

The molecular and clinical features of dengue during outbreak in Jambi, Indonesia in 2015

Sotianingsih Haryanto^{1,4†}, Rahma F. Hayati^{2†}, Benediktus Yohan², Lanceria Sijabat¹, Ifo F. Sihite¹, Sukmal Fahri³, Febrina Meutiawati², Jonathan A. N. Halim⁵, Stefanie N. Halim⁵, Amin Soebandrio², R. Tedjo Sasmono² 

¹Siloam Hospital, Jambi, Indonesia, ²Eijkman Institute for Molecular Biology, Jakarta, Indonesia, ³Health Polytechnic, Jambi Provincial Health Office, Jambi, Indonesia, ⁴Faculty of Medicine, Jambi University, Jambi, Indonesia, ⁵Faculty of Medicine, Diponegoro University, Semarang, Indonesia

Dengue is hyperendemic in Indonesia. In 2015, reported cases of dengue fever doubled those of 2014 in the Jambi municipality of Sumatra. We examined viral aetiology and its relationship with disease outcome in Jambi. Dengue-suspected patients' sera were collected and NS1 detection and IgM/IgG serology were performed. Dengue virus (DENV) serotyping was performed using real-time RT-PCR. Envelope genes were sequenced to determine the genotypes of DENV. Clinical, haematologic, and demographic data were recorded. Of 210 dengue-suspected patients, 107 were confirmed. The disease manifested as Dengue Fever (62%), Dengue Haemorrhagic Fever (36%), and Dengue Shock Syndrome (2%). The serotypes of 94 DENV were determined. All DENV serotypes were detected with DENV-1 as the predominant serotype (66%). Genotypically, the DENV-1 viruses belong to Genotype I, DENV-2 was of Cosmopolitan genotype, DENV-3 as Genotype I, and DENV-4 belonged to Genotype II. Comparison with historical data revealed serotype predominance switched from DENV-3 to DENV-1, and the replacement of Genotype IV of DENV-1 with Genotype I. In summary, DENV-1 predominated during the 2015 dengue outbreak in Jambi. The full spectrum of dengue disease occurred and was characterized by a switch in predominant serotypes.

Keywords: Dengue, Indonesia, Jambi, Genotype, Serotype

Introduction

Dengue disease is an acute febrile illness caused by dengue virus (DENV), a member of Flaviviridae family. Four DENV serotypes (DENV-1, -2, -3, and -4) circulate in tropical and subtropical regions and are transmitted by *Aedes* mosquito vectors. The clinical manifestations of dengue range from asymptomatic or mild flu-like syndrome known as classic Dengue Fever (DF) to more severe form known as Dengue Haemorrhagic Fever (DHF) and the often fatal Dengue Shock Syndrome (DSS). The DENV genome consists of ~10.7 kb single-stranded positive-sense RNA genome which encodes three structural (C, prM/M, and E) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins.¹ Each serotype of DENV further harbors extensive genetic diversity in the form of phylogenetically distinct clusters termed genotypes. These genotypes differ in both their geographical distributions and fitness and virulence.²

Dengue is hyperendemic in Indonesia with frequent epidemic cycles. Currently, all 34 provinces of Indonesia

have reported dengue cases.³ Major dengue outbreaks have been reported such as those in 1998 in Palembang, South Sumatra⁴ and 2004 in Jakarta.⁵ The Indonesia Ministry of Health reported that in 2014, 100,347 cases were reported with an incidence rate (IR) of 39.80/100,000 and 907 deaths (Case Fatality Rate – CFR 0.90%). Many provinces in Indonesia recently experienced increases in dengue cases, including Jambi province on the island of Sumatra in western Indonesia. In 2013, 638 cases were reported in Jambi (IR 19.64/100,000) and increased to 1308 cases in 2014 (IR 38.33/100,000).⁶ Citing Jambi Health Authority data, local newspapers also reported the surge of dengue cases in Jambi during December 2014. Despite the increasing case numbers of dengue in the province, no data on the DENV serotypes associated with the outbreak has emerged. The current study undertaken represents the first virological characterization of DENV from this area of Sumatra.

Dengue disease virological and epidemiological investigation of this surge in cases may yield important clinical and public health trends, similar to investigations elsewhere.⁷ This study was thus aimed to: (i) determine disease aetiology and serotypes of circulating DENV; (ii) perform phylogenetic and amino acid analyses of DENV in Jambi; and (iii) understand the clinical manifestations of

Correspondence to: R. Tedjo Sasmono, Eijkman Institute for Molecular Biology, Jl. Diponegoro 69, Jakarta 10430, Indonesia, Email: sasmono@eijkman.go.id

[†]These authors contributed equally to this work.

This article was originally published with errors. This version has been corrected. Please see Corrigendum (<http://dx.doi.org/10.1080/20477724.2016.1207306>).

the disease in Jambi and examine its possible relationships with DENV genetic characteristics.

Materials and methods

Study site, patient recruitment, and sample collection

This cross-sectional study was conducted in Jambi, a city located in the southern part of Sumatra Island and populated by about 531,857 people in 2010 (Indonesia Central Statistical Bureau) with total area of about 205 km². This study was approved by the Eijkman Institute Research Ethic Commission. Dengue-suspected patients were recruited from Siloam Hospital, Jambi after obtaining informed consent during the period of December 2014–March 2015. Patients with fever >38 °C accompanied by at least one of clinical signs of dengue such as malaise, arthralgia, rash, retro-orbital pain, signs of DHF or DSS were recruited. A 3–5 mL of blood was obtained by venepuncture from 210 patients during the acute phase on first five days of fever. Demographic data of patient age, gender, home address, and relevant clinical history were recorded.

Dengue diagnosis and serotyping

Dengue diagnosis was performed by detection of NS1 antigen using NS1 rapid test kit (Standard Diagnostics®) and serologically using Panbio Dengue Duo IgM and IgG ELISA™ (Alere®). Primary vs. secondary dengue infection was determined using the ELISA results according to manufacturer protocols. Briefly, the positive IgM (>11 Panbio Units) and negative IgG (<22 Panbio Units) indicated primary infection while positive IgG (>22 Panbio Units), which may be accompanied by elevated IgM levels, indicated secondary infection. DENV detection and serotyping was performed using Simplexa™ Dengue real-time RT-PCR (Focus Diagnostics).⁸ Virus RNA was extracted from 200 µl serum using MagNA Pure LC RNA Isolation Kit I (Roche) in MagNA Pure LC 2.0 extraction system (Roche). The RNA products were subjected to Simplexa™ Dengue RT-PCR assay. Strict controls were applied on RNA extraction and RT-PCR procedures to prevent cross-contamination. All dengue positive cases were categorized clinically either as DF, DHF, DSS, or expanded dengue syndrome according to criteria described by the WHO-SEARO.⁹

DENV Envelope gene sequencing and phylogenetic analyses

Genotyping was performed on E gene. DENV RNA extracted directly from patients' sera was reverse-transcribed into cDNA using Superscript III RT (Invitrogen-Life Technologies) and then PCR-amplified using *Pfu* Turbo Polymerase (Stratagene-Agilent Technologies). PCR products were purified from 0.8% agarose gel using QIAquick gel extraction kit (Qiagen) and used in cycle sequencing reactions performed using six overlapping

primers for each serotype from both strands and BigDye Dideoxy Terminator sequencing kits v3.1 (Applied Biosystems-Life Technologies), as described previously.¹⁰ Purified DNA was subjected to capillary sequencing performed on 3130xl Genetic Analyzer (Applied Biosystems). The complete E gene sequences of 68 isolates have been deposited in GenBank with accession numbers KU529691 – KU529758.

