RINGKASAN

POLA DISTRIBUSI SEROTIPE VIRUS DENGUE PADA BEBERAPA DAERAH ENDEMIK DI JAWA TIMUR DENGAN KONDISI GEOGRAFI BERBEDA

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Infeksi virus Dengue telah menjadi masalah kesehatan yang serius pada banyak negara tropis dan subtropis, oleh karena terjadi peningkatan jumlah penderita, menyebar luasnya daerah yang terkena wabah dan manifestasi klinis berat yang merupakan keadaan gawat darurat yaitu ***Dengue Haemorrhagic Fever*** (DHF) dan ***Dengue Shock Syndrome*** (DSS). Penyakit DBD di Indonesia telah menyebar ke seluruh Propinsi, tidak saja di daerah urban, tetapi sudah menyebar ke daerah rural, bersifat endemis dan cenderung mengalami kejadian luar biasa/(KLB).

Penelitian ini bertujuan untuk mempelajari epidemiologi penyakit Demam Berdarah Dengue (DBD) dari sisi hubungan antara kondisi geografi suatu daerah terhadap serotipe virus Dengue dan tingkat keparahan penyakit, agar dapat digunakan sebagai strategi dalam pencegahan, pengobatan dan pemberantasan virus penyakit Demam Berdarah Dengue di Indonesia. Jenis penelitian ini adalah penelitian eksploratif laboratoris yaitu eksplorasi terhadap serotipe virus Dengue pada penderita DBD dari berbagai daerah yang berbeda kondisi geografinya dengan menggunakan tehnik *reverse transcriptase-polymerase chain reaction* (RT-PCR) sekaligus dilakukan pemeriksaan DNA sequencing untuk mengetahui susunan nukleotidanya. Rancangan penelitian yang digunakan dalam penelitian ini adalah *cross-sectional study*, dengan wilayah penelitian di Jawa Timur meliputi wilayah Surabaya, Malang, dan Jember yang berbeda kondisi geografinya (didasarkan data Badan Meteorologi dan Geofisika Wilayah III Juanda Surabaya).

Penelitian ini menggunakan sampel darah pasien yang telah didiagnosis positif menderita DBD di Wilayah Jawa Timur dengan mengambil tiga daerah penelitian yaitu Surabaya, Malang, dan Jember, dengan penegakan diagnosis yang didasarkan

pada kriteria WHO 1997. Pengumpulan sampel dilakukan di RSUD wilayah penelitian, dengan mengambil serum darah penderita DBD, kemudian dilakukan isolasi virus Denguenya di laboratorium, dengan menggunakan tehnik RT-PCR dilakukan identifikasi serotipe virusnya, selanjutnya dilakukan sequencing DNA untuk mengetahui susunan nukleotidanya. Hasil pengumpulan sampel, diperoleh 28 sampel dari Rumah Sakit Dr. Soetomo Surabaya, 21 sampel dari Rumah Sakit Dr. Syaiful Anwar Malang, dan 31 sampel dari Rumah Sakit Dr. Soebandi Jember.

Sebagai indikator untuk menetapkan adanya antibodi anti Dengue adalah hasil pemeriksaan kadar IgM dan IgG dengan metode *Captured* ELISA, indikator untuk menetapkan serotipe virus adalah hasil pemeriksaan ***semi nested-****Polymerase Chain Reaction* **(*sn*-PCR)** serum penderita, dan hasil pemeriksaan klinis oleh dokter di Rumah sakit wilayah penelitian sebagai indikator manifestasi klinis penderita DBD berdasar kriteria WHO 1997. Hasil penelitian ini dianalisis dengan menggunakan analisis deskriptif, yaitu analisis yang didasarkan pada deskripsi data hasil penelitian.

Berdasarkan hasil pemeriksaan IgM dan IgG dan ***sn*-PCR** pada penderita DBD dari wilayah Surabaya, Malang, dan Jember, menunjukkan bahwa infeksi sekunder dengan jenis virus Dengue serotipe Den-2 mendominasi kasus DBD di wilayah penelitian, sehingga dapat dinyatakan bahwa endemisitas di tiga wilayah daerah tersebut cukup tinggi. Sementara ditemukan seorang penderita DBD dari Surabaya terinfeksi virus ganda, Den-2 dan Den-3. Berdasarkan hasil sekuensing dapat dilihat bahwa isolat dari Jawa Timur yaitu dari Surabaya, Malang, Jember dan Pacitan terdapat homologi lebih dari 80% dan bila dibandingkan dengan isolat dari Jakarta, USA dan Jamaica homologinya kurang dari 73%. Artinya bahwa virus Dengue serotipe Den-2 di Jawa Timur masih tinggi kesamaannya, sedangkan bila dibanding serotipe Den-2 Jakarta, USA dan Jamaica kesamaannya berkurang. Untuk menentukan pola kekerabatan serotipe virus Dengue tersebut perlu penelitian lanjutan berupa *"Phylogenetic analysis"*.

