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Phylogenetic Analysis of Dengue Virus Type 3 Strains Primarily Isolated in 2013 from Surabaya, Indonesia

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Dengue fever (DF) and its severe form, dengue hemorrhagic fever (DHF), are mosquito-borne viral diseases that occur in tropical and subtropical regions. They are caused by 4 serotypes of dengue viruses (DENV) 1–4 (1). Each serotype can be further divided into several genotypes (2). Epidemiological studies have shown an increased risk of developing DHF after a secondary heterotypic infection, highlighting the urgent need for a better understanding of how the virus spreads as well as its epidemiological trend (3).

In Indonesia, more than 100,000 cases of DF/DHF have been annually reported in recent years (4). Phylogenetic analysis revealed that particular types of DENV that spread throughout the region had their origin in Indonesia (5,6). Although the country is considered to be an important dengue-endemic area, sequence information available for the circulating dengue virus strains is limited. Furthermore, the largest number of Japanese dengue cases was found in Indonesia during 2006–2010 (7). Therefore, conducting dengue research in Indonesia is of paramount importance.

In 2007, we started DENV surveillance in Surabaya, the second largest city in Indonesia. The surveillance data revealed co-circulation of DENV-1, -2, and -4, with a predominance of DENV-1 from 2008 onward (till June 2013); this followed the predominant circulation of DENV-2 in 2007 (8) (Kotaki et al., unpublished data). DENV-3, which was previously the predominant dengue serotype in many parts of Indonesia (9–12) and thereafter evolved independently. Both clades chief-

In order to investigate the possible route of virus entry and its evolution.

Blood samples were collected from patients with suspected DF or DHF visiting Soerya Maternal and Child Health Hospital. Approval was obtained from the Institutional Ethics Committees of the Institute of Tropical Disease, Airlangga University (Ethics Committee Approval No.: 784). Viral isolation and sequence analysis of the envelope (E) protein coding region were performed according to a previous report (8).

The 3 strains of DENV-3 isolated in Surabaya displayed nucleotide identities of 96.7%, and 2 of the strains (D3/SBY45/13 and D3/SBY49/13) were completely identical. Therefore, 2 representative strains (D3/SBY45/13 and D3/SBY49/13) were used for subsequent analyses. There were 49 nucleotide differences between these isolates, which translated into 6 amino acid substitutions. Phylogenetic analysis was conducted using MEGA 5.2 and BEAST v1.5.3 softwares (13–15). The analysis revealed that the 2 DENV-3 isolates were of genotype I, which has been reported to spread from Indonesia (6) (Fig. 1). This genotype includes most Indonesian strains isolated between 1973 and 2004 as well as strains isolated from Malaysia, East Timor, and Philippines (6).

The 3 strains of DENV-3 isolated in Surabaya were further divided into different clades with a 99% bootstrap value. The most recent common ancestor of these 2 strains possibly emerged approximately 20 years ago and thereafter evolved independently. Both clades chief-

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Fig. 1. Phylogenetic tree of the E gene sequence of DENV3 constructed using the neighbor joining (NJ) method and Bayesian Markov Chain Monte Carlo (MCMC) method. GenBank accession number, country, and year of isolate are shown in order. The Surabaya isolates are indicated in bold. Phylogenetic tree using the NJ method with a Kimura 2-parameter model was described using MEGA 5.2 software. The tree derived from the Bayesian MCMC method was constructed using BEAST software v1.5.3. Clades including the Surabaya isolates are enclosed and numbered as clade 1 and clade 2. Bootstrap values ≥ 90 are shown. The coalescent times of some key nodes, their 95% HPD values and posterior probability values are shown.

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<tr>
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<td>DHF</td>
<td>DF</td>
<td>DHF</td>
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<tr>
<td>Travel history before infection</td>
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<td>No</td>
<td>unavailable</td>
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</tbody>
</table>

1: Demographical and clinical information was provided by the hospital.
2: Travel history was obtained by their interview.
serotypes circulating in Surabaya for a decade (8). Therefore, the emergence of DENV-3 potentially increases the incidence of secondary heterotypic infection, leading to severe symptoms. Previous studies have shown that the emergence of DENV3 following the circulation of heterotypic DENV caused outbreaks of DF/DHF (19,20). In addition, a cohort study of adult DHF Cuban cases showed that infection with DENV-1 followed by DENV-3 has significantly higher nucleotide substitution rates than other DENV serotypes, resulting in the emergence of DENV variants with increased transmissibility and/or virulence (6).

In conclusion, we isolated strains of DENV-3 genotype I that potentially cause endemic outbreaks in Surabaya. There is a possibility that DENV-3 is already established in the population and that it will replace the currently circulating strains, leading to an increase in the incidence and rate of severe DHF cases. Continuous epidemiological surveillance is required for monitoring the incursion and spread of this virus.

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Conflict of interest None to declare.

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