 THAI 1993 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 PUO-280 THAI 1980 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 D80-100 THAI 1980 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 D81-081 THAI 1981 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 MM-1 MYANMAR 1987 . . A . . . . . G . . . . . G . . . . . U . . . . . U . . . . . C .....  
 MM-2 MYANMAR 1987 . . A . . . . . G . . . . . G . . . . . U . . . . . U . . . . . C .....  
 MM-3 MYANMAR 1987 . . A . . . . . G . . . . . G . . . . . U . . . . . U . . . . . C .....  
 KD92-021 THAI 1992 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 KO142 THAI 1994 . . A . . . . . G . . . . . U . . . . . U . . . . . C .....  
 CO235 THAI 1995 . . A . . . . . G . . . . . G . . . . . U . . . . . U . . . . . C .....  
 D90-206 THAI 1990 . . A . . . . . U . . . . . U . . . . . U . . . . . C .....

57S VIETNAM 1987 . . A . . . . . G . . . . . U . . . . . G . . . . . A . . . . . A . . . . . C . . . . . C . . . . . UA .....  
 24H VIETNAM 1987 . . A . . . . . G . . . . . U . . . . . G . . . . . A . . . . . A . . . . . C . . . . . UA .....  
 8110827 JAMAICA 1981 . . . . . G . . . . . G . . . . . G . . . . . C . . . . . C . . . . . U .....  
 JAM1409 JAMAICA 1983 . . . . . G . . . . . G . . . . . G . . . . . C . . . . . C . . . . . U .....  
 516 THAI 1983 . . C . . . . . G . . . . . G . . . . . A . . . . . A . . . . . C . . . . . U .....  
 D80-141 THAI 1980 . . A . . . . . G . . . . . G . . . . . G . . . . . U . . . . . CU . . . . . A .....  
 1329 JAMAICA 1982 . . A . . . . . G . . . . . G . . . . . G . . . . . U . . . . . C . . . . . U .....  
 JAH JAMAICA 1982 . . . . . G . . . . . G . . . . . G . . . . . C . . . . . U .....

1232 INDONESIA 1978 . . . . . U . . . . . G . . . . . U . . . . . U .....  
 8720 INDONESIA 1973 . . . . . G . . . . . U . . . . . U .....  
 8730 SEYCHELLES 1977 . . . . . G . . . . . U . . . . . U .....  
 1349 B FASO 1982 . . . . . G . . . . . U . . . . . U .....  
 ArA6894 B FASO 1986 . . . . . G . . . . . U . . . . . U .....  
 975 SRI LANKA 1985 U . . . . . G . . . . . U . . . . . U .....  
 1334 SRI LANKA 1981 U . A . . . . . G . . . . . U . . . . . U .....  
 1353 SRI LANKA 1982 U . A . . . . . G . . . . . U . . . . . U .....  
 DS31/291005 BRUNEI 2005 . . A . . . . . G . . . . . U . . . . . U .....  
 DS11/221105 BRUNEI 2005 . . A . . G . . G . . . . . GG . . . . . U . . . . . U .....  
 DS04/221205 BRUNEI 2005 . . A . . . . . G . . . . . U . . . . . U .....  
 DS09/280106 BRUNEI 2006 . . A . . G . . . . . GG . . . . . U . . . . . U .....  
 DS17/130606 BRUNEI 2006 . . A . . G . . . . . GG . . . . . U . . . . . U .....  
 BA05i INDONESIA 2004 . . A . . . . . GG . . . . . U .....  
 TB16i INDONESIA 2004 . . A . . . . . G . . . . . A . . . . . U .....  
 MY96-5176 MALAYSIA 1996 . . A . . . . . GG . . . . . U . . . . . C .....  
 MY98-17933MALAYSIA 1998 . . A . . . . . G . . . . . U . . . . . U .....  
 MY99-19586MALAYSIA 1999 . . A . . . . . G . . . . . U . . . . . U .....  
 LF5-99 SARAWAK 1999 . . A . . . . . G . . . . . U . . . . . U .....

PM33974 GUINEA 1981 . . . . . GC . . . . . A . . . . . A . . . . . C . . . . . A . . . . . G . . . . . C . . . . .  
 ArA510 IVORY COAST 1980 . . . . . G . . . . . A . . . . . A . . . . . C . . . . . A . . . . . G . . . . . C . . . . .  
 UV2039 B FASO 1980 . . . . . G . . . . . A . . . . . A . . . . . C . . . . . A . . . . . G . . . . . C . . . . .  
 ArA2022 B FASO 1980 . . A . . G . . . . . A . . . . . A . . . . . C . . . . . A . . . . . A . . . . . G . . . . . C . . . . .  
 DakA578IVORY COAST 1980 . . . . . G . . . . . A . . . . . U . . . . . AA . . . . . C . . . . . A . . . . . G . . . . . C . . . . .  
 ArD20761 SENEGAL 1974 . . . . . G . . . . . A . . . . . A . . . . . C . . . . . A . . . . . G . . . . . C . . . . .  
 HD10674 SENEGAL 1970 . . . . . G . . . . . A . . . . . A . . . . . C . . . . . A . . . . . A . . . . . G . . . . . C . . . . .

SEQUENCE ANALYSIS OF E/NS1 GENE JUNCTION OF DEN-2

2431

2490

PR159 PUER R 1969 UGCGUUGUGAGCUGGAAGAACAAGAACUAAAAUGGGCAGUGGAAUUAUCGUCACAGAU  
 PR159/S1 PUER R 1969 .....  
 1251 TONGA 1974 .....  
 28741 TAHITI 1971 .....  
 348600 COLOMBIA 1986 .....  
 NC9163 N CALEDONIA 1972 .....  
 044 MEXICO 1983 .....  
 VEN2 VENEZUELA 1987 .....  
 350447 COLOMBIA 1987 .....C  
 351863 COLOMBIA 1988 .....C  
 1318 PUER R 1981 .....G  
 TR1751 TRINIDAD 1954 .....G...C...A...G...U.....

766635 TAIWAN 1987 .....A.....G.....G..U..A.....C  
 PL-046 TAIWAN 1981 .....A.....G.....G..U..A.....C  
 028 PHILIPPINES 1988 .....A.....G.....U.....G..U..A.....C  
 PL-001 TAIWAN 1981 .....A.....G.....U.....GU..U..A.....C  
 PhH2172PHILIPPINES 1983 .....A.....G.....U.....GU..U..A.....C  
 D80-038 THAI 1980 .....A.....G.....C.....G..U..A.....C  
 NGC N GUINEA 1944 .....A.....G.....G.....G..U..A.....C  
 16681 THAI 1964 .....A.....G.....G.....G..U..A.....C  
 ThNH-7/93 THAI 1993 .....A.....G.....G.....G..U..A.....C  
 ThNH-28/93 THAI 1993 .....A.....G.....G.....G..U..A.....C  
 ThNH-52/93 THAI 1993 .....A.....G.....G.....G..U..A.....C  
 ThNH-p11/93 THAI 1993 .....C.....A.....G.....U.....A.....C  
 PUO-280 THAI 1980 .....A.....A.....G.....G..U.....C  
 D80-100 THAI 1980 .....A.....G.....G.....U.....U..C  
 D81-081 THAI 1981 .....A.....G.....C.....G..U..A.....C  
 MM-1 MYANMAR 1987 .....A.....G.....G.....U..U.....C  
 MM-2 MYANMAR 1987 .....U.....A.....G.....G.....U..U.....C  
 MM-3 MYANMAR 1987 .....A.....G.....G.....U..U.....C  
 KD92-021 THAI 1992 .....A.....G.....G.....U..A.....C  
 KO142 THAI 1994 .....A.....G.....G.....U..A.....C  
 CO235 THAI 1995 .....A.....G.....G.....U..A..U.....C  
 D90-206 THAI 1990 .....A.....G.....G.....U..A.....C

57S VIETNAM 1987 .....A.....A..U.....G.....C..G..C..A.....  
 24H VIETNAM 1987 .....A.....A.....G.....C..G..C..A.....  
 8110827 JAMAICA 1981 .....A..U.....C..G.....A..U.....  
 JAM1409 JAMAICA 1983 .....A..U.....G.....C..G..C..A.....  
 516 THAI 1983 .....A.....G.....C..G..U..A.....  
 D80-141 THAI 1980 .....A.....C..G..U..A.....  
 1329 JAMAICA 1982 .....A..U.....G.....C..G..C..UA.....  
 JAH JAMAICA 1982 .....A..U.....C..G..C.....