Diagnosis klinis penderita DBD pada penelitian ini dilakukan oleh dokter yang merawat di tiga daerah penelitian dengan menggunakan kriteria WHO 1997 untuk mengurangi terjadinya *over diagnosis*. Tingkat keparahan penyakit

diklasifikasikan menjadi 4 grade mulai dari yang paling ringan ke yang paling berat yaitu grade-1, grade-2, grade-3 dan grade-4.

Hasil pemeriksaan terhadap 28 penderita DBD dari daerah penelitian Surabaya, menunjukkan hasil diagnosis klinis grade-1 sebanyak 14 penderita (50%), grade-2 sebanyak 11 penderita (39,3%), grade-3 sebanyak 2 penderita (7,1%) dan grade-4 sebanyak 1 penderita (3,6%). Hasil pemeriksaan serologi terhadap 14 penderita dengan diagnosis klinis grade-1 menunjukkan positif 3 penderita (21,4%), equivocal 2 penderita (14,3%) dan negatif 9 penderita (64,3%). Pada 11 penderita grade-2 menunjukkan positif 7 penderita (63,6%), negatif 4 penderita (36,4%). Dari 2 penderita grade-3 menunjukkan positif semuanya (100%). Pada seorang penderita grade-4 menunjukkan positif semuanya (100%). Hasil pemeriksaan PCR terhadap 14 penderita dengan diagnosis klinis grade-1 menunjukkan positif 5 penderita (35,7%) virus Den-2 dan 9 penderita (64,3%) negatif. Pada 11 penderita dengan diagnosis klinis grade-2 menunjukkan 2 penderita (18,2%) positif virus Den-2 dan 9 penderita (81,8%) negatif. Pada 2 penderita grade-3 menunjukkan seluruhnya (100%) positif virus Den-2. Pada seorang penderita dengan diagnosis klinis grade-4 menunjukkan positif (100%) virus Den-2 dan Den-3 (infeksi ganda). Hasil temuan ini menunjukkan bahwa penderita yang didiagnosis klinis grade-1 dan grade-2 tingkat positifnya ditemukan virus Dengue rendah yaitu 71% dan 82%, sedangkan penderita dengan diagnosis klinis grade-3 dan grade-4 100% positif bahkan 1 kasus grade-4 mengalami infeksi ganda dengan virus Den-2 dan Den-3, artinya infeksi ganda tersebut memperparah perjalanan penyakit DBD sampai terjadi DSS dan mayoritas ditemukan virus Dengue serotipe Den-2, serotipe Den-3 pada satu penderita yang mengalami infeksi ganda Den-2 dan Den-3.

Hasil pemeriksaan terhadap 21 penderita DBD dari daerah penelitian Malang menunjukkan hasil diagnosis klinis dengan grade-1 sebanyak 3 penderita (14,3%), grade-2 sebanyak 15 penderita (71,4%), grade-3 sebanyak 1 penderita (4,8%) dan grade-4 sebanyak 2 penderita (9,5%). Hasil pemeriksaan serologi terhadap 3 penderita dengan diagnosis klinis grade-1 menunjukkan semuanya (100%) positif. Pada 15 penderita grade-2 menunjukkan 12 penderita (80%) positif, 2 penderita

(13,3%) equivocal dan 1 penderita (6,7%) negatif. Pada seorang penderita grade-3 menunjukkan semuanya (100%) positif. Pada 2 penderita grade-4 menunjukkan semuanya (100%) positif. Hasil pemeriksaan PCR terhadap 3 penderita dengan diagnosis klinis grade-1 menunjukkan seluruhnya (100%) negatif. Pada 15 penderita grade-2 menunjukkan 7 penderita (46,7%) positif virus Den-2 dan 8 penderita (53,3%) negatif. Pada 1 penderita grade-3 menunjukkan negatif (100%). Pada 2 penderita grade-4 menunjukkan 1 penderita (50%) positif virus Den-2 dan 1 penderita (50%) negatif. Virus yang ditemukan ternyata seluruhnya serotipe Den-2.

