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Transovarial Infection of Dengue Virus in *Aedes aegypti* and *Aedes albopictus*

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Abstract. At present, Dengue Haemorrhagic Fever (DHF) is still a significant health problem, including in Kupang City. The existence of transovarial infection of Dengue virus is essential to know to support the prevention to be more effective and efficient. This study aimed to determine the existence of transovarial infections and the serotypes variability of Dengue virus in *Aedes sp* in Kupang City. This observational research was conducted in 9 villages in Kupang City Year 2017, where 20 houses in every village are observed to collect *Aedes sp* eggs. Cluster sampling was conducted to choose houses with DHF cases and surrounding areas 100 m from the cases. Ovitrap and Ovistrip were used to collect *Aedes sp* eggs, then *Aedes sp* eggs are reared in Parasitology Laboratory UGM. Adults mosquitoes from rearing were observed the transovarial Infection and Dengue Virus Serotype by One Step RT-PCR followed by nested PCR. This research found a transovarial infection in *Aedes aegypti* and *Aedes albopictus* and for Denvir-2 and Denvir-3. This finding is a change from previous findings in the same place where only Denvir-1 was transmitted transovarially in *Aedes sp*.

Keywords: *Aedes sp*, Dengue, Transovarial, Serotypes

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is a mosquito-borne viral disease that still a significant problem globally in public health (Murray, Quam, & Wilder-Smith, 2013; Yan et al., 2018). As a golbally problem, Dengue cases found mostly in tropical and subtropical areas (Ali, Asha, & Aneesh, 2014). The DHF is a significant disease transmitted by the *Aedes sp* vectors (Harapan, Michie, Mudatsir, Sasmono, & Imrie, 2019). High mortality, poverty, and social burdens are still problems caused by DHF (Harapan et al., 2019).

Dengue has become a threat for global health, and it is estimated that about half of the population in the world is at risk to get Dengue virus infection (Deng et al., 2020). Since DHF was founded in 1968 in Indonesia, DHF cases were getting higher and caused the irregular pattern of DHF outbreaks (Harapan et al., 2019). Incidence of DHF Indonesia in 2018 was 24.75 per 100.000 population. The data is different from Kota Kupang, where IR in 1999-2015 ranged from 11.1 to 266.5 cases per 100,000. This figure was always the highest in NTT and always higher than the national average in 2000 – 2015 (Kemenkes RI, 2019).

The DHF prevention and control activities in Kupang City have been done every year include eradicating mosquito nests, epidemiological investigations, focus fogging, and counseling (Kemenkes RI, 2019). However, the DBF incidence rate is always high and exceeds the national figure. The larva free rate is always low (<95%). Outbreaks often also occur in several cities/regencies in NTT province, including in Kupang City. The prevention and control of DHF in Kupang City have not sufficient to the decreasing of cases of DHF, so DHF cases getting higher and always can be found mortality of DHF cases every year.

It is known that DHF incidence is related to the environmental condition, season, high mobilization, density in the house and also in the community, housing development, and also community behavior (Kemenkes RI, 2019). Dengue virus as agent and mosquito as DHF vector are also essential factors in DHF incidence. Disease incidence will continue to occur if only taking the medication without controlling the risk factors according to existing nodes. This interaction varies from place to place and causes the degree of endemicity to differ between places and times. The DHF agents, namely the dominant dengue virus (Denvir), can differ from one region to another (Martín et al., 2010). Dengue viruses have four serotypes (Denvir-1, Denvir-2, Denvir-3, and Denvir-4) that can be found in the DHF patient and also in *Aedes sp* mosquito. The high number of dengue cases including in Indonesia is also supported because all four serotypes can be found. The mode of transmission of the dengue virus in also affects the increase in dengue cases, where dengue can also be transmitted vertically and horizontally. The transovarial infection in *Aedes sp* is essential because it causes the *Aedes sp* mosquito (*Aedes aegypti* and *Aedes albopictus*) can transmit the virus throughout its life and its descendants (Esteva & Vargas, 2000). For dengue treatments, there is no specific medicine recently, so for prevention need to focus on vaccine development and also vector control (Deng et al., 2020; Yan et al., 2018). For this reason, this study aims to determine the existence of transovarial infections and serotypes variability of Dengue virus in *Aedes sp* mosquitoes in Kupang City.