1232 INDONESIA 1978 .....U.....A.....G.....C.....GG..U..UA.....C  
 8720 INDONESIA 1973 .....U.....A.....G.....G.....U..UA.....C  
 8730 SEYCHELLES 1977 .....U.....A.....U.....U.....C  
 1349 B FASO 1982 .....U.....A.....U.....U.....C  
 ArA6894 B FASO 1986 .....U.....A.....G.....G.....U..U.....C  
 975 SRI LANKA 1985 .....U.....A.....G.....U..U.....C  
 1334 SRI LANKA 1981 .....U.....A.....U.....UA.....C  
 1353 SRI LANKA 1982 .....C.....U.....A.....G.....U..UA.....C  
 DS31/291005 BRUNEI 2005 .....A.....G.....G.....U..UA.....C  
 DS11/221105 BRUNEI 2005 .....U.....A.....G.....G.....U..UA.....C  
 DS04/221205 BRUNEI 2005 .....A.....G.....G.....U..UA.....C  
 DS09/280106 BRUNEI 2006 .....U.....A.....G.....G.....U..UA.....C  
 DS17/130606 BRUNEI 2006 .....U.....A.....G.....G.....U..UA.....C  
 BA05i INDONESIA 2004 .....U.....A.....G.....G.....U..UA.....C  
 TB16i INDONESIA 2004 .....U.....A.....G.....G.....U..U.....C  
 MY96-5176 MALAYSIA 1996 .....U.....A.....G.....G.....U..UA.....C  
 MY98-17933MALAYSIA 1998 .....U.....A.....G.....G.....U..UA.....C  
 MY99-19586MALAYSIA 1999 .....U.....A.....G.....G.....U..UA.....C  
 LF5-99 SARAWAK 1999 .....U.....A.....G.....G.....U..UA.....C

PM33974 GUINEA 1981 .....U.....G.....A.....G..C..U..A..U..C  
 ArA510 IVORY COAST 1980 .....U.....A.....G.....A.....G..C..U..A..U..C  
 UV2039 B FASO 1980 .....U.....A.....G.....A.....G..C..U..A..U..C  
 ArA2022 B FASO 1980 .....U.....A.....G.....A.....G..C..U..A..U..C  
 DakA578IVORY COAST 1980 .....U.....A.....G.....A.....G..C..U..A..U..C  
 ArD20761 SENEGAL 1974 .....A..A.....A..G.....A.....G..C..U..A..U..C  
 HD10674 SENEGAL 1970 .....A..A.....A..G.....A.....G..C..U..A..U..C

		2491	2550
PR159	PUER R	1969	AACGUGCAUACAUGGACAGAACAUAACAAGUCCAACCAGAAUCCCUUCAAACUGGCU
PR159/S1	PUER R	1969	.....
1251	TONGA	1974	.....
28741	TAHITI	1971	.....
348600	COLOMBIA	1986	.....
NC9163 N	CALEDONIA	1972	.....C
044	MEXICO	1983	.....G.....
VEN2	VENEZUELA	1987	.....G.....
350447	COLOMBIA	1987	.....U.....
351863	COLOMBIA	1988	.....U.....
1318	PUER R	1981	.....C.....G.....
TR1751	TRINIDAD	1954	.....
766635	TAIWAN	1987	.....C.....
PL-046	TAIWAN	1981	.....U.....C.....
028	PHILIPPINES	1988	.....C.....A.....
PL-001	TAIWAN	1981	.....U.....C.....U.....U.....
PhH2172	PHILIPPINES	1983	.....U.....C.....U.....
D80-038	THAI	1980	.....C.....U.....A.....
NGC	N GUINEA	1944	.....C.....A.....
16681	THAI	1964	.....C.....A.....
ThNH-7/93	THAI	1993	.....C.....A.....
ThNH-28/93	THAI	1993	.....C.....A.....
ThNH-52/93	THAI	1993	.....C.....A.....
ThNH-p11/93	THAI	1993	.....C.....A.....C
PUO-280	THAI	1980	.....C.....A.....
D80-100	THAI	1980	.....CU.....C.....A.....
D81-081	THAI	1981	.....C.....U.....
MM-1	MYANMAR	1987	.....C.....U.....G.....A.....
MM-2	MYANMAR	1987	.....C.....A.....
MM-3	MYANMAR	1987	.....C.....A.....
KD92-021	THAI	1992	.....C.....A.....
KO142	THAI	1994	.....C.....A.....
CO235	THAI	1995	.....C.....A.....
D90-206	THAI	1990	.....C.....
57S	VIETNAM	1987	.....C.....U.....A.....
24H	VIETNAM	1987	.....C.....A.....
8110827	JAMAICA	1981	.....C.....U.....
JAM1409	JAMAICA	1983	.....C.....U.....A.....
516	THAI	1983	.....C.....U.....A.....
D80-141	THAI	1980	.....C.....U.....A.....
1329	JAMAICA	1982	.....C.....U.....A.....G.....
JAH	JAMAICA	1982	.....C.....U.....
1232	INDONESIA	1978	.....A.....G.....A.....G.....
8720	INDONESIA	1973	.....C.....A.....G.....
8730	SEYCHELLES	1977	.....A.....A.....G.....
1349	B FASO	1982	.....A.....C.....U.....C.....G.....
ArA6894	B FASO	1986	.....A.....C.....A.....U.....G.....
975	SRI LANKA	1985	.....U.....C.....A.....G.....C.....G.....
1334	SRI LANKA	1981	.....U.....A.....C.....A.....C.....G.....
1353	SRI LANKA	1982	.....U.....A.....C.....A.....U.....C.....G.....A.....C
DS31/291005	BRUNEI	2005	.....C.....A.....G.....
DS11/221105	BRUNEI	2005	.....C.....A.....G.....
DS04/221205	BRUNEI	2005	.....C.....A.....G.....
DS09/280106	BRUNEI	2006	.....A.....C.....A.....G.....
DS17/130606	BRUNEI	2006	.....A.....C.....A.....G.....
BA05i	INDONESIA	2004	.....A.....C.....A.....G.....
TB16i	INDONESIA	2004	.....C.....G.....A.....G.....G.....
MY96-5176	MALAYSIA	1996	.....C.....A.....G.....
MY98-17933	MALAYSIA	1998	.....C.....A.....G.....
MY99-19586	MALAYSIA	1999	.....C.....A.....G.....
LF5-99	SARAWAK	1999	.....C.....A.....G.....
PM33974	GUINEA	1981	.....U.....C.....C.....G.....A.....U.....C.....G.....
ArA510	IVORY COAST	1980	.....U.....C.....U.....G.....A.....U.....C.....G.....
UV2039	B FASO	1980	.....U.....C.....U.....G.....A.....U.....C.....G.....
ArA2022	B FASO	1980	.....U.....C.....U.....G.....A.....U.....C.....U.....G.....
DakA578	IVORY COAST	1980	.....U.....C.....U.....G.....A.....U.....C.....G.....
ArD20761	SENEGAL	1974	.....U.....C.....C.....G.....A.....C.....G.....
HD10674	SENEGAL	1970	.....U.....C.....C.....G.....A.....C.....U.....G.....CG.

Fig 3–Nucleotide sequence alignment of the E/NS1 gene junction of Brunei DEN-2 isolates and DEN-2 strains of genotype I - V. Dots indicate nucleotides identical for all the isolates to the reference strain, PR159. Numbering of the nucleotide position is according to Hahn *et al* (1988).

