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Continuous dengue type 1 virus genotype shifts followed by co-circulation, clade shifts and subsequent disappearance in Surabaya, Indonesia, 2008–2013

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ABSTRACT

Four serotypes of dengue virus (DENV-1 to DENV-4) and their genotypes are distributed in tropical and subtropical regions. Indonesia has been recently suggested as the origin of some dengue virus genotypes. In Surabaya, the second biggest city of Indonesia, we previously reported a shift of the predominantly circulating serotype from DENV-2 to DENV-1 in November 2008, followed by a genotype shift of DENV-1 from genotype IV (GIV) to genotype I (GI) in September 2009, based on nucleotide sequences in the envelope protein coding region. Since then, GI strains had predominantly circulated until December 2010. In this report, we investigated further DENV-1 transitions in Surabaya during 2011–2013 in order to comprehend dengue dynamics during 2008–2013 in more detail. From January 2011 through December 2011, only GIV strains were isolated, indicating that a genotype shift again took place from GI to GIV. In January 2012, GI and GIV strains started co-circulating, which continued until June 2013. To further investigate this phenomenon, analysis was performed at a clade level. GI and GIV strains isolated in Surabaya formed four and three distinct clades, respectively. Concomitant with co-circulation, new clade strains appeared in both genotypes. In contrast, some previously circulating clades were not isolated during co-circulation, indicating clade shifts. Among our Surabaya isolates, nucleotide and amino acid differences in the E region were, respectively, 1.0–2.3% and 0.2–1.0% for GI isolates and 2.0–6.3% and 0.0–1.8% for GIV isolates. Several characteristic amino acid substitutions in the envelope ectodomain were observed in some clades. After July 2013, DENV-1 strains were not isolated and were replaced with DENV-2. This study showed that continuous shifts of more than one genotype resulted in their co-circulation and subsequent disappearance and suggested the relevance of clade replacement to genotype co-circulation and disappearance in Surabaya.

Abbreviations: DENV, dengue virus; DF, dengue fever; DHF, dengue hemorrhagic fever; E, envelope; G, genotype; ADE, antibody-dependent enhancement; MAb, monoclonal antibody; NJ, neighbor-joining; MCMC, Markov Chain Monte Carlo; BEAST, Bayesian Evolutionary Analysis by Sampling Trees; TMRCA, the most recent common ancestor; HPD, highest posterior density; C, clade.

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1. Introduction

Infection with any of four types of dengue virus (generally designated serotypes; DENV-1, DENV-2, DENV-3 and DENV-4) causes globally important arthropod-borne diseases, dengue fever (DF) and dengue hemorrhagic fever (DHF) (Halstead, 2007); or dengue and severe dengue recently classified by World Health Organization (WHO, 2012). Since its primary isolation in Japan in 1943 (Kimura and Hotta, 1944; Hotta, 1952), more than 10,000 DENV strains had been registered in GenBank as of 3rd March 2014. Phylogenetic analysis of nucleotide sequences in the envelope (E) coding region demonstrated that each serotype is further divided into several genotypes (Weaver and Vasilakis, 2009). For DENV-1, there are five genotypes, genotypes I–V (GI–GV), distributed in Southeast Asia, China and the Middle East for GI; Thailand (limited to 1950s–1960s) and Indonesia (limited to 2012) for GII; Malaysia for GIII (sylvatic strain); Southeast Asia, China, the Western Pacific islands, Australia, the Indian Ocean and Central America for GIV; and the Americas, West Africa and Asia for GV (Chen and Vasilakis, 2011; Villabona-Arenas and Zanotto, 2013; Fahri et al., 2013). Recently, three genotypes, GI, GIV, and GV, have accounted for most DENV-1 infections in humans.

DENVs are maintained in a natural transmission cycle between amplifier humans and vector mosquitoes in urban areas (Weaver, 2005). Anthropophilic mosquitoes, *Aedes aegypti* and *Aedes albopictus*, are the principal and secondary vectors, respectively. Many factors are involved in transmission efficiency and thus viral dynamics in a given area (Chen and Vasilakis, 2011; Andraud et al., 2012). Population size and the density of human and mosquito hosts are principally important for contact frequency between the hosts. In addition, vector competence is a major determinant of virus transmission from mosquitoes to humans, whereas viremia levels are the most critical factor for transmission from humans to mosquitoes. The viremia level depends on levels of infection-neutralizing or -enhancing antibodies in the circulation of the human host. Specifically, neutralizing antibody suppresses viremia, while enhancing antibody (serotype cross-reactive, non- or sub-neutralizing antibody) is thought to increase viremia levels. The enhancing antibody is the cause of antibody-dependent enhancement (ADE) of infection, a hypothetical mechanism behind increased disease severity from DF to DHF (Halstead and O'Rourke, 1977). Additionally, the viremia level correlates with disease severity (Vaughn et al., 2000). It is important that neutralizing antibody may show enhancing activity at sub-neutralizing doses (Pierson et al., 2007; Rothman, 2011).

E protein is the major surface protein of DENV virions and thus the main target of neutralizing and enhancing antibodies (Heinz and Stiasny, 2012). The E protein structure consists of three domains, I, II and III (Modis et al., 2003). Domain III is believed to be responsible for attachment to host cells (Crill and Roehrig, 2001), and studies using mouse monoclonal antibodies (MAbs) indicated that most neutralizing antibodies target domain III (Shrestha et al., 2010; Sukupolvi-Petty et al., 2010; Brien et al., 2010; Sukupolvi-Petty et al., 2013). However, human antibodies binding to domain III have been shown not to play a significant role in neutralization (Wahala and Silva, 2011; Williams et al., 2012). From the aspect of serotype specificity/cross-reactivity, serotype-specific neutralizing epitopes are mainly contained in domain III, whereas other domains are relevant to cross reactive epitopes (Lai et al., 2008). On the other hand, recent studies using dengue-immune human sera reported that domains I and II also contain serotype-specific, strong neutralizing epitopes against DENV, especially in the hinge region between these domains (de Alwis et al., 2012; Messer et al., 2014).

In Indonesia, more than 100,000 cases of DF/DHF have been reported annually (Setiati et al., 2006). Currently, all four serotypes and their genotypes circulate in Indonesia (Fahri et al., 2013; Suwandono et al., 2006). In addition, Indonesia is proposed as a source of some genotypes (e.g. DENV-1 GIV, DENV-3 GI, DENV-4 GII) (Villabona-Arenas and Zanotto, 2011, 2013; Araújo et al., 2009). Previously, we reported the displacement of the circulating dengue serotype from DENV-2 to DENV-1 with a subsequent genotype shift from GIV to GI during 2008–2010 in Surabaya, the second biggest city of Indonesia (Yamanaka et al., 2011).

In the present study, we investigated further DENV transition in Surabaya during 2011–2013. Analysis was performed along with the data of 2008–2010 (Yamanaka et al., 2011) so as to comprehend the DENV dynamics in more detail during 2008–2013. In January 2011, a genotype shit of DENV-1 again took place, from GI to GIV. Interestingly, in January 2012, GI and GIV strains started co-circulating, which continued until June 2013. In clade-level analysis, co-circulation coincided with the appearance and disappearance of some clade strains in both genotypes. After July 2013, DENV-1 was not isolated and was replaced with DENV-2. The clade shift may be relevant to the co-circulation of GI and GIV and subsequent disappearance of DENV-1 in Surabaya.

2. Materials and methods

2.1. Patient samples

Blood samples were obtained in 2011–2013 from patients clinically diagnosed as DF or DHF at Dr. Soetomo Hospital and Soerya Maternal and Child Health Hospital, major medical facilities covering Surabaya, East Java, Indonesia. Collected blood was subjected to virus isolation. This study was approved by the Ethics Committees of Airlangga University (Ethics Committee Approval Number: 24-934/UN3.14/PPd/2013) as well as Kobe University Graduate School of Medicine (Ethics Committee Approval Number: 784). Blood was collected after receiving signed consent from the patients or their parents.

2.2. Virus isolation

Serum specimens diluted 1:10 with culture medium were inoculated into a 96-well plate seeded with Vero cells (Yamanaka et al., 2011). The cultures were incubated at 37 °C in 5% CO_2–95% air for seven days. After three blind passages, cells were subjected to immunostaining with flavivirus group cross-reactive MAb (D1-4G2; American Type Culture Collection, Manassas, VA) to examine the presence of viral antigens as described previously (Konishi et al., 2010).

2.3. RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from immunostaining-positive Vero cells using TRIzol Reagent (Invitrogen, Carlsbad, CA), and then RT-PCR was carried out in order to determine the virus serotype using a primer set described by Lanciotti et al. (1992). RT-PCR-positive samples were then subjected to another RT-PCR for sequence analysis using gene-specific sense primer designed in the pre-membrane protein coding region [5′-GGGAGAGTTATGTG AGG-3′; corresponding to nucleotides 559–575 of the Mochizuki strain (GenBank accession number AB074760)] and antisense primer designed in the non-structural protein 1 coding region (5′-CCCAGCTTTTCCACGAG-3′: nucleotides 2774–2758). RT-PCR products (2216 nt) were purified using illustra ExoProStar (GE Healthcare, Little Chalfont, UK) and then directly sequenced using the Big Dye v.1.1 terminator (Applied Biosystems, Foster City, CA) and ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

2.4. Phylogenetic analysis

Sequence similarity analysis of the DENV E gene sequence (1485 nt) was performed using Genetyx ver. 10 (Genetyx, Tokyo, Japan). A phylogenetic tree derived from the neighbor-joining (NJ) method with a Kimura 2-parameter model was constructed using MEGA5.2 software (Tamura et al., 2011).

The Bayesian Markov Chain Monte Carlo (MCMC) method was used, and is available in the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software package v1.5.3 (Drummond and Rambaut, 2007). This analysis used a strict molecular clock, a General Time Reversible + Γ4 model of nucleotide substitution for each codon position and a Bayesian skyline coalescent model (five coalescent-interval groups): details were described previously (Yamanaka et al., 2011). All chains were run for a sufficient length of time, with 100 million generations and multiple time points to ensure convergence, with 10% removed as burn-in. This analysis allowed us to estimate the time when the most recent common ancestor (TMRCA) diverged. The degree of uncertainty in each parameter estimate was provided by the 95% highest posterior density (HPD) values, while posterior probability values provided an assessment of the degree of support for each node on the tree.

3. Results

3.1. Serotype composition of isolated viruses

Overall, 891, 1009 and 1143 serum samples were collected in Surabaya in 2011, 2012 and 2013, respectively, of which, 246, 75 and 69 strains of DENVs were isolated, again respectively. The monthly number of isolated strains in each DENV serotype during June 2008 to December 2013 is shown in Fig. 1. Before October 2008, DENV-2 dominated in Surabaya, as shown in previous reports (Yamanaka et al., 2011; Aryati, 2006). DENV-1 predominance started from November 2008 and continued until June 2013. After November 2008, sporadic circulation of DENV-2 was observed during January to February 2009, May to September 2011 and May 2012 to January 2013. Circulation of DENV-3 was limited to January to February 2013 (Kotaki et al., 2014), while DENV-4 was isolated only during January to February 2009 and in October 2011. After July 2013, only DENV-2 was isolated, suggesting the disappearance of DENV-1 and a serotype shift. The number of isolates and serotype composition in each year is shown in Supplementary Table S1.

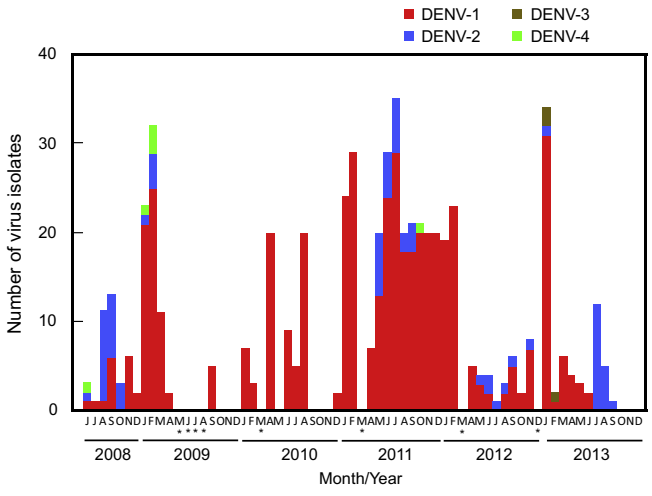

Fig. 1. The monthly number of dengue virus isolates in 2008–2013. The number of isolates is indicated monthly for each dengue virus (DENV) serotype between June 2008 and December 2013. Asterisk mark (*) indicates the month in which sample collection failed for a practical reason. The number of isolates is shown in red for DENV-1, blue for DENV-2, brown for DENV-3 and green for DENV-4. The monthly number of dengue virus isolates in 2008–2010 has been previously reported (Yamanaka et al., 2011).

3.2. Phylogenetic analysis

The complete E gene nucleotide sequences of 62 randomly selected DENV-1 strains were determined: the 62 consisted of 40 isolated in 2011, 11 in 2012 and 11 in 2013. Of the 62 isolates, one representative was used for identical sequences. Finally, 23 strains were used for phylogenetic analyses (6 strains in 2011, 9 in 2012 and 8 in 2013). The number of identical sequences and their GenBank accession numbers are shown in Supplementary Table S2. First, we constructed a preliminary NJ tree using Surabaya isolates along with 2774 DENV-1 strains registered in the GenBank database before December 31st, 2013, which include our previously reported Surabaya isolates before 2010: 39 strains consisting of 25 strains in 2009 and 14 in 2010 (GenBank accession number; AB550408–AB550429, AB597966–AB597980, and AB624553–AB623554; the NJ tree is available upon request). To simplify the tree, most strains were then deleted so as not to significantly affect the topology and to preferentially show the strains which were included in Surabaya clade or the clade next to Surabaya clade. Finally, the phylogenetic tree of these strains was re-constructed using the MCMC method, which was similar to that created by the NJ method. In this tree, a clade was defined as the minimum clade which included Surabaya isolates with statistical support (posterior probability value $\geqslant 0.9$). The Surabaya isolates that were included in the same clade and isolated in the same year were represented by a single strain to show 14 Surabaya strains in the phylogeny (Fig. 2); 1 strain in 2009, 2 in 2010, 1 in 2011, 5 in 2012 and 5 in 2013.

The 15 Surabaya isolates were grouped into two genotypes (GI and GIV), according to the generally accepted classification system (Goncalvez et al., 2002). The Surabaya GI strains were further divided into 4 clades (GI-C1 to GI-C4), clustering with Thai or Singaporean strains: Malaysian, Vietnam, Chinese and previously reported Indonesian strains were closely related to the present Surabaya isolates and shared TMRCA approximately 15 years ago (95% HPD of 13–18 years). On the other hand, GIV strains isolated in Surabaya were further divided into 3 clades (GIV-C1 to GIV-C3), clustering with Chinese or previously reported Indonesian strains: Vietnam strains were closely related to our isolates. They shared TMRCA approximately 56 years ago (95% HPD of 50–63 years).