DENV genotype analysis

Genotype classification was based on classifications by Goncalvez et al.¹¹, Twiddy et al.¹², Lanciotti et al.¹³, Lanciotti et al.¹⁴ for DENV-1, -2, -3 and -4, respectively. Multiple sequence alignment of E gene was performed using MUSCLE in MEGA 5.0 software. Data-set for each serotype was prepared using BEAUTi v.1.8.2 and followed by phylogenetic reconstruction and evolutionary rate analysis using Bayesian Markov chain Monte Carlo (MCMC) method as implemented in BEAST v.1.8.2 using GTR + Γ4 model with invariant sites, relaxed uncorrelated lognormal molecular clock and Bayesian skyline prior, with 100 million generations and sampled for every 1000th iteration. MCMC trace was analyzed using Tracer v.1.5.0 to ensure adequate effective sampling size (ESS) for all parameters. Maximum clade credibility (MCC) tree was created using TreeAnnotator v.1.8.2 and visualized in FigTree v.1.4.0.

Results

Dengue incidence, patient demography, and serotype distribution

Serum samples from 210 dengue-suspected patients were collected during the rainy season between December 2014 and March 2015. Most cases occurred during January 2015 (Fig. 1(A)). A total of 107 patients (50.9% of those sampled) were confirmed as dengue fever by NS1 antigen detection and/or DENV RNA detection by real-time RT-PCR. Among these patients, 55 (51%) were male and 52 (49%) were female. These cases occurred predominantly in children below 10 years of age (48%) (Fig. 1(B)). Serology indicated the majority (80%) of patients were experiencing a primary infection (Table 1).

A total 94 of the 107 confirmed cases were positive for DENV nucleic acid detection by real-time RT-PCR. Analysis of those amplicons revealed DENV-1 as the predominant serotype (62 cases) followed by DENV-2 (23 cases), DENV-4 (5 cases), and DENV-3 (2 cases) (Fig. 1(C)). Two of the 94 cases examined had both DENV-1 and DENV-2 serotypes (Fig. 1(C)). Of the 103 dengue-negative cases, other viruses were detected, including Zika¹⁵ and Chikungunya viruses (data not shown, manuscript in preparation).

Clinical manifestations and virological analyses

Out of 94 dengue-confirmed patients, most of them (62%) were found to be DF, followed by DHF (36%) and DSS

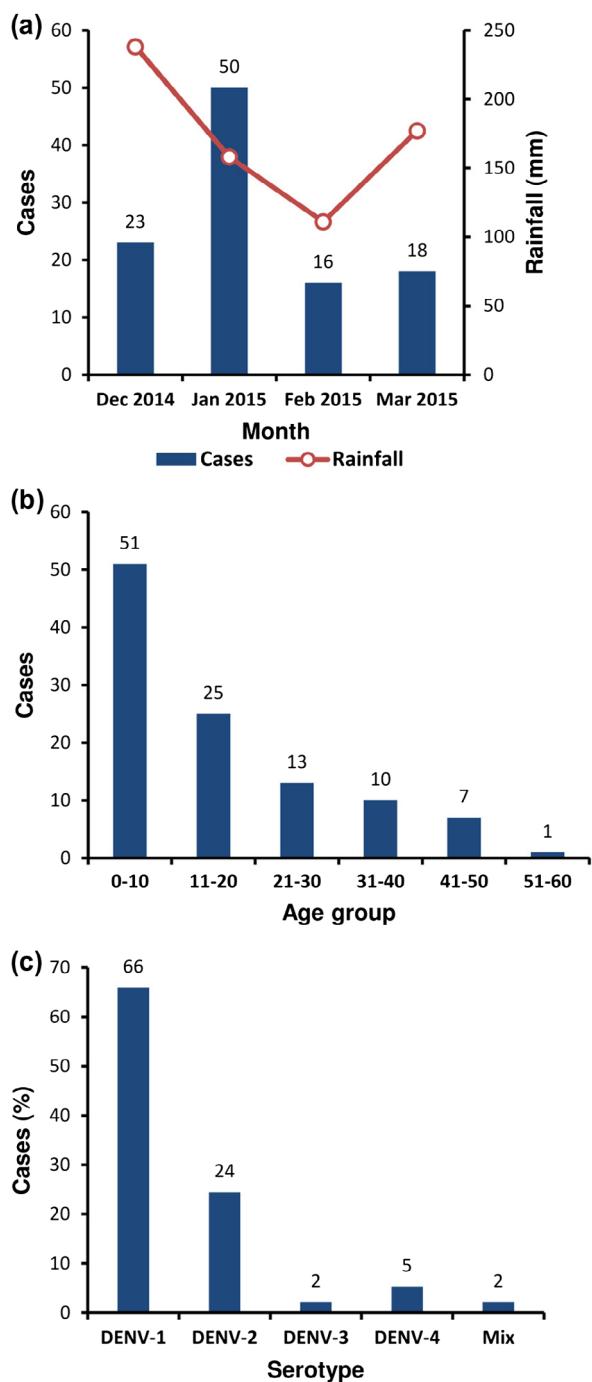


Figure 1 The characteristics of dengue in Jambi with regard to temporal distribution and rainfall data (a), patients' age distribution (b), and DENV serotype distribution (c). Rainfall data were obtained from Jambi Meteorology Agency. DENV serotypes were determined using real-time RT-PCR as described in the Methods section.

(2%) (Table 1). Expanded dengue syndrome did not appear. Of the DF patients ($n = 58$), the majority (55.2%) were children aged 1–10 years old. This age group accounted for only 32.4% of the DHF patients (11 of 34 cases). Both DSS patients were children. Thrombocytopenia (<100,000/L) occurred among most patients (70.2%), with an observed range of 4000–273,000/L. Thirty-eight (40.4%) patients experienced severe thrombocytopenia (<50,000/L). The

average of thrombocyte counts according to clinical classification is shown in Table 2.

The possible relationships of the infecting serotypes with demography, hematology, and clinical manifestation were analyzed on 94 patients with known infecting serotypes (Table 1). No significant distribution of serotypes appeared. Moreover, most of dengue-confirmed patients also showed the typical symptoms of dengue such as headache, lethargy, arthralgia, myalgia, leukopenia, rash, and drowsiness.

