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Research Report

## SERO-EPIDEMIOLOGY OF DENGUE VIRUS INFECTION IN 4 CITIES OF INDONESIA

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### ABSTRACT

*Background:* Dengue Virus Infection is major public health problem in Indonesia. *Aedes aegypti* is widespread in both urban and rural areas, where multiple virus Serotype are circulating. On 2013 outbreak of dengue virus infection occur in East Java. Therefore study seroepidemiology in Bangkalan and Lombok had been done. *Aim:* to find a mutated strain of Dengue Virus in 4 cities of Indonesia. *Method:* On 2011 and 2012 seroepidemiology study had been done in Dr. Soetomo Surabaya and Soerya Sidoarjo Hospital; and on 2013 study had been done in Surabaya, Bangkalan and Lombok Hospital. *Diagnosis of Dengue Virus Infection* was based on Criteria WHO - 2009. Virus isolation in Surabaya, Sidoarjo, Bangkalan and Lombok had been done. *Result:* a total of 349 isolate were obtained from dengue patients sera collected in Surabaya and Sidoarjo, 2011–2012 showed that Den VI (182), Den V2 (20) Den V4 (1) were found in Surabaya on 2011 and Den V 1 (79), Den V 2 (7) were found in Surabaya on 2012; Den VI (40), Den V 2 (3) were found in Sidoarjo on 2011 and Den V 1 (17) were found in Sidoarjo on 2012; Virus isolation in Surabaya on 2013, January: 237 serum sample were collected, found Den V 1 (8), Den V 3 (2) and Den V 4 (5). And PCR stereotyping of isolated viruses in Madura found Den V 1 (1) and Den V 4 (23). In Lombok found Den V 4 (4). It is possible to shift predominant strain in Surabaya, Genotype or Serotype shift might increase the number of dengue patients. *Conclusion:* there were shift predominant strain in Surabaya especially Den V 1. Therefore to continuous surveillance of circulating viruses is required to predict the risk of DHF and DF.

**Key words:** Seroepidemiology, Den V, predominant strain, serotype, RNA

### ABSTRAK

*Latar belakang:* Sampai sekarang infeksi virus dengue masih merupakan penyakit infeksi yang menjadi problem kesehatan masyarakat Indonesia. Infeksi virus dengue tersebar luas di kota dan desa, dimana banyak serotip virus dengue tersebar dalam darah penderita infeksi virus dengue. Tahun 2013 di Jawa Timur dan Jakarta terjadi penyelidikan kasus infeksi virus dengue secara dekat. Oleh karena itu perlu dilakukan sero epidemiologi. Penderita infeksi virus dengue di Surabaya, Sidoarjo, Bangkalan dan Mataram. *Tujuan:* Menemukan materi berdasarkan virus dengue di 4 kota di Indonesia. *Metode:* Pada tahun 2011 dan 2012 penulis sero epidemiologi telah dilakukan di RSUD Dr. Soetomo Surabaya dan RS Soerya Sidoarjo, dan pada tahun 2013 penelitian dilakukan di Surabaya, Sidoarjo, Bangkalan dan Mataram. *Diagnose* penderita infeksi virus dengue berdasarkan criteria WHO 2009, sekarang dilakukan isolasi virus dengue. *Hasil:* Jumlah kasus yang diketahui sebanyak 349, berasal dari penderita infeksi virus dengue yang dirawat di Surabaya dan Sidoarjo. Seperti pada tahun 2011–2012 menunjukan hasil sebagai berikut: Den VI (182), Den V2 (20), Den V4 (1), temuan ini di Sidoarjo pada tahun 2011 dan Den VI (79), Den V2 (7), ditemukan di Surabaya pada tahun 2012: Den VI (40), Den 2 (3). Ditemukan di Sidoarjo tahun 2011: Den V1 (17), ditemukan di Sidoarjo tahun 2012. Isolasi virus dengue di Surabaya tahun 2013 pada bulan Januari terkumpul 237 serum, hasil yang positif terdeteksi Den VI (8), Den V3 (2) dan Den V4 (5). Hasil isolasi virus dengue dari Madura ditemukan Den V1 (1) dan Den V4 (23), dan dari Lombok ditemukan Den 4 (4). Jika temuan ini dicermati perubahannya stain virus dengue terjadi di Surabaya terutama Den VI. Perubahan genotif dan serotif memungkinkan peningkatan

