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Letter to the editor: molecular genotyping of Dengue Virus Types 2 and 4 from the Guatemalan and Honduran Epidemics of 2007 using the envelope glycoprotein gene

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Abstract Eight serum specimens collected from dengue patients in Guatemala and Honduras during the Central American epidemic of 2007 were analyzed. Virus identification and serotyping performed by a nested RT-PCR assay revealed two DENV-1 isolates from Guatemala, four DENV-2 isolates, two each from Guatemala and Honduras, and two DENV-4 isolates from Honduras. Viral genotyping determined by phylogenetic analysis of the complete envelope gene sequences demonstrated that the DENV-2 isolates from Guatemala and Honduras fell into the American/Asian Genotype III, and were most closely related to DENV-2/NI/BID-V2683-1999 isolated from a dengue case in Nicaragua in 1999; and the DENV-4 F07-076 isolate from Honduras belonged to genotype II, and was most closely related to DENV-4/US/BID-V1093/1998 isolated from Puerto Rico in 1998. Our results suggest that the 2007 dengue outbreaks in Guatemala and Honduras were most likely caused by the re-emergence of earlier, indigenous DENV strains rather than by newly introduced strains and there were at least three serotypes of DENV co-circulating during the 2007 Central American epidemics.

Keywords Dengue virus · Serotyping · Sequencing · Phylogenetic analysis

Dengue (DEN) is the most important mosquito-borne viral disease affecting millions of people in the tropics and subtropics caused by four serotypes of DEN viruses (DENVs), which can infect humans resulting in a wide spectrum of DEN disease ranging from acute febrile dengue fever (DF) to life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) [1–3]. DEN has been spreading rapidly across the world in the past three decades. South-east Asia and the Western Pacific are the most seriously affected areas [4]. However, recently in the America the incidence of DEN cases has increased dramatically accompanied by an increase in severe forms of the disease [5]. During 2007 in the Americas there were 900,782 reported cases of DEN with 26,413 DHF and 317 deaths. In Central America and Mexico, from 2001 to 2007, there were 545,049 reported cases of DEN with 35,746 cases of DHF and 209 deaths. All four DENV serotypes are co-circulating in Central America with a predominance of DENV-1 and -2 [5].

Four genetically distinct but antigenically related serotypes of DENV are further sub-typed into genotypes. So far, four genotypes have been defined for DENV-1, six for DENV-2 (one of which is found only in non-human primates), four for DENV-3 and four for DENV-4 [6–10]. Virus serotyping, genotyping, and epidemiological

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analyses are critical for DEN surveillance as well as understanding of DENV evolution and its impact on virus transmission and disease severity. The envelope (E) gene of DENV is the most frequently selected target for genotyping DENVs through phylogenetic analyses. Serum specimens sampled from DEN patients in Guatemala and Honduras during the Central American epidemic of 2007 were submitted to our laboratories for virus identification, serotyping, and genotyping. We report here the results from that study (Fig. 1).

Eight DEN positive specimens (four from Guatemala and four from Honduras) were identified from serum specimens collected from the patients with DEN-like febrile illnesses during the Central American epidemic of 2007, by the nested RT-PCR assay in the field, then the extracted viral RNA samples were sent to the Division of Viral Diseases, Walter Reed Army Institute of Research for genotyping. Detailed information for each specimen is presented in Table 1. The complete E gene sequence (1485 nt) of reference strains of DENVs, 21 from DENV-2 and 9 from DENV-4 retrieved from GenBank, representing all genotypes of these two serotypes circulating worldwide, were used for sequence comparison and phylogenetic analysis (Table 1).

Viral RNA was extracted from sera using the ZYMO Mini RNA Isolation II kit (ZYMO Research, Orange, CA). Virus identification and serotyping were performed by the nested RT-PCR assay described by Lanciotti et al. [11] in local clinical laboratories in Guatemala and Honduras. Of eight viral RNA samples, four were degraded during shipping, only four complete E genes, (DENV-2 F07-030 from Guatemalan, and DENV-2 F07-073, F07-075 and DENV-4 F07-076 from Honduran), were sequenced for further genotyping. One-step RT-PCR was carried out using the Superscript™ One-Step RT-PCR with Platinum® *Taq* (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The generated RT-PCR products were purified using the QIAquick Gel Extraction Kit (QiaGen, Valencia, CA). For used primer sequences in the E gene sequencing see supplementary material Tables S1 and S2. The BigDye® Terminator v3.1 Cycle Sequencing Kit (ABI, Foster City, CA) was used for cycle sequencing. Cycle sequencing products were purified using The BigDye® XTerminator™ Purification Kit (Foster City, CA).

