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Short Communication

Molecular and Virological Analyses of Dengue Virus Responsible for Dengue Outbreak in East Timor in 2005

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SUMMARY: A severe dengue outbreak occurred in East Timor in 2005. The dengue virus genome was detected by TaqMan RT-PCR in 40 serum samples, as follows: dengue virus type-3 (DENV-3) in 37 samples, DENV-2 in 2 samples, and DENV-1 in one sample. One DENV-1 genome, one DENV-2 genome, and 5 DENV-3 genomes were sequenced, and these specimens were aligned with the previously determined envelope (E) gene sequences. The DENV-1 strain belonged to genotype IV and was close to those previously isolated in Indonesia and Australia. The DENV-2 strain belonged to genotype I and was close to those previously isolated in Indonesia, Australia, the Far East, and India in 1993–2001. The DENV-3 strain belonged to genotype I and was close to those previously isolated in Indonesia. The results indicate that the dengue outbreak was caused mainly by DENV-3, with DENV-1 and DENV-2 as minor serotypes, and suggest that these strains of 3 serotypes of DENV entered East Timor from neighboring countries, co-circulated, and caused the dengue outbreak in 2005.

Dengue virus causes dengue fever (DF) and dengue hemorrhagic fever (DHF). There are 4 serotypes, dengue virus types 1–4 (DENV-1–4). It is estimated that up to 100 million cases of DF and 500,000 cases of DHF occur every year in Southeast and South Asia, Central and South America, the Caribbean, and Africa (1).

A dengue outbreak occurred in Timor-Leste (East Timor) and affected 9 out of 13 districts from January to May, 2005. The total number of reported cases was 1,067, with 39 deaths (case fatality rate [CFR], 3.6%). The majority of the cases lived in two of the most populated districts of East Timor, Dili (55.7% of the total number of cases) and Baucau (26.7%). Of a total of 1,067 cases, 446 (56.7%) were clinically diagnosed as DF, and 623 (43.3%) as DHF or dengue shock syndrome (DSS). Nearly half of the cases were children under 5 years of age with a CFR of 4.4%. The CFR under 5 years was approximately 2 times higher than that of the rest of the population (2.9%) (Fig. 1). No severe dengue outbreak had been previously reported in East Timor.

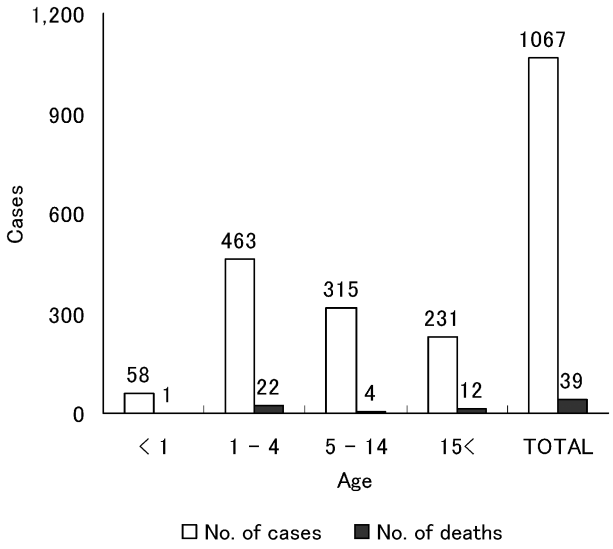

Fig. 1. Distribution of dengue cases by age during the dengue outbreak in East Timor, January–May 2005.

In response to the outbreak in 2005, the WHO Global Outbreak Alert and Response Network dispatched a field mission to Dili. Serum samples were collected for laboratory diagnosis from 124 patients suspected of having DF, DHF, or DSS in the National Hospital, Dili, from February 12 to March 2, 2005. The patients were 7 months to 54 years old. Samples were collected twice from 27 patients at acute and convalescent stages. These serum samples were examined for dengue virus-specific IgM and IgG by the DENGUE DUO IgM and IgG RAPID CASSETTE TEST (rapid test) (Pambio, Brisbane, Australia) and IgM-capture, enzyme-linked immunosorbent assay (IgM-ELISA) (Focus, Cypress, Calif., USA). Assays were performed according to the