METHODS

This research is an observational descriptive with a cross-sectional design conducted in Kupang City. As samples research were nine villages in Kupang City that consist of 3 villages as the sporadic area of DHF and six villages are DHF endemics. Variables in this research are the existence of transovarial infection and Dengue serotype variability in *Aedes sp*. This research's subject was eggs of *Aedes sp* in houses with dengue cases and houses surrounding dengue fever sufferers with a radius of 100 meters. Eggs of *Aedes sp* collected from 20 houses in each village so that the total number of houses surveyed was 180 houses from 9 sub-districts. The houses surveyed were taken using cluster sampling since few DHF cases spread across several urban villages (Medical Research Institute & Dengue Coordination Unit, 2011; Usman & Akbar, 2009). Research is carried out in 2017 in the field or community in Kupang City to collect *Aedes sp* eggs and the parasitology laboratory UGM to observe the Dengue virus's existence in *Aedes sp* mosquitoes.

Aedes sp egg collected using ovitrap and ovistrip after one week installed inside and outside the dark and humid house, thought to have the potential to become the nesting place for the *Aedes sp*. Dengue virus examination was conducted in adult mosquitoes that emerged from *Aedes sp* eggs after reared around one week. The mosquitoes that come out of the pupa given a sugar-water solution, then all adult mosquitoes at least two days old are checked for the existence of transovarial infections and any Dengue virus serotypes.

Isolation of Dengue Virus RNA in Adult Mosquitoes aims to obtain pure RNA, which will be used for RT-PCR examination. Dengue Virus Serotype Determination by One Step RT-PCR followed by nested PCR. Serotype determination aims to detect the presence of Dengue virus genetic material using RT-PCR. The next step is electrophoresis. The electrophoresis result is said to be positive for Dengue virus one if it shows a diagnostic band at 483bp, positive for Dengue 2 if the diagnostic band is 119bp, positive for Dengue 3 if the diagnostic band is at 290bp, and positive for Dengue 4 if the diagnostic band is at 392bp (Lanciotti, Calisher, Gubler, Chang, & Vorndamt, 1992).

All the collected data be shown in the simple distribution table, figure, and also map. The data will then be analyzed descriptively to show the existence of the transovarial infection of Dengue Virus in *Aedes sp*. It also shows the serotype of the Dengue virus in Aedes Mosquitoes. The transovarial infection existence is also shown in Minimum Infection Rate (MIR) with the formula: one per total mosquitoes in one pool with constant 1000.

RESULTS AND DISCUSSION

This study conducted rearing from *Aedes sp* eggs into adult mosquitoes. The rearing results show that *Aedes albopictus* mosquitoes hatched from eggs more than *Aedes aegypti*, 70 *Aedes albopictus* can be found in 8 villages, while *Aedes aegypti* can be found in 1 village. Table 1 also shown that transovarial infection of Dengue virus in *Aedes aegypti* can be found in one village (Oetete) and *Aedes albopictus* in 4 villages (Manulai II, Maulafa, Naimata, and TDM).

Table 1. The Transovarial Infection Existence of Dengue Virus in *Aedes sp* Mosquitoes in Kupang City

Village	*Aedes aegypti*		Dengue virus	*Aedes albopictus*		Dengue virus
	n	%		n	%	
Pasir Panjang	0	0	-	9	100	-
Manulai II	0	0	-	10	100	+
Sikumana	0	0	-	5	100	-
Maulafa	0	0	-	8	100	+
Naimata	0	0	-	11	100	+
Kolhua	0	0	-	14	100	-
Lasiana	0	0	-	9	100	-
TDM	0	0	-	4	100	+
Oetete	5	100	+	0	0	-
Total	5	7	-	70	93	-

Notes: (+) means there is Dengue virus in mosquitoes; (-) means there is no Dengue virus

Based on the mosquito species, MIR on *Aedes aegypti* is 200‰, while in *Aedes albopictus* is 90 - 250‰, as shown in Table 2. The MIR for Denvir-2 was 100– 125‰, while for Denvir-3, it was 90–250‰. Table 2 also shown that transovarial infection can be found in the serotype of Denvir-3 for *Aedes aegypti* . On the other hand, Denvir-2 and Denvir-3 also can be found in *Aedes albopictus*. In adult *Aedes sp*, the presence of transovarial infection of Denvir-2 from rearing eggs was detected in 2 villages from 9 villages in Kupang City that be observed (22.2%), and Denvir-3 can be found in 3 villages (33.3%). In contrast, from 4 urban villages (44, 5%), no dengue virus was found, as shown in Table 2.

