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ACCURATE DETECTION OF VIRAL SEROTYPE DENGUE HEMORRHAGIC FEVER THROUGH *AEDES SP* MOSQUITOES USING *REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)*

Erlin Yustin Tatontos[a*] ; Fihiruddin[b] ; Nurul Inayati[c]

[a, b,c] *Poltekkes Kemenkes Mataram ; Prabu Rangkasari street ; Dasan Cermen ; Mataram 83232 ; Indonesia*

Abstract

Dengue Hemorrhagic Fever (DHF) caused Dengue virus which transmitted through the bite of the *Aedes sp*. Detection of dengue virus serotypes is essential for epidemiology as well as potential disease pathogens. RT-PCR method was more effective in mosquitoes, because the virus contained in the mosquito body is passed on to the next generation (trans ovarian). The purpose of this research is to know the serotype of DHF virus accurately through vector mosquito using RT-PCR method in Mataram City, so that vector control, early diagnosis and management of DHF disease could be done quickly and precisely. This research was a laboratory explorative research with cross sectional design that determines serotype of DHF virus through vector mosquito using RT-PCR method in Mataram City. The population of this study were Aedes aegypti female mosquitoes from eggs. Collected from houses located in Community Health Center Mataram city worked area with high dengue cases: Pagesangan, Karang Taliwang, Karang Pule, Tanjung Karang, Mataram, and Selaparang especially around the house's sufferers. The results showed that only the mosquito group from the worked area of the Mataram Community Health Center showed the ribbon image with the base pair in accordance with serotype 1 (DENGUE 1), the other negative.

Keywords: *Viral serotype ; Dengue hemorrhagic fever ; Aedes sp ; RT-PCR*

1. Introduction

Dengue Hemorrhagic Fever is an infectious disease caused by dengue virus that is transmitted through the bite of *Aedes aegypti* mosquito which the main vector and Ae. albopictus which the potential vector. Dengue virus Dengue Hemorrhagic Fever (DHF) is an infection is a major public health problem in the tropics and sub tropics. Two and a half billion people living in more than 100 countries at risk of dengue virus infection, with cases of 20 million sufferers each year and resulting in 24 million deaths (WHO, 2011).

The dengue virus belongs to the Arthropod-Borne Virus (Arboviruses) B group known as the genus Flavivirus, the Flaviviridae family. There are four serotypes DENGUE (DEN) 1, DEN 2, DEN 3, and DEN 4. Infections due to one serotype will cause antibodies against the serotype concerned not to the other serotype (Depkes RI, 2004).

DHF disease in Indonesia was first reported in 1968 as an outbreak in Surabaya with 58 cases and 24 deaths. But definite confirmation through the isolation of new viruses could be done in 1970 and in 1980 its spread extends to various provinces, so until now DHF is still one of endemic disease (InfoDatin Kementerian Kesehatan, 2016; Wahyuni et al., 2005; Yudhastuti & Vidiyani, 2005).

DHF was a public health problem in NTB province because of its rapid spread, potentially death and all districts or cities have been infected with DHF. By 2014 the number of dengue cases found is 872 cases, rising very significantly to 1340 cases or an increase of 53.67% in 2015. Most cases

*) Corresponding Author (Erlin Yustin Tatontos)
E-mail: erlintatontos64@gmail.com

are reported in Sumbawa, East Lombok and Mataram (Dinas Kesehatan Provinsi NTB, 2015).

The Case of Dengue Hemorrhagic Fever in Mataram City was first reported in 1986 and peaked in 1998 with 715 cases and a Case Fatality Rate of 1.4%(Yustin Tatontos, 2008). Dengue cases in Mataram city since January to April 2016 as many as 412 cases with 6 deaths and until now cases of dengue fever still continue to appear. Dengue fever in Mataram City has been endemic throughout Mataram City with the highest case in Community Health Center worked area, Karang Pule, Karang Taliwang, Tanjung Karang, Mataram and Selaparang (Dikes & Mataram, 2016; Ridwan M Fauzi (Republika), 2016).

The four serotypes of dengue virus were found in various regions of Indonesia and the dominant ones were DEN-3 (Karyanti & Hadinegoro, 2016). The dominant serotype of Dengue virus in Java was DEN-3, whereas in Medan the dominant was DEN-2 (Mashoedi, 2007; Nurfadly, 2009). Serotype of dengue virus in two urban villages in Mataram, Inayati research result almost all serotype of dengue virus exist except DE N 4 (Inayati et al., 2011).

