

## Dengue Virus Detection Using Rt-Pcr Method In Ternate City, North Maluku, Indonesia

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### Abstract

The Dengue disease is caused by a virus from the family Flaviviridae and there are four distinct, but closely related, virus serotypes that cause dengue (DENV-1, DENV-2, DENV-3 and DENV-4). Dengue virus detection is very important to determine the virus serotype that develops in an area. There are several methods to detect dengue virus based on different targets, namely nucleic acids, viral antigens, and antibodies. This study aims to determine the type of dengue virus serotype in four working areas of the Public Health Center in Ternate City. The sample in this study was the eggs of *Aedes sp* mosquitoes caught in the homes of DHF sufferers and the houses around them. Collection of mosquito eggs *Ae. Aegypti* was carried out in four Puskesmas working areas in Ternate City. Ovitrap installation was carried out in 200 houses, with a total of 400 ovitraps. Rearing eggs and dengue virus detection were carried out at the Microbiology Laboratory, LITBAGKES Banjarnegara. The method used in this research is the RT-PCR test. The data analysis of the research results was carried out descriptively. Based on the identification results, the mosquito used in this study was the *Aede aegypti* mosquito. The results of electrophoresis produced viral RNA with a base length of 100 bp, while the target RNA that had been determined were Den1 = 342 bp, Den2 = 251 bp, Den3 = 538 bp. Den4 = 752 bp. The results of the RT-PCR examination showed that the *Ae. aegypti* in the four working areas of the Puskesmas did not contain the dengue virus (virus negative).

**Keywords:** Detection, Dengue Virus, RT-PCR, Ternate City

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### I. INTRODUCTION

Dengue Fever Disease (DBD) is a contagious disease caused by dengue virus transmitted through the bite of infected *Aedes aegypti* and *Aedes albopictus*. DBD is an environmental based disease as a result of unhygienic environmental sanitation that does not qualify health requirements.[1] DBD is found distributed in tropical and subtropical regions, especially in Southeast Asia.[2,3] Dengue Fever Disease is caused by Dengue virus composed of four virus serotypes: den-1, den-2, den-3 and den-4. The dengue virus serotypes are found infecting humans throughout Indonesia.[4,5] Dengue virus transmission is influenced by three important factors: human, virus, and intermediate vector.[6] Dengue virus transmission may occur when the vector sucks human blood that is having viremia and then mo

ves to suck healthy human blood.[5] Dengue virus detection is important in order to identify the virus serotypes developing in an area. There are some methods to detect dengue virus based on different targets, namely nucleic acid, viral antigen, and antibody.

[7] Specific viral antigen and antibody can be found with ELISA method or rapid test kit. Nucleic acid, meanwhile, can be detected using PCR (Polymerase Chain Reaction) method after genome isolation.[7] ELISA and PCR methods have been used to detect Dengue virus in 2000s, replacing the immunology method.[8] This research used the RT-PCR (Real Time Polymerase Chain Reaction) method. The RT-PCR method is recommended by WHO and can quite sensitively detect Dengue virus.[9] (WHO, 2021). Wowor used the RT-PCR method to detect Dengue virus from serum or plasma in 2011.[10] Meanwhile, Sorisi also used the RT-PCR method to detect Dengue virus from mosquitoes in 2013.[11] Putri *et al.* used the RT-PCR method to detect Dengue virus transovarially in 2018.[12] Later, Putri *et al.* stated that the use of RT-PCR method to detect Dengue virus more quickly using mosquito larvae that were directly collected from the nature.[12] Accurate information of the types of Dengue virus serotype in Ternate City is not yet known clearly. DBD disease can be controlled through its vector through fogging for adult mosquitoes and temefos (abate) for larvae. It is necessary to detect dengue virus serotypes for correct and spot on preventive efforts. This research aimed at examining the types of Dengue virus serotype in endemic region, that was distributed in four working areas of Community Health Centers (*Puskesmas*) in Ternate City using the RT-PCR method.

## II. METHODS

This research used descriptive method with laboratory test. this research was conducted from September to December 2021. This research consists of two stages: First; egg collection in four Community Health Centers' working areas in Ternate City (Community Health Center in Gambesi, Kalumata, Kota and Siko). (Figure 1) Second; Egg rearing, identification of types of mosquitoes and virus detection using the RT-PCR method at the Microbiology Laboratory of Health Research and Development Office (*Balai LITBANGKES*) in Banjarnegara.



