Molecular Surveillance of Dengue Virus in Bangkalan, Madura Island, Indonesia, 2012-2014

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Abstract: The dengue virus (DENV), which belongs to the family Flaviviridae and the genus Flavivirus was endemic in tropical or sub-tropical areas and was transmitted to human by *Aedes aegypti*. Bangkalan is one of the largest cities in the dengue endemic region in East Java Province, Indonesia. This study, aimed to understand the dynamic of dengue cases in Bangkalan, were performed a molecular surveillance and serotyping. Dengue detection and serotyping were observed using Reverse Transcriptase-Polymerase chain reaction (RT-PCR). A total of 359 samples suspected with dengue infection were collected in Bangkalan Madura Island in 2012, 2013, and 2014, which successfully isolated 17 viruses with positive dengue infection. Serotyping study revealed the predominance of DENV-1, presented in 9 isolated viruses, DENV-2 presented in 7 isolated viruses, and DENV-3 presented in 1 isolated virus.

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1 INTRODUCTION

Dengue is a kind of infectious disease that was distributed in the tropical and sub-tropical areas (Green and Rothman, 2006). Dengue was transmitted to human by *Aedes aegypti*. More than 250,000-500,000 dengue infection cases occurred in the world every years (Wilder-Smith et al. 2010). Dengue causes various clinical manifestations, ranging from dengue fever (DF) to more severe forms of the disease, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Martina et al. 2009). Four distinct serotypes were reported, DENV-1, DENV-2, DENV-3, and DENV-4.

Indonesia is one of the largest countries in the dengue endemic region worldwide (Gubler, 2002). Indonesia is a vast archipelago country that is regularly affected by the disease. Dengue occurs in all 34 provinces in the country annually and periodic major outbreaks occur regularly (Nusa et al. 2014).

Bangkalan is a city located in the Madura island, Indonesia, located 37.8 Km from Surabaya. Dengue cases were reported in Bangkalan every year (Department of Health East Java Province, 2013). In this study, we performed a molecular surveillance study to understand the dynamic of dengue cases in Bangkalan. We reported information about the prevalence of the dengue disease, as well as the DENV serotype distribution in the city.

2 EXPERIMENTAL

2.1 Samples Collection

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). A total of 359 selected patients having febrile, grading severity of dengue infection followed by WHO criteria 2011. Blood samples were collected from patients, aseptically processed for the separation of serum and

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keep in -20 ℃ deep freeze at hospital. After 2 week were transferred to -80 ℃ deep freezer in Institute of Tropical Disease (ITD) Universitas Airlangga till processed futher for virus isolation in Vero cell (Sucipto et al. 2018).

2.2 Virus Isolation in Vero Cell

Serum specimens diluted with Vero culture medium (1:10) were inoculated to Vero cell monolayer (Yamanaka et al. 2011) at 37℃ in a 5% CO_2 for seven days. After three blind passages, cells were subjected to immunostaining with flavivirus group cross-reactive monoclonal antibody (D1–4G2; American Type Culture Collection, Manassas, VA) to examine the presence of viral antigens (Konishi et al. 2010). Antigen-positive cells were subjected to RNA extraction by using the QIAamp® viral RNA Mini Kit (Qiagen, Cat. No. 52906).

2.3 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Serotyping using multiplex RT-PCR complied by Lanciotti et al., 1992 (Lanciotti et al. 1992). Reverse transcriptase-PCR was performed in a Superscript III (Invitrogen). The PCR amplification reaction was performed in r-Taq Polymerase (Toyobo) 0.1 µL. The PCR program was 94°C for 30 second, 94°C for 1 minutes, 50°C for 1 minutes, 72°C for minutes, 72°C for 7 minutes.

3 RESULT AND DISCUSSION

A total of 359 samples from suspected dengue infection cases were collected in Bangkalan, Madura Island in 2012, 2013, and 2014. RT-PCR confirmations were performed to detect the presence of virus on those 359 serum samples and successfully isolated viruses from 17 positive dengue infections (Table 1). The result of serotyping identified the presence of all DENV serotypes in Bangkalan, with DENV-1 as the predominant serotype, followed by DENV-2 and also DENV-3. Of the 17 isolated viruses, serotyping study revealed the predominance of DENV-1, presented in 9 isolated viruses. DENV-2 was presented 7 isolated viruses. DENV-3 was presented in 1 isolated virus.

The DENVs were detected very low in the samples. This is due to the storage of serum in the hospital is not at -80 °C, causing the virus to be unstable and broken (Young et al. 2000).

The predominant DENV-1 serotypes in Bangkalan found in this study, is currently reported in other cities in East Java Province-Indonesia including, in Surabaya (Kotaki et al. 2014; Yamanaka et al. 2011). The previous study suggests the possibility that a DENV-1 outbreak in Bangkalan, with 459 dengue cases reported in 2013 by Department of Health of East Java, is similar to that observed in Surabaya in the same year (Department of Health East Java Province, 2013). It may be caused by travellers from Bangkalan that visit Surabaya for leisure and Bangkalan is one of the regions in Madura Island that have been supplying workers for cities Surabaya. Based on data from the governor decision of East Java Province states that the pay of workers in Bangkalan is less than in Surabaya.

In Indonesia, dengue occurred for the first time as an outbreak in Surabaya in 1968 (Hotta et al. 1970; Sumarmo, 1987). Dengue fever had spread to all regions of the province with increasing number of cities infected. In Jakarta from 1975 until 1978, dengue cases were reported. DENV-3 was predominantly presented, followed by DENV-2, DENV-1, and DENV-4 (Sumarmo et al. 1983). In 2004, there was an outbreak of dengue again in Jakarta, with DENV-3 being the most predominant serotype, similar to be outbreak in 1975 until 1978, followed by DENV-4, DENV-2, and DENV-1 (Suwandono et al. 2005). In 2000 to 2002, it was reported that dengue cases in Bandung, West Java Province, in which DENV-2 was the predominant serotype identified (Porter et al. 2005). In Sukabumi, West Java Province, serotyping revealed the predominant of DENV-2, followed by DENV-1, and also DENV-4. DENV-3 was not detected (Nusa et al. 2014). In the Central Java Province, Semarang city, serotyping identified the presence of all DENV serotypes, which DENV-1 genotype I and II as the predominant serotypes, followed by DENV-2 with cosmopolitan genotype, and also DENV-3 with genotype I (Fahri et al. 2013). In Surabaya East Java Province, DENV-1 was reported as predominant in November 2008 until June 2013, while DENV-2 was predominantly reported before November 2008 and after June 2013 (Kotaki et al. 2014; Yamanaka et al, 2011).

Table 1. The result of serotyping study in 17 positive dengue-infected samples from 2012 to 2014

No.	Code of Samples	Serotype	Years
1.	M43	DENV-1	2012
2.	M48	DENV-1	
3.	M50	DENV-1	
4.	M54	DENV-1	
5.	M56	DENV-1	
6.	M60	DENV-1	
7.	M105	DENV-2	2013
8.	M107	DENV-2	
9.	M145	DENV-2	
10.	M152	DENV-2	
11.	M158	DENV-2	
12.	M163	DENV-3	
13.	M280	DENV-1	2014
14.	M282	DENV-2	
15.	M298	DENV-1	
16.	M340	DENV-2	
17.	M348	DENV-1	

4 CONCLUSIONS

This study has observed the first molecular data of DENV in Bangkalan, Madura Island, Indonesia. Serotype predominance is similar to that from molecular surveillance study in Surabaya, suggesting the endemic nature of the infecting virus that caused a high prevalence of dengue in Bangkalan. This study shows the importance of continuous virus surveillance in dengue endemic areas better understanding in the dynamic of dengue infection disease in Indonesia.

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