Dengue virus detection can be done through culture method, immunohistochemistry of *Streptavidin Biotin Peroxidase Complex (SBPC)* and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method. Dengue virus was generally very labile to heat (thermolabile), special precautions were needed to prevent virus inactivation due to heat so that the culture method becomes complicated and expensive, the result takes one week (Genis, 2007; Yong et al., 2007). Immunohistochemical methods could detect only the presence of dengue virus transmission in mosquitoes and did not specify virus serotypes (Trovancia et al., 2016). The RT-PCR method could detect dengue virus in patients within four hours, but the virus could be detected in humans only during viremia. The RT-PCR method was more effective in mosquitoes, because the virus contained in the mosquito body was lowered in the next generation (transovarian) (Hasmiwati, Dahelmi, 2009; Joshi V1, Mourya DT, 2002).

Viral serotype studies in Mataram City in areas with the highest cases of DHF were conducted so that more effective eradication goals, early diagnosis and management of DHF diseases could be done quickly and accurately. Previous research was limited to two sub-districts in one sub-district of Mataram City(Inayati et al., 2011).

The results of this study indicate that only *Aedes aegypti* from the worked area of Mataram Community Health Center which shows the ribbon image with the base pair accordance to serotype 1 (DEN1) the other negative. Form a band image mosquito group from five other worked areas of Community Health Center in Mataram city did not accordance to the base pair of dengue virus serotype 1, 2, 3 or 4.

2. Method

This study was a descriptive study with a crosssectional design. Population and samples was Aedes sp mosquitoes. Data collection of mosquito eggs was done by laying the ovitrap in the worked area of six Puskesmas of Mataram City with the highest case of DHF. Ovitrap was placed in the house of DHF patients and other houses within 100 meters (Perich MJ1, Kardec A, Braga IA, Portal IF, Burge R, Zeichner BC, Brogdon WA, 2003).

Colonization of *Aedes sp* by soaking filter paper containing mosquito eggs in a plastic cup according to the location then stays for 1-2 days until it hatches into a larvae. Then the larvae that have become pupa moved to paper cup that has been in the water content, then covered with gauze. An adult mosquito will appear after 2 days.The mosquito wasbe silenced for 7 days, then transferred to the eppendorf tube and stored at -25° C (Inayati et al., 2011).

Determination of the DHF vector in Mataram by identifying colonized *Aedes sp* adult female mosquitoes. Identification using a magnifying glass and mosquito identification key. If there is a longitudinal white ribbon image on the mesonotum of the mosquito then the species is *Aedes albopictus.* If there is a picture of a white band shaped like a harp instrument on the mesonatum of the mosquito then the spesies is *Aedes aegypti.* Dengue virus serotype data were processed and analyzed descriptively based on the size of the fragment image (band) in accordance base pair (bp) after the test using RT-PCR method.

3. Results and Discussion

Determination of serotype DBD virus RT-PCR method using samples of mosquitoes that have been colonized from mosquito eggs collection of six worked areas Community Health Center with the highest case of DHF. One-Step Multiplex RT-PCR checking, using Dcon primer,

D1, D2, D3, and D4 the results as shown in Figure 2.

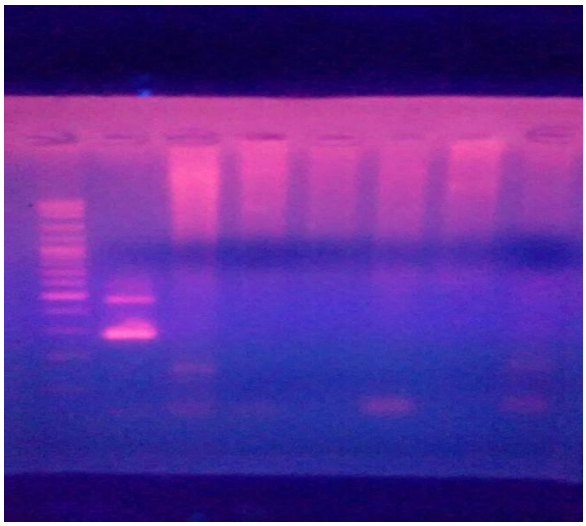

Figure 1. *Serotype DHF virus using RT-PCR method in Mataram*

Result of determination of DHF virus in Mataram City of RT-PCR method, showed the formation of band image accordance to the base pair of dengue virus serotype 1 (DEN1) in the mosquito group from the worked area of Mataram Community Health Center. Form a band image mosquito group from five other worked areas of Community Health Center Mataram city did not accordance to the base pair of dengue virus serotype 1, 2, 3 or 4.

Mataram city was a developing city and was one of the national and international tourist destinations. With dengue cases increasing every year and the widespread spreading area of almost all Mataram City, the determination of DHF vector and dengue virus detection was needed.

Negative results in the mosquito group from the five working areas of the Community Health Center with the highest dengue fever cases could be caused by the overpopulation worked area of the Community Health Center that oversees two and several sub-districts (kelurahan), although the mosquito eggs have been attempted to target dengue fever cases.

The dengue virus belongs to the Arthropod-Borne Virus (Arboviruses) B group known as the genus Flavivirus, the Flaviviridae family. There are four serotypes, namely: DEN 1, DEN 2, DEN 3, and DEN 4 (Hadinegoro, 2004). The dengue virus isolation survey conducted by the Agency for Research and Development of the Department of Health and the NAMRU-2 laboratory in Jakarta shows that from 1972 to 1992 in Indonesia the four dengue serotypes were found in mild to severe dengue cases treated.

On the seventeen-year observation, dengue virus serotype 3 and 2 was the dominant serotype, followed by serotypes 1 and 4. In 2004, the Ministry of Health Research and Development conducted viral isolation with RT-PCR during the extraordinary period of DHF. Serotype of dengue virus 3 was still the most dominant serotype (57%), followed by serotype 2 (13%), serotype 4 (20.7%) and serotype 1 (5.6%), while dengue virus infection mixed from serotype 3 and 1 found a number of 3.7% (Karyanti & Hadinegoro, 2016).

Determination of dengue virus serotypes was important for epidemiology and also determines the potential pathogens of the disease to the population. Recent studies to determine the serotype of circulating dengue virus in each region need to be done to see the potential pathogenicity of dengue virus in the area and time(Andriyoko et al., 2011).

Double infection of heteroserotypes of dengue viruses in field populations of Aedes aegypti and Aedes albopictus in Thailand. The incidence of multiple serotypes of dengue virus in *Ae. aegypti* and *Ae. albopictus* in the same area points toward a high risk for an epidemic of DHF (Thavara et al., 2006).

RT-PCR method used in samples of suspect dengue patients was expensive and samples must be taken on the fifth day when viremia, so the detection of dengue virus with RT-PCR method is easier on vector mosquito sample. The difficulty faced by this research was the collection of mosquito eggs in the houses of the inhabitants, because not all residents allow their houses to be installed ovitrap even often discarded. So it takes a long time to convince residents in dengue endemic area of Mataram City.

The limitation of this study is that the dengue virus serotype was not detected in *Aedes aegypti* mosquitoes in five other Community Health Center working areas. Therefore, further research is needed to detect dengue virus serotypes in all working areas Community Health Center of the Mataram City. In addition, to prevent the occurrence of the DHF Outbreak, it is necessary to monitor with a good information system. As has been implemented in the city of Semarang with an outbreak monitoring information system based on dengue fever Geographic Information System (GIS) (Masrochah et al., 2017).

4. Conclusion and Suggestion

Serotype of Dengue virus in Mataram City detected from *Aedes aegypti* mosquitoes with RT-PCR method was serotype 1 (DEN 1). Serotype of Dengue virus that could be detected only from *Aedes aegypti* mosquito group from the worked area of Mataram Community Health Center. Further research needed to detect the serotype of dengue virus, in each worked area of Community Health Center with high dengue cases in Mataram City by dividing the smaller area.

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6. References

Andriyoko, B., Parwati, I., Tjandrawati, A., & Lismayanti, L. (2011). Penentuan Serotipe Virus Dengue dan Gambaran Manifestasi Klinis serta Hematologi Rutin pada Infeksi Virus Dengue Dengue Virus Serotyping and Its Clinical Manifestation and Routine Haematology in Dengue Infections. *Mkb*, *44*(4), 253 – 260. http://journal.fk.unpad.ac.id/index.php/mkb/article/view/138/pdf_42

Dikes, S. P. B., & Mataram, K. (2016). *Perkembangan Sebaran Kasus DBD di Kota Mataram*.

Dinkes NTB. (2015). *Profil Dinas Kesehatan Provinsi Nusa Tenggara Barat*. http://www.depkes.go.id/

Genis, G. (2007). *Demam Berdarah - Dr Genis Ginanjar - google books*.

Hadinegoro, S. R. H. (2004). *Tata Laksana DBD* (3rd ed.). DepKes RI. https://silahuddinm.files.wordpress.com/2013/02/bk2007-g4.pdf

Hasmiwati, Dahelmi, and N. (2009). Deteksi Virus Dengue dari Nyamuk Vektor Aedes aegypti di Daerah Endemik Demam Berdarah Dengue (DBD) di Kota Padang dengan Metode Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). In *Gudang Penyimpanan Data Ilmiah Universitas Andalas*. http://repository.unand.ac.id/id/eprint/739

Inayati, N., Supargiyono, & Umniyati, S. R. (2011). The Differences of the Prevalences and Serotypes of Dengue Virus on Aedes Aegypti Mosquitoes from Pagutan and PagutanTimur in the Sub District of Mataram. *Tmj*, *02*(1), 1–11.

