



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

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Abstract

Nowadays, 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 infectious and the serotypes variability of Dengue virus in *Aedes* sp in Kupang City. This observational research took place in nine villages in Kupang City in 2017. Where 20 houses in each 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.

Introduction

Dengue Haemorrhagic Fever (DHF) is a mosquito-borne viral disease that is still a significant problem globally in public health (Murray, Quam, & Wilder-Smith, 2013; Yan et al., 2018). As a global problem, Dengue cases are 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 to 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 an irregular pattern of DHF outbreaks (Harapan et al., 2019). The incidence of DHF in 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 from 2000 to 2015 (Kemenkes RI, 2019).

The DHF prevention and control activities in Kupang City have been done every year including 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 been sufficient to the decreasing of cases of DHF, so DHF cases getting higher and always can be found mortality of DHF cases every year.

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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 be differ from one region to another (Martín et al., 2010). Dengue viruses have four serotypes (Denvir-1, Denvir-2, Denvir-3, and Denvir-4). They can be found in the DHF patient and *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 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). No specific medicine recently for dengue treatments. So prevention needs to focus on vaccine development and 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. Research samples were from nine villages in Kupang City. They consisted 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 surrounding dengue fever sufferers with a

radius of 100 meters. Eggs of *Aedes sp* were collected from 20 houses in each village so that the total number 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 ovitrip 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 rearing around one week. The mosquitoes that come out of the pupa are 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 is 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).

Collected data are shown in the simple distribution table, figure, and map. It will then be analyzed descriptively to find 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 a constant of 1000.

Results and Discussion

This study was conducted by rearing *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 eight villages,

while *Aedes aegypti* can be found in one village. Table 1 also shows 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

Villages	<i>Aedes aegypti</i>			<i>Aedes albopictus</i>		
	n	%	Dengue virus	n	%	Dengue virus
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
Source: Primary Data, 2017

Based on the mosquito species, MIR on *Aedes aegypti* is 200%. While in *Aedes albopictus*, it 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 indicates 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 two of nine villages observed in Kupang City (22.2%). Meanwhile, Denvir-3 can be found in three 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

Villages	Serotypes		Mosquitoes / pool	MIR (%)
	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>		
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

Source: Primary Data, 2017

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 the Dengue virus has not been distributed in all villages.

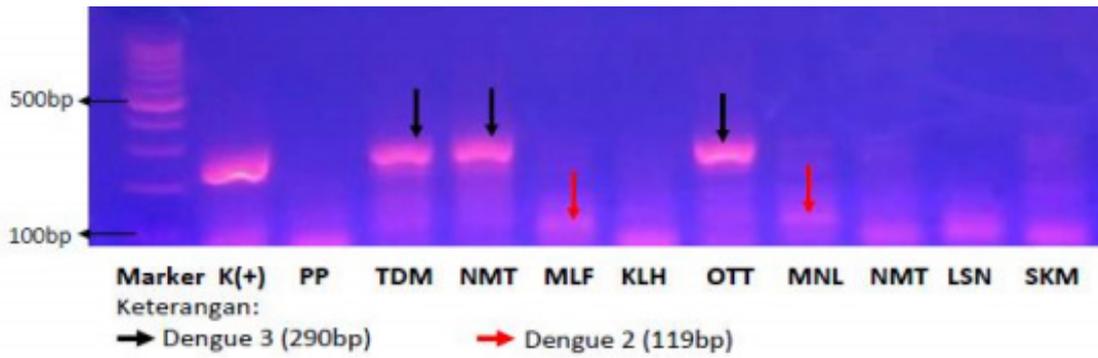


Figure 1. RT-PCR Dengue Virus on Adult Aedes sp Mosquitoes in Kupang City

This research finds 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 shows that Denvir-3 was founded only in an endemic area, and Denvir-2 was founded in the endemic and sporadic area.

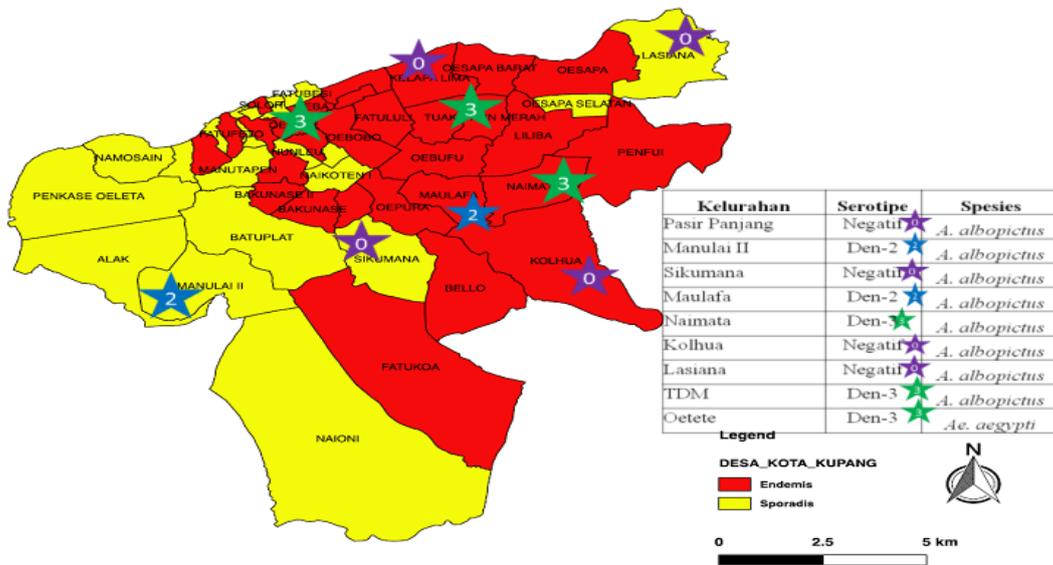


Figure 2. Distribution of Transovarial Infection of Dengue virus in Aedes sp Mosquitoes in Kupang City Year 2017

The rearing results showed that Aedes albopictus mosquitoes hatched from eggs compared to Aedes aegypti. Many things can cause why the eggs cannot hatch at all. The ovistraps 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 the ovistrap and possibly eat the mosquito eggs in the ovistrap. So in the study, the number of mosquitoes

obtained was also very small and possibly did not follow 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 is 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%, which 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 higher than in *Aedes albopictus*. Even though *Aedes albopictus* also can transmit the Dengue virus, thus result showed that *Aedes aegypti* is more potential as a vector of DHF than *Aedes albopictus*. Other research also proved that the 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 for *Aedes aegypti* is 36.5‰ and *Aedes albopictus* is 6.66‰ (Rohani et al., 2014). It is also higher than in Delhi, India, with 5.8‰ (Vikram et al., 2015). The more MIR, the more density of the virus. It can indicate the transovarial infection rate. The higher rate of transovarial infection can contribute to the high cases of DHF because 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 transovarial transmission presence 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 (Cecilio 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 *Aedes albopictus*. It has been known as a secondary vector of DHF in Indonesia. It showed *Aedes albopictus* also have competency as a dengue vector because it can transmit the virus Dengue-2 and Dengue-3 transovarially. It has been found that *Aedes albopictus* density, either in the house or outside, is lower than *Aedes aegypti* density (Wanti et al., 2017). But since *Aedes albopictus* can transmit Dengue virus transovarially, research on a competency DHF vector needs to be examined further. The evaluation of local mosquito populations for their competence in transmitting the Dengue virus is vital and will help program managers in pointing specific vector populations for vector control programs (Richards, Anderson, & Alto, 2012). Similar to the study in Caribbean that both *Aedes aegypti* and also *Aedes mediovittatus* are competent to transmit Denvir-1, Denvir-2, