COMPARATIVE NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCE OF THE ENVELOPE GLYCOPROTEIN GENE AMONG THREE DENGUE VIRUS TYPE 2 STRAINS ISOLATED FROM PATIENTS WITH DIFFERENT DISEASE SEVERITIES IN MAHA SARAKHAM, NORTHEAST THAILAND

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Abstract. The nucleotide (nt) sequence of the envelope glycoprotein (E) gene of dengue virus type 2 was determined by the primer-extension dideoxy chain-termination method for 3 dengue virus type 2 (D2) strains which had been isolated from patients with dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), in Maha Sarakham, Northeast Thailand, in 1986-1987. Their nt sequences were essentially the same except for a single silent nt replacement in each DHF and DSS strain compared with DF strain. Therefore, these 3 strains possessed identical deduced amino acid (AA) sequences in their E protein. The result indicated that the primary structure of the E protein of D2 virus is not related to the clinical severity of the infected patients. Eleven nt replacements which resulted in 4 amino acid replacements were found to be unique to these 3 Northeast Thai strains. Sequence similarity showed that the 3 Northeast Thai strains were closest to the DSS isolate (H) followed by the DHF isolate (D) identified in Bangkok in 1980.

INTRODUCTION

Dengue viruses with 4 different serotypes (D1, D2, D3, D4) belong to the family Flaviviridae (Westaway et al, 1985), and are transmitted by mosquito bites, of which Aedes aegypti has been documented as the major vector species. Most of the dengue virus infected cases show acute febrile illness accompanied by joint-muscle pain and rash (DF), however, some patients manifest severe clinical symptom of hemorrhage (DHF) and shock (DSS) which is fatal if not properly treated (reviewed by Monath, 1986). Besides appearance of DHF/DSS, increasing numbers of patients and enlarged epidemic areas have currently made dengue virus infection a major health problem in many tropical countries including Thailand (Halstead, 1992). It has been proposed that immunopathological mechanisms play important roles in

the development of DHF/DSS (Halstead, 1988).

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Particular attention has been paid to the immune enhancement of dengue virus growth in human monocytes by pre-existing non-neutralizing antibodies, which could be produced by the previous infection by heterologous dengue virus type in the case of secondary infection (Halstead, 1981), or could be conferred as maternal antibodies in the case of primary infection (Kliks et al, 1988). On the other hand, heterogeneity of epidemic dengue virus strains has been shown by serological tests and at the molecular level. However, it is not clear which genomic change may be related with the virulence of a particular strain. Recently, Morens et al (1991) reported that D2 strains isolated from severe cases multiplied better in human leukocyte cultures and their growth rate was elevated by enhancing antibodies compared with the strains isolated from mild cases. Because the E protein is the major antigenic component on the surface of dengue virion and D2 was shown to be the most frequently associated serotype with DHF/DSS in Thailand (Sangkawibha et al, 1980), we analyzed nt sequence of the E gene for three D2 strains which

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had been isolated from DF, DHF and DSS cases in Maha Sarakham, Northeast Thailand. Although similar comparative sequence analysis has been reported for strains isolated in Bangkok Metropolitan area, none of the strains isolated in other parts of Thailand have so far been analyzed.

MATERIALS AND METHODS

Viruses

The D2 strains were isolated from patients hospitalized between 1986 and 1987, in Maha Sarakham Provincial Hospital, about 450 km northeast from Bangkok. The MK 42-86 strain was isolated from a DF case, MK 244-87 from a DHF case, and MK 116-87 from a DSS case, respectively. Each patient's clinical severity was classified according to WHO (1986) guidelines. These strains were isolated from patients' sera by inoculation into an Aedes albopictus cloned C6/36 cell line (Igarashi, 1978), determined for their serotypes by type-specific monoclonal antibodies (Henchal et al, 1982), and stored at -70°C at the Virus Research Institute, Department of Medical Sciences, Ministry of Public Health, Thailand. An aliquot of each strain was transferred to the Department of Virology, Institute of Tropical Medicine, Nagasaki University, Japan, and inoculated to C6/36 cells to prepare seed virus, which was aliquoted and stored at -70°C.

Preparation of virus RNA

Seed virus was first amplified by inoculation into stationary culture C6/36 cells in 500 ml bottles and infected culture fluid was harvested 6 days after incubation at 28°C. The amplified virus of 50 ml volume was then inoculated into spinner culture C6/36 cells of 1 liter volume and the infected culture fluid was collected 6 days after incubation at 28°C. Virions were concentrated by polyethylene glycol precipitation (6%) and purified by 30-50% sucrose gradient sedimentation (Srivastava et al, 1987). RNA was extracted from the purified virion with ISOGEN reagent (Molecular Research Center Inc) and resuspended in 40 µl distilled water.

Primers and sequence information

Six primers used in this study were synthesized

by an Applied Biosystems DNA Synthesizer Model 392. Their sequences shown in Table 1 were selected according to the published sequences of D2 Jamaica and S2 strains. The purity of the product was examined by an ion exchange chromatography (Gen-pack, Waters). When more than 1% of incomplete oligomers were found, the product was discarded and the primer was newly synthesized. The sequence information on D2 strains was obtained from the following sources: Jamaica (Deubel et al, 1986); PR-159 (S1) (Hahn et al, 1988); New Guinea C and PUO-218 (Gruenber et al, 1988); M1, M2, and M3 (Samuel et al, 1989 a, b, c); 16681, D, F, G, H, a, b and c (Blok et al, 1989); TH-36 (Shiu et al, 1992).

Synthesis of cDNA from viral RNA

Gubler and Hoffman's method (1983) was used with some modifications. The virion RNA, approximately 10 µg, was heat-denatured at 90°C for 3 minutes in annealing buffer (100 mM Tris-HCl, pH 7.5, 50 mM EDTA, 500 mM KCl) containing 100 pmoles antisense primer D2-2474C (Table 1), followed by quenching on ice for 30 minutes. Then, 500 mM Tris-HCl, pH 8.3, 80 mM MgCl₂, 15 mM DTT, 20 mM dNTPs, 60 U reverse transcriptase (Life Science Inc), and 145 U RNase inhibitor (Takara) were added. The mixture was incubated at 42°C for 1 hour for the 1st strand cDNA synthesis, and extracted by phenol-chloroform. The 2nd strand cDNA was synthesized by adding 20 mM BNAD, 20 mM dNTPs, 100 mM DTT, 8 U RNase H (Takara), 20 U Escherichia coli ligase (Takara) and 28 U DNA polymerase I (Takara) in 5X buffer containing 250 mM Tris-HCl, pH 7.5, 25 mM MgCl₂, 50 mM ammonium sulfate. 250 mM KCl, and 250 μg/ml BSA. The mixture was incubated at 12°C for 1 hour, followed by 22°C for 1 hour, extracted with phenol-chloroform, and kept at -80°C until use. The doublestranded cDNA was blunt-ended by 2 U of T4 DNA polymerase (Takara) mixed with 20 mM dNTPs in 10X buffer containing 100 mM NaCl, 100 mM MgCl₂, 100 μg/ml BSA and 500 mM Tris-HCl, pH 7.9, by incubation at 37°C for 15 minutes, and extracted with phenol-chloroform. The size of the cDNA product was estimated by 0.9% agarose gel electrophoresis followed by ethidium bromide staining.