InfoDatin Kementerian Kesehatan. (2016). *Situasi DBD di Indonesia*. InfoDATIN. https://doi.org/ISSN 2442-7659

Joshi V1, Mourya DT, S. R. (2002). Persistence of dengue-3 virus through transovaria mosquitoes. *Am J Trop Med Hyg. 2002 Aug;67(2):158-61.*, *Aug;67(2)*, 158-61.

Karyanti, M. R., & Hadinegoro, S. R. (2016). Perubahan Epidemiologi Demam Berdarah Dengue Di Indonesia. *Sari Pediatri*, *10*(6), 424. https://doi.org/10.14238/sp10.6.2009.424-32

Mashoedi, I. D. (2007). Hubungan antara Distribusi Serotipe Virus Dengue dari Isolat Nyamuk Aedes spesies dengan Tingkat Endemisitas Demam Berdarah Dengue: Studi Kasus di Kota Semarang [Universitas Diponegoro Semarang]. In *Epidemiology*. http://eprints.undip.ac.id/5258/1/Imam_Djamaluddin_Mashoedi.pdf

Masrochah, S., Susanto, E., & Irmawati, I. (2017). Sistem Informasi Pemantauan Kejadian Luar Biasa (Klb) Demam Berdarah Berbasis Geographic Information System (Gis) Di Kota Semarang. *Jurnal Riset Kesehatan*, *5*(2), 53. https://doi.org/10.31983/jrk.v5i2.798

Nurfadly. (2009). Deteksi dan penentuan serotipe virus dengue tipe 1 dari nyamuk Aedes aegypti dengan menggunakan Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) di Kota Medan. In *USU Repository*.

Perich MJ1, Kardec A, Braga IA, Portal IF, Burge R, Zeichner BC, Brogdon WA, W. R. (2003). Field evaluation of a lethal ovitrap against dengue vectors in Brazil. *Med Vet Entomol*, *17 (2)*, 205–210. https://onlinelibrary.wiley.com/doi/10.1046/j.1365-2915.2003.00427.x

Ridwan M Fauzi (Republika). (2016, April 19). *Ada 426 Kasus DBD di Mataram , Wali Kota*. *April*, 2016.

Thavara, U., Siriyasatien, P., Tawatsin, A., Asavadachanukorn, P., Anantapreecha, S., Wongwanich, R., & Mulla, M. S. (2006). Double infection of heteroserotypes of dengue viruses in field populations of Aedes

aegypti and Aedes albopictus (Diptera: Culicidae) and serological features of dengue viruses found in patients in southern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *37*(3), 468–476.

Trovancia, G., Sorisi, A., & Tuda, J. S. B. (2016). Deteksi transmisi virus dengue pada nyamuk wild Aedes Aegypti betina di Kota Manado. *Jurnal E-Biomedik (EBM)*, *4*(2).

Wahyuni, C., Keman, S., & Fathi, F. (2005). Peran Faktor Lingkungan dan Perilaku terhadap Penularan Demam Berdarah Dengue di Kota Mataram. *Jurnal Kesehatan Lingkungan Unair*, *2*(1), 3944.

WHO. (2011). Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. In *WHO Regional Publication SEARO*. https://doi.org/10.1017/CBO9781107415324.004

Yong, Y. K., Thayan, R., Chong, H. T., Tan, C. T., & Sekaran, S. D. (2007). Rapid detection and serotyping of dengue virus by multiplex RT-PCR and real-time SYBR green RT-PCR. *Singapore Medical Journal*, *48*(7), 662 – 668. http://www.ncbi.nlm.nih.gov/pubmed/17609830

Yudhastuti, R., & Vidiyani, A. (2005). Hubungan Kondisi Lingkungan, Kontainer, Dan Perilaku Masyarakat Dengan Keberadaan Jentik Nyamuk Aedes Aegypti di Daerah Endemis Demam Berdarah Dengue Surabaya. *Jurnal Kesehatan Lingkungan Unair*, *1*(2), 3942. https://www.mendeley.com/catalogue/d2787957-a1ce-3d8f-977e-ccb2f3853b2d/?utm_source=desktop&utm_medium=1.19.4&utm_campaign=open_catalog&userDocumentId=%7B3338be6d-1dfe-38ea-8fd0-5b881a0cd6d9%7D%0D

Yustin Tatontos, E. (2008). *Uji Resistensi Larva Aedes sp dari daerah Endemis Demam Berdarah Dengue Kota Mataram dan Larva Aedes aegypti Koloni Lembaga Penyakit Tropis Universitas Airlangga Surabaya terhadap temefos* [Airlangga, surabaya]. https://fk.unair.ac.id/archives/2008/08/10/uji-resistensi-larva-aedes-sp-dari-daerah-endemis-demam-berdarah-dengue-kota-mataram-dan-larva-aedes-aegypti-koloni-lembaga-penyakit-tropis-universitas-airlangga-surabaya.html