Hasil pemeriksaan terhadap 31 penderita DBD dari daerah penelitian Jember menunjukkan hasil diagnosis klinis dengan grade-1 sebanyak 15 penderita (48,4%), grade-2 sebanyak 12 penderita (38,7%), grade-3 sebanyak 3 penderita (9,7%) dan grade-4 sebanyak 1 penderita (3,2%). Hasil pemeriksaan serologi terhadap 15 penderita dengan diagnosis klinis grade-1 menunjukkan 5 penderita (33,3%) positif, equivocal 1 penderita (6,7%) dan 9 penderita (60%) negatif. Pada 12 penderita dengan diagnosis klinis grade-2 menunjukkan 10 penderita (83,3%) positif dan 2 penderita (16,8%) negatif. Pada 3 penderita grade-3 menunjukkan semuanya (100%) positif, grade-4 sebanyak 1 penderita menunjukkan positif (100%). Hasil temuan ini menunjukkan bahwa mayoritas diagnosis klinis penderita adalah grade-1 (48,4%) dan grade-2 (38,7%) dan hasil pemeriksaan serologi grade-2 ditemukan 83,3% positif, sedang grade-3 dan grade-4 100% positif. Hasil pemeriksaan PCR terhadap 15 penderita dengan diagnosis klinis grade-1 menunjukkan 1 penderita (6,7%) positif virus Den-2, 14 penderita (93,3%) negatif. Pada 12 penderita grade-2 menunjukkan 5 penderita (41,7%) positif virus Den-2 dan 7 penderita (58,3%) negatif. Pada 3 penderita grade-3 semuanya negatif (100%). Pada seorang penderita grade-4 juga negatif (100%). Virus yang ditemukan seluruhnya serotipe Den-2.

Hasil penelitian ini menunjukkan bahwa: 1) Daerah Surabaya, Malang dan Jember yang secara geografis berbeda ternyata ditemukan semuanya virus Dengue serotipe Den-2 kecuali Surabaya ditemukan satu kasus infeksi ganda dengan serotipe Den-2 dan Den-3. 2) Infeksi ganda oleh Den-2 dan Den-3 menyebabkan DSS (grade-4). 3) Karakterisasi molekuler serotipe Den-2 yang ditemukan di daerah Surabaya,

Malang dan Jember ternyata memiliki homologi lebih dari 80%, sedangkan bila dibandingkan dengan isolat Den-2 dari Jakarta, USA dan Jamaica homologinya kurang dari 73%. 4) Pola serologi menggunakan *Captured* ELISA menunjukkan mayoritas adalah infeksi sekunder bila dibanding infeksi primer yaitu di Surabaya 35,7% , Malang 76,2% dan Jember 54.8%. Artinya diketiga daerah tersebut menunjukkan endemis penyakit DBD. Didapatkan hasil serologi negatif, namun pada pemeriksaan PCR ditemukan adanya virus Dengue, artinya meskipun hasil serologi negatif tidak menyingkirkan adanya infeksi virus Dengue.

Berdasarkan hasil penelitian ini, maka dapat disampaikan beberapa saran: 1) Untuk menurunkan angka kesakitan dan kematian penyakit DBD perlu kerjasama lintas sektor. 2) Perlu dilakukan penelitian berkesinambungan tentang penyakit DBD ini antara lain untuk menemukan metoda yang efektif untuk pemberantasan vektor, menemukan metoda untuk diagnosis dini yang lebih tepat, menemukan kandidat vaksin Dengue yang lebih sesuai dengan karakteristik virus Dengue di masing masing daerah, mempelajari patogenesis dan imunologi penyakit DBD yang hingga saat ini masih belum jelas benar, termasuk biologi molekuler tentang kemungkinan adanya mutasi genetik virus.

SUMMARY
DISTRIBUTION PATTERN OF DENGUE VIRUS SEROTYPE IN SOME ENDEMIC AREAS IN EAST JAVA WITH DIFFERENT GEOGRAPHICAL CONDITION

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Dengue virus infection has became a serious health problem in many tropical and sub-tropical countries, due to the increasing number of victims, the spreading of endemic areas and severe clinical manifestation which can be categorized as emergency case that is Dengue Haemorrhagic Fever (DHF) and Dengue Shock syndrome (DSS). Dengue Haemorrhagic Fever disease in Indonesia has been spread out all over provinces, not only in urban areas, but also in rural areas, having endemic nature and tends to provoke unusual event.