Cloning and nt sequencing

The double-stranded and blunt-ended cDNA

Table 1									
Nt sequence of the D2 primers									

Code	Sequence $(5' - > 3')$	Position*
D2-810S	AACCTGGATCTTGAGACATC	829-810
D2-1120C	TAATCCACATCCATTTCC	1257-1120
D2-1535C	CCAAGCTTTGTCTTCCATCT	1555-1535
D2-1830C	TTCCTGTACACATAGAGTAT	1849-1830
D2-2143C	TGTGCACGTTATCTGTGATGAAGATCC	2163-2143
D2-2474C	TGTGCACGTTATCTGTGATGAAGATCC	2500-2474

^{*}Position of nt in entire D2 genome sequence

fragment was ligated to the pUC18-Smal/BAP (Pharmacia) using DNA ligation kit (Takara). Resulting recombinant plasmid was used to transform E. coli strain JM 109 cells (Hanahan, 1983), and transformant colonies were screened by boiling methods (Sambrook et al, 1989). The nt sequence of the inserted cDNA was determined by the primerextension dideoxy chain-termination method (Sanger et al, 1977) using the Taq Dye-Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc). Briefly, a mixture of 3.2 pmoles of each D2 primer (Table 1), 1 µg of cDNA template, 4 µl of 5X Terminator Ammonium Cycle Sequencing (TACS) buffer, each 1 µl fluorescent-labeled dideoxynucleotides (G, A, T, C), Dye-Deoxy Terminators and 4 U of Taq polymerase was prepared and overlaid with one drop of mineral oil. The reaction tubes were placed in a thermal cycler (Techne Ltd) preheated at 90°C, and subjected to a total 25 thermal cyclings (96°C 15 seconds, 50°C 1 second, and 60°C 4 minutes). Then, the excess Dye-Deoxy terminator was removed from the reaction mixture by filtration through a Quick Spin Column (Boehringer, Germany), and the specimen was dried in vacuum. The dried reaction product was dissolved in 6 µl each of a 5:1 mixture of deionized formamide and 50 mM EDTA, pH 8.0, heated at 90°C for 2 minutes before loading onto an Applied Biosystems DNA Sequencer model 373, according to the instruction manual. The obtained nt sequence was analyzed by DNA-SIS version 4.0 software (Hitachi Co, 1991) and converted into AA sequence.

RESULTS

Fig 1 shows the nt sequence of the E protein gene

determined for MK 42-86 (DF strain), MK 244-87 (DHF strain), and MK 116-87 (DSS strain), in comparison with the nt sequence of New Guinea C strain as a reference. The 3 strains isolated in Mahasarakham, Northeast Thailand, possessed essentially identical nt sequences to each other, except that a single nt replacement was found for each DHF (A -> G at 873) and DSS (G -> T at 882) strain compared with the DF strain. Since both replacements were silent mutations, those deduced AA sequence of E protein was exactly identical for these 3 Northeast Thai strains, as shown in Fig 2. When the nt sequences of these 3 Northeast Thai strains were compared with the published sequences of other D2 strains, there were 11 unique nt replacements. Most of them (8/11) were transitions and resulted in 2 AA replacements (M - > I at 96 and T - > A at 359), and the remaining 7 transitional replacements were silent mutations (Table 2). On the other hand, two transversion replacements (T -> A at 249 andG -> T at 480) resulted in two AA replacements (N-> L at 83 amd K-> N at 160). Moreover, 5 other nt replacements, one of which resulted in an AA replacement (V -> A at 491), were shared by these 3 Northeast Thai strains and either one or both of the 2 other Thai strains isolated from DHF (D strain) or DSS (H strain) cases in Bangkok in 1980, as shown in Table 3 (Blok et al. 1989). Overall numbers of different nt and AA residues in the E protein gene sequence among the published D2 strains are summarized in Table 4. The results showed that the 3 Northeast Thai strains which were analyzed in this study possessed closest similarity to the H strain followed by the D strain and Jamaica strain. Sequence comparison also revealed that among 3 Malaysian