*jumlah penderita virus dengue. Kesimpulan: Ditemukan perubahan strain dengue virus di Surabaya terutama Den VI, oleh karena itu surveillance virus dengue, perlu di teliti dan di cari hubungannya dengan perjalanan klinis DBD dan demam dengue.*

**Kata kunci:** Seroepidemiology, Den V, predominant strain, serotype, RNA

## INTRODUCTION

Dengue virus (DENV) infection can make a major public health problem; > 50 million persons are infected each year worldwide,<sup>1</sup> and the incidence of severe, sometimes lethal, forms of the disease is increasing.<sup>2</sup> Dengue viruses are mosquito-borne flaviviruses with a single-stranded, nonsegmented, positive-sense RNA genome ~11 kb in length. Four antigenically distinct serotype, DENV types 1 to 4, exist.<sup>3</sup> Infection with any serotype can lead to disease, ranging from mild infection, dengue fever (a generally mild disease with complete recovery), to severe forms (dengue hemorrhagic fever and dengue shock syndrome). Molecular epidemiology studies have investigated the possibility of a link between particular DENV genotype or cluster or particular clinical form of disease.<sup>4,5</sup> Consequently, finding new viral genotypes in areas where they had been absent could be of epidemiologic and clinical interest.

Emerging Infectious Diseases dengue epidemic are expanding rapidly. This emerging diseases continues to baffle and challenge epidemiologists and clinicians to study. Despite endemicity of 3 or more different dengue viruses, why does severe dengue occur in some populations and not in other? Why are children principally affected in some areas and adults in others? How can severe dengue reliably be recognize early enough to permit appropriate therapy to be applied?<sup>6</sup>

During an infection there are 4 dengue viruses, the principal threat to human health resides in the ability of the infecting virus can causes an acute febrile syndrome characterized by clinically significant vascular permeability, dengue hemorrhagic fever (DHF). However, because at onset vascular permeability exhibits only subtle changes, how can a diagnostic be made early enough to begin life-saving intravenous treatment? In person with light skin color, the standard syphgmomanometer cuff tourniquet test has been widely used to screen children in outpatient settings; a positive result in an early warning of incipient DHF. Because of genetic diversity among humans, the tourniquet test as a screening tool requires widespread evaluation and validation.<sup>6</sup>

Human are not uniformly susceptible to the DHF syndrome. HLA gene distribution correlates with increased susceptibility as well as with increased resistance.<sup>7</sup> In addition, a powerful resistance gene is found in blacks.<sup>8</sup> Importantly, susceptibility to vascular permeability during a dengue infection is age-related. The susceptibility of young children to DHF precisely paralleled age-related changes in microvascular permeability measured in normal children and adult.

Dengue fever syndrome in susceptible adults may be contrasted to the innate susceptibility of children for vascular permeability syndrome during a secondary dengue virus infection.

In Southeast Asia, the epicenter of DHF epidemics, dengue infection rates are falling in children, resulting in changing epidemiologic patters of DHF. In Thailand, for example, the modal age at which children are hospitalized for DHF has steadily increased over the past decades.

In addition, because an increasing number of persons experience their first dengue infection at an older age, dengue fever cases are now appearing in adults.<sup>2</sup>

## MATERIALS AND METHODS

### Population Study

Seroepidemiology study had been done in Surabaya, Sidoarjo, Bangkalan and Mataram. Surabaya and Sidoarjo are part of East Java including in Java Island, and Bangkalan is part of Madura Island. Mataram city is part of West Nusa Tenggara province. There are 392 serum specimens from patients which had a clinical manifestation of dengue infection based on Criteria WHO-2009 from 2011 until January 2013.

349 isolate were obtained from dengue patients sera in Surabaya (Dr. Soetomo Hospital) and Sidoarjo (Soerya Hospital) on 2011–2012; and 43 serum sample were collected in Surabaya, Bangkalan, and Mataram on January 2013.

### Sample Collection and Diagnosis of Dengue

Human sera were obtained from 392 patients presenting clinical manifestations of dengue and tested for anti-dengue IgM antibodies (Becton-Dickinson). The clinical samples corresponded with dengue cases reported during 2011–2013. Diagnosis of dengue virus infected was based on Criteria WHO 2009. Dengue-infected samples were obtained during the first five days of the onset of fever and were processed for anti dengue IgM detection using IgM capture ELISA (MAC-ELISA) as described by Vorndam *et al.*

As a routine practice and with the idea of recording epidemic data, the suspected dengue sample already clinically diagnosed in community health centers were sent to Institute of Tropical Disease Airlangga University, Surabaya, Indonesia. In the laboratory, the presence of dengue virus was confirmed by MAC-ELISA and RT-PCR.

### Dengue Virus Isolates

Dengue virus isolated by using *Aedes albopictus* C6/36 cells were grown in 48-well tissue culture plates as described by Irgarashi.<sup>9</sup> Briefly,  $2 \times 10^5$  cells were plated in 1 ml of minimum essential medium (Gibco BRL, Grand Island, N.Y.) supplemented with 7% fetal bovine serum (Sigma Chemical Co., St Louis, Mo) and 1% glutamine, vitamins and nonessential amino acids. After 24 hours of cultures, 100  $\mu$ l of every sera diluted 1:10 was added to the corresponding well. The mixture was then gently shaken and incubated for 60 minutes at room temperature. Cells were then washed with serum-free medium and cultured at 28° C with complete medium for at least 10 days. Cells were harvested for RT-PCR diagnosis.

### RNA extraction

Total RNA was extracted either from 100  $\mu$ l of serum or from cultured cells by using Trizol LS (GIBCO BRL, Gaithersburg, MD.) according to the manufacturers recommendations. Ethanol-precipitated RNA was recovered by centrifugation and air-dried. The RNA pellet was re-suspended in 50  $\mu$ l of Diethyl-pyrocabonate (Sigma)-treated water (DEPC water) and used as template for RT-PCR.

### RT-PCR

Synthetic oligonucleotide primer pairs were designed based on published sequence data for each of the four serotypes of dengue.<sup>10,11</sup> Four fragments of an expected size of 392bp (DEN-1), 392 bp (DEN-4), 290 bp (DEN-3) and 119 bp (DEN-2) were obtained by using the SuperScrip<sup>TM</sup> One Step RT-PCR kit in conjunction with Platinum<sup>R</sup>Taq polymerase (Invitrogen, Life Technologies). A mixture of 5  $\mu$ l of RNA, 25  $\mu$ M of sense and anti-sense PCR primers, and DEPC water to a total volume of 50  $\mu$ l was incubated at 85° C for 5 minutes and then chilled on ice. The tube-reaction mixture containing 2x PCR buffer containing 0.4 mM MgSO<sub>4</sub> and SuperScrip<sup>TM</sup>RT/platinum<sup>R</sup>Taq Mix, as recommended by the manufacturer (Invitrogen TM Life Technologies), was added to the RNA and primers-containing tube. The reverse transcription reaction was performed at 50° C for 30 minutes. Thermocycling began with a hot start at 9° C for 2 minutes followed by 40 cycles of annealing at 55° C for 30 seconds, and extension at 72° C for one minutes and denaturing at 94° C for 15 minutes.

The PCR conditions for serotype assessment were as follows: 40 cycles of denaturing at 94° C for 30 seconds, annealing at 55° C for 1 minute, and extension at 72° C for 7 minutes. The reaction mixtures were electrophoresed and visualized under UV light after ethidium bromide staining of the gels.

### RESULTS

Project 1. Virus isolation was collected from Surabaya and Sidoarjo on 2011–2012.

**Table** A total of 349 isolates sera were collected from Surabaya and Sidoarjo, 2011–2012

	2011		2012	
	Surabaya	Sidoarjo	Surabaya	Sidoarjo
D1	182	40	79	17
D2	20	3	7	0
D3	0	0	0	0
D4	1	0	0	0



**Project 2.** 43 viruses isolation were collected from Surabaya, Bangkalan\_Madura and Mataram\_Lombok on January 2013

**Table** PCR serotyping of isolated viruses

Site	D1	D2	D3	D4
Surabaya	8	0	2	5
Bangkalan	1	0	0	23
Mataram	0	0	0	4

### DISCUSSION

Seroepidemiology of Dengue Virus Infection is a science of transferring Dengue Virus to other host of human being by biting of *Aedes aegypti* mosquito which has been supported by biotic and abiotic factor: such as an increasing population of *Aedes aegypti* in urban and sub urban environment due to the changing of raining season to sunrise.

Dengue virus infection in human being can be found if the interaction among etiologic, host and environment has occurred: <sup>(1)</sup>The etiologic is dengue virus which usually life in *Aedes aegypti* mosquito where it like life in clean water. <sup>(2)</sup>Host is human being who has decreasing immunity due to very tired and getting more virulent virus from biting of *Aedes aegypti* mosquito which is supported by <sup>(3)</sup>changing humidity of environment.