The objective of this research was to study DHF epidemiology seeing from the correlation between geographical condition of an area with Dengue virus serotype and the illness degree, so that it can be used as a strategy for DHF prevention, treatment, and recovery in Indonesia. This research was a laboratory explorative study, exploring the serotype of Dengue virus in DHF patients from several regions with different geographical condition by using reverse transcriptase-polymerase chain reaction (RT-PCR) technique. The design used in this research was cross-sectional study. The research covered areas in East Java from Surabaya, Malang and Jember, that are different in their geographical condition (based on the data of Meteorology and Geography Institution Regional III Juanda Surabaya).

This research used blood samples from patients diagnosisd with DHF positive in East Java regions consisting of Surabaya, Malang, and Jember, with diagnosis reference based on WHO criteria 1997. Sample collecting was conducted at Regional Hospital of each research region, by taking blood serum of DHF patient, then isolating dengue virus in laboratory, followed by using RT-PCR technique to identify the virus serotype. Afterwards, the DNA sequencing was conducted in order to find the nucleotide form. Based on the data taken from the samples, we obtained 28 samples from Dr. Soetomo

Hospital Surabaya, 21 samples from Dr. Syaiful Anwar Hospital Malang, and 31 samples from Dr. Soebandi Hospital Jember.

Indicator to determine the existence of anti Dengue antibody was the result of IgM and IgG scale with captured ELISA method. Indicator to determine virus serotype was the result of ***Semi nested***-Polymerase Chain Reaction ***(sn*-PCR)** from the patients' serum, and clinical examination result conducted by clinicians at the hospital in each region as indicator of clinical manifestation of DHF in the patients was based on WHO criteria 1997. These results were analyzed by using descriptive analysis, an analysis that was based upon the description of results data.

Based on the examination of IgM and IgG and ***sn*-PCR** on DHF patients from Surabaya, Malang and Jember, it was shown that secondary infection with Dengue virus serotype Den-2 predominated DHF cases in these regions. Therefore, it could be concluded that endemic status in those three regions were high enough. Meanwhile, we found a DHF patient from Surabaya who has been infected by double viruses, Den-2 and Den-3. Based on sequencing result, it could be seen that isolates from East Java (Surabaya, Malang, and Jember) contained homology of more than 80% compared to isolates from Jakarta, USA and Jamaica which was less than 73%. It means Dengue virus serotype Den-2 in East Java was high in its equality compared to serotype Den-2 Jakarta, USA and Jamaica, which had lower level of equality. To determine to the brotherhood pattern of Dengue virus serotype, it needs further research in the form "phylogenetic analysis". Sequencing from many regions of genome Den was meant to determine genetic variation of Dengue virus and to characterize the sub-type in each serotype. Sub-type characterization is useful in molecular epidemiology that it may be used to monitor the distribution of serotype and sub-type circulating in endemic area.

Clinical diagnosis of DHF patient in this research was conducted by clinician who carried out the treatment in three research areas using the WHO criteria 1997 to reduce overdiagnosis. Illness degree was classified into 4 grade starting from mild to severe, i.e., grade-1, grade-2, grade-3 and grade-4.

Examination result on 28 DHF patients from Surabaya showed clinical diagnosis grade-1 for 14 patients (50%), grade-2 for 11 patients (39.3%), grade-3 for 2 patients (7.1%) and grade-4 for 1 patient (3.6%). Serology examination to 14 patients with clinical diagnosis grade-1 showed positive for 3 patients (21.4%), equivocal 2 patients (14.3%) and negative 9 patients (64.3%). Eleven grade-2 patients showed 7 patients (63.6%) positiv and, 4 patients (36.4%) negative. Two grade-3 patients were all positive (100%). One grade-4 patient was positive (100%). PCR examination to 14 patients with clinical diagnosis grade-1 showed 5 patients (35.7%) was positive having Den-2 virus, and 9 patients (64.3%) negative. Among eleven patients with clinical diagnosis grade-2, 2 patients (18.2%) were positively having Den-2 virus and 9 patients (81.8%) negative. Two patients of grade-3 were all (100%) positive having Den-2 virus. One grade-4 patient (100%) was found to have Den-2 and Den-3 virus (double infection). These findings showed patients with clinical diagnosis grade-1 and grade-2. It was noticed that the positive degree for Dengue virus was found to be low, 71% and 82%, while patients with clinical diagnosis grade-3 and grade-4 were found to be all positive, and even 1 grade-4 case was found to have double infection with Den-2 and Den-3 virus, which means that the double infection had worsened DHF to become DSS occurred and in majority Dengue virus serotype Den-2, serotype Den-3 were found in one patient having double infection with Den-2 and Den-3.