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New Guinea C	ATGCGTTGCATAGGAATATCAAATAGAGACTTTGTAGAAGGGGTTTCAGG	50		TGGGGAAATGGATGTGGATTATTTGGAAAAGGAGGCATTGTGACCTGTGC	350
Jamaica		50	Jamaica PR-159 (SI)	G	350 350
PR-159 (S1) PUO-218	C	50 50	PK-159 (SI)		350
M1	A	50	M1		350
M2		50	M2		350
M3		50	M3		350
16681		50	16681		350
D		50	D F		350 350
r		50 50	ć		350
н		50	н		350
MX 42-86		50	MK 42-86		350
MK 244-87		50	MK 244-87		350
MK 116-87	T	50	MK 116-87		350
			N 0		400
	AGGAAGCTGGGTTGACATAGTCTTAGAACATGGAAGCTGTGTGACGACGA	100	New Guinea C Jamaica	TATGTTCACATGCAAAAAGAACATGAAAGGAAAAGTCGTGCAACCAGAAA	400
Jamaica PR-159 (S1)	GTTT	100	PR-159 (S1)	C	400
PUO-218		100	PUO-218		400
M1		100	Ml		400
H2		100	M2		400
M3	T	100	M3	TCTGT.TT	400
16681		100	16681		400
D		100	D	T	400
Ğ		100	G	C	
н	T	100	н	T	
MX 42-86		100	MK 42-86	T	400
HOK 244-87	T	100	MX 244-87		400
MIK 116-87	TT	100	MIK 116-87	T	
New Guinea C	TGGCAAAAACCAACATTGGATTTTGAACTGATAAAAACAGAAGCC	150 150	New Guinea (
Jamaica PR-159 (S1)	TCC	150	Jamaica PR-159 (S1)	.T	1 450 . 450
PUO-218		150	PUO-218		450
M1		150	M1		450
M2		150	M2		450
H3		150	M3	.T	
16681		150	16681		. 450
D		150	D	.T	450
F	·	150	F		3 450
G		150	G	- · <u>·</u> · · · · · · · · · · · · · · · · ·	450
H		150	н	. <u>T</u>	T 450
MX 42-86 MX 244-87		150	MX 42-86 MX 244-87	.T	
MX 244-87		150 150	MK 116-87	.T	T 450
HW 110-01	· · · · · · · · · · · · · · · · · · ·				
		130	HK 110-07		
New Cuines C	AAACAACCTTCCCACTTCTTAAACAACTTCTTCTTTTTTT				
New Guinea C	AAACAACCTGCCACTCTAAGGAAGTACTGTATAGAGGCAAAGCTGACCAA	200	New Guinea (GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACA	500
Jamaica		200	New Guinea C Jamaica	GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACA	N 500
Jamaica PR-159 (S1) PUO-218	C	200	New Guinea (Jamaica PR-159 (S1) PUO-218	GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACA	500 500
Jamaica PR-159 (S1) PUO-218 M1		200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1	GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACA	500 500
Jamaica PR-159 (S1) PUO-218 M1 M2		200 200 200 200	New Guinea (Jamaica PR-159 (SI) PUO-218 M1 M2	C GTCGGANATGACACAGGANAACATGGCAAGGANATCANAATAACACCACACACACACACACACACACACAC	500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3	C CT T.A	200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3	C GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACA A. T. T. A. G. G	500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681	C .CT	200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681	### T	500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3	C CT T.A. G C A. G C A. G C T.C A. T. G C C T.C A. C. G C T. C A. T. A. T. A.	200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3	C GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACACAAAAAAAA	500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681	C CTA	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D	- G	500 500 500 500 500 500 500 500
Jamaica PR-159 (SI) PWO-218 M1 M2 M3 16681 D F G H	C CT T.A. G C A. G C A. G C T.C A. T. G C C T.C A. C. G C T. C A. T. A. T. A.	200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681	C GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACACAAA T	500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MK 42-86	C CT T.A. G C A. G C A. T. G C C A. T. G C C A. T. G C C T.C.A. C. G C T. C A. T. G C C A. G T. C A. T. G C C A. G T. C A. T. G C C A. A. T. A. T.	200 200 200 200 200 200 200 200 200 200	New Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F	T A G G A G A G A G A G A A A G A A C A A A G A A C A A A G A A C A A A G A A C A A A A C A A A A A C A	500 500 500 500 500 500 500 500
Jamaica PR-159 (SI) PUO-218 H1 H2 H3 16681 D F G H HX 42-86 HK 244-87	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 IA681 D F G H MK 42-86 MK 244-87	C GTCGGAAATGACACAGGAAAACATGGCAAGGAAATCAAAATAACACCACACACA	\$ 500 500 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MK 42-86	C .CT .AA	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 14681 D F G H	T A G G A G A G A G A G A A A G A A C A A A G A A C A A A G A A C A A A G A A C A A A A C A A A A A C A	500 500 500 500 500 500 500 500 500 500
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Jamaica PR-159 (S1) PUO-218 M1 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 PP-159 (S1) PUO-218 M1 H1 H6681 D F G HMX 42-86 MX 42-87 MX 116-87	CC CT T.A. G C C A. G C A. G C A. G C A. G C C A. A. G C C C A.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 H3 H6681 D F G G H K 42-86 HK 42-86 HK 44-87 HK 116-87	### COMPANY CO	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M3 16681 D F G HMX 42-86 MX 42-86 MX 244-87 MX 116-87 Jamaica PR-159 (S1) PUO-218 M1 H1 H2 H3 H3 H4 H4 H4 H4 H5 H6 H5 H6 H6 H8 H4 H7 H7 H8 H1 H6 H8 H1 H6 H8 H8 H1 H8 H8 H8 H8 H8 H8 H8 H8 H8 H8 H8 H8 H8	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87	### CONTROL	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 M1 H1 M6 MX 42-86 MX 116-87	CC CT T.A. A. C. C. A. T. A. C. C. A. T. C. C. A. T. C. C. C. A. T. A. C. C. C. C. C. A. T. A. C. C. C. C. C. C. A. T. A. C. C. C. C. C. C. A. T. A. C. C. C. C. C. C. C. A. A. A. C.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1	### COMMITTEE CO	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K244-87 HK 116-87 HK 116-87 HK 116-87 HK 42-86 HK 44-86 HK 44-86 HK 44-86 HK 44-86 HK HK 44-86 HK 44-87 HK 116-87	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H H4 H4 H5 H6 H7 H7 H7 H7 H8	### CONTROL	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 MX 42-86 MX 44-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) D F G G JMX 42-86 MX 44-87 MX 116-87	CC CT T.A. A.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 1681 D F G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 H3 H6681 D F G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D F G G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D F G G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D Jamaica PR-159 (S1) PUO-218 H1 D Jamaica PR-159 (S1) PUO-218 H1 D D D D D D D D D D D D D D D D D D	### COMMITTEE CO	\$ 500 500 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K244-87 HK 116-87 HK 116-87 HK 116-87 HK 42-86 HK 44-86 HK 44-86 HK 44-86 HK 44-86 HK HK 44-86 HK 44-87 HK 116-87	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 16681 D F G H H 42 H3 16681 D F C H H 10-218 H1 16681 D F C H H 10-218 H1 PUO-218 H1 PUO-218 H1 PR-159 (S1) PUO-218	### COMMITTEE CO	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H MX 42-86 HX 244-87 HX 116-87	CC CT T.A. A.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 1681 D F G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 H3 H6681 D F G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D F G G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D F G G H HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D Jamaica PR-159 (S1) PUO-218 H1 D Jamaica PR-159 (S1) PUO-218 H1 D D D D D D D D D D D D D D D D D D	### COLUMN COLUM	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 M1 MX M3 MX	CC CT T.A. A.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 PUO-218 H1 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 H3 H6681 D F G G HK 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 D F G G H K 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 Jamaica PR-159 (S1) PUO-218 H1 H2	### COLUMN COLUM	\$ 500 \$
Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H MX 42-86 HX 244-87 HX 116-87	CC CT T.A. A.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 16681 D F G H H 42-86 HK 244-87 HK 116-87 New Cuinea (Jamaica PR-159 (S1) PUO-218 H1 PUO-218 H1 PUO-218 H1 H1 PUO-218 H1 H2 H3 H4	### CONTRACTOR C	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87	CC CT T.A. A. C. G. C. A. T.A. C. G. C. A. T. A. C. G. C. A. T. C. C. A. T. A. C. C. G. C. A. T. A. C. C. G. C. A. T. A. C. G. C. C. A. T. A.	200 200 200 200 200 200 200 200 200 200	New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 424-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 H3 H6681 D F	### CTOGGAATGACACAGGAAAACATGACACACACACACACACACA	\$ 500 500 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G N K 42-86 MK 244-87 MK 116-87	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea () Jamaica PR-159 (S1) PUO-218 H1 H2 H3 1-6681 D F G H K 42-86 MK 244-87 MK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 10681 D F G H K 42-86 HK 42-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 HI H1 H2 H3 H6681 D F G H H689 G H H789 H799 H799 H799 H799 H799 H799 H7	### CTOGGAATGACACAGGAAAACATGACACACACACACACACACA	\$ 500 500 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 116-87 New Guinea (M3 M4 M4 M5 M6 M7 M8	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea () Jamaica PR-159 (S1) PUO-218 H1 PUO-218 H1 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G G H K 44-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 PUO-218 H1 D F G G H K 244-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 D F G G H H C H H D F G G H H C H H H C H H H H C H	### CONTRACTOR C	\$ 500 500 500 500 500 500 500 500
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G N K244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MY 116-87	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea () Jamaica PR-159 (S1) PUO-218 H1 H2 H3 1-6681 D F G H K 42-86 MK 244-87 MK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 16681 D F G H K 42-86 MK 42-86 MK 42-86 MK 42-86 MK 42-86 MK 42-86	### COMMITTEE CO	\$ 500 500 500 500 500 500 500 500 500 50
Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 116-87 New Guinea (M3 M4 M4 M5 M6 M7 M8	C CT	200 200 200 200 200 200 200 200 200 200	New Guinea () Jamaica PR-159 (S1) PUO-218 H1 PUO-218 H1 16681 D F G H K 42-86 HK 244-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G G H K 44-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 PUO-218 H1 D F G G H K 244-87 HK 116-87 New Guinea () Jamaica PR-159 (S1) PUO-218 H1 D F G G H H C H H D F G G H H C H H H C H H H H C H	### CONTRACTOR C	\$ 500 500 500 500 500 500 500 500