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Table 1. Oligonucleotide primers used for genome amplification and sequencing of dengue virus type 1 (DENV-1), dengue virus type 2 (DENV-2), and dengue virus type 3 (DENV-3)

Primer name	Nucleotide sequence (5′–3′)	Position in genome			
		Sense		Reference	
DENV-1					
D1s858	ACATCCATYACCCAGAAAGG	+	858–877	M87512	12
D1Env491s, geno1–6	ATAACACCTCAAGCTCCYAC	+	1420–1439		
D1Env934s, geno1–6	GTGGCTGAGACCCAGCATG	+	1865–1872		
D1s 2128	CAACCGCCCGAGGAGCACGAA	+	2128–2148		
D1c1487	ATCTCATTAAAGTCCAGCCCA	−	1507–1487		
D1c2425	ATTTGAGTTCTCTGCCCTTCC	−	2445–2425		
D1Env641c, geno1–6	ARAAACCATTGTTTGTGGAC	−	1542–1561		
D1Env1058c, geno1–6	GTTATCAATCTCCCATTCTG	−	1978–1959		
DENV-2					
D2 0826F	CATCCAGGCTTCACCATAAT	+	826–845	AY702039	13
D2, 844s, geno1–5	ATGGCMGCAATCCTGGCATA	+	844–863		
D2F1219	TCCATGGTAGACAGAGGATG	+	1219–1238		
D2F1588	TTACCATGGCTGCCCGGAGC	+	1588–1607		
D2F2044	GAACCTCCATTCGGAGACAG	+	2044–2063		
D2R1296	GTGAACATTGCACAGGTCAC	−	1295–1276		
D2, 1517c, geno1–5	TTGAAGTCGAGGCCYGTTCT	−	1517–1498		
D2R1709	CCTTCTTGGGATCCTAAAAC	−	1709–1690		
D2, 2045c, geno1–5	TCTGCTTCTATGTTGACTGG	−	2045–2026		
D2R2115	CTTAAACCAGCTGAGCTTCAG	−	2115–2095		
D2, 2555R	GCT GAA GCT AGT TTT GAA GG	−	2555–2536		
DENV-3					
D3, 846s	CCCTATTTCTTGCCCATTACA	+	846–866	M93130	14
D3, 1381s	CCAGGTGGGAAATGAAACGCA	+	1381–1401		
D3, 1725s	GAGCTACAGAGATCCAAACCT	+	1725–1745		
D3, 1459c	TCTAGCCCGAGGGTTCCATAT	−	1479–1459		
D3, 1927c	TCCGTGGAGAAAGGAATCTTG	−	1947–1927		
D3, 2422c	CCTTTCCAGTTTATGACACAC	−	2442–2422		

manufacturers' instructions. These samples were also examined for dengue virus genome by fluorogenic real-time reverse transcriptase-polymerase chain reaction (TaqMan RT-PCR) (ABI Prism 7000; PE Applied Biosystems, Foster City, Calif., USA), as previously reported (2) (Table 1).

Serum samples from 124 clinically suspected patients were tested by TaqMan RT-PCR, rapid test, and IgM-ELISA as described above. Dengue virus infection was confirmed in 74 (60%) of 124 suspected cases. Fifty-eight (78%) of these 74 confirmed patients were less than 10 years old (data not shown).

The virus genome was detected by TaqMan RT-PCR in 40 serum samples, as follows: DENV-3 in 37 samples, DENV-2 in 2 samples, and DENV-1 in one sample. Among them, one DENV-1 genome, one DENV-2 genome, and 5 DENV-3 genomes were sequenced and aligned with the envelope (E) gene sequences previously deposited in the GenBank. The E gene was used for analysis because it was the most frequently analyzed among the dengue genes (3). The direct sequencing and phylogenetic analysis were done according to Tajima et al. (4) and Ito et al. (5), respectively. The primers used for PCR amplification and direct sequencing are shown in Table 1.

DENV-1, VIP01 (GenBank accession no. AB219136) belonged to genotype IV, according to the genotype classification by Goncalvez et al. (6), and to a subcluster with the strains isolated in Indonesia and Australia in 1983–2002 with the nucleotide homologies of 96.9–97.6% (Figure 2A). The results suggest that VIP01 is close to those strains isolated in Indonesia or Australia.