Examination results in 21 DHF patients from Malang showed clinical diagnosis grade-1 in 3 patients (14.3%), grade-2 in 15 patients (71.4%), grade-3 in 1 patient (4.8%) and grade-4 in 2 patients (9.5%). Serology examination result in 3 patients with clinical diagnosis grade-1 showed all positive (100%). Among fifteen grade-2 patients, 12 patients (80%) were positive, 2 patients (13.3%) equivocal and 1 patient (6.7%) negative. One patient of Grade-3 showed (100%) positive. Two grade-4 patients showed all (100%) positive. Majority of clinical diagnosis in Malang revealed grade-2 (71.4%) and in fact serological results showed that majority (80%) was positive. It was also happened to patient with clinical diagnosis grade-1, grade-3 and grade-4. In

fact, all of them (100%) had positive serology. PCR examination on 3 patients with clinical diagnosis grade-1 showed all of them (100%) were negative. In 15 patients of grade-2, 7 patients (46.7%) had positive virus den-2 and 8 patients (53.3%) negative. One patient of grade-3 was negative (100%). Two patients of grade-4 showed that 1 patient (50%) positively having virus Den-2 and 1 patient (50%) negative.

Examination results on 31 DHF patients in Jember showed clinical diagnosis grade-1 was in 15 patients (48.4%), grade-2 in 12 patients (38.7%), grade-3 in 3 patients (9.7%) and grade-4 in 1 patient (3.2%). Serological examination in 15 patients with clinical diagnosis grade-1 showed 5 patients (33.3%) positive, 1 patient (6.7%) equivocal and 9 patients (60%) negative. In 12 patients with clinical diagnosis grade-2, 10 patients (83.3%) positive and 2 patients (16.8%) negative. Three patients of grade-3 were all (100%) positive, and 1 patients of grade-4 was also positive (100%). These findings showed that majority of the patients' clinical diagnosis were grade-1 (48.4%) and grade-2 (38.7%) and the result of grade to serological examination was found to be 83.3% positive, while grade-3 and grade-4 were all positive. PCR examination result in 15 patients with clinical diagnosis grade-1 showed 1 patient (6.7%) had positive Den-2 virus, and 14 patients (93.3%) negative. In 12 grade-2 patients 5 patients (41.7%) had positive Den-2 virus and 7 patients (58.3%) negative. In 3 grade-3 patients all were negative (100%). In one grade-4 patient it was also negative (100%). The virus found were all Den-2 serotype.

This research result showed that : 1) Surabaya, Malang and Jember, which are different in geographical condition had Dengue virus of serotype Den-2, except for Surabaya where there was one double infection case with serotype Den-2 and Den-3. 2) The existence of double infection of Den-2 and Den-3 caused DSS (grade-4). 3) Molecular characterization of Den-2 serotype found in Surabaya, Malang and Jember in fact had homology more than 80%, compared to Den-2 isolate from Jakarta, USA and Jamaica which had homology of less than 73%. 4) Serological pattern using *Captured* **ELISA** showed that the majority was secondary infection as compared to primary infection, which was

in Surabaya 35.7%, Malang 76.2% and Jember 54.8%. It means the three areas showed an endemic of DHF disease. It was found a negative serological result, yet PCR examination revealed the existence of Dengue virus. This indicated that even if the serological result was negative, it did not get rid off Dengue virus infection.

Based on the research result, we would like to convey several suggestions: 1) to reduce the incidence and morbidity rates of DHF, we need intersectoral coordination 2) It is necessary to conduct further research on this DHF disease with the purpose to find effective method on vector control, to find method for early appropriate diagnosis, to find a more suitable candidate Dengue vaccine with the characteristics of Dengue virus in each region, to explore the pathogenesis and immunology of DHF which is still not clearly defined, including the molecular biology about the probability of virus genetic mutation.