**Fig 1.** The Working Areas of Gambesi, Kalumata, Kota and Siko Health Centers.

Egg collection of *Aedes spp* was performed by applying ovitrap to 50 houses (totally 200 houses) in four *Community Health Center* working areas. The sample houses for ovitrap application were those of patients and surrounding houses. There were 400 ovitraps applied outdoor and indoor.[7] The egg samples of each *Community Health Center* working areas were sent to the Microbiology Laboratory of Health Research and Development Office in Banjarnegara for rearing and identification, for later test using the RT-PCR method.[7] The identification results of the Dengue virus serotypes (Den1, Den 2, Den 3 and Den 4) were analyzed descriptively and presented in table and figure

## III. RESULT AND DISCUSSION

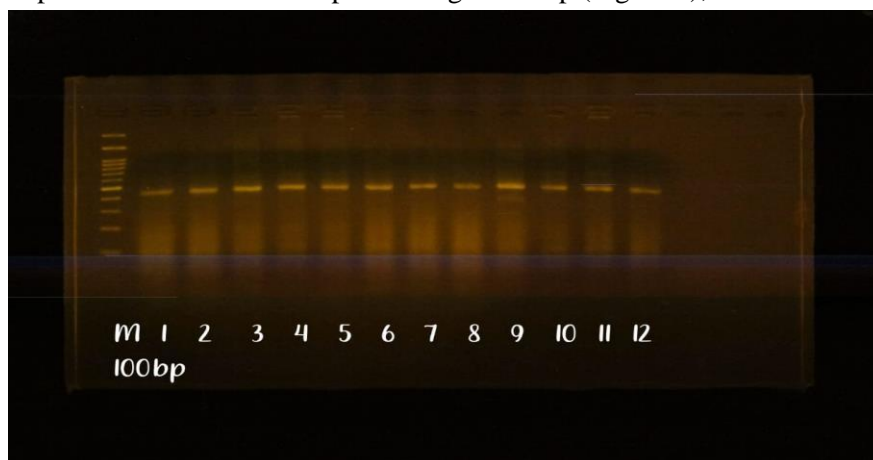
The results of RT-PCR test in the endemic region in the four *Community Health Center* working area in Ternate City on *Ae aegypti* show that there was no mosquito found positive dengue virus. For detail, see Table 1 below: Results of Dengue virus detection in mosquitoes using the RT-PCR method

**Tabel 1. Results of Dengue virus detection in mosquitoes using the RT-PCR method**

No	Sample Code	mosquito species	Number	Positive Poll	Description
1	0297/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
2	0298/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
3	0299/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
4	0300/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
5	0301/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
6	0302/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
7	0303/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
8	0304/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
9	0305/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
10	0306/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
11	0307/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative
12	0308/0020-02-32-P/BAK/03/II/2021	<i>Ae. aegypti</i>	30	0	Negative

Based on the research results (Table 4) the mosquitoes found at the research location were *Ae. aegypti*. Virus detection was carried out at 12 points of observation in the four *Community Health Center* working areas in Ternate City that were endemic. There were 360 adult mosquitoes used from the result of rearing at the Microbiology Laboratory of Health Research and Development Office in Banjarnegara. There were 30 mosquitoes used for each observation point and each *Community Health Center* consisted of 3 pools (pools

1-3 from Gambesi Community Health Center working area, pools 4-6 from Kalumata Community Health Center working area, pools 7-9 from City Community Health Center working area and pools 9-12 from Siko Community Health Center working area). The Dengue virus detection in this research was carried out in two stages. First stage, modification of virus's RNA into cDNA and general amplification of Dengue virus. Second stage, amplification of virus's cDNA with the specific primary for those four serotypes of Dengue virus. The sample amplification resulted in amplicon length 100 bp (Figure 2);



**Fig 2.** The result of agarose gel electrophoresis from amplification result using the Single-Tube Multiplex RT-PCR Method on the sample mosquitoes from Ternate city.