SEQUENCE ANALYSIS OF E/NS1 GENE JUNCTION OF DEN-2

739

778

PR159	PUER R	1969	IGVITWIGMNSRSTSLVSVSLVLVGVITLTLVGMVQADSG
PR159/S1	PUER R	1969	.....
1251	TONGA	1974	.....
28741	TAHITI	1971	.....
348600	COLOMBIA	1986	.....
NC9163 N	CALEDONIA	1972	.....
044	MEXICO	1983	.....
VEN2	VENEZUELA	1987	.....
350447	COLOMBIA	1987	.....
351863	COLOMBIA	1988	.....
1318	PUER R	1981	.....T.....
TR1751	TRINIDAD	1954	.....
766635	TAIWAN	1987	.....V.....V.....
PL-046	TAIWAN	1981	.....V.....V.....
028	PHILIPPINES	1988	.....V.....V.....
PL-001	TAIWAN	1981	.....V.....V.....
PhH2172	PHILIPPINES	1983	.....V.....V.....
D80-038	THAI	1980	.....V.....A..L.....
NGC	N GUINEA	1944	.....V.....
16681	THAI	1964	.....T.....
ThNH-7/93	THAI	1993	.....
ThNH-28/93	THAI	1993	.....
ThNH-52/93	THAI	1993	.....
ThNH-p11/93	THAI	1993	.....
PUO-280	THAI	1980	.....P.....
D80-100	THAI	1980	.....I.....
D81-081	THAI	1981	.....M.....
MM-1	MYANMAR	1987	.....
MM-2	MYANMAR	1987	.....
MM-3	MYANMAR	1987	.....
KD92-021	THAI	1992	.....
KO142	THAI	1994	.....
CO235	THAI	1995	.....V.....
D90-206	THAI	1990	.....
57S	VIETNAM	1987	.....VI.....
24H	VIETNAM	1987	.....V.....
8110827	JAMAICA	1981	.....V.....A.....
JAM1409	JAMAICA	1983	.....V.....A.....
516	THAI	1983	.....V.....
D80-141	THAI	1980	.....I.....V.....A.....
1329	JAMAICA	1982	.....V.....
JAH	JAMAICA	1982	.....V.....
1232	INDONESIA	1978	...V.....F..V.....
8720	INDONESIA	1973	...V.....V.....
8730	SEYCHELLES	1977	.....V.....
1349	B FASO	1982	.....V.....
ArA6894	B FASO	1986	.....V.....
975	SRI LANKA	1985	...V.....V.....
1334	SRI LANKA	1981	...V.....V.....
1353	SRI LANKA	1982	...V.....V.....
DS31/291005	BRUNEI	2005	...V.....V.....
DS11/221105	BRUNEI	2005	...F.....V.....
DS04/221205	BRUNEI	2005	...V.....V.....
DS09/280106	BRUNEI	2006	...V.....V.....
DS17/130606	BRUNEI	2006	...V.....V.....
BA05i	INDONESIA	2004	...V.....V.....
TB16i	INDONESIA	2004	...V.....V.....
MY96-5176	MALAYSIA	1996	...V.....V.....
MY98-17933	MALAYSIA	1998	...V.....V.....
MY99-19586	MALAYSIA	1999	...V.....V.....
LF5-99	SARAWAK	1999	...V.....T.....V.....
PM33974	GUINEA	1981	...V.....
ArA510	IVORY COAST	1980	T..V.....T.....
UV2039	B FASO	1980	...V.....T.....
ArA2022	B FASO	1980	...V.....T.....
DakA578	IVORY COAST	1980	...V.....T.....F..Q.....
ArD20761	SENEGAL	1974	...V.....T.....
HD10674	SENEGAL	1970	...V.....T.....

