

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

3

4 **LETTERS**

5

6 **Dear editor:**

7 Dengue diseases are transmitted by *Aedes* mosquitoes. Dengue virus (DENV)
8 belongs to the family *Flaviviridae*, genus *Flavivirus*¹, and has 4 serotypes:
9 DENV-1, DENV-2, DENV-3, and DENV-4. Over the past 50 years dengue has
10 spread extraordinarily; only 9 countries were reported to have dengue
11 transmission prior to 1970, whereas over 100 countries have been reported in
12 2014 (WHO, 2014). Vietnam is a dengue endemic country, especially southern
13 Vietnam a hyper-endemic area². The in-house report of the National Institute of
14 Hygiene and Epidemiology (NIHE) showed that there were about ten-fold more
15 dengue cases in southern Vietnam than in central Vietnam annually (Table S1).
16 Yearly, about 10 thousand dengue cases have been reported from central
17 Vietnam. DENV-4 has been found in each area of Vietnam, but DENV-4-
18 dominated outbreak has not been known in this country since 2001. Over 50

19 DENV-4 strains isolated in Vietnam (northern, central, southern) were available
20 in GenBank, and all of them belonged to genotype I (Figure 1).

21

22 In 2013, a large DENV outbreak occurred with 204,661 clinical cases in central
23 Vietnam. During the dengue season, September – December 2013, a total of
24 1532 collected blood samples were screened by dengue NS1 Ag ELISA in Hue
25 central hospital. Out of the 702 samples positive by NS1 Ag ELISA, 501 samples
26 were positive by serotype specific realtime - reverse transcription (RT) -PCR
27 method³ (Table S2). As a result, DENV-4 was the dominant serotype (245 cases,
28 48.9%), followed by DENV-1 (141 cases, 28.1%), DENV-3 (63 cases, 12.6%)
29 and the last DENV-2 (52 cases, 10.4%). None of dengue negative specimens
30 showed Chikungunya positive by RT-PCR (data not shown)⁴. The DENV-4
31 positive samples were subjected to virus isolation, and 21 DENV-4 strains were
32 isolated. Infected culture fluid-derived RNA was used for the sequence (Table
33 S3.) The complete nucleotide sequences of the envelope (E) -protein coding
34 region for those isolates were determined and deposited in GenBank (accession
35 no. KP292911-KP292931).

36

37 In 2013, DENV-3 outbreaks were reported in Solomon islands⁵ and in China⁶,
38 whereas DENV-4- dominated outbreak has not been reported in the neighboring
39 countries around Vietnam. In central Vietnam, nearly 20-fold more DENV-4
40 cases were found in 2013 than in 2012, and the percentage of DENV-4 cases
41 also increased from 22.6% (2012) to 48.9% (2013) (Table S4). The number of
42 severe cases were not dramatically increased, and there have been no recorded
43 climate changes such as heavy rainfall, higher temperature in central Vietnam in
44 2013 (data not shown). In 2012, many dengue cases were reported from
45 southern Vietnam, and the dominant serotype was DENV-1, which was also
46 dominant in central Vietnam (Table S4). It is possible that the epidemic serotype
47 of DENV may be similar between central and southern Vietnam.

48

49 There are several possible considerations for the sudden increase of DENV-4,
50 such as, introduction of other genotype, or virus genome acquired mutations for
51 adaptation in hosts, or the change of immunity in human population. Interestingly,
52 the other genotype of DENV-4 was not found from this outbreak according to the
53 phylogenetic analysis (Figure 1). Currently, we consider research on the in vitro

54 and in vivo properties of virus isolates, adaption to mosquitos or humans, and

55 immunity in human population.

56

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67 **Competing interests:**

68 The authors have declared that no competing interests exist.

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70 **Ethical approval:**

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81 **References**

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- 83 1. Gubler DJ, Kuno G, Markoff L. *Field's Virology*. Philadelphia: Wolters Kluwer
84 Lippincott Williams and Wilkins; 2007.
- 85 2. Ha DQ, Tien NT, Huong VT, Loan HT, Thang CM. Dengue epidemic in southern Vietnam,
86 1998. *Emerging infectious diseases*. 2000;6:422-5.
- 87 3. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a
88 fourplex real-time reverse transcriptase PCR assay. *Journal of clinical microbiology*.
89 2005;43:4977-83.
- 90 4. Grywna K, Kupfer B, Panning M, Drexler JF, Emmerich P, Drosten C, et al. Detection of
91 all species of the genus Alphavirus by reverse transcription-PCR with diagnostic sensitivity.
92 *Journal of clinical microbiology*. 2010;48:3386-7.
- 93 5. Nogareda F, Joshua C, Sio A, Shortus M, Dalipanda T, Durski K, et al. Ongoing outbreak
94 of dengue serotype-3 in Solomon Islands, January to May 2013. *Western Pacific surveillance
95 and response journal : WPSAR*. 2013;4:28-33.
- 96 6. Zhang FC, Zhao H, Li LH, Jiang T, Hong WX, Wang J, et al. Severe dengue outbreak in
97 Yunnan, China, 2013. *International journal of infectious diseases : IJID : official publication
98 of the International Society for Infectious Diseases*. 2014;27:4-6.

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100 **Figure 1 Legend**

101 ML-phylogenetic tree. Bootstrap values over 700 > of 1000 repeats are shown at

102 the nodes. Labels of strains conform to the following format: (GenBank
103 accession nos)_(Strain name)_(Country-region)_(Year of isolation). The shaded
104 strains are isolates from central Vietnam in 2013.

105

Fig. 1