Four full length E gene sequences obtained in this study along with 32 additional E gene sequences (23 DENV-2 and 9 DENV-4) retrieved from GenBank (the accession numbers presented in Table 1) were compared and

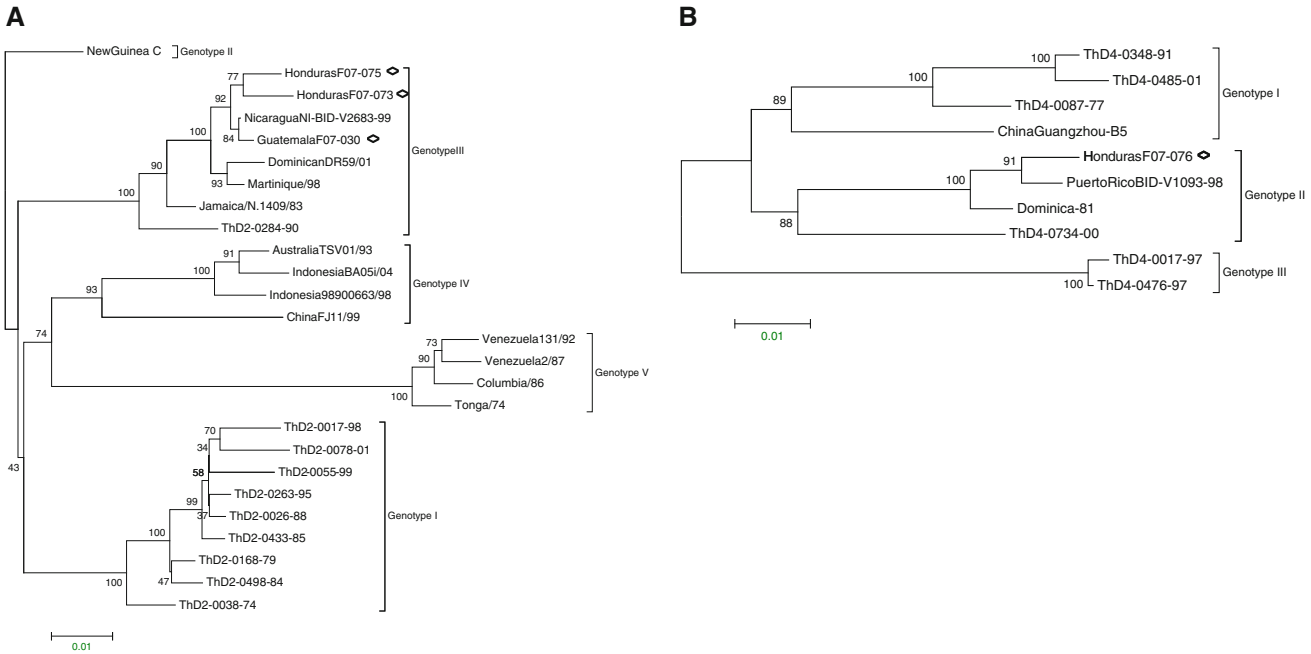

Fig. 1 Neighbor-joining (NJ) phylogenetic trees of the complete E gene sequence of 26 DENV-2 (*Panel A*) and 10 DENV-4 (*Panel B*) strains. The trees were drawn by MEGA 4 with 100 bootstrap replicates and all horizontal branch lengths were drawn to scale. Panel A derived from three DENV-2 sequences sampled from Guatemala and Honduras (marked with *hollow diamond*) along with 23 other DENV-2 retrieved from GenBank, representing all five genotypes circulating worldwide. The tree was rooted by one strain of genotype II (New Guinea C). Three DENV-2 isolates fell into American/Asian Genotype III (marked with *hollow diamond*). Panel B derived from one DENV-4 E gene sequence sampled from Honduras (HondurasF07-076, marked with *hollow diamond*) and nine DENV-4 E gene retrieved from GenBank, showing the HondurasF07-076 fell into Genotype II of DENV-4