Table 2. Minimum Infection Rate and Denvir Serotype in *Aedes sp* in Kupang City

Village	Serotypes		Mosquitoes / pool	MIR (‰)
	Ae. aegypti	*Ae. albopictus*		
Pasir Panjang	-	-	9	100
Manulai II	-	Denvir-2	10	-
Sikumana	-	-	5	125
Maulafa	-	Denvir-2	8	90.9
Naimata	-	Denvir-3	11	-
Kolhua	-	-	14	-
Lasiana	-	-	9	250
TDM	-	Denvir-3	4	200
Oetete	Denvir-3	-	5	131.6

Figure 1 shows the DNA band appears at the 119bp position in the Maulafa and Manulai II Villages, which means positive Denvir-2, and the 290bp position in the Tuak Daun Merah, Naimata, and Oetete Villages, which means positive Denvir-3. Dengue virus was not found in Pasir Panjang, Sikumana, Kolhua, and Lasiana Villages. It means that Dengue virus not been distributed in all villages.

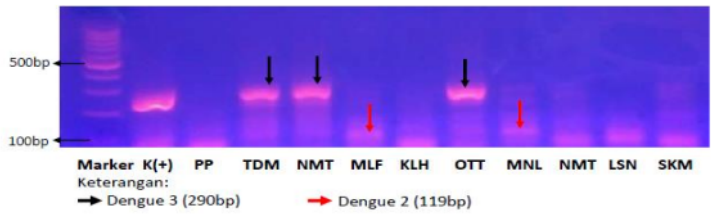

Figure 1. RT-PCR Dengue Virus on Adult *Aedes sp* Mosquitoes in Kupang City

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This research find the presence of transovarial infection of Dengue virus in *Aedes sp* is not only in DHF endemic areas but also in sporadic areas. However, viruses are found mainly in dengue endemic areas. Figure 1 also shown that Denvir-3 was founded olny in endemic area, and Denvir-2 was founded in endemic and sporadic area.

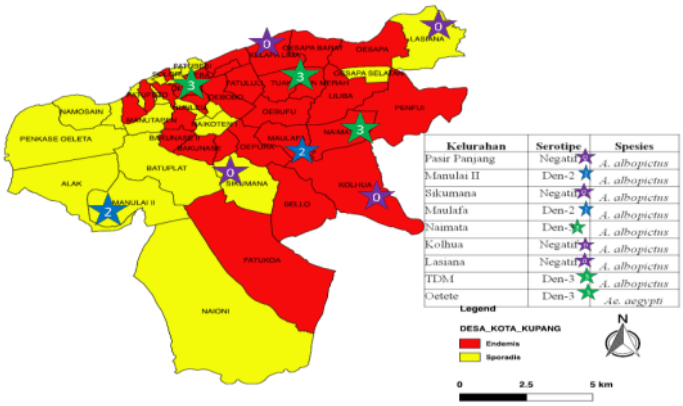

Figure 2. Distribution of Transovarial Infection of Dengue virus in *Aedes sp* Mosquitoes in Kupang City Year 2017

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And eventhough the dengue serotipe using same symbol but inside the symbol there is number to show the dengue serotipe

The results of rearing showed that *Aedes albopictus* mosquitoes hatched from eggs compared to *Aedes aegypti* . Many things can cause why the eggs cannot hatch all. The ovistrips are storing too long and in conditions that are not completely dry and still damp, causing mold and damage to mosquito eggs. The research is conducted in the rainy season. The air is more humid than in the dry season. The humid conditions can also make small animals live comfortably on ovistrip and possibly eat the mosquito eggs in the ovistrip. So that in the study, the number of mosquitoes obtained was also very small and possibly not following the actual mosquito density at the research location.

This research can prove the existence of transovarial infection of Dengue virus in *Aedes sp* in Kupang City, that is, one village for *Aedes aegypti* and four villages for *Aedes albopictus*. It means that *Aedes albopictus* also vital as a DHF vector, even though just known as a secondary vector. Based on the mosquito species, the MIR on *Aedes aegypti* is 200‰, while in *Aedes albopictus* is 90‰ - 250‰, means that transovarial infection can be found in 200 from 1,000 *Aedes aegypti* mosquitoes and in 90-250 from 1,000 *Aedes albopictus* mosquitoes. It means the transovarial infection in *Aedes aegypti* is higer than *Aedes albopictus*. Eventhough *Aedes albopictus* also can transmit Dengue virus, thus result showed that *Aedes aegypti* is more potencial as vector of DHF than *Aedes*

albopictus. Other research also proved that Dengue virus is also can transmit venerially by male *Aedes aegypti* with the potency of their behavior of polygamy (Putri, Widya, Sugeng, & Sitti, 2018). Infected male mosquitoes can transmit the virus to female mosquitoes through infected sperm cells during mating.

This MIR figure in Kota Kupang is higher than in Malaysia's previous study, which found MIR for *Aedes aegypti* is 36.5‰ and in *Aedes albopictus* is 6.66‰ (Rohani et al., 2014) and also higer than in Delhi India with MIR 5.8‰ (Vikram et al., 2015). The more MIR the more density of virus, and can indicates the transovarial infection rate. The higher rate of transovarial infection can contribute to the high cases of DHF because it makes *Aedes sp* mosquito can transmit the dengue virus for its life to humans and its offspring. This transovarial infection allows the dengue virus to maintain its presence in the mosquito's body if nature conditions do not allow it to breed (Angel & Joshi, 2008). The presence of transovarial transmission is not only found in adult *Aedes* mosquitoes, but can also be found in the larval stage. A study in Brazil found that from 54 pools of *Aedes sp* larvae, 4 pools were found to be positive for dengue virus (Cecílio et al., 2015).

This study also found that Denvir-2 and Denvir-3 viruses can be transmitted transovarially in *Aedes sp*, which previous research in the same location only Denvir-1 confirmed in the *Aedes sp* mosquito (Wanti, Sila, Irfan, & Sinaga, 2016). Study in Mexico also found that Denvir-1 can transmitted transovarially (Martínez et al., 2014), but like in Kupang City that now maybe already changed not only Denvir-1 but also Denvir-2, Denvir-3 and Denvir-4. This research shows that Denvir-1, Denvir-2, and Denvir-3 can be transmitted by transovarial in both *Aedes aegypti* and *Aedes albopictus*. The change and the addition of virus serotypes that infect Kupang City could be due to increased population mobility due to easier transportation facilities between districts and between islands. This has an impact on increasing the risk of dengue virus transmission from outside the area brought into Kupang City. Other study in Malaysia show there were closely related of strain and genotype from Denvir-1 dan Denvir-2 to strain and genotype in other countries around Malaysia. This is predicted because of the human movement of people from abroad and this change of strain and genotype can increase possibility in Dengue cases outbreak (Chew, Rahman, & Hussin, 2015).

Transovarial infection of Dengue virus in this study occurred in *Aedes aegypti* as the primary vector of DHF and in *Aedes albopictus*, which has been known as a secondary vector of DHF in Indonesia. It is showed *Aedes albopictus* also have competency as a dengue vector because it can transmit the virus Dengue-2 and Dengue-3 transovarially. It have been found that Aedes albopictus density either in house or outside the house is lower than *Aedes aegypti* density (Wanti et al., 2017), but since *Aedes albopictus* can transmit Dengue virus transovarially, a deep research of a competency DHF vector needs to be examined further. The evaluation of local mosquito populations for their competence in transmitting Dengue virus is important and will help program manager in pointing specific vector populations for vector control program (Richards, Anderson, & Alto, 2012). Similar with study in Caribean that both *Aedes aegypti* and also *Aedes mediovittaus* competent to transmit Denvir-1, Denvir-2, Denvir-3 and also Denvir-4 (Poole-Smith et al., 2015).

Next research in Kupang City should be can prove on what the Dengue virus can transmit biologically is by reaching the mosquito proboscis and releasing it to humans when it is sucking human blood. If the virus cannot reach the proboscis and cannot release to human means that this vector is not competent as vector of DHF. Research in Taiwan found that *Aedes aegypti* was more competent in transmitting Denvir-1 than *Aedes albopictus*. The Dengue virus never infected that statement, evidence by probosci's tissue in *Aedes albopictus* than *Aedes aegypti* was frequently infected (Chen, Wei, Hsu, & Chen, 1993).

The existence of transovarial infection in Kupang is one of the causes of DHF cases throughout the year in Kupang City. The incidence is always high every year dan the cases are found even in the dry season when the mosquito density is low. Transovarial infection in Aedes sp makes mosquitoes can infected of Dengue virus for their whole life. Dengue virus in egg embryos can last a long time in dry conditions, and when exposed to water, the eggs can still hatch, and adult mosquitoes that come out will transmit the dengue virus when they suck blood for the first time.

