

1 A Dengue virus serotype 4- dominated outbreak in central Vietnam,  
2 2013

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4 **Supplementary data**

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6 **Materials and Methods.**

7 This study was approved by the Institutional Review Board of NIHE, Vietnam  
8 (No.15-HDD, January 18, 2011).

9 During the dengue season, September – December 2013, single blood samples  
10 were collected from 1532 dengue-suspected patients admitted at Hue Central  
11 Hospital, a tertiary medical care center and the biggest hospital in central  
12 Vietnam. Blood collection was done during the acute phase of illness after a  
13 written informed consent was given by each patient or the accompanying  
14 parents or legal guardians. The serum samples obtained were stored at -70°C  
15 for further analysis.

16 All the collected blood samples were screened by SD dengue NS1 Ag ELISA  
17 (Standard Diagnostics), and 702 samples were found positive. Positive samples  
18 were tested by serotype specific realtime - reverse transcription (RT) -PCR  
19 method (the primers used for realtime RT-PCR are indicated in Table S2).

20 The DENV-4 positive samples were subjected to virus isolation by using C6/36

21 cells and Vero cells, and a total of 21 DENV-4 strains were isolated.

22 The Infected culture fluid-derived RNA was used for the sequence as described

23 previously (Takamatsu *et al.* Virology Journal 2013 10:280). The primers for

24 PCR and sequence are indicated in Table S3. The complete nucleotide

25 sequences of the envelope (E)-protein coding region for the 21 DENV-4 isolates

26 were determined and deposited in GenBank (accession no.

27 KP292911-KP292931). The sequences were aligned by MAFFT v7.215 and

28 phylogenetic trees were constructed by ML method using PHYML 3.0.1

29 (Guindon, S. *et al.* Syst Biol 59 307-321). The substitution models were selected

30 by jmodeltest-2.1.7 and GTR+I+G was used as the model. Tree was drawn by

31 FigTree software, version 1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>).

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34 **Table S1.** Number of clinical dengue cases in central and southern Vietnam

35 during 2008-2013

36 (Data kindly provided by the Department of Virology, NIHE, Hanoi, Vietnam)

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Region	Number of dengue cases					
	2008	2009	2010	2011	2012	2013
Central	8,871 (7)	11,519 (8)	35,865 (24)	3,421 (2)	6,619 (7)	204,661 (18)
South	82,592 (90)	73,890 (74)	74,040 (80)	60,574 (59)	153,182 (41)	29,955 (21)

38 \*The number of fatal cases is enclosed in the brackets.

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40 **Table S2.** Primer information used for realtime RT-PCR (Johnson BW *et al.*

41 Journal of clinical microbiology. 2005 Oct;43(10):4977-83.)

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<b>Primer or Probe</b>	<b>Sequence</b>
DENV-1 Primer Forward	CAAAGGAAGTCGTGCAATA
DENV-1 Primer Reverse	CTGAGTGAATTCTCTACTGAACC
DENV-2 Primer Forward	CAGGTTATGGCACTGTCACGAT
DENV-2 Primer Reverse	CCATCTGCAGCAACACCATCTC
DENV-3 Primer Forward	GGACTGGACACACGCACTCA
DENV-3 Primer Reverse	CATGTCTCTACCTTCTCGACTTGTCT
DENV-4 Primer Forward	TTGTCCTAATGATGCTGGTCCG
DENV-4 Primer Reverse	TCCACCTGAGACTCCTTCCA
Probe DENV-1	CATGTGGTTGGGAGCACGC
Probe DENV-2	CTCTCCGAGAACAGGCCTCGACTTCAA
Probe DENV-3	ACCTGGATGTCGGCTGAAGGAGCTTG
Probe DENV-4	TTCCTACTCCTACGCATCGCATTCCG

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45 **Table S3.** Primer information used for RT and PCR

<b>Primer name</b>	<b>Orientation</b>	<b>Sequence</b>
Random primer	reverse transcription	
VNR primer	reverse transcription	AGAACCTGTTGATTCAACAGCACCATTTCCA
DC-S	PCR-Forward, sequence	TCAATATGCTGAAACGCGCGAGAAACCG
DC-C	PCR-Reverse, sequence	TTGCACCAACAGTCAATGTCTTCAGGTTC
DENV-4-1R	sequence	CAATCCCATTCTGAATGTGG
DENV-4-2R	sequence	GCACATGTCACAACCTCTCC
DENV-4-3R	sequence	GCTGTGTTTCTGCCATCTC
DENV-4-4R	sequence	CCACAGACCCCATCTTTGTG
DENV-4-5R	PCR-Reverse, sequence	TCCAGCCTCGTGGTTGATCT
DENV-4-6R	PCR-Reverse, sequence	CTCTTCGTTTCATTGGGGCATTG
DENV-4-7R	PCR-Reverse, sequence	AACATGCCAAATCCATAGTC
DENV-4-8R	PCR-Reverse, sequence	CCATGTTCTCTTCTTTTTCA
DENV-4-9R	PCR-Reverse, sequence	GTTGTCATGGCCATTCTAT
DENV-4-1F	sequence	GAACCCCTCATGATAGTGG
DENV-4-2F	sequence	ACCACATTCAGGAATGGGATTG
DENV-4-3F	sequence	CCTCATGCCAAGAGACAGGATG
DENV-4-4F	sequence	GATGTTTGAGTCCACATACAG

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48 **Table S4.** Dengue serotype confirmed cases in central and southern Vietnam

49 during 2012 – 2013.

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year	Number of dengue confirmed cases (%) in central Vietnam				
	DENV1	DENV2	DENV3	DENV4	total
2012	<b>25</b> <b>(40.3)</b>	18 (29.0)	5 (7.9)	14 (22.6)	62
2013	141 (28.1)	52 (10.4)	63 (12.6)	<b>245</b> <b>(48.9)</b>	501

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year	Number of dengue confirmed cases (%) in southern Vietnam				
	DENV1	DENV2	DENV3	DENV4	total
2012	<b>237</b> <b>(36.3)</b>	155 (23.8)	45 (6.9)	215 (33.0)	652
2013	NA*	NA	NA	NA	NA

52 The number and percentage of cases for the dominant serotypes are in red font.

53 \*NA: not available. The data of southern Vietnam in 2013 were not available.