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			Have Continue C	TGGAACAATAGTTATCAGAGTACAATATGAAGGGGACGGTTCTCCATGTA	1000
		650	Jamaica	C	
Jameica PR-159 (81)	G.C	650 650	PR-159 (S1)		
PUO-218	T	650	PUO-218 M1	C	1000
H1	γ	650	M2	c	1000
M2 M3	G.C	650 650	H3		1000
16681		650	16681 D		1000
D	G.C	650	ř	G	1000
F G	A	650 650	Ġ		1000
н	G.C	650	H	<u>e</u>	
MK 42-86	A	650	MX 42-86		1000
MX 244-87	A	650	MX 244-87 MX 116-87		1000
MK 116-87	G.C	650	NA 110-07		
New Guinea C	GTTGCCATGGCTGCCCGGAGCGGACACACAAGGATCAAATTGGATACAGA	700	New Guinea C	AGATCCCTTTTGAGATAATGGATTTGGAAAAAAGACATGTTTTAGGTCGC	
Jameica	A	700	Jamaica PR-159 (S1)		1050
PR-159 (S1)		700	PUO-218	.A	1050
PUO-218 M1	A. A	700 700	M1	A	1050
H2 -	AA	700	M2	.AC	1050
M3	A	700	M3 16681	ACC	1050
16681 D	AT	700 700	D		1050
F	A	700	7	.A	
Ğ	A	700	G	.λ	
H	····ý······ý······	700	H HDK 42-86		1050
MK 42-86 MK 244-87	AA	700 700	HOK 244-87	c	
MOK 116-87	A	700	MOK 116-87	c	
New Guinea C		750		CTGATTACAGTCAACCCAATCGTAACAGAAAAAGATAGCCCAGTCAACAT	
Jamaica PR-159 (S1)	CAC	750 750	Jamaica PR-159 (S1)	cc	
2UO-218	A	750	PK-159 (S1) PUO-218	C	
M1	A	750	M1	C	1100
M2	h	750	H2 H3	CTGGCG.	1100
H3 16681		750 750	M3 16681		
D	c	750	D		1100
F	A	750	F	C	1100
G	A	750 750	G H	CATGA	
MK 42-86		750 750	MK 42-86		1100
HIK 244-87		750	MX 244-87		
MSK 116-87		750	MK 116-87	GGG	1100
			New Guinea C	AGAAGCAGAACCTCCATTCGGAGACAGCTACATCATCATAGGAGTAGAGC	1150
New Guinea C		800	Jamaica		
Jamaica PR-159 (S1)		800	PR-159 (S1) PUO-218		
PUO-218		800	M1		
MI	CA	800	H2		1150
M2 M3	CA	800 800	H3	A	
16681		800	16681 D		
D		.800	F		
F G	t	800 800	G		
н	CAA	800	H MIK 42-86		
MK 42-86		800	MX 244-87		
MK 244-87 MK 116-87	AT	800	MK 116-87		1150
MK 116-67		800	New Guinea C	CGGGACAATTGAAGCTCAACTGGTTTAAGAAAGGAAGTTCTATCGGCCAA	1200
New Guinea C	CACAGAAATCCAGATGTCATCAGGAAACTTACTGTTCACAGGACATCTCA	850	Jamaica		
Jamaica		850	PR-159 (S1)	. A	1200
PR-159 (S1) PUO-218	TG	850 850	PUO-216		
M1		850	M1 M2		
M2		850	H3		
M3	.G	850	16681	c	
16681 D		630			1200
	c	850	D •		1200 1200
F		850	D F G	C	1200 1200 1200
F G		850 850	F G H		1200 1200 1200 1200 1200
G H	A	850 850 850	F G H MK 42-86		1200 1200 1200 1200 1200 1200
		850 850	F G H MX 42-86 MX 244-87		1200 1200 1200 1200 1200 1200
G H MIK 42-86	A	850 850 850 850	F G H MK 42-86		1200 1200 1200 1200 1200 1200
G H MK 42-86 MK 244-87 MK 116-87	A	850 850 850 850 850 850	F G H MX 42-86 MX 244-87 MX 116-87	A	1200 1200 1200 1200 1200 1200 1200
G H MK 42-86 MK 244-87 MK 116-87	A	850 850 850 850	F G H MX 42-86 MX 244-87 MX 116-87		1200 1200 1200 1200 1200 1200 1200
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1)	A T. A. T. G. C	850 850 850 850 850 850 850	F G H MX 42-86 MX 244-87 MX 116-87 New Guinea C Jamaica	C	1200 1200 1200 1200 1200 1200 1200
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218	A C C C C C C C C C C C C C C C C C C C	850 850 850 850 850 850 850 900 900	F G H MX 42-86 MX 244-87 MX 116-87		1200 1200 1200 1200 1200 1200 1200 1250 125
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1	A C C C C C C C C C C C C C C C C C C C	850 850 850 850 850 850 900 900 900 900	F G H MX 42-86 MX 244-87 MX 116-87 How Guinea C Jameica PR-159 (S1) PUO-218 M1	A C	1200 1200 1200 1200 1200 1200 1200 1250 125
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218	A C C C C C C C C C C C C C C C C C C C	850 850 850 850 850 850 850 900 900	F G H MX 42-86 MX 244-87 MX 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218	A	1200 1200 1200 1200 1200 1200 1200 1250 125
G H HX 42-86 HX 244-87 HX 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3	A	850 850 850 850 850 850 900 900 900 900 900 900	F G H MX 42-86 MX 244-87 MX 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2	A	1200 1200 1200 1200 1200 1200 1200 1250 125
G H HK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D	A	850 850 850 850 850 850 900 900 900 900 900 900	F G HMX 42-86 HMX 244-87 HMX 116-87 How Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D	A	1200 1200 1200 1200 1200 1200 1200 1250 125
G HMK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681	A	850 850 850 850 850 850 900 900 900 900 900 900	F G H HX 42-86 HX 244-87 HX 116-87 Hew Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F	A	1200 1200 1200 1200 1200 1200 1200 1250 125
G H HK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F	A	850 850 850 850 850 850 850 900 900 900 900 900 900 900 900 900	F G H HX 42-86 HX 244-87 HX 116-87 Hew Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H	A C C C C C C C C C C C C C C C C C C C	1200 1200 1200 1200 1200 1200 1200 1250 125
G H HX 42-86 MX 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	MX 42-86 MX 42-86 MX 244-87 MX 116-87 How Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G HX 42-86	A C C C C C C C C C C C C C C C C C C C	1200 1200 1200 1200 1200 1200 1250 1250
G H HX 42-86 MX 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M3 16681 D F G G MX 42-86 MX 244-87	A	850 850 850 850 850 850 850 900 900 900 900 900 900 900 900 900	F G H HK 42-86 HK 244-87 HK 116-87 How Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G HK 42-86 HK 42-86		1200 1200 1200 1200 1200 1200 1200 1250 125
G H HX 42-86 MX 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	MX 42-86 MX 42-86 MX 244-87 MX 116-87 How Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G HX 42-86	A C C C C C C C C C C C C C C C C C C C	1200 1200 1200 1200 1200 1200 1200 1250 125
G H H 42-86 MX 244-87 MX 116-87 MS 116-87 MS 116-87 MS 116-87 MS 116-87 MX 116-87	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H MX 42-86 MX 244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 16681 D F G H K 42-86 MX 244-87 MX 116-87 MX 116-87 MX MAY Guinea C	A	1200 1200 1200 1200 1200 1200 1200 1250 125
G H MX 42-86 MX 244-87 MX 16-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 MN MW Guinea C Jamaica	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H 42-86 HK 42-87 HK 116-87 HW Guinea C Jamaica PR-159 (S1) PUO-216 H1 H2 H3 16681 D F G HK 42-86 HK 244-87 HK 116-87 HW Guinea C Jamaica C Jamaica		1200 1200 1200 1200 1200 1200 1250 1250
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica 9R-159 (S1) PUO-218 H1 M2 H3 16681 D F G H MK 42-86 MK 42-86 MK 116-87 New Guinea C Jamaica PR-159 (S1)	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H MX 42-86 MX 244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 16681 D F G H MX 42-86 MX 244-87 MX 116-87	A C C C C C C C C C C C C C C C C C C C	1200 1200 1200 1200 1200 1200 1200 1250 125
G H MX 42-86 MX 244-87 MX 16-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 MN MW Guinea C Jamaica	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H 42-86 HX 244-87 HX 116-87 HW Guinea C Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H K 42-86 HX 244-87 HX 116-87 HW Guinea C Jamaica PR-159 (S1) PUO-218 H1	A C	1200 1200 1200 1200 1200 1200 1200 1250 125
G H MX 42-86 MX 244-87 MX 16-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 M2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218	A	850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G HX 42-86 HX 244-87 HX 116-87 HX 116-87 HX 116-87 HX 116-87 HX 116-81 HZ HX 116-81 HX 116-87		1200 1200 1200 1200 1200 1200 1250 1250
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 16681 D F G MK 42-86 MK 116-87 MK 116-87 MK 116-87 MF 116-87 MI M1 M2 M1	A	850 850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H MX 42-86 MX 244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 116-81 M1 M2 MX 42-86 MX 244-87 MX 116-87 MX 116	A C	1200 1200 1200 1200 1200 1200 1200 1250 125
G H MX 42-86 MX 244-87 MX 116-87 MX	A	850 850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G HX 42-86 HX 244-87 HX 116-87 HX 116-87 HX 116-87 HX 116-87 HX 116-81 HZ HX 116-81 HX 116-87		1200 1200 1200 1200 1200 1200 1200 1200
G H MK 42-86 MK 244-87 MK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1 M2 MK 42-86 MK 424-87 MK 116-87 MK 116-87 MF 19-018 MI MA 1 MM 1 MM 1 MM 1 MM 1 MM 1 MM 1 M	A	850 850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H MX 42-86 MX 244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 16681 D F G H MX 244-87 MX 116-87 MW Guinea C Jamaica PR-159 (S1) PUO-218 M1 16681 D F MX 16681 D F MX 16681 D F MX 16681 D F F MX 16681 D F		1200 1200 1200 1200 1200 1200 1200 1250 125
G H MX 42-86 MX 244-87 MX 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 M1 16681 D F G H MX 44-87 MX 116-87 MX 116-87 MX 116-87 H M1 16681 D PR-159 (S1) PUO-218 H M1 16-87 MX 116-87 MX	A	850 850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H MX 42-86 MX 244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 116-81 M1 H2 M3 16681 D F G H MX 42-86 MX 244-87 MX 116-87	. A	1200 1200 1200 1200 1200 1200 1200 1200
G H K 42-86 HK 42-87 HK 116-87	A	850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	F G H MX 42-86 MX 244-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 116-87 MX 16681 D F G H MX 244-87 MX 116-87 MW Guinea C Jamaica PR-159 (S1) PUO-218 M1 16681 D F MX 16681 D F MX 16681 D F MX 16681 D F F MX 16681 D F		1200 1200 1200 1200 1200 1200 1200 1200
G H HK 42-86 HK 244-87 HK 116-87 New Guinea C Jamaica PR-159 (S1) PUO-218 H1 16681 D F G HK 244-87 HK 116-87 HK 244-87 HK 116-87 HK 116-	A	850 850 850 850 850 900 900 900 900 900 900 900 900 900 9	MX 42-86 MX 42-86 MX 244-87 MX 116-87 New Guinea C Jamaica PR-1:59 (S1) PUO-218 M1 M2 M3 16681 D F G H K 42-86 MX 244-87 MX 116-87 Mew Guinea C Jamaica PR-1:59 (S1) PUO-218 M1 M2 M3 16681 D F G H MW 116-87	. A	1200 1200 1200 1200 1200 1200 1200 1200