Based on the determinant criteria of virus Den1= 342 bp, Den2= 251 bp, Den3= 538 bp and Den4= 752 bp, the amplification results show that there was no Dengue virus detected (negative). Dengue Fever (DBD) Case always occurs in Ternate City annually. DBD endemic region determination increases from 4 endemic sub-districts in 2017 to 33 endemic sub-districts (42.8%), 27 Sporadic sub-districts (35%) and 5 potential sub-districts (96.5%) out of 77 sub-districts in Ternate City in 2021.[13]The result of mosquito identification at the Microbiology Laboratory of Health Research and Development Office in Banjarnegara on mosquito eggs from the four *Community Health Center* areas in Ternate City was *Ae. Aegypti*, since positive ovitrap was more dominant with the ovitrap applied in the houses. According to the Ministry of Health of the Republic of Indonesia in 2017 *Ae. aegypti* was found more in clear water located in the house. *Ae. aegypti* always lives close to human.[2] The electrophoresis in this research results in virus's RNA with amplicon length 100 bp. The result is different from the structure of target RNA's amplicon length used (Figure 2). The RT-PCR test result shows that *Ae. aegypti* egg in the *Community Health Center* working areas do not contain dengue virus (virus negative). This is the case may be because of the influence of limited sample collection, insufficient viral titer that it is undetectable by RT-PCR.[14] The other possibility is that, *Ae. aegypti* egg obtained is not from mosquito that contains Dengue virus serotype. Besides, it may be influenced by the long timing of sampling from the DBD case, and use of control through fogging and use of temefos (abate) by the Health Department during the DBD case in the patients' houses.

Unsuccessful detection of Dengue virus in the mosquitoes, in the opinion of Sasmanto *et al.* in 2012, can be influenced by temperature at the time of research. It is said that high or low temperature may disturb virus's resistance in mosquito's body. The use of chemicals (insecticides) and relatively short time of viremia is expected to influence Dengue virus isolation in mosquito's body.[14] Dengue virus's degraded RNA in mosquito that inhibits DNA amplification process disturbs detection of Dengue virus serotype.[15] The sample mosquitoes (eggs, larvae and adult mosquitoes) were taken from patients' houses after occurrence and KLB after fogging, that was one of the reasons to the unsuccessful detections of virus in mosquito's body. [15] According to Sorisi (2013), not all mosquitoes *Ae. aegypti* contain Dengue virus since not all mosquitoes can become DBD vector. This is related to vector vulnerability, vector age, biting habit, and food preference.[11] Besides, infection through egg is different between areas, thus low-dose viral infection will influence virus detection result. [11]Endemic area in Ternate City keeps increasing, but Dengue virus detection result via egg in Ternate City using RT-PCR method is negative. This is the case since that Dengue virus transmission process in Ternate City is expected to be through vector from sick to healthy people (Horizontal).[9] Factors influencing horizontal transmission include uncontrolled

urbanization, people mobility, climate change, socio-economic factor, population density and mode of transportation. [16,17] The same is also stated by Wijayanti that endemicity of an area is caused more by demographic and environmental factors such as population density, urban environment, rainfall, temperature, high humidity and potential population mobility and import case.[5] additional endemic areas in Ternate City may potentially be the new source of Dengue virus transmission.

Ternate City is a city trade and transit center for those traveling in and out of North Maluku Province. Mode of transportation and population density are quite high. This condition influences Dengue virus distribution in Ternate City. The data reveal that DBD case in Ternate City occurs more in densely populated area. This is one factor facilitating Dengue virus transmission by vector, because of vector's quite limited flying distance. [18] Dengue virus transmission can also be influenced by container (vector habitat) that is very close to human. People's awareness of reducing contact with *Ae. aegypti* is still lacking.[19] High contact frequency between mosquito and human will influence mosquito's biting frequency.[20] In addition, *Ae. aegypti* is anthropophilic that they like human blood, and their blood sucking behavior make them have high capacity in transmitting Dengue virus. [20] Dengue virus transmission also often occurs from pregnant mother to her child.[1] According to Basurko *et al.*, transmission from mother to child occurs both at the early and end periods of pregnancy. The research found 52 pregnancies infected by DBD, including three newborn babies showing neonatal dengue fever, requiring thrombocyte transfusion. [1]The overall negative result of the research in the four Community Health Center working areas in Ternate City does not mean that *Ae. aegypti* in Ternate City does not contain Dengue virus, since DBD cases are fluctuating and endemic regions increase annually. With no Dengue virus found via egg using RT-PCR method, it may be detectable using patients' blood serum or through directly catching adult mosquitoes from the field.

#### IV. CONCLUSION

The mosquitoes found during the research were *Ae. aegypti*. The electrophoresis resulted in virus's RNA with amplicon length 100 bp, while target RNA included Den1= 342 bp, Den2= 251 bp, Den3 = 538 bp. Den4 = 752 bp. The RT-PCR examination results show that *Ae. Aegypti*'s eggs from the four Community Health Centers' working areas in Ternate City (Community Health Center Gambesi, Kalumata, Kota and Siko) did not contain dengue virus (virus negative).It is necessary to do further research using patients' blood serum or through directly catching adult mosquitoes from the field for Dengue virus detection.

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