PR159	PUER R	1969	CVVSWKNKELKCGSGIFVTDNVHTWTEQYKFPESP SKLA
PR159/S1	PUER R	1969	.....
1251	TONGA	1974	.....
28741	TAHITI	1971	.....
348600	COLOMBIA	1986	.....
NC9163 N	CALEDONIA	1972	.....
044	MEXICO	1983	.....
VEN2	VENEZUELA	1987	.....
350447	COLOMBIA	1987	.....
351863	COLOMBIA	1988	.....
1318	PUER R	1981	.....
TR1751	TRINIDAD	1954	.....N.....
766635	TAIWAN	1987	.....I.....
PL-046	TAIWAN	1981	.....I.....
028	PHILIPPINES	1988	.....I.....
PL-001	TAIWAN	1981	.....F.I.....LL.....
PhH2172	PHILIPPINES	1983	.....F.I.....F.....L.....
D80-038	THAI	1980	.....I.....L.....
NGC	N GUINEA	1944	.....I.....P.....
16681	THAI	1964	.....I.....
ThNH-7/93	THAI	1993	.....I.....
ThNH-28/93	THAI	1993	.....I.....
ThNH-52/93	THAI	1993	.....I.....
ThNH-p11/93	THAI	1993	.....I.....
PUO-280	THAI	1980	.....M.....
D80-100	THAI	1980	.....S.....
D81-081	THAI	1981	.....I.....
MM-1	MYANMAR	1987	.....I.....
MM-2	MYANMAR	1987	.....I.....
MM-3	MYANMAR	1987	.....I.....
KD92-021	THAI	1992	.....I.....
KO142	THAI	1994	.....I.....
CO235	THAI	1995	.....I.....
D90-206	THAI	1990	.....I.....
57S	VIETNAM	1987	.....I.....
24H	VIETNAM	1987	.....I.....
8110827	JAMAICA	1981	.....I.....
JAM1409	JAMAICA	1983	.....I.....
516	THAI	1983	.....I.....
D80-141	THAI	1980	.....I.....
1329	JAMAICA	1982	.....I.....
JAH	JAMAICA	1982	.....I.....
1232	INDONESIA	1978	.....V.I.....R.....
8720	INDONESIA	1973	.....I.....
8730	SEYCHELLES	1977	.....I.....
1349	B FASO	1982	.....P.....
ArA6894	B FASO	1986	.....I.....
975	SRI LANKA	1985	.....I.....
1334	SRI LANKA	1981	.....I.....
1353	SRI LANKA	1982	.....I.....
DS31/291005	BRUNEI	2005	.....I.....
DS11/221105	BRUNEI	2005	.....I.....
DS04/221205	BRUNEI	2005	.....I.....
DS09/280106	BRUNEI	2006	.....I.....
DS17/130606	BRUNEI	2006	.....I.....
BA05i	INDONESIA	2004	.....I.....
TB16i	INDONESIA	2004	.....I.....
MY96-5176	MALAYSIA	1996	.....I.....
MY98-17933	MALAYSIA	1998	.....I.....
MY99-19586	MALAYSIA	1999	.....I.....
LF5-99	SARAWAK	1999	.....I.....
PM33974	GUINEA	1981	.....I.....
ArA510	IVORY COAST	1980	.....D.....
UV2039	B FASO	1980	.....D.....
ArA2022	B FASO	1980	.....D.....
DakA578	IVORY COAST	1980	.....D.L.....
ArD20761	SENEGAL	1974	.....M.....D.....
HD10674	SENEGAL	1970	.....M.....D.L...R.....

Fig 4-Deduced amino acid sequence alignment of the E/NS1 gene junction of Brunei DEN-2 isolates and DEN-2 strains of genotype I - V. Dots indicate identical amino acids for all the isolates with the reference strain, PR159. Numbering of the amino acid position is according to Hahn *et al* (1988).