Table 1 DENV serotyping results and sequence information used for phylogenetic analysis in this study

Serotype	Country of origin	Isolation (years)	Genotype	GenBank accession #
DENV-1	GuatemalaF07-008	**2007**	–[b]	–[c]
	GuatemalaF07-012	**2007**	–[b]	–[c]
DENV-2	GuatemalaF07-019	**2007**	–[b]	–[c]
	ThD2-0168/79[a]	1979	Asian I	DQ181805
	ThD2-0038/74[a]	1974	Asian I	DQ181806
	ThD2-0498/84[a]	1984	Asian I	DQ181804
	ThD2-0263/95[a]	1995	Asian I	DQ181800
	ThD2-0017/98[a]	1998	Asian I	DQ181799
	ThD2-0055/99[a]	1999	Asian I	DQ181798
	ThD2-0078/01[a]	2001	Asian I	DQ181797
	ThD2-0433/85[a]	1985	Asian I	DQ181803
	ThD2-0026/88[a]	1988	Asian I	DQ181802
	ThD2-0284/90[a]	1990	Asian/American (III)	DQ181801
	GuatemalaF07-030	**2007**	Asian/American (III)	**GU586492**
	HondurasF07-073	**2007**	Asian/American (III)	**GU586122**
	HondurasF07-075	**2007**	Asian/American (III)	**GU586123**
	Jamaica N1409-83	1983	Asian/American (III)	M20558
	Nicaragua NI-BID-V2683-99	1999	Asian/American (III)	GQ199895
	Martinique/98	1998	Asian/American (III)	AF208496
	DominicanDR59/01	2001	Asian/American (III)	AB122022
	AustraliaTSV01/93	1993	Cosmopolitan (IV)	AY037116
	Indonesia98900663/98	1998	Cosmopolitan (IV)	AB189122
	ChinaFJ11/99	1999	Cosmopolitan (IV)	AF359579
	IndonesiaBA05i/04	2004	Cosmopolitan (IV)	AY858035
	Tonga/74	1974	American (V)	AY744147
	Columbia/86	1986	American (V)	AY702040
	Venezuela2/87	1987	American (V)	AF100465
	Venezuela131/92	1992	American (V)	AF100469
	NewGuineaC	1944	Asian II	AF038403
DENV-4	**HondurasF07-061**	**2007**	–[b]	–[c]
	HondurasF07-076	**2007**	**II**	**GU586124**
	PuertoRicoBID-V1093-98	1998	II	EU854296
	ThD4-0087/77[a]	1977	I	AY618991
	ThD4-0348/91[a]	1991	I	AY618990
	ThD4-0017/97[a]	1997	III	AY618989
	ThD4-0476/97[a]	1997	III	AY618988
	ThD4-0734/00[a]	2000	II	AY618993
	ThD4-0485/01[a]	2001	I	AY618992
	China Guangzhou B5	NA[d]	I	AF289029
	Dominica/81	1981	II	AF326573

[a] Thailand

[b] Strains were not genotyped due to unavailable viral RNAs

[c] No accession numbers for these strains due to no available sequences for submission to GenBank

[d] Year of isolation is not available. Isolates in bold indicate the DENVs obtained from this study

analyzed by BLAST. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 4 (Tamura et al.) [12].

The results of the nested RT-PCR assay revealed two DENV-1 isolates from Guatemala, four DENV-2 isolates, two for each from Guatemala and Honduras, and two