The transition of genotype and clade in DENV-1 during 2011–2013 along with the results for 2008–2010 is shown in Table 1. DENV-1 GIV predominantly circulated during November 2008–September 2009, followed by DENV-1 GI from January 2010 to December 2010 in Surabaya (Yamanaka et al., 2011). In 2011, only GIV strain was isolated from January to December, indicating a genotype shift from GI to GIV. In addition, both GI and GIV strains were isolated from January 2012 through June 2013, indicating the co-circulation of genotype I and IV strains. When GI and GIV started co-circulating in January 2012, new clade strains of both GI (GI-C2) and GIV (GIV-C3) appeared that had not been isolated before December 2011, suggesting that the emergence of new clades might be relevant to co-circulation in Surabaya. In addition, another new GI clade (GI-C4) and GIV clade (GIV-C2) appeared after April 2012. On the other hand, strains of one of two previously circulating GI clades (GI-C1) were not isolated after January 2012. Similarly, strains of the only previously circulating GIV clade (GIV-C1) were not isolated after April 2012.

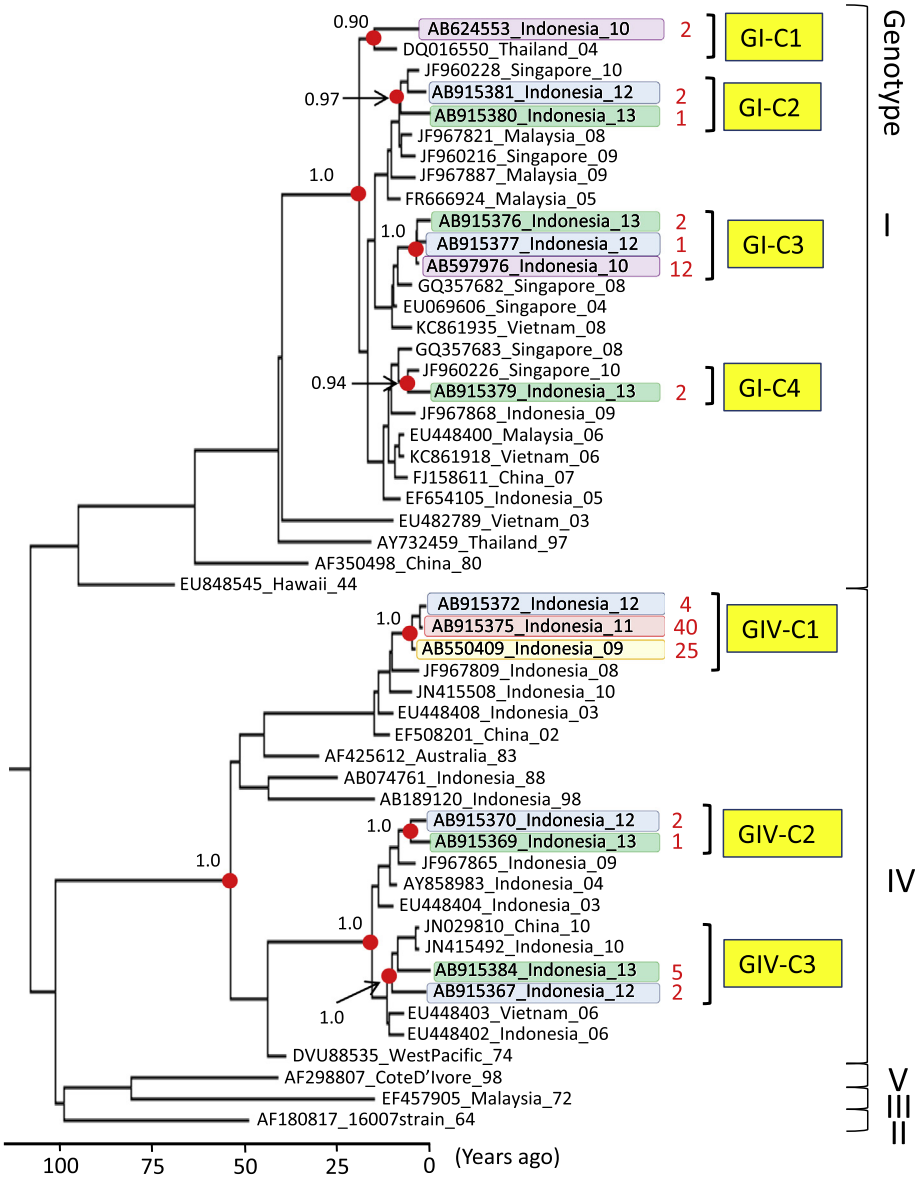

Fig. 2. Maximum clade credibility tree of the DENV-1 E gene sequence containing Surabaya isolates in 2009–2013. The strain codes are presented as the GenBank accession number, country, and year of isolate in order. The genotype and clade code, which includes Surabaya isolates, are shown in a box highlighted in yellow. The Surabaya isolates are enclosed individually in orange (for 2009), purple (for 2010), red (for 2011), blue (for 2012) and green (for 2013) squares. More than one Surabaya isolate, included in one clade and isolated in the same year, is represented by a single isolate, and the number of total isolates is shown in red next to the strain code. Horizontal branches are drawn to the scale of the estimated year of divergence with tip times reflecting the sampling date (years ago). The tree is automatically rooted under the assumption of a molecular clock. Red filled circle indicates statistical support at some key nodes; posterior probability value $\geqslant 0.9$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Thus, clade replacement in GI (from GI-C1 to GI-C2 and GI-C4) and GIV (from GIV-C1 to GIV-C2 and GIV-C3) occurred at some time point between December 2010 and April 2012. GI-C3, which predominantly circulated throughout 2010, re-emerged on April 2012 with 15-month disappearance, probably due to silent transmission or re-introduction.