Kupang City in the year 2013 only found Denvir-1 that can be transmitted transovarially, but it changed based on the discovery in this research. The recent research proves Denvir-2 also can be transmitted transovarially in *Aedes aegypti* , and Denvir-3 also be transmitted transovarially in *Aedes albopictus*. Other research found that Denvir-3 also can be transmitted through venereal from male to female *Aedes aegypti* (Putri et al., 2018). These two modes of transmission lead to increased transmission of the Dengue virus between mosquitoes and between mosquitoes and humans. The existence of vertical transmission of the dengue virus in *Aedes sp* can make the circulation of arboviruses sustainable in nature (Ferreira-de-Lima & Lima-Camara, 2018). Several studies it has been proven that the dengue virus can be transmitted by transovarial transmission, such as in Brazil, Trinidad, Tobago, Peru, Bolivia, Argentina, Costa Rica, Mexico, India, Myanmar, Thailand, Malaysia, Philippines, Singapore and including in

Indonesia (Ferreira-de-Lima & Lima-Camara, 2018). Transovarial transmission makes mosquitoes in order to be infected with the Dengue virus do not have to first suck the blood of people who have the virus in their blood because mosquitoes infected with the virus will still be found with the virus throughout their life and will be able to reduce them to their offspring.

This existence of transovarial infection of the Dengue virus in Kupang City can be used as an early warning in Kupang City about new cases of DHF and outbreaks of DBD. People who have had primary dengue infection may only have immunity to Denvir-1, so if there is secondary infection with dengue with different serotypes, for example, by Denvir-2 and Denvir-3, the community will still get DHF again. With the discovery of transovarial of Denvir-3, it is necessary to be aware of severe cases because Denvir-3 infection has the highest disease severity level followed by Denvir-2, Denvir-1 follow it, and Denvir-4. Besides, if you have had a primary infection with serotypes that are different from secondary infections, the chances of getting a more severe dengue infection are more significant. Study in Singapore also has found that infecting dengue by different serotype and genotype may an important role in disease severity among dengue patients (Yung et al., 2015).

Infection by the Dengue virus any serotype will affect body produce active immunity against specific serotype. The immunity that arises from primary dengue virus infection is lifelong (long life immunity), but this specific immunity cannot prevent secondary infection by other virus serotypes. The chances of getting a more severe dengue infection are more significant if other serotypes infect people. By looking at the Dengue virus's transovarial transmission in the *Aedes sp* mosquito and the discovery of Denvir-2 and Denvir-3 in *Aedes aegypti* and *Aedes albopictus* mosquitoes in Kupang City, it is necessary to take measures to prevent dengue transmission and control the dengue mosquitoes more intensively. The peak of transovarial infection in mosquitoes is estimated to occur four months before the peak of DHF transmission (Thongrungkiat, Maneekan, Wasinpiyamongkol, & Prummongkol, 2011), so this requires more intensive vector control from the primary stage to prevent a high increase in case outbreaks. Further research is needed on the benefits of recognizing transovarial infections in predicting of increased incidence and occurrence of outbreaks.

The recognition of this transovarial infection indicates the need for vector control to be carried out from the primary stage before the virus multiplies in adult mosquitoes before it can transmit to humans. Draining activities, such as cleaning water reservoirs with brushing the inside part of reservoir and also replacing water with a new water; tightly closing the water reservoir; bury or eliminating water storage places that are no longer used; and monitoring activities at least once a week for other controlling activities dengue mosquitoes. Drums have been proven to be related to the incidence of dengue fever in Kupang City (Wanti et al., 2019), so that the action of cleaning and eliminating mosquito breeding places is also focused on drum type landfills, in addition to general types. This monitoring is necessary to eradicate eggs, larvae, and mosquitoes found in each water storage. Here, the intervention is for water reservoirs and dengue mosquitoes both in the imago and pre-imago stages. For this reason, it is suggested to the health office or primary health center to improve entomological surveillance and laboratory surveillance in order to be alert to the presence of primary and secondary infections of dengue and dengue outbreaks due to the presence of new Denvir-2 and Denvir-3 serotypes. It is also necessary to strengthen the DHF program by carrying out advocacy and outreach about dengue disease to related agencies such as education, tourism, and public relations office.

CONCULUSSION

This research found a transovarial infection not only in *Aedes aegypti* mosquitoes but also in *Aedes albopictus* . This research also found that Denvir-2 can be transmitted transovarially in *Aedes aegypti* and *Aedes albopictus* in Kupang City. Besides Denvir-3, it can be transmitted transovarially in *Aedes albopictus* . This finding is a change from previous findings in the same place where only Denvir-1 was transmitted transovarially in *Aedes sp*.

Further research is needed on the benefits of recognizing transovarial infections in predicting of increased incidence and occurrence of outbreaks. It is suggested to the health office or primary health center to improve entomological surveillance and laboratory surveillance to be alert to the presence of primary and secondary infections of dengue and dengue outbreaks. It is also necessary to strengthen the DHF program by carrying out advocacy and outreach about dengue disease to related agencies.

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