DENV-4 isolated from Honduras (Table 1). The nt BLAST search showed that DENV-2 F07-030 had a 99% identity with Nicaragua strain (DENV-2/NI/BID-V2683-1999), and DENV-2 F07-073 and DENV-2 F07-075 had a 99% identity (1481/1485) with Nicaragua strain (DENV-2/NI/BID-V535-2005), respectively; while DENV-4 F07-076 possessed a 99% identity with DENV-4/US/BID-V1093/1998 isolated from Puerto Rico in 1998. Molecular genotyping of complete viral E gene sequences for Guatemalan isolate (DENV-2 F07-030) and Honduran isolates (DENV-2 F07-073 and F07-075 and DENV-4 F07-076) demonstrated that three DENV-2 isolates from Guatemala and Honduras fell into the American/Asian Genotype III, and were most closely related to DENV-2/NI/BID-V2683-1999 isolated from a DEN case in Nicaragua in 1999. DENV-2 F07-073 and F07-075 from Honduras had 99.0 and 98.9% nt sequence identity, respectively, with DENV-2/NI/BID-V2683-1999; whereas, DENV-2 F07-030 from Guatemala had 99.7% nt sequence identity with DENV-2/NI/BID-V2683-1999 (Supplementary Material Table S3). At the aa level, DENV-2 F07-030 from Guatemala, and DENV-2 F07-073 and F07-075 from Honduras exhibited 99.6, 99.2, and 99.6%, respectively, similarity with the Nicaragua strain DENV-2/NI/BID-V2683-1999 (Supplementary Material Table S3). Comparison of nt and aa sequence similarity indicated the presence of some silent mutations in the E gene of the two Honduran DENV-2 viruses. The DENV-4 isolate from Honduras (F07-076) belonged to genotype II, and was most closely related to DENV-4/US/BID-V1093/1998 isolated from Puerto Rico in 1998, with a 98.7% nt and 99.8% aa sequence identity, respectively (Supplementary Material Table S4).

There were serious DEN outbreaks in Guatemala and Honduras in 2007 [5], with DENV-1, DENV-2, and DENV-4 reported in both countries. That we detected only DENV-1 and DENV-2 in Guatemala, and DENV-2 and DENV-4 in Honduras may reflect our limited sample size.

In conclusion, our results provide direct evidence that at least three serotypes of DENV, i.e., DENV-1, DENV-2, and DENV-4, were co-circulating in the 2007 central American epidemic, and that the 2007 DEN outbreaks in Guatemala and Honduras were most likely caused by the re-emergence of earlier, indigenous DENV strains rather than by newly introduced strains.

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References

1. D.J. Gubler, Novartis Found. Symp. **277**, 3–16 (2006). discussion 16–22, 71–13, 251–253
2. D.J. Gubler, Clin. Microbiol. Rev. **11**, 480–496 (1998)
3. WHO, TDR *Report of Dengue Scientific Working Group*, TDR/SWG/08, Geneva (2000), http://wwwwhoint/tdr/publications/publications/swg_dengue_2htm. Accessed July 2008
4. WHO, Dengue and dengue haemorrhagic fever (2010), (http://wwwwhoint/mediacentre/factsheets/fs117/en/)
5. PAHO, Dengue in the Americas: 2007 Summary (2010), (http://wwwpahoorg/english/ad/dpc/cd/eid-eer-2007-09-26htm)
6. C. Klungthong, C. Zhang, M.P. Mammen Jr., S. Ubol, E.C. Holmes, Virology **329**, 168–179 (2004)
7. C. Zhang, M.P. Mammen Jr., P. Chinnawirotpisan, C. Klungthong, P. Rodpradit, P. Monkongdee, S. Nimmannitya, S. Kalayanarooj, E.C. Holmes, J. Virol. **79**, 15123–15130 (2005)
8. C. Zhang, M.P. Mammen Jr., P. Chinnawirotpisan, C. Klungthong, P. Rodpradit, A. Nisalak, D.W. Vaughn, S. Nimmannitya, S. Kalayanarooj, E.C. Holmes, J. Gen. Virol. **87**, 873–883 (2006)
9. S.C. Weaver, N. Vasilakis, Infect. Genet. Evol. **9**, 523–540 (2009)
10. C. Klungthong, R. Putnak, M.P. Mammen, T. Li, C. Zhang, J. Virol. Meth. **154**, 175–181 (2008)
11. R.S. Lanciotti, C.H. Calisher, D.J. Gubler, G.J. Chang, A.V. Vorndam, J. Clin. Microbiol. **30**, 545–551 (1992)
12. K. Tamura, J. Dudley, M. Nei, S. Kumar, Mol. Biol. Evol. 24: 1596–1599 (2007) (Publication PDF at http://wwwkumarlabnet/publications)