DENV genotypes distribution

Full-length E genes from 68 isolates among the 107 patients were successfully sequenced and genotyped. Of 62 DENV-1 isolates, 48 (77%) their E gene was successfully PCR-amplified. Phylogenetic analysis revealed that all of these 48 viruses were Genotype I (Fig. 2). Another DENV-1 genotype (Genotype IV) which was known to circulate in Indonesia did not occur among the patients examined at Jambi.

For DENV-2, 15 of 23 (65%) isolates were successfully genotyped. Phylogenetic analysis revealed that all belonged to the Cosmopolitan genotype (Fig. 3). Genotyping of one isolate of DENV-3 was for Genotype I (Fig. 4). Among five confirmed-DENV-4 isolates, four were successfully genotyped as Genotype II (Fig. 5). We found two cases of DENV-1 and DENV-2 combined infections. One of these was successfully genotyped as Genotype I of DENV-1 and Cosmopolitan of DENV-2.

E gene amino acid comparison and disease severity

Figure 6(A) illustrates a comparative analysis of amino acid (AA) sequences of 48 DENV-1 isolates from Jambi. Of 495 AA residues within the E glycoprotein, 33 (6.6%) AAs were variable. Sixteen isolates possessed unique AA sequences, 4 AA sequences patterns were shared by 2 isolates each, one AA sequence was shared by 3 isolates, and another one AA sequence was shared by 21 isolates. For DENV-2, among 15 isolates analyzed, there were 8 variable AA residues out of 495 (1.6%) (Fig. 6(B)). There were four DENV-2 isolates with unique AA sequences patterns, and two patterns were shared by four and seven isolates each. As shown in Fig. 6, no unique AA pattern was specifically related with DF, DHF, or DSS. We did not analyze the AA sequences of DENV-3 and -4 isolates because the numbers were too small.

Discussion

We conducted the first virological investigations of a dengue fever outbreak in southern Sumatra and learned patterns of serotypes/genotypes in the context of clinical manifestations. A sharp rise in case numbers at Jambi may be largely explained by the appearance of a new dominant genotype, but most symptomatic infections occurred as primary exposures in young children.

Table 1 DENV serotypes correlation with gender, clinical manifestation, and laboratory data

Parameters	N (%)	DENV-1 (n=62)	DENV-2 (n=23)	DENV-3 (n=2)	DENV-4 (n=5)	Mixed (n=2)	p value ^a
<i>Gender</i>							
Male	50 (53)	33	11	1	4	1	0.786
Female	44 (47)	29	12	1	1	1	
<i>Infection type</i>							
Primary	76 (80)	48	21	1	4	2	0.434
Secondary	18 (20)	14	2	1	1	0	
<i>NS1 antigen detection</i>							
Positive	85 (90)	54	22	2	5	2	0.650
Negative	9 (10)	8	1	0	0	0	
<i>Severity</i>							
DF	58 (62)	37	17	1	2	1	0.878
DHF	34 (36)	23	6	1	3	1	
DSS	2 (2)	2	0	0	0	0	
<i>Hematology data^b</i>							
Thrombocyte (10 ⁹ /L)	NA	81±62	81±61	48±53	57±33	116±62	0.743 ^c
Haematocrit (%)	NA	38.0±5.6	39.4±3.5	43.6±5.0	37.8±3.8	33.9±0.8	0.353 ^c
WBC count (10 ⁹ /L)	NA	4.8±5.1	3.3±1.5	4.4±2.7	3.7±0.9	2.6±0.6	0.679 ^c

^aPearson chi-squared test.^bMean ± STDEV.^cOne way ANOVA test.**Table 2 Relationship of clinical manifestation, gender, infection status, NS1 detection, and hematology data**

Parameters	N (%)	DF (N=58)	DHF (N=34)	DSS (N=2)	p value ^a
<i>Gender</i>					
Male	50 (53)	33	17	0	0.255
Female	44 (47)	25	17	2	
<i>Infection type</i>					
Primary	76 (80)	48	28	0	0.013
Secondary	18 (20)	10	6	2	
<i>NS1 antigen detection</i>					
Positive	85 (90)	53	30	2	0.794
Negative	9 (10)	5	4	0	
<i>Hematology data^b</i>					
Thrombocyte (10 ⁹ /L)	NA	103.1±63.1	44.2±29.5	17.0±14.1	0.000 (7.4×10^{-7}) ^{c,d}
Hematocrit (%)	NA	37.85±5.76	39.43±4.56	35.10±2.83	0.274 ^c
WBC count (10 ⁹ /L)	NA	4.59±5.31	3.72±1.66	7.07±1.04	0.434 ^c

^aPearson chi-squared test.^bMean ± STDEV.^cOne way ANOVA test.^dStatistical calculation using log₁₀-normalized data; Tukey HSD post hoc test result: p = 0.0000060 (DHF-DF), 0.0044627 (DSS-DF), and 0.2058640 (DSS-DHF).

Dengue cases were increased significantly in Jambi during the study, with the cases peaked in January 2015 (Fig. 1(A)). This coincides with the decreasing rainfall, which suggests that post-monsoon condition is favorable for *Aedes* mosquitoes to actively breed, as heavy rainfall may wash away the breeding sites.

Historical data are important in understanding the dynamic of the disease. As there's no historical data of dengue in Jambi, we used the data from a study in nearby city of Palembang (located about 280 km from Jambi) conducted in 1998⁴ for comparison. Compared to that report, we observe distinct clinical and virological features between two cities. While the majority of clinical manifestation in Palembang was DHF (66%); in Jambi most patients were DF (63%) (Table 1). In Palembang, the case fatality rate was 4.1%; but in our study, no death was reported. The patients' age also differs. In Jambi, majority of cases (55.2%) was children under 10 years old, while in Palembang the population aged 10–19 years accounted for the largest hospitalized cases. It is possible that the predominant of children less than

10 years of age may correlate with the higher proportion of primary infections (Table 1). The higher proportion of primary infection may also related to the relatively less-severe clinical manifestation in Jambi, as secondary infection is known as one of the risk factors for severe dengue.¹⁶ On hematology data, only thrombocytopenia that was significantly different between DF, DHF and DSS, finding that is consistent with the WHO classification.⁹

Previous studies reported the association of DENV serotype with clinical manifestation,^{17,18} in which particular serotypes have been correlated with the severity of the disease. As depicted in the Table 1, we did not observe any direct correlation of particular serotype with the disease severity. However, one limitation that may influence the results was the unequal distribution of serotypes in this study. The absence of direct correlation of serotypes with clinical manifestation was also observed in previous studies in Indonesia.^{3,19}

In Jambi, all four serotypes circulated, with DENV-1 predominant (66%) (Table 1). This is different from that in

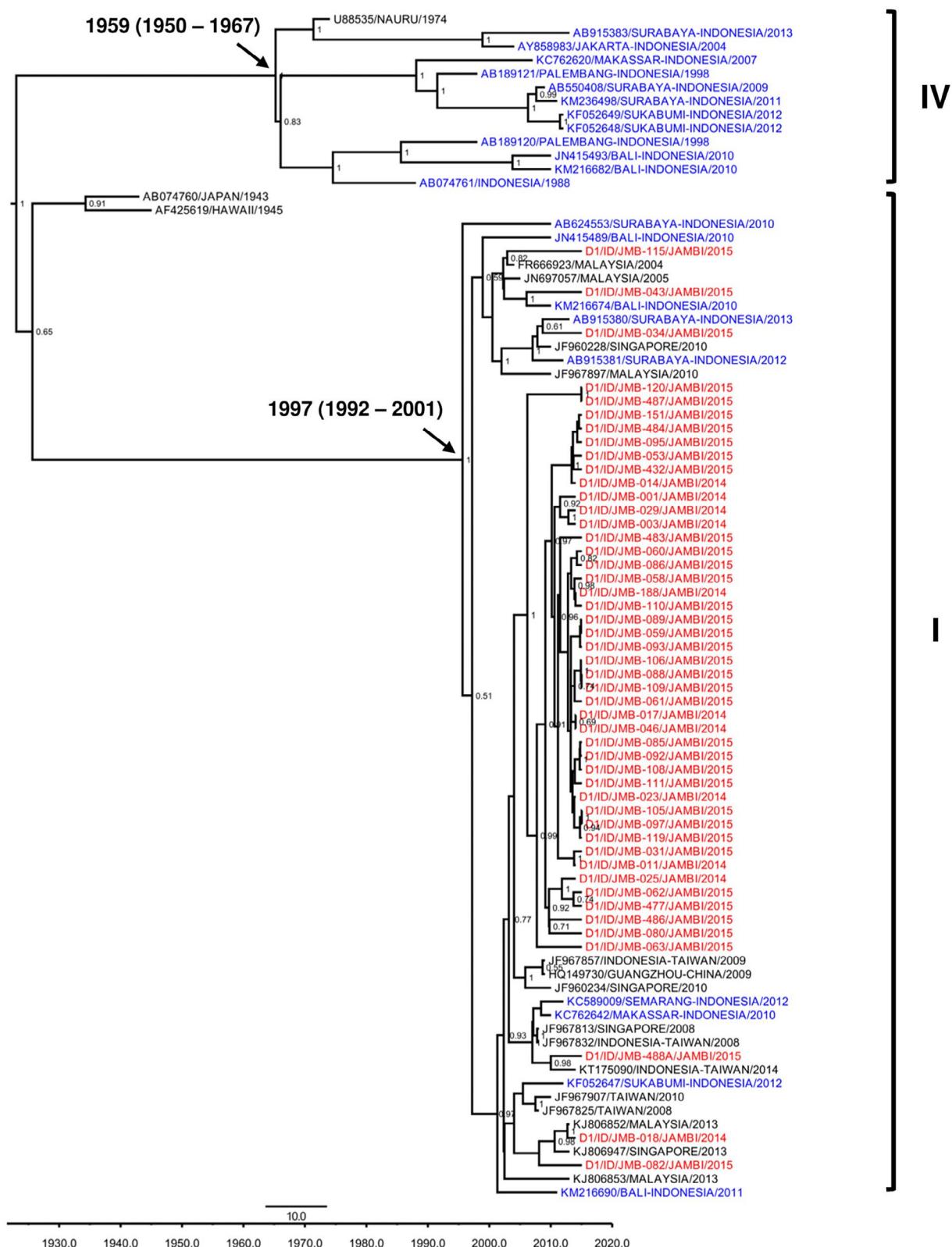


Figure 2 Maximum Clade Credibility (MCC) tree of DENV-1 Genotypes I and IV grouping generated by Bayesian inference method as implemented in BEAST using GTR evolution model and gamma parameter rates from the E gene sequences. The Jambi isolates (red font) were grouped into Genotype I based on classification by Goncalvez et al.¹¹, together with isolates from other cities in Indonesia (blue font). The posterior probabilities of the clades were indicated as numbers in the node labels.

Palembang, in which DENV-3 was predominant (43%),⁴ and thus demonstrated the dengue disease dynamic in the region. DENV serotypes have been known to show cyclical predominant pattern which may correlate with

herd immunity profile.^{20, 21} Historically, DENV-3 has been reported as the predominant serotype in Indonesia,³ therefore, the predominance DENV-1 in this and other recent studies in Indonesia^{10, 19} suggested that the

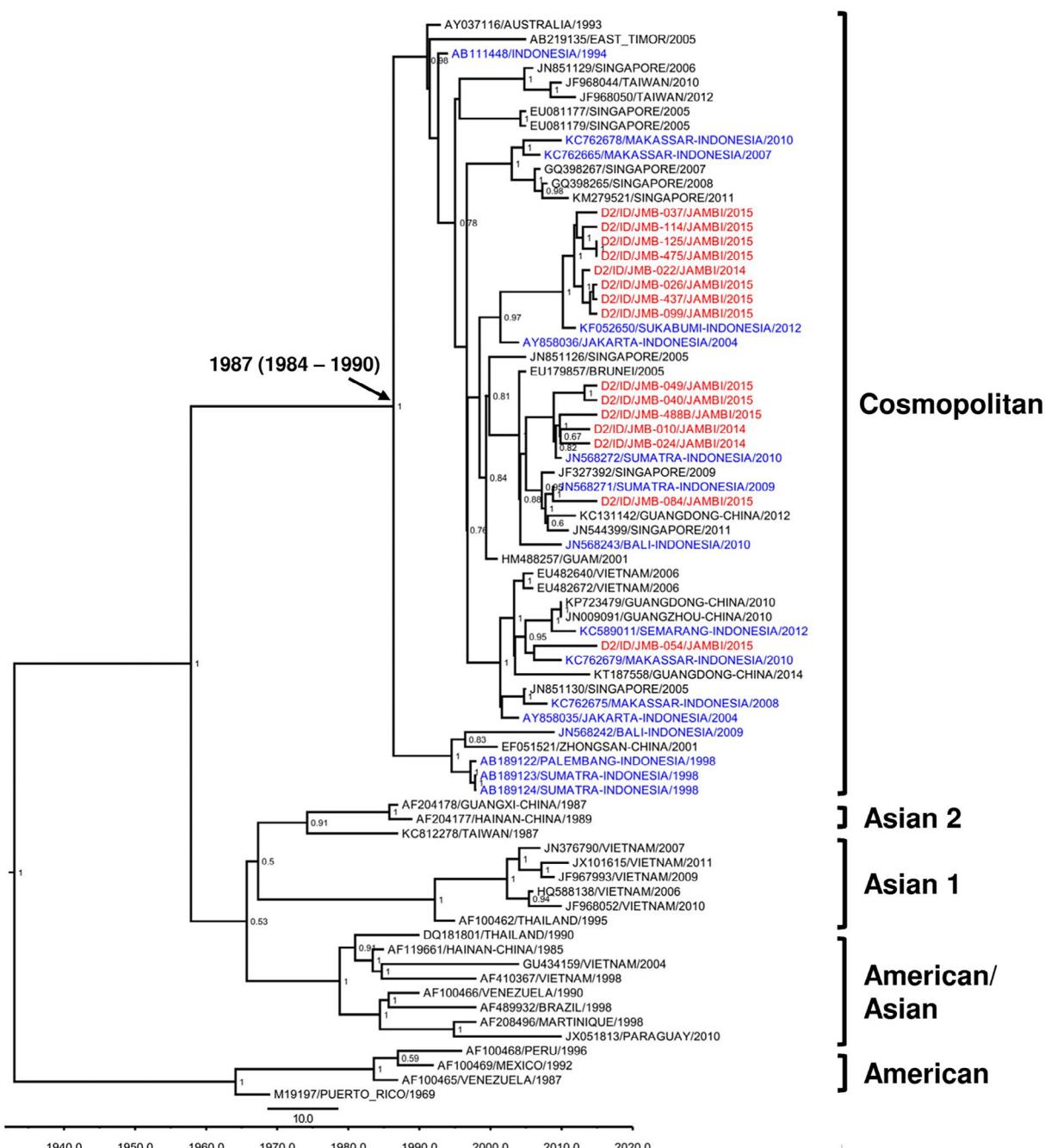


Figure 3 MCC tree of DENV-2 genotypes grouping generated by Bayesian inference method as implemented in BEAST using GTR evolution model and gamma parameter rates from the E gene sequences. The Jambi isolates (red font) were grouped into Cosmopolitan Genotype based on classification by Twiddy et al.¹², together with isolates from other cities in Indonesia (blue font). The posterior probabilities of the clades were indicated as numbers in the node labels.

cyclical serotype predominance is currently occurring in Indonesia. The study in Palembang observed that DENV-1 was principally responsible for less severe dengue illness; therefore, it is likely that the less severe dengue clinical outcome in Jambi is indeed correlated with the predominance of DENV-1.

Genetically, each of the four DENV serotypes comprised several genotypes. One aspect of DENV evolution is the presence of lineage turnover, in which a particular genotype of circulating viruses is replaced by a new genotype.^{22,23} This lineage/genotype replacement was apparently occurred in Jambi region. The DENV-1 viruses in

Jambi were Genotype I. Meanwhile, the DENV-1 viruses isolated in nearby city of Palembang in 1998 were Genotype IV (Fig. 2). The time to most common recent ancestor/TMCRA for DENV-1 genotype IV was the year 1959 (95% Highest Posterior Density/HPD = 1950–1967), while genotype I of Indonesia DENV-1 had more-recent time (year 1997, with 95% HPD = 1992–2001). Of 62 DENV-1 isolates from Jambi, none belongs to Genotype IV. This clearly indicated that DENV-1 genotype replacement occurred in Jambi and that Genotype I succeed in displacing Genotype IV. The better transmissibility of Genotype I than Genotype IV was also observed in our

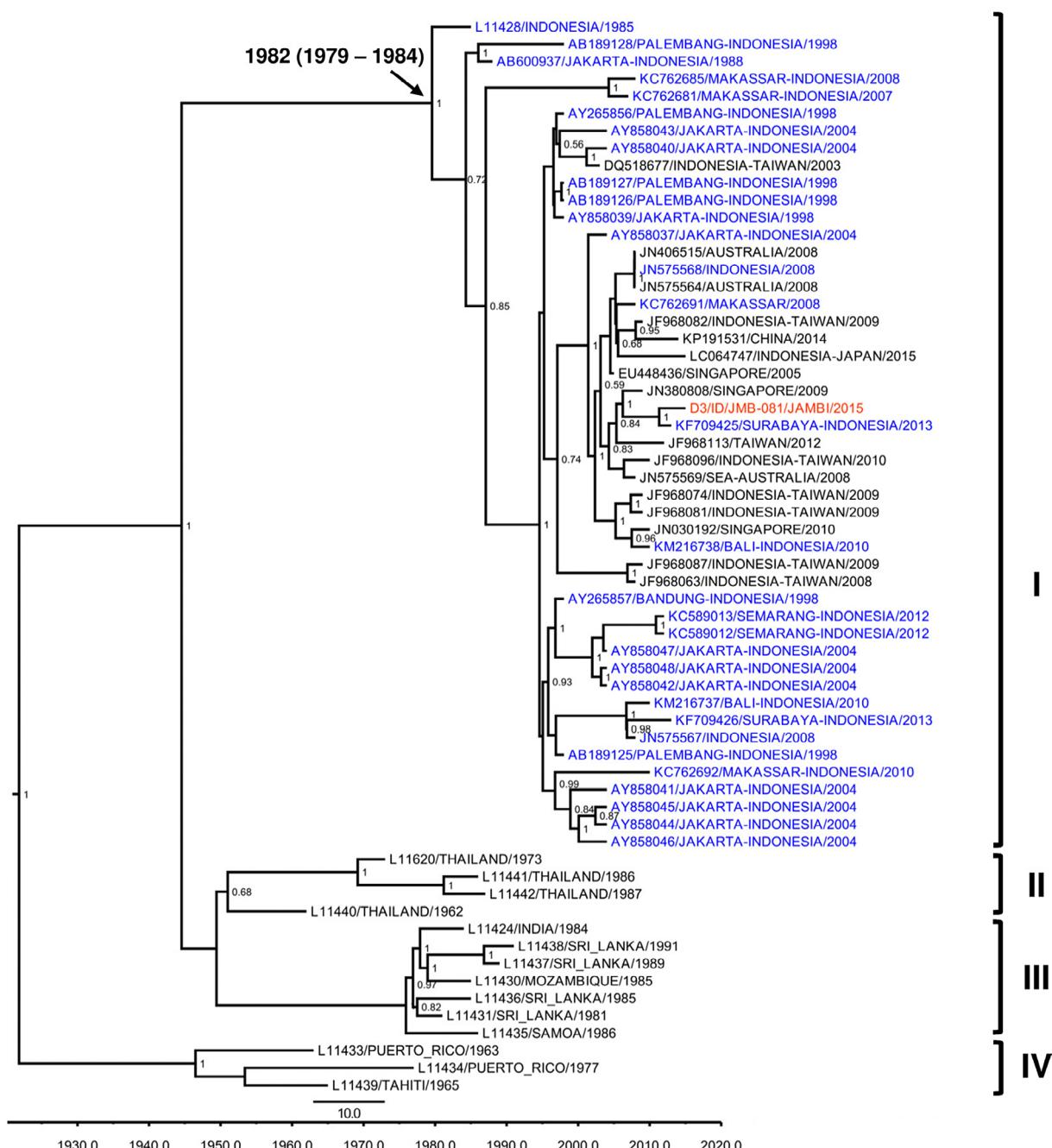


Figure 4 MCC tree of DENV-3 genotypes grouping generated by Bayesian inference method as implemented in BEAST using GTR evolution model and gamma parameter rates from the E gene sequences. The Jambi isolate (red font) was grouped into Genotype I based on classification by Lanciotti et al.¹³, together with isolates from other cities in Indonesia (blue font). The posterior probabilities of the clades were indicated as numbers in the node labels.

study in Makassar.¹⁰ Altogether, our data suggested the occurrence of DENV-1 genotype replacement in Jambi.