DENV-2, OPD30 (GenBank accession no. AB219135) belonged to genotype I, according to the genotype classification by Twiddy et al. (7) and Wang et al. (3), and to a subcluster with the strains isolated in Indonesia, Australia, the Far East, and India in 1993–2001 with the nucleotide homologies of 98.6–99.0% (Figure 2B). OPD30 also shared high nucleotide homologies of 99.0% with the strain AB111450 isolated from a traveler to East Timor in 2000. The results suggest that the East Timor DENV-2 originated from neighboring countries and circulated in East Timor for at least 6 years.

DENV-3, OPD07, OPD18, OPD29, PED109, and PED129 (GenBank accession numbers: AB219130, AB219131, AB219132, AB219133, and AB219134, respectively) belonged to genotype I, according to the genotype classification by Wittke et al. (8), and made an independent cluster (Figure 2C). The nucleotide sequence homologies among these 5 isolates were 99.4–99.8%. They shared nucleotide homologies of

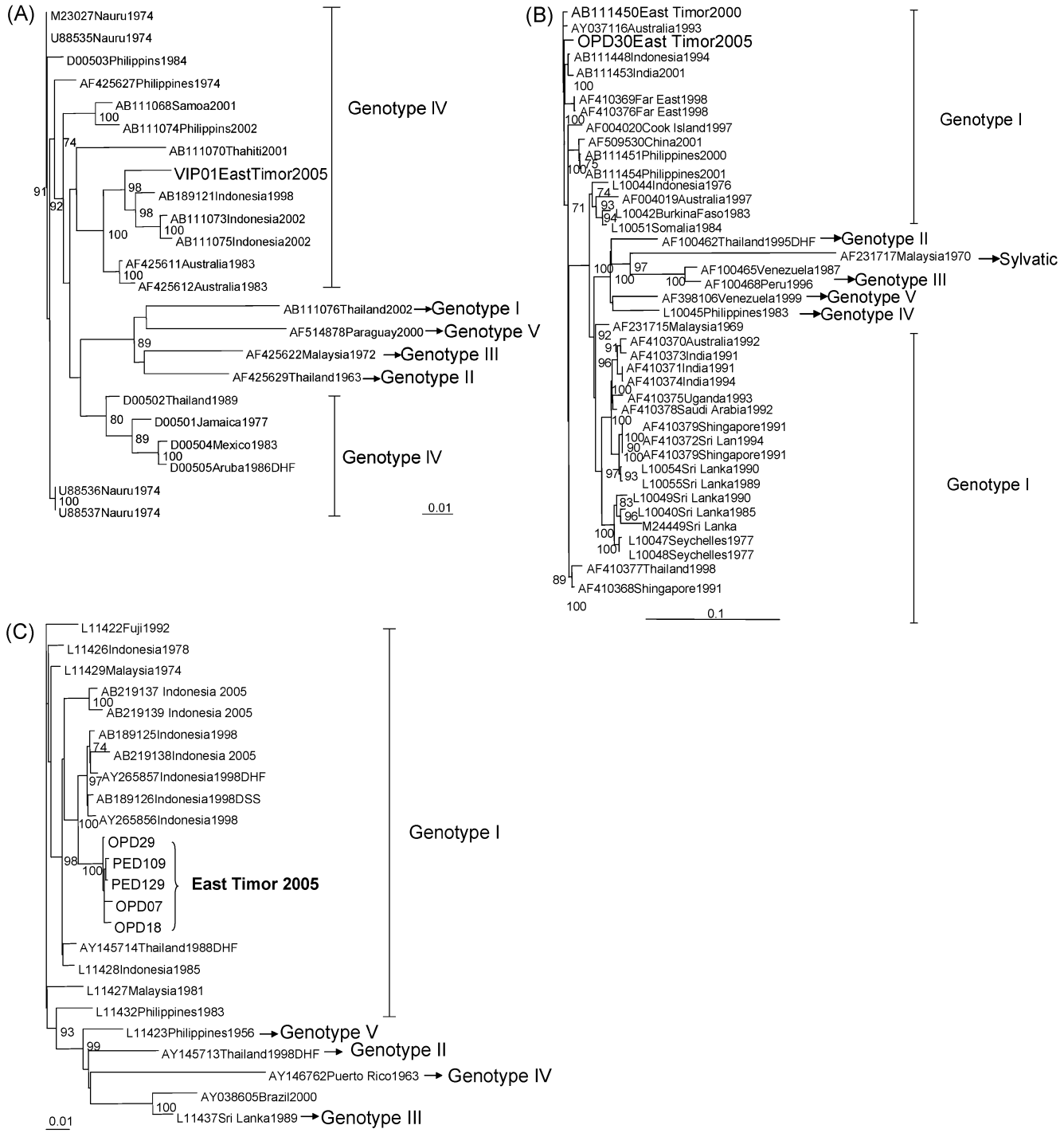

Fig. 2. Phylogenetic tree of dengue virus type 1 (DENV-1) (A), dengue virus type 2 (DENV-2) (B), and dengue virus type 3 (DENV-3) (C). Phylogenetic analyses were done according to Ito et al. (5). Briefly, the neighbor-joining method was taken using the Clustal X program. The bootstrap probability of each node was calculated using 100 replicates. Bootstrap values greater than 70% were regarded as the criteria for phylogenetic grouping. Neighbor joining tree shows the phylogenetic relationships and genetic subtypes based on the entire envelope (E) gene sequences from 23 DENV-1 strains, 41 DENV-2 strains, and 24 DENV-3 strains. The E genes of DENV-1, DENV-2, and DENV-3 are 1485, 1485, and 1479 nucleotides long, respectively. The genotypes are labeled according to Goncalvez et al. (6) for DENV-1, Twiddy et al. (7) and Wang et al. (3) for DENV-2, and Wittke et al. (8) for DENV-3. Roman numerals denote the different genotypes of dengue virus circulating in the world. Neighbor joining values (100 replications) are shown for nodes.

96.2–96.8% with Indonesian isolates (AB219137 and AB219139) in January 2005, and 97.2–98.2% with other Indonesian isolates in 1998 (AB189125, AB189126, AY265857, and AY265856) and in 2005 (AB219138). The results suggest that the East Timor DENV-3 isolates were close to, but different from Indonesian isolates.