3.3. Nucleotide and amino acid differences in the E gene

The nucleotide difference in the E coding region of 101 DENV-1 strains isolated in Surabaya from 2009 to 2013 was 0.1–9.8% with amino acid differences of 0.0–4.0%. Differences between GI and GIV strains were 8.1–9.8% for nucleotides and 2.0–4.0% for amino acids. On the other hand, differences among four GI clades were 1.0–2.3% for nucleotides and 0.2–1.0% for amino acids, and those among three GIV clades were 2.0–6.3% for nucleotides and 0.0–1.8% for amino acids. Differences among GIV clades were greater than those among GI clades, consistent with the larger distance in phylogeny in GIV than GI clades (Fig. 2).

Table 2 shows amino acid substitutions in the E ectodomain found to be characteristics of individual clades of each genotype isolated in Surabaya. The criteria of the amino acids shown in Table 2 are that: (i) all strains composing the clade have the difference; and (ii) the proportion of the difference is $\leqslant$20% of all 52 strains shown in Fig. 2. A greater number of amino acids that met these criteria in GIV (five) than GI (two) strains were consistent with a larger distance in phylogeny in GIV than GI clades (Fig. 2) and greater numbers of nucleotide and amino acid differences among GIV than GI clades. These six amino acid substitutions were distributed in three domains of E.

Table 1
Transition of the DENV-1 genotype and clade from November 2008 through June 2013.

Genotype[a]	Clade[a]	Number of DENV-1 isolates (% of total in each time period)				
		November 2008–September 2009	January 2010–December 2010	January 2011–December 2011	January 2012–February 2012	April 2012–June 2013
GI	Clade 1	0 (0%)	**2 (12%)**[b]	0 (0%)	0 (0%)	0 (0%)
	Clade 2	0 (0%)	0 (0%)	0 (0%)	**2 (28.6%)**	**1 (6.7%)**
	Clade 3	0 (0%)	**15 (88%)**	0 (0%)	0 (0%)	**3 (20%)**
	Clade 4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**2 (13.3%)**
Subtotal		0 (0%)	17 (100%)	0 (0%)	2 (28.6%)	6 (40%)
GIV	Clade 1	**30 (100%)**	0 (0%)	**40 (100%)**	**4 (57.1%)**	0 (0%)
	Clade 2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**3 (20%)**
	Clade 3	0 (0%)	0 (0%)	0 (0%)	**1 (14.3%)**	**6 (40%)**
Subtotal		30 (100%)	0(0%)	40 (100%)	5 (71.4%)	9 (60%)
Total		30	17	40	7	15