Since 1968 Dengue Virus Infection has been found in Indonesia, especially at Surabaya and Jakarta city . Firstly

management of dengue virus infection very difficult to improve, therefore the higher mortality nearly 41,4% had been found; but on the following years in five decades the mortality rates was becoming to decrease until 1,27 % on 2011.

On January 2013, the outbreak of Dengue Virus Infection has been occurred and many cases of Children under five years has been found and one of them is a 21 days old baby suffered from DSS has been found, as a younger baby case.

Beside it many cases babies below one years old has been found as severe dengue virus infection which shown clinical manifestation of bleeding and encephalopathy, therefore some of them cannot be help. It should be studied!, what kind Serotype of dengue virus was becoming predominant strain as a cause of severity?

As we know in the first decade the Serotype of Dengue 2 and 3 were predominant strain Den 2 tended to be neutralized stronger than other viruses. It was found in Surabaya before 2008 but on the year 2009, 2010, 2011 and 2012 there were changing of Serotype and found Den 1 genotype 1V showed a severity clinical manifestation with primary infection but on 2013 Serotype Den 1 and IV were found at Surabaya, Bangkalan and Mataram; especially Bangkalan and Mataram showed more Den IV.

Phylogenetic analysis on Den V 1 in Surabaya showed that genotype 1 and IV consisted at the same time in 2012 although only genotype IV were isolate in 2011. There were differences between D 1 genotype I and IV. Analyzing the difference of genotype is important for vaccine development.

Based on this result of study, monitoring the emergence of imported or mutated strain of Dengue Virus in human being and mosquito should always be continued. Therefore, continuous surveillance of circulating viruses is required to predict the risk of DHF and DF.

This idea is very important for making procedure Update management of Dengue Virus Infection Cases to decrease Case Mortality in the outbreak situation and try to do new prevention method before Outbreak Occur.

## CONCLUSSION

Until now, there were changing of serotype of dengue virus based on time, place and humadity.

## REFERENCE

1. McBride WJ, Bielefeldt-Ohmann H. Dengue viral infections: pathogenesis and epidemiology. *Microbes Infect.* 2002; 2: 1041–50.
2. Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Disease* 2002; 2: 33–42.
3. Rice CM. Flaviviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, et al., editors *Virology*. Philadelphia: Lippincott-Raven; 1996. P. 931–1034.
4. Riccp-Hesse R. Microevolution and virulence of dengue viruses. *Adv Virus Res.* 2003; 59: 315–41.
5. Messer WB, Gubler DJ, Harris E Sivananthan K, de Silva AM. Emergence and global spread of a dengue serotype 3, subtype III virus. *Emerg Infect Dis.* 2003; 9: 800–9.
6. Scoott B. Halstead. More Dengue, More Questions, Maryland, USA, 2005
7. Stephens HA, Klaythong R, Sirikong M, Vaughn DW, Green S, Kalayanaroj K, et al HLA-A and-B allele associations with secondary dengue virus infections correlate with disease severity and infecting viral serotype in ethnic Thais. *Tissue Antigens.* 2002; 60: 309–18.
8. Guzman MG, Kouri GP, Bravo J, Soler M, et all. Dengue Hemorrhagic Fever in Cuba, 1981: a retrospective epidemiological study. *Am J Trop Med Hyg.* 1990; 42: 179–84.
9. Igarashi A. Mosquito cell cultures and study of antropod-borne togaviruses. *Advance in Virus Research*, 1985, 30: 21–39.
10. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ and Vorndam V. Rapid detection and typing of dengue viuses from clinical sample by using reverse trancriptase-polymerase chain reaction. *Journal of Clinical Microbiology*, 1992, 30: 545: 551.
11. Haris e. A low cost approach to PCR. ED. Oxford University Press, USA, 1998. 96–105.
12. Christophe NP, Boris AMP, Mael B, Patrick G, Fabienne T, Patricia CP, Jenny M, Patricia HA, Raymond S, Marc G and Hugues JT. Dengue Type 3 Virus, Saint Martin, 2003–2004.
13. A. Cineros-Solano, M. M. B. Moreno-Altamirano, U. Martinez-Soriano, F. Jimenez-Rojas, A. Diaz-Badillo and M. L Munoz. Sero-epidemiological and Virological Investigation of Dengue Infection in Oaxaca, Mexico, during 2000–2001.



Picture. a 21 days old baby suffered from DSS