New Guinea C	AGGCTCTCCACCAAGTTTTCGGAGCAATCTATGGGGCTGCCTTCAGTGGG	1350	New Guinea C	AATGAATTCACGCAGCACCTCACTTTCTGTGTCACTAGTATTGGTGGGAG	1450
Jamaica	······································	1350	Jamaica		
PR-159 (S1)		1350	PR-159 (S1)	CTAG	
PUO-218	.AC		PUO-218		
M1	.A		MI		
M2	.A		M2	T	1450
M3			M3	T	
16681			16681		1450
	.A		D	T	1450
F	.A		6		
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H	······································		ŭ	AT	
MK 42-86			MK 42-86	T	1450
MK 244-87	······································		MIK 244-87		1450
MIK 116-87	······································		MK 116-87	T	1450
		1,,,,	110 07		1450
New Guines C	GTCTCATGGACTATGAAAATCCTCATAGGAGTCATTATCACATGGATAGG	1400	New Guinea C	TCGTGACGCTGTATTTGGGAGTTATGGTGCAGGCC	1500
Jamaica		1400	Jamaica	ACCC	1500
PR-159 (S1)		1400	PR-159 (S1)	AC	1500
PUO-218		1400	PUO-218	.7AA	1500
M1	T		HT.		1500
H2	T	1400	H2	.TA	1500
M3		1400	H3	AACGC	1500
16681	T	1400	16681	.TA	
D	T	1400	D		1500
F					
	T	1400	F	.TA	1500
G	т		F G	.TA	1500
G H		1400	F G H	.TA	1500
G H MK 42-86		1400	F G H MK 42-86	-TACCACACACACCACCA	1500 1500 1500
G H MK 42-86 MK 244'-87	A	1400 1400 1400	MK 42-86	.TA	1500 1500 1500
		1400 1400 1400		-TACCACACACACCACCA	1500 1500 1500 1500

Fig 1—Nt sequence of the E protein gene of D2 strains. Abbriviation of strains and sources of the sequence informations was shown in the Materials and Methods.

strains, the M3 isolated from a DF possessed the highest similarity to the 3 Northeast Thai strains.