There is some evidence that DENV genotypes differ in their fitness and virulence.² Infection by certain genotype sometime associated with severe disease outcomes.²⁴ In Jambi, we observed more DF patients (63%) than DHF or DSS, suggesting that the circulating DENV causes a mild disease manifestation in the region. In regard to the replacement of DENV-1 Genotype IV with Genotype I and the different clinical manifestation between Jambi and Palembang, it is likely that the DENV-1 Genotype I indeed have better transmissibility compared to Genotype IV. In

compensation, the Genotype I only causes mild disease to the population. This may be explained with the trade-off hypothesis in which to increase their chances of transmission to other hosts, organisms will limit their replications or virulence so that it will not kill their hosts.²⁵

Phylogenetically, the Jambi DENV-2 viruses were grouped into Cosmopolitan genotype. This genotype is commonly found in Indonesia and surrounding countries such as Singapore, Malaysia, Brunei, and Australia, as well as other Asian countries such as China, Taiwan, and Vietnam. The isolates were grouped together with isolates from other cities in Indonesia and surrounding countries

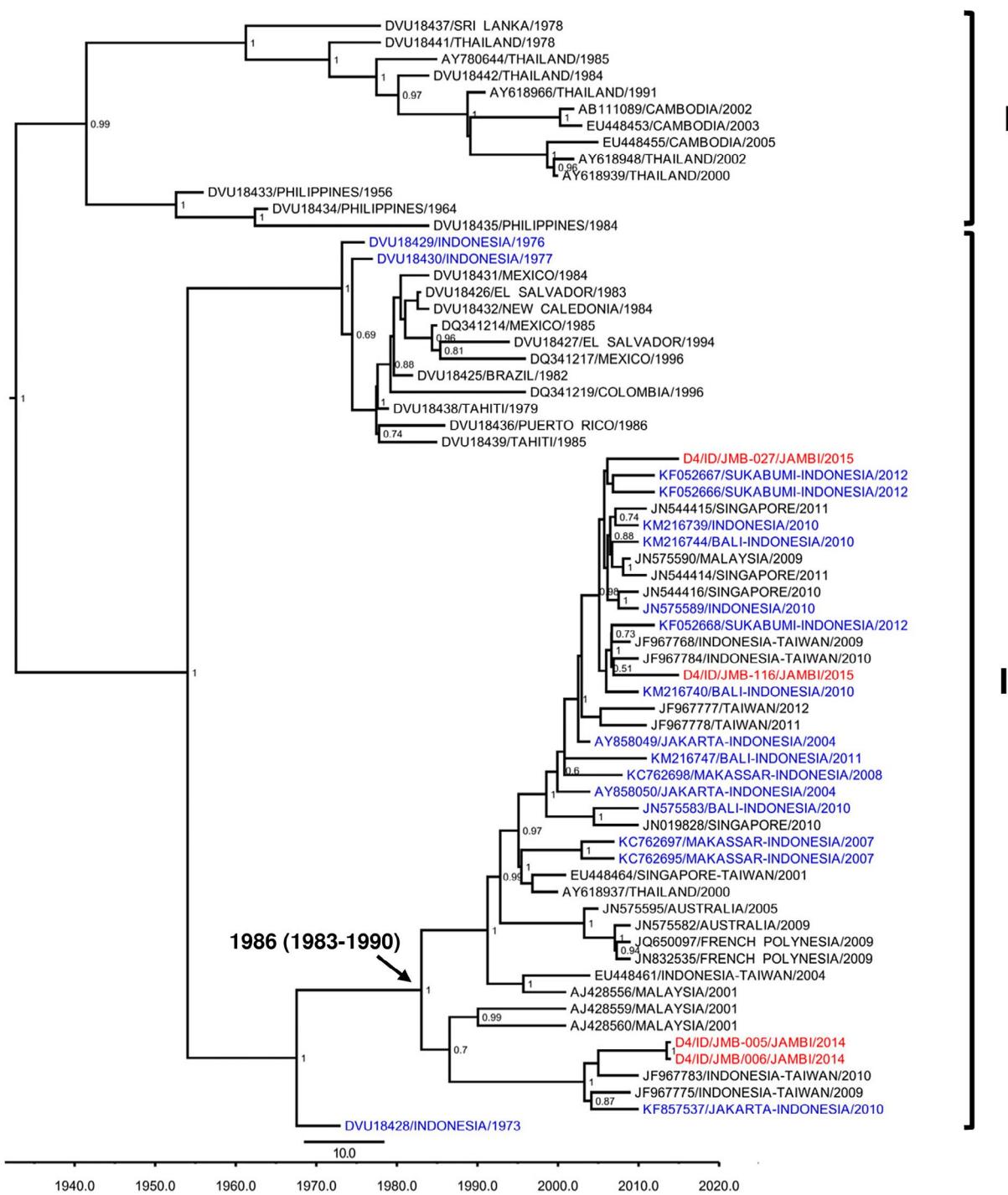


Figure 5 MCC tree of DENV-4 genotypes grouping generated by Bayesian inference method as implemented in BEAST using GTR evolution model and gamma parameter rates from the E gene sequences. The Jambi isolates (red font) were grouped into Genotype II based on classification by Lanciotti et al.¹⁴, together with isolates from other cities in Indonesia (blue font). The posterior probabilities of the clades were indicated as numbers in the node labels.

(Fig. 3). Indonesia DENV-2 Cosmopolitan genotype had been circulating for approximately three decades, suggesting their endemicity. One isolate of DENV-3 was grouped into Genotype I, together with other isolates from cities in Indonesia. The isolate was closely related to an isolate from Surabaya, East Java²⁶ (Fig. 4). The DENV-4 Jambi viruses were grouped into Genotype II and closely related to isolates from Jakarta and Sukabumi (Fig. 5). Similar to DENV-2 situation, the Indonesia DENV-3 and DENV-4 had been circulating for approximately three decades (Figs.

4 and 5). Together, the phylogenetic analyses of DENVs in Jambi revealed the endemicity and the close relationship between viruses in Sumatra and Java islands of Indonesia. Transportation routes by air and sea between these islands and the movement of population due to economic and urbanization purposes may be the possible causes.