SEQUENCE ANALYSIS OF E/NS1 GENE JUNCTION OF DEN-2

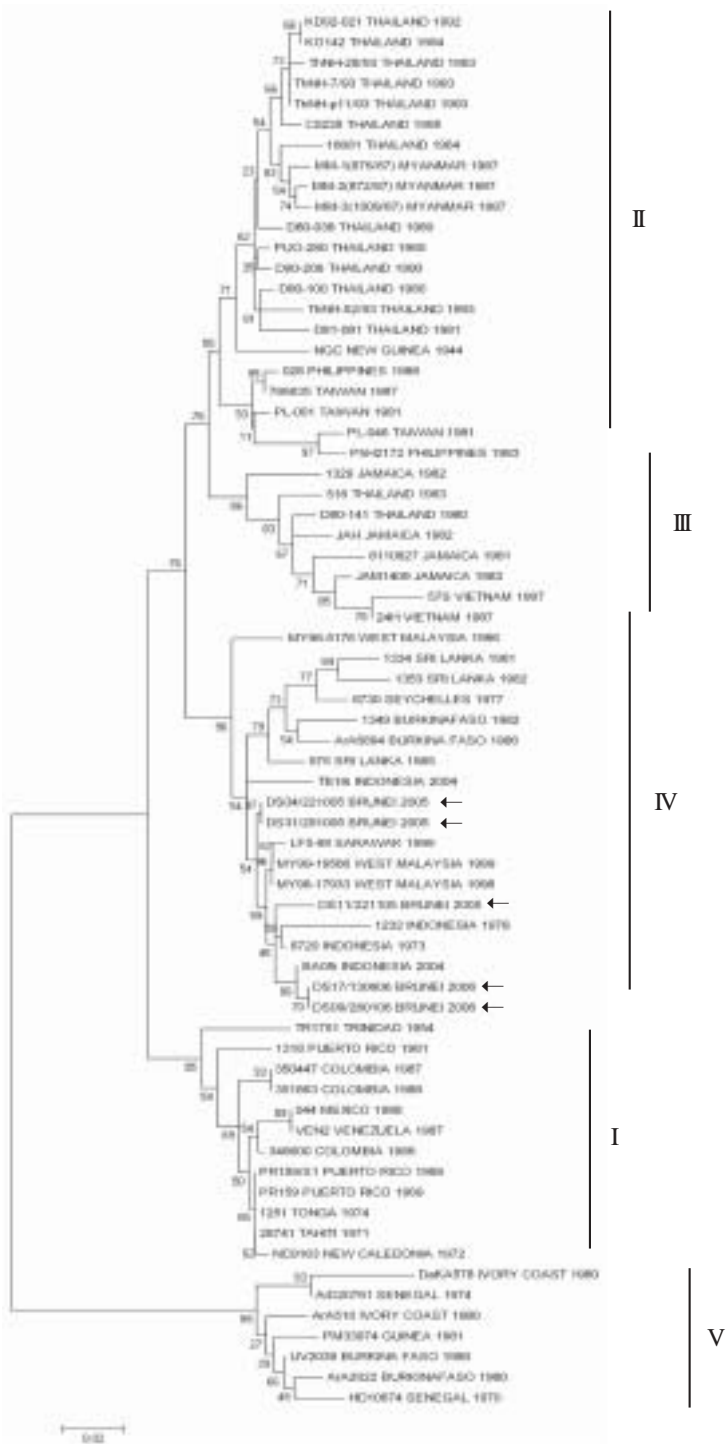


Fig 5–The evolutionary relationship tree inferred using the neighbor-joining method. The distances were computed using Tamura-Nei method with unit of the number of base substitutions per site. Bootstrap value is shown above the branch that connects the genotypic groups. Arrows in black indicate the five Brunei isolates.

pairwise comparison of the nucleotide sequences using CLUSTAL W, it was observed that DEN-2 virus in Brunei showed high homology ranging from 97.5-100 %, and with maximal divergence of 2.5 % the virus is showing minimal evolution. However they have diverged significantly from prototype strains New Guinea C and PR159/S1 that were clustered in different genotypes. Based on the E/NS1 nucleotide sequences differences, the phylogenetic tree built had subtyped DEN-2 into five genotypic groups. This was similar to the classification determined by Rico-Hesse (1990). All DEN-2 Brunei isolates were classified in the genotype IV together with strains from Indonesia (strains 1232, 8720, BA05i and TB16i), Malaysia (strains MY96-5176, MY98-17933, MY99-19586 and LF5-99), Seychelles (strain 8730), Sri Lanka (strains 975, 1334 and 1353) and Burkino Faso (strains 1349 and ArA6894).

Comparison with a DEN-2 strain from Lundu, Sarawak isolated in 1999, the closest neighbor to Brunei, showed nucleotide sequence homology ranging from 97% to 99%. Similar ranges in nucleotide sequence homology were attained when comparing with strains from Peninsular Malaysia isolated in 1996, 1998 and 1999 and Indonesia viruses isolated in 1973, 1978 and 2004. However to date there are no dengue virus sequences from other neighboring countries of Sabah and Kalimantan, available in the GenBank to be used in this study. These high homologies of the Brunei strains with those of its neighbors could implicate as to the origin of the strain and may elucidate any evolutionary trait of these strains on the island of Borneo and in the region.

In conclusion, this study has generated useful molecular data for future use in the field of molecular epidemiology of dengue virus in Brunei and also in Borneo Island. The data presented here suggests that DEN-2 viruses circulating in Brunei from 2005 to 2006 are primarily of genotype IV and are closely re-

lated to older isolates from Indonesia, Malaysia, Sri Lanka, Seychelles and Burkina Faso. Only by constant surveillance of the circulating dengue virus genotype can any development of genetic variation within the virus be detected and the necessary action be taken to curb possible outbreak of the severe form of dengue in the future.

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