[a] The genotype and clade were determined by phylogenetic analysis of E gene sequences.
[b] Positive number of isolates is shown in bold.

Table 2
Amino acid substitutions in the E ectodomain characteristic of individual clades of each genotype.

Amino acid substitutions among GI clades						Amino acid substitutions among GIV clades				
Position	Domain	Clade 1	Clade 2	Clade 3	Clade 4	Position	Domain	Clade 1	Clade 2	Clade 3
E227	II	S	S	S	**T**[a]	E37	I	**N**	D	D
E365	III	V	V	V	**I**	E55	II	**I**	V	V
						E163	I	**I**	T	T
						E346	III	**I**	T	T

[a] Characteristic amino acid substitutions conserved in all strains composing the clade but only observed in ⩽ 20% of all the 52 strains used for phylogenetic analysis (Fig. 2) are shown in bold.

4. Discussion

Two genotypes of DENV-1 have been isolated in Surabaya since 2008; GI and GIV. Our Surabaya GIV strains clustered or were closely related with previously isolated Indonesian strains. It is highly probable that our Surabaya GIV strains were introduced from other regions of Indonesia and/or silently transmitted for a long time in Surabaya. On the other hand, our GI strains seem to have been introduced into Surabaya from other Southeast Asian countries, including Thailand, Singapore, Vietnam and Malaysia within the last 15 years.

Several shifts of the predominantly circulating DENV serotype/genotype/clade occurred in Surabaya during 2008–2013. Many potential factors are involved in this phenomenon, including antibodies against natural infection with DENV in human populations (Chen and Vasilakis, 2011). Antibodies may control viremia levels and accordingly the efficiency of viral transmission to vector mosquitoes. Since people are usually protected from subsequent infection with the same serotype (homoserotypic infection) (Sabin, 1952; Wikramaratna et al., 2010) and since viremia levels are generally believed to correlate with disease severity (Vaughn et al., 2000), viremia is considered to remain minimal upon homoserotypic infections. Neutralizing antibody seems to play a major role in reducing viremia levels. Thus, the first predominantly circulating serotype becomes difficult to efficiently transmit in the human population. On the other hand, neutralizing antibody induced by infection with one serotype is not able to protect against infection with other serotypes (heteroserotypic infection), although it is protective for a short period after infection (Montoya et al., 2013). Furthermore, non- or sub-neutralizing, cross-reactive antibody may cause ADE, leading to increased viremia levels and facilitated virus transmission (Ferguson et al., 1999). This is the most probable explanation for the serotype shift where the first predominantly circulating serotype is replaced by a second serotype.

Currently, all four DENV serotypes are co-circulating in most dengue endemic areas. Serial introductions of a new serotype in a particular area probably caused shifts of the predominantly circulating serotype in the early phase and eventually established co-circulation (Gubler, 1988; Olkowski et al., 2013). In this process, ADE provides advantages to human-to-mosquito transmission of co-circulating virus serotypes (Chen and Vasilakis, 2011; Cummings et al., 2005). Maintenance in nature of one particular serotype against which most people have a neutralizing antibody can be explained by the continuous availability of susceptible populations (Rabaa et al., 2013) and the mechanism of transovarial transmission in vector mosquitoes (Chen and Vasilakis, 2011; Mulyatno et al., 2012).