Here, we also looked at further detail the genetic of DENV. It has been reported that specific mutation in the E gene is responsible for viral virulence.²⁵ Most of the AA changes found in Jambi DENVs were conservative

(a)	No	Sample ID	Amino Acid Position		Severity																									
			36	51	66	83	95	116	128	139	149	163	165	171	191	209	227	233	247	255	280	303	324	337	338	346	359	388	389	413
1	JMB-001	KISVTFKVHTTTLHTRKTAIFSETTKLSAIVI	DF																											
2	JMB-043	KTSVTFKVHTTTLHSRKTAIFSETTRLNAAIVV	DF																											
3	JMB-477	RTSVTFKVHTTTLHTRKRTATVFSETTKLSAIVI	DF																											
4	JMB-483	KTSVTFKVHTTTLQTRKTSATIFSETTKLSAIVI	DF																											
5	JMB-486	RTSATFKVHTTTLHTRKTAIFSETTKLSAIVI	DF																											
6	JMB-488	KTSVTFKVHTTTLHTRKTAIFSETTKLSAIVI	DF																											
7	JMB-080	RTSVTFKVHTTTLHTRKTAIFSETIKLSAIVI	DF																											
8	JMB-025	RTSVTFKVYHTTTIHTRKTAATVFSEATKLSAIVI	DHF																											
9	JMB-062	RTLVTFKVHTTTLHTRKTAATVFSETTKLSAIVI	DHF																											
10	JMB-115	KTSVTFKVHTTTLHFRAKTAIFLETTKLSAIVI	DHF																											
11	JMB-034	KTSVTFKVHTTTLHFRAKTAIFSGTTKLSAIVI	DF																											
12	JMB-082	KTSVTFKIHHTSTLHTKKTAIFSEITKLSAIVI	DF																											
13	JMB-061	KTSVTFKVHTTTLHTRKTAATIFLETTKLSAIVI	DF																											
14	JMB-095	KTSVTIKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
15	JMB-018	KTSVTFNVHTTTLHTRKSATIFSETTKLSAIVI	DF																											
16	JMB-053	KTSVTFKVHTTTLHTRKTAARIFSETTKLSAIVI	DF																											
17	JMB-003	KTSVTFKVHIATTLHTRKTAATIFSETTKLSAIVI	DF																											
18	JMB-029	KTSVTFKVHIATTLHTRKTAATIFSETTKLSAIVI	DSS																											
19	JMB-031	KTSVMFKVHTTTLHTRKTAATIFSETTKLSVVVI	DHF																											
20	JMB-011	KTSVVMFKVHTTTLHTRKTAATIFSETTKLSVVVI	DF																											
21	JMB-487	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAII	DF																											
22	JMB-120	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAII	DF																											
23	JMB-097	KTSVTFKVHTTTLHTRKTAATIYSSETTKLSAIVI	DHF																											
24	JMB-105	KTSVTFKVHTTTLHTRKTAATIYSSETTKLSAIVI	DHF																											
25	JMB-110	KTSVTFKVHTTAAHTRKTAATIFSETTKLSAIVI	DF																											
26	JMB-188	KTSVTFKVHTTAAHTRKTAATIFSETTKLSAIVI	DF																											
27	JMB-058	KTSVTFKVHTTAAHTRKTAATIFSETTKLSAIVI	DHF																											
28	JMB-046	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
29	JMB-063	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
30	JMB-086	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
31	JMB-093	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
32	JMB-089	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
33	JMB-108	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
34	JMB-109	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
35	JMB-111	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
36	JMB-484	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DF																											
37	JMB-014	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
38	JMB-017	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
39	JMB-023	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
40	JMB-059	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
41	JMB-060	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
42	JMB-085	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
43	JMB-088	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
44	JMB-092	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
45	JMB-119	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
46	JMB-151	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
47	JMB-432	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DHF																											
48	JMB-106	KTSVTFKVHTTTLHTRKTAATIFSETTKLSAIVI	DSS																											

(b)	No	Sample ID	AA Position		Severity																					
			160	162	203	320	362	432	469	484																
1	JMB-010	TINIDENV	DF																							
2	JMB-114	KISIEHSI	DF																							
3	JMB-054	KVNVDIENV	DF																							
4	JMB-084	KINIDENV	DF																							
5	JMB-040	KINIDENV	DF																							
6	JMB-049	KINIDENV	DF																							
7	JMB-488	KINIDENV	DF																							
8	JMB-024	KINIDENV	DHF																							
9	JMB-022	KISIEINI	DF																							
10	JMB-037	KISIEINI	DF																							
11	JMB-099	KISIEINI	DF																							
12	JMB-437	KISIEINI	DF																							
13	JMB-475	KISIEINI	DF																							
14	JMB-026	KISIEINI	DHF																							
15	JMB-125	KISIEINI	DHF																							

Figure 6 E protein AA sequence comparison of DENV-1 (A) and DENV-2 (B). Only AA changes are shown with the residue positions depicted above the sequences.

and most likely will not change the protein conformation (Fig. 6). One of the AA substitution involved proline (P) in residue 227, structurally different than that of threonine (T) as it had cyclic group in its backbone chain. However, this

substitution was found in both DF and DHF (Fig. 6(A)), therefore, this most likely not associated with disease severity. Overall, we did not observe any DHF-specific changes or motifs. Most of the AA changes were shared

by isolates that cause both DF and DHF. This finding suggests that the dengue clinical manifestation in Jambi is not merely determined by DENV genetic characteristics, but may depend on multi-factors including the hosts' immune response against DENV infections and pre-existing immunity. Previous studies also observed the absence of AA changes associated with disease severity.^{27–29} As this study only focused on E protein AA sequences, further study comparing the DENV whole genome or polyprotein will be useful in finding the DENV genetic determinants for disease severity.

In summary, our study provides information on the clinical and virological features of dengue in Jambi, Sumatra, Indonesia. The clinical and virological features were different from the historical dengue features in Sumatra, characterized by the serotype switch and genotype replacement. No association between DENV genetic characteristics with disease severity was observed. This study highlights the dynamic of the dengue disease in region and the importance of continuous DENV surveillance to further understand the molecular epidemiology of Dengue in Indonesia.

Acknowledgements

We would like to thank patients and physicians involved in this study. We greatly appreciate the help of Dr. Harijanto Solaeman, Dr. Samsirun Halim, Dr. Andri Budiman, Dr. Arif Sejati, and Dr. Benutomo Rumondor from Siloam Hospital, Jambi for their supports in patients' recruitment. We thank Prof. J. Kevin Baird for the critical review of the manuscript.

Funding

This work was supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia through APBN grant to Eijkman Institute.