Forty serum samples positive for dengue virus genome by TaqMan RT-PCR were also used for virus isolation by inoculation onto *Aedes albopictus* mosquito cell line C6/32 cells according to the procedure described by Tajima et al. (4). Dengue virus was isolated from 14 serum samples. Of the 14 isolates, one was DENV-2 and 13 were DENV-3 (data not shown).

The results suggest that the epidemic in East Timor in

2005 was caused mainly by DENV-3, with DENV-1 and DENV-2 as minor serotypes. The E gene sequence of the DENV-2 isolated in 2005 had a high nucleotide homology with those isolated in 2000. It is likely that this DENV-2 strain had been circulating for the 6 years prior to 2005 in this region. However, the information was limited and it was difficult to perform further comparison. Dengue virus infection was serologically confirmed in soldiers returning from East Timor to Australia from December 1999 to March 2000: 6 cases with DENV-2 and 2 with DENV-3. However, no dengue cases were reported among the East Timor local population at that time (9).

In the present study, our results indicated that the epidemic in 2005 was mainly caused by DENV-3. This epidemic was probably the most serious one in East Timor history, based on the highest number of reported cases, the severity of clinical manifestation, and the number of confirmed fatal cases. It is possible that the DENV-3 strain responsible for this outbreak was more virulent than the DENV strains that caused previous outbreaks; however, further studies are needed to confirm or deny this. Large DENV-3 epidemics were reported in Brazil (10), New Caledonia, and Tahiti. There is little information available about past dengue epidemics in East Timor. Dengue virus infection of soldiers returning from East Timor to Australia were serologically confirmed in 1999–2000 and caused mainly by DENV-2 (9). Porter et al. also reported that DENV-2 infection was detected most frequently among symptomatic cases in Indonesia from 2000 to 2002 (11). The DENV-1, DENV-2, and DENV-3 strains analyzed in the present study were all genetically related to those isolated in Indonesia and Australia. It is possible that DENV-1, DENV-2, and DENV-3 entered East Timor from neighboring countries and co-circulated in East Timor.

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