DISCUSSION

Heterogeniety of the epidemic D2 strains at the molecular level has been analyzed by oligonucleotide fingerprinting (Trent et al, 1983, 1989), cDNA-RNA hybridization (Blok et al, 1984), antigen signature analysis (Monath et al, 1986), hybridization using synthetic deoxyoligonucleotides (Kerschner et al, 1986), restriction enzyme mapping (Walker et al, 1988), and more recently by nt sequencing (Blok et al, 1989; Rico-Hesse, 1990). These studies demonstrated that D2 strains could be classified into several geographical types (or topotypes). However, it is still not clear whether any particular molecular changes are associated with the pathogenicity of DHF/DSS. Comparative sequence analysis of the E protein gene of several D2 strains by Blok et al (1989) indicated that certain AA replacements, such as I at 308 and A at 491, were observed in 2 DHF/DSS strains (D and H), while V was found at these positions in remaining 2 DHF/DSS and 6 DF strains. In our analysis, all 3 Northeast Thai strains possessed I at 308 and A at 491, indicating that these AA replacements may be characteristic of certain local strains but not of DHF/DSS strains. The fact that a Malaysian DF strain, M3 and jamaica strains, also possessed I at 308 supports this possibility. Therefore, it is difficult to imagine that a certain AA replacement in the E protein is related with

severe or mild clinical manifestations of the D2 virus-infected patients. If the clinical severity of the D2 infection depends on the growth capacity and the enhanceability of each viral strain in human leukocyte culture, (Morens et al, 1991), such biological characteristics may be determined by viral gene sequences other than that of the E protein gene. All 3 Northeast Thai D2 strains which were analyzed in our study had conserved potential glycosylation sites (NTT at 67-69; NDT at 153-155), as well as conserved type-specific hypervariable domain (GADTQGSN at 223-230; Shiu et al, 1992). Among 4 AA replacements unique to the Northeast Thai strains, 2 mutations (N -> K at 83; M - > I at 96) were present in the R1 region (AA 1-122) which was postulated by Nowak and Wengler (1987) for West Nile flavivirus. While the remaining mutations (K -> N at 160; T -> A at 359) exist in L1 and L2 loops, respectively, the implication of these AA replacements for the antigenicities or biological characteristics of D2 strains is unknown. However, they may elicit strainspecific antibody responses which could be observed only in certain epidemic regions like Northeast Thailand. Another AA mutation (V - > A at 491)was shared by these 3 Northeast and 2 other Bangkok strains isolated in Thailand, but may not exert so much effect on the antigenicities, because it is present in the transmembrane domain.