The present study showed DENV-1 genotype shifts between GI and GIV during 2009–2011, followed by their co-circulation. Despite several reports on the co-circulation of different genotypes in a single serotype (Jiang et al., 2012; Khan et al., 2008), no studies have shown continuous genotype shifts and subsequent co-circulation, to the best of our knowledge. As mentioned above, antibodies play a significant role in serotype shifts and co-circulation. The E coding region is crucial for the induction of antibodies, since it is a major target of neutralizing and enhancing antibodies (Heinz and Stiasny, 2012). The amino acid difference of the E region among DENV-1 to -4 is approximately 30% and this is related to the serotype-specificity/cross-reactivity of antibodies and serotype shifts/co-circulation. In contrast, the amino acid difference among genotypes in one serotype is about 3–4%. In fact, the present DENV-1 Surabaya isolates (GI and GIV) had amino acid differences of 2.0–4.0%. Since viremia seems to be suppressed more efficiently upon subsequent heterogenotypic than heteroserotypic infections, it remains unclear if the 3–4% amino acid difference can cause the shift and co-circulation of the genotype in the same manner as that of the serotype. However, previous reports have clarified genotype differences in neutralization using monoclonal antibodies (Shrestha et al., 2010;

Sukupolvi-Petty et al., 2010; Brien et al., 2010; Sukupolvi-Petty et al., 2013).

When GI and GIV started co-circulating in January 2012, new clade strains of both GI (GI-C2) and GIV (GIV-C3) appeared. Later, clade replacement in GI (from GI-C1 to GI-C2 and GI-C4) and GIV (from GIV-C1 to GIV-C2 and GIV-C3) occurred. Since amino acid differences in the E region among these clades were only 0.2–1.0% in GI and 0.0–1.8% in GIV, it seems unlikely that antibodies worked as a selection pressure. However, several characteristic amino acid substitutions were found in GI-C4 (S227T, V365I) and GIV-C1 (D37N, V55I, T163I, T346I) on the E protein, including domains I and II, which may contain epitopes relevant to neutralizing antibody induction in humans. In particular, E55 and E163 located within the hinge region between domains I and II may be important, since these are suggested as a part of serotype specific neutralizing epitope of DENV-3 and DENV-1, respectively (Fibriansah et al., 2014; Messer et al., 2014). In addition, E37 was suggested as a part of cross-reactive neutralizing epitope (Aaskov et al., 1989; Roehrig et al., 1990; Myat Thu et al., 2005). Furthermore, an amino acid substitution occurring at E365 of DENV-4 was associated with the serotype shift from DENV-1 to DENV-4 in the Pacific region (Li et al., 2010). Thus, the characteristic amino acid substitutions observed in Surabaya strains might be involved in the clade shift and co-circulation. Failure to isolate DENV-1 after July 2013 suggests that all diverged clade strains could no longer significantly grow in most humans potentially because of the induced neutralizing antibody. This speculation is supported by a report showing that clade replacement within a DENV-1 genotype was associated with the decline of DENV-1 prevalence (Zhang et al., 2005).

On the other hand, factors other than the antibody may also be involved in these clade shifts. Specifically, it is suggested that density and biting activity of vector mosquitoes play an important role (Myat Thu et al., 2005; Chen and Vasilakis, 2011). The present study showed the genetic shifts at one year interval in January (e.g. 2010, 2011 and 2012). January has seen the maximum rainfall based on the monthly average for 30 years (1961–1990) in Surabaya (World Meteorological Organization, 2014). Accordingly, the adult mosquito density and survival rate have been expected to be high in January (Halstead, 2008).

In conclusion, this study described an example showing continuous shifts of DENV-1 genotypes followed by their co-circulation, concomitant with clade shifts and subsequent disappearance, which occurred in Surabaya during 2008–2013. It was suggested that clade replacement was relevant to the co-circulation of GI and GIV strains and their disappearance. Further studies of detailed viral dynamics will be needed, as well as its relation to the antibody status in human populations and other environmental factors for DENV to co-circulate in nature.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.09.002.

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