ORCID

R. Tedjo Sasmono  <http://orcid.org/0000-0003-0986-2590>

References

- Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, et al. Dengue: a continuing global threat. *Nat Rev Microbiol.* 2010;8 Suppl 12:S7–16. doi:<http://dx.doi.org/10.1038/nrmicro2460>.
- Holmes EC. RNA virus genomics: a world of possibilities. *J Clin Invest.* 2009;119(9):2488–95. doi:<http://dx.doi.org/10.1172/JCI38050>.
- Setiati TE, Wagenaar JF, de Kruif MD, Mairuhu AT, van Gorp EC, Soemantri A. Changing epidemiology of dengue haemorrhagic fever in Indonesia. *Bull WHO.* 2006;30:1–14.
- Corwin AL, Larasati RP, Bangs MJ, Wuryadi S, Arjoso S, Sukri N, et al. Epidemic dengue transmission in southern Sumatra, Indonesia. *Trans R Soc Trop Med Hyg.* 2001;95(3):257–65.
- Suwandono A, Kosasih H, Nurhayati, Kusriastuti R, Harun S, Ma'rœf C, et al. Four dengue virus serotypes found circulating during an outbreak of dengue fever and dengue haemorrhagic fever in Jakarta, Indonesia, during 2004. *Trans R Soc Trop Med Hyg.* 2006;100(9):855–62.
- Indonesia Ministry of Health. Indonesia health profile year 2014. 2015.
- Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de C, et al. Dengue virus structural differences that correlate with pathogenesis. *J Virol.* 1999;73(6):4738–47.
- Sasmono RT, Aryati A, Wardhani P, Yohan B, Trimarsanto H, Fahri S, et al. Performance of Simplexa dengue molecular assay compared to conventional and SYBR Green RT-PCR for detection of dengue infection in Indonesia. *PLoS ONE.* 2014;9(8):e103815. doi:<http://dx.doi.org/10.1371/journal.pone.0103815>.
- WHO-SEARO. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded. New Delhi: World Health Organization; 2011.
- Sasmono RT, Wahid I, Trimarsanto H, Yohan B, Wahyuni S, Hertanto M, et al. Genomic analysis and growth characteristic of dengue viruses from Makassar, Indonesia. *Infect Genet Evol.* 2015;32:165–77. doi:<http://dx.doi.org/10.1016/j.meegid.2015.03.006>.
- Goncalvez AP, Escalante AA, Pujol FH, Ludert JE, Tovar D, Salas RA, et al. Diversity and evolution of the envelope gene of dengue virus type 1. *Virology.* 2002;303(1):110–9.
- Twiddy SS, Farrar JJ, Vinh Chau N, Wills B, Gould EA, Gritsun T, et al. Phylogenetic relationships and differential selection pressures among genotypes of Dengue-2 virus. *Virology.* 2002;298(1):63–72.
- Lanciotti RS, Lewis JG, Gubler DJ, Trent DW. Molecular evolution and epidemiology of Dengue-3 viruses. *J Gen Virol.* 1994;75(Pt 1):65–75.
- Lanciotti RS, Gubler DJ, Trent DW. Molecular evolution and phylogeny of Dengue-4 viruses. *J Gen Virol.* 1997;78(Pt 9):2279–84.
- Perkasa A, Yudhaputri F, Haryanto S, Hayati RF, Ma'rœf CN, Antonjaya U, et al. Isolation of Zika virus from febrile patient, Indonesia. *Emerg Infect Dis.* 2016;22(5):924–5. doi:<http://dx.doi.org/10.3201/eid2205.151915>.
- Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. *Arch Virol.* 2013;158(7):1445–59. doi:<http://dx.doi.org/10.1007/s00705-013-1645-3>.
- Balmaseda A, Hammond SN, Perez L, Tellez Y, Saborio SI, Mercado JC, et al. Serotype-specific differences in clinical manifestations of dengue. *Am J Trop Med Hyg.* 2006;74(3):449–56.
- Fried JR, Gibbons RV, Kalayanarooj S, Thomas SJ, Srikiatkachorn A, Yoon I-K, et al. Serotype-specific differences in the risk of dengue hemorrhagic fever: an analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLoS Negl Trop Dis.* 2010;4(3):e617. doi:<http://dx.doi.org/10.1371/journal.pntd.0000617>.
- Fahri S, Yohan B, Trimarsanto H, Sayono S, Hadisaputro S, Dharmana E, et al. Molecular surveillance of dengue in Semarang, Indonesia revealed the circulation of an old genotype of dengue virus serotype-1. *PLoS Negl Trop Dis.* 2013;7(8):e2354. doi:<http://dx.doi.org/10.1371/journal.pntd.0002354>.
- Thu MH, Lowry K, Jiang L, Hlaing T, Holmes EC, Aaskov J. Lineage extinction and replacement in dengue type 1 virus populations are due to stochastic events rather than to natural selection. *Virology.* 2005;336(2):163–72. doi:<http://dx.doi.org/10.1016/j.virol.2005.03.018>.
- Adams B, Holmes EC, Zhang C, Mammen MP, Nimmannitya S, Kalayanarooj S, et al. Cross-protective immunity can account for the alternating epidemic pattern of dengue virus serotypes circulating in Bangkok. *Proc Natl Acad Sci U S A.* 2006;103(38):14234–9. doi:<http://dx.doi.org/10.1073/pnas.0602768103>.
- Zhang C, Mammen MP, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P, et al. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. *J Virol.* 2005;79(24):15123–30. doi:<http://dx.doi.org/10.1128/JVI.79.24.15123-15130.2005>.
- Vu TTH, Holmes EC, Duong V, Nguyen TQ, Tran TH, Quail M, et al. Emergence of the Asian 1 genotype of dengue virus serotype 2 in viet nam: *in vivo* fitness advantage and lineage replacement in South-East Asia. *PLoS Negl Trop Dis.* 2010;4(7):e757. doi:<http://dx.doi.org/10.1371/journal.pntd.0000757>.
- Watts DM, Porter KR, Putvatana P, Vasquez B, Calampa C, Hayes CG, et al. Failure of secondary infection with American genotype Dengue 2 to cause dengue haemorrhagic fever. *Lancet.* 1999;354(9188):1431–4.
- Rico-Hesse R. Dengue virus virulence and transmission determinants. *Curr Top Microbiol Immunol.* 2010;338:45–55. doi:http://dx.doi.org/10.1007/978-3-642-02215-9_4.
- Kotaki T, Yamanaka A, Mulyatno KC, Labiqah A, Sucipto TH, Churrotin S, et al. Phylogenetic analysis of dengue virus type 3 strains primarily isolated in 2013 from Surabaya, Indonesia. *Jpn J Infect Dis.* 2014;67(3):227–9.

27. Blok J, Samuel S, Gibbs AJ, Vitarana UT. Variation of the nucleotide and encoded amino acid sequences of the envelope gene from eight Dengue-2 viruses. *Arch Virol.* 1989;105(1–2):39–53.
28. Lee E, Gubler DJ, Weir RC, Dalgarno L. Genetic and biological differentiation of Dengue 3 isolates obtained from clinical cases in Java, Indonesia, 1976–1978. *Arch Virol.* 1993;133(1–2):113–25.
29. Roche C, Cassar O, Laille M, Murgue B. Dengue-3 virus genomic differences that correlate with *in vitro* phenotype on a human cell line but not with disease severity. *Microbes Infect.* 2007;9(1):63–9. doi:<http://dx.doi.org/10.1016/j.micinf.2006.10.010>.