ACKNOWLEDGEMENTS

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amaica	MRCIGISHRDFVEGVSGGSWVDIVLEHGSCVTTMAKHKPTLDFELIKTEA	50	Jamaica	VVLGSQEGAMHTAL TGATE I OMSSGNLLFTGHLKCRLRMDKLQLKGMSYS
ma i Ca		50	PR-159 (S1)	
-159 (S1)		50	PR-159 (51) PUO-218	
0-218		50		
•		50	M1	
		50	M2	
		50	M3	
81	M	50	16681	
		50	D	
		50	F	
		50	G	
		50	H	
42-86		50	MK 42-86	
244-87	······	50	MX 244-87	
116-87		50	MX 116-87	
- Guinea C	KQPATLRKYCI EAKLTNTTTDSRCPTQGEPSLNEEQDKRFVCKHSHVDRG	100	New Guinea C	
maica		100	Jamaica	
-159 (S1)	T	100	PR-159 (S1)	
0-218	EE	100	PUO-218	
	L	100	HI	E
	E	100	M2	EDN
	S.FELLL.	100	M3	IF
681		100	16681	
901			D	
	L	100	F	
		100	Ġ	
	L	100	н	
	EE	100		I
42-86		100	MK 42-86	
244-87		100	MK 244-87	
116-87	K	100	MK 116-87	I
√ Guinea C	MGNGCGLFGKGGIVTCAMFTCKKNNKGKVVOPENLEYTIVITPHSGEEHA	150		LITVNPIVTEKDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQ
maica	EE	150	Jamaica	
-159 (S1)	VV	150	PR-159 (S1)	.T
)-218			PUO-218	
-418	<u>E</u> y	150	M1	
		150	H2	v
	vv	150	M3	YH.LDIH.L
	EF.N	150	16681	
681	EE	150	D	
		150	F	***************************************
	F	150	Ġ	
	vv	150	ü	
		150	M	
42-86	F. T.	150	HOK 42-86	······································
		150	MIX 244-87	
244-87	E I	150 150 150		
244-87 116-87	E.I	150 150	MK 244-87 MK 116-87 New Guinea	AAAA
244-87 116-87 w Guinea C	E. I. E. I. VONDTGKHGKEIKITPOSSITEAELTGYGTVTMECSPRTGLDFNEMVLLQ	150 150	MK 244-87 MK 116-87 New Guinea Jamaica	. A A HETTHRGAKRMAILGOTAMDFGSLGGVFTSIGKALHQVFGAIYGAAPSG .F
244-87 116-87 w Guinea C maica	E. I. E. I. WONDTGKHGKEIKITPOSSITFAELTGYGTYTMECSPRTGLDFNEMULLQ	150 150 200 200	MK 244-87 MK 116-87 New Guinea Jamaica PR-159 (S1)	A. A. HIETTHRGAKRMAILGOTAWDFGSLGGVFTSIGKALHQVFGAIYGAAPSG F. F.
244-87 116-87 • Guinea C maica -159 (S1)	E. I. E. I. WONDTCKHCKEIKITPOSSITFAELTGYGTVTMECSPRTGLDFNEMALLQ V.	150 150 200 200 200	MK 244-87 MK 116-87 New Guinea Jamaica	. A A HETTHRGAKRMAILGDTAMDFGSLGGVFTSIGKALHQVFGAIYGAAPSG .FFF.
244-87 116-87 W Guinea C Maica -159 (S1)	E. I. E. I. VGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. V	150 150 200 200 200 200	MK 244-87 MK 116-87 New Guinea Jamaica PR-159 (S1)	. A. A. A. HIETTHRGAKRMAILGOTAMOFGSLGGVFTSIGKALHQVFGAIYGAAPSG .FFFFFFFFFF
244-87 116-87 W Guinea C Maica -159 (S1)	E. I. B. WONDTCKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMALLQ V. V.	150 150 200 200 200	MK 244-87 MK 116-87 New Guinea Jamaica PR-159 (S1) PUO-218	. A A HETTHRGAKRMAILGDTAMDFGSLGGVFTSIGKALHQVFGAIYGAAPSG .FFF.
244-87 116-87 W Guinea C Maica -159 (S1)	E. I. WONDTGKHGKEIKITPOSSITFAELTGYGTVTMECSPRTGLDFNEMULLQ V. V. D.	150 150 200 200 200 200	MK 244-87 MK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 M2	. A
244-87 116-87 W Guinea C maica -159 (S1) 0-218	E. I. WONDTGKHGKEIKITPOSSITFAELTGYGTVTMECSPRTGLDFNEMULLQ V. V D	150 150 200 200 200 200 200 200	MK 244-87 MK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3	. A. A. A. HIETTHRGAKRMAILGOTAMOFGSLGGVFTSIGKALHQVFGAIYGAAPSG .FFFFFFFFFF
244-87 116-87 W Guinea C Maica -159 (S1) O-218	E. I B. 1 WGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMULLQ V. D. L. Q. (150 150 200 200 200 200 200 200 200	MK 244-87 MK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 M2 H3 16681	. A. C HIETTWRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSG F
244-87 116-87 W Guinea C Maica -159 (S1) 0-218	E. I. E. I. WIGHTIGKHIGKEIKITPOSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. T.	200 200 200 200 200 200 200 200 200 200	MK 244-87 MK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D	. A A A HETTHRGAKRMAILGOTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG .FFFFFFFFFF
244-87 116-87 W Guinea C Maica -159 (S1) W-218	E. I B. 1 WGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMVLLQ V. D. D. L. Q. I	150 150 200 200 200 200 200 200 200 200 200	MK 244-87 MK 116-87 New Guinea (Jamaica PR-159 (S1) PUO-218 M1 H2 H3 16681 D	. A
244-87 116-87 W Guinea C Maica -159 (S1) W-218	E. I. E. I. WGMDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. T. V. A	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F	. A A A A HETTHRGAKRMAILGOTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG . F F F I F IR IR F IR IR F
244-87 116-87 W Guinea C Maica -159 (S1) W-218	E. I E. I WGNDTGKNGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMVLLQ V D L Q I V V V V V V V V V V	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 M2 M3 16681 D F G	A C HIETTHRGAKRHAILGDTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG F F F IR F F F F F F F F F F F F F
244-87 116-87 W Guinea C maica (-159 (SI) (O-218	E. I. E. I. WGMDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. V. V. V. V. V. V. V. V	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H	. A A A A HETTHRGAKRMAILGOTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG . F F F I F IR IR F IR IR F
(244-87 (116-87 SW Guinea C Smaica 1-159 (S1) 20-218 (22-86	E. I E. I WONDTCKHGKEIKITPOSSITFAELTCYGTVTMECSPRTGLDFNEMULLQ V. D. L. V V V V V V V V V N	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 M2 M3 16681 D F G	A C HIETTHRGAKRHAILGDTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG F F F IR F F F F F F F F F F F F F
: 244-87 : 116-87 ow Guinea C maica :-159 (S1) 00-218 :: :: :: :: :: :: :: :: :: :: :: :: ::	E. I. E. I. B. I. VGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. N.	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 H2 H3 16681 D F G H	. A
: 244-87 : 116-87 ow Guinea C maica :-159 (S1) 00-218 :: :: :: :: :: :: :: :: :: :: :: :: ::	E. I E. I WONDTCKHGKEIKITPOSSITFAELTCYGTVTMECSPRTGLDFNEMULLQ V. D. L. V V V V V V V V V N	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Culinea Jamaica PR-159 (S1) PJO-218 H1 H2 H3 16681 D F G HMX 42-86 MX 244-87 MX 116-87	A A C HISTTHRGAKRHAILGOTAMDFGSLGGVFTSIGKALHQVFGAITGAAFSG F F F F F F F F F F F F F F F F F F
244-87 116-87 W Guinea C maica -159 (S1) O-218 681	E. I. E. I. E. I. VONDTGKHGKEIKITPOSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. V. V. V. V. V. V. V. V. N. N	150 150 200 200 200 200 200 200 200 200 200 2	MK 244-87 MK 116-87 New Guinea Jamaica PR-159 (S1) P/30-218 H1 H2 H3 16681 D F G H HK 42-86 HK 42-86 MK 244-87 MK 116-87	
244-87 116-87 w Guinea C maica -159 (S1) O-218 6681 (42-86 (244-87 (116-87	E. I. E. I. E. I. VGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. V. V. V. V. V. V. N. N.	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea: Jamaica PR-159 (S1) PXO-218 H1 H2 H3 16681 D F G H K 42-86 MX 244-87 MX 116-87 New Guinea Jamaica	A A A I ETTHRGAKRMAILGOTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG F F F F I F I F I F I F I F I F I F I
244-87 116-87 w Guinea C maica -159 (Si) 0-218 	E. I. E. I. WINDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMVLLQ V. D. L. Q. I. V. V	150 150 200 200 200 200 200 200 200 200 200 2	MK 244-87 MK 116-87 Mew Guinea Jamaica PR-159 (S1) P/20-218 H1 H2 H3 16681 D F G H MK 42-86 MK 244-87 MK 116-87 New Guinea Jamaica PR-159 (S1)	. A. A. C. HIETTWRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSG .F
244-87 116-87 w Guinea C maica -159 (S1) 0-218 681 42-86 : 244-87 : 116-67 ov Guinea C maica	E. I. E. I. WINDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. V. V. V. V. V. V. V. V.	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PJO-218 M1 M2 M3 16681 D F G G MX 44-86 MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PJO-218	A A C HIETTHRGAKRHAILGOTAMOPGSLGGVFTSIGKALHQVFGAITGAAPSG F F F F F F F F F F F F F F F F F F F
244-87 116-87 w Guinea C macica -159 (S1) 0-218 (42-86 244-87 116-87 116-87 116-87 116-97 116	E. I. E. I. VGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMULLQ V. D. L. Q. I. V. V. V. V. N. N. N. MENKAMLVHRQMFLDLPLPMLPGADTQGSMMIQKETLVTFKNPHAKKQDV D. KD.	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 MW Guinea Jamaica PR-159 (S1) P)/0-218 H1 M2 M3 16681 D F G H MK 42-86 MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) P/00-218 M1	A. I HIETTWRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSG F
244-87 116-87 w Guinea C maice 159 (S1) O-218 (42-86 (244-87 (116-87 ew Guinea C simaica 159 (S1) O-218	E. I. E. I. WINDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. V. V. V. V. V. V. N. HENKAMLVHRQMFLDLPLPWLPGADTQGSMMIQKETLVTFKNPHAKKQDV D. KD.	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PJO-218 H1 H3 16681 D F G HMX 42-86 HMX 42-86 HMX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 H2	A A C HIETTHRGAKRHALLGOTAMOPGSLGGVFTSIGKALHQVFGALTGAAPSG F F F F F F F F F F F F F F F F F F F
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244-87 116-87 w Guinea C maice -159 (S1) 0-218 681 42-86 1244-87 116-87 w Guinea C maica 1-159 (S1) 10-218	E. I. E. I. WINDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. V. V. V. V. V. N. HENKAMLVHRQMFLDLPLPWLPGADTQGSMMIQKETLVTFKNPHAKKQDV D. KD. KD.	200 200 200 200 200 200 200 200 200 200	MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PJO-218 H1 H3 16681 D F G HMX 42-86 HMX 42-86 HMX 116-87 New Guinea Jamaica PR-159 (S1) PUO-218 H1 H2	A A C HIETTHRGAKRHALLGOTAMOPGSLGGVFTSIGKALHQVFGALTGAAPSG F F F F F F F F F F F F F F F F F F F
244-87 116-87 w Guinea C maice -159 (S1) 0-218 681 42-86 1244-87 116-87 w Guinea C maica 1-159 (S1) 10-218	E. I. E. I. WGMDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMVLLQ V. D. D. L. V. V. V. V. V. V. V. N. N. N. MENKAMLVHRQMFLDLPLPWLPGADTQGSNMIQKETLVTFKNPHAKKQDV D. KDKLD.	200 200 200 200 200 200 200 200 200 200	MX 244-87 MX 116-87 MW Guinea Jamaica PR-159 (S1) P)UO-218 H1 M2 M3 16681 D F G H MK 42-86 MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) P)UO-218 M1 M2 M3	A A C HIETTHRGAKRHAILGOTAMDFGSLGGVFTSIGKALHQVFGAIYGAAFSG F F F F F F F F F F F F F F F F F F
244-87 116-87 w Guinea C maice -159 (S1) 0-218 681 42-86 1244-87 116-87 w Guinea C maica 1-159 (S1) 10-218	E. I. E. I. WINDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V. V. V. V. V. V. N. HENKAMLVHRQMFLDLPLPWLPGADTQGSMMIQKETLVTFKNPHAKKQDV D. KD. KD.	200 200 200 200 200 200 200 200 200 200	MX 244-87 MX 116-87 MW Guinea Jamaica PR-159 (S1) PVO-218 H1 H2 H3 16681 D F G H MX 42-86 MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PVO-218 H1 H2 H3 16681 D R-159 (S1) PVO-218	
244-87 116-87 w Guinea C maice 159 (S1) 00-218 (42-86 (42-86 (244-87 (116-87) ow Guinea C maice (159 (S1) 10-218	E. I. E. I. WGMDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMVLLQ V. D. D. L. V. V. V. V. V. V. V. N. N. N. MENKAMLVHRQMFLDLPLPWLPGADTQGSNMIQKETLVTFKNPHAKKQDV D. KDKLD.	200 200 200 200 200 200 200 200 200 200	MX 244-87 Mew Guinea Jamaica PR-159 (S1) PJO-218 H1 H3 16681 D F G HK 42-86 HK 42-86 HK 116-87 New Guinea Jamaica PR-159 (S1) PJO-218 H1 H2 H3 16681 D F	A A C HIETTHRGAKRHAILGOTAMOPGSLGGVFTSIGKALHQVFGAITGAAPSG F F F F F F F F F F F F F F F F F F F
244-87 116-87 w Guinea C maice 159 (S1) 00-218 (42-86 (42-86 (244-87 (116-87) ow Guinea C maice (159 (S1) 10-218	E. I. E. I. B. I. VGMDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMVLLQ V. D. L. V. V. V. V. N. N. MENKAMLVHRQMFLDLPLPWLPGADTQGSNMIQKETLVTFKNPHAKKQDV D. KD. KLD. D.	200 200 200 200 200 200 200 200 200 200	MX 244-87 MX 116-87 New Guinea 1 Jamaica PR-159 (S1) P10-218 H1 H2 H3 16681 D F G H K42-86 HX 244-87 HX 116-87	A. C HIETTWRGAKRMAILGDTAMDFGSLGGVFTSIGKALHQVFGAITGAAFSG F
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amaica R-159 (SI) UO-218 1 2 3 6681	E. I E. I E. I VGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEMULLQ V. V. D. L. Q. II V.	150 150 200 200 200 200 200 200 200 200 200 2	MX 244-87 MX 116-87 Mew Guinea 1 Jamaica PR-159 (S1) P10-218 H1 H2 H3 H681 D F G H MX 42-86 MX 244-87 HX 116-87	A. C HIETTWRGAKRMAILGDTAMDFGSLGGVFTSIGKALHQVFGAITGAAFSG F
2 244-87 i 116-87 simucica t-159 (S1) 00-218 i 2 3 3 6681 C 42-86 C 42-87 K 116-87 ew Guinea C ammaica ew Guinea C ammaica 12 10-218 13 12 12 12 12 12 12 12 12 12 12 12 12 12	E. I. E. I. WGNDTGKHGKEIKITPQSSITFAELTGYGTVTMECSPRTGLDFNEHVLLQ V. D. L. Q. I. V T. V A. V A. V V. N N. HENKAWLVHRQWFLDLPLPWLPGADTQGSNWIQKETLVTFKNPHAKKQDV .D. KD. KLD. .D.	200 200 200 200 200 200 200 200 200 200	MX 244-87 Mew Guinea Jamaica PR-159 (S1) PJO-218 M1 M3 16681 D F G MX 42-86 MX 244-87 MX 116-87 New Guinea Jamaica PR-159 (S1) PJO-218 M1 M2 M3 16681 D F G G H K G G H K G G H K G G H K G G H K G G H K G G H K G G H K G G H K G G H G G G H G G G G	. A. A

Fig 2—AA sequences of the E proteins of D2 strains. AA sequences were deduced from the nt sequences in Fig 1.

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Table 2

Nt and AA replacements unique to 3 northeast
Thai D2 strains.

Nt	Position*	AA	Position*		
$\overline{A->G}$	79	-			
G -> A	174	-			
T -> A	249**	N - > K	83		
G -> A	288	M - > I	96		
T - > C	429	-			
A -> G	441	-			
C -> A	453**	-			
G->T	480**	K -> N	160		
A -> G	483	-			
C - > T	765	-			
A -> G	1,075	T - > A	359		

^{*} Position in the E protein gene (nt), or E protein (AA)

Table 3

Nt and AA replacements unique to 3 northeast and 2 Bangkok D2 strains.

Nt	Position*	AA	Position* Bangkok D2 strains
$\overline{C->1}$	Γ 201	_	Н
A - > 0	G 297	-	D, H
G - > A	A 735	-	Н
T - > A	** 1472	V->	A 491 D, H
C - > 1	1485		D

^{*} Position in the E protein gene (nt), or E protein (AA)

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Table 4
Number of different nt in E protein gene and AA in E protein among different D2 strains.

Strain	New Guinea C	Jamaica	PR-159 (S1)	PUO-218	Ml	M 2	M 3	16681	D	F	G	Н	MK 42-86	MK 244-87	MK 116-87
New Guinea C	-	59	121	57	79	86	107	49	71	69	69	61	69	70	71
Jamaica	8	-	133	79	94	100	72	76	53	86	90	24	27	28	29
PR-159 (S1)	12	14	-	137	154	160	164	130	146	147	140	141	135	136	135
PUO-218	6	8	12	-	48	57	115	34	61	32	34	80	84	83	87
M1	15	17	19	11	-	31	121	56	71	43	44	94	99	98	101
M2	20	22	24	16	17	-	135	63	78	55	57	99	106	105	108
M3	31	27	34	30	35	43	-	111	86	120	119	70	76	75	78
16681	8	10	14	6	15	20	33	-	62	44	49	76	83	82	85
D	10	6	14	10	15	22	26	12	-	60	60	46	55	56	57
F	11	13	15	7	15	19	34	10	11	-	22	82	94	93	96
G	11	13	15	7	12	19	33	11	9	8	-	80	94	93	96
Н	9	5	12	9	16	21	28	11	5	12	10	_	26	27	28
MK 42-86	11	7	15	11	18	23	30	13	7	14	14	6	-	1	2
MK 244-87	11	7	15	11	18	23	30	13	7	14	14	6	0	-	3
MK 116-87	11	7	15	11	18	23	30	13	7	14	14	6	0	0	_

Numbers above the diagonal represent the nt, and those below the diagonal the AA, respectively. Abbreviation of the strains: see Materials and Methods in the text.

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^{**} Transversion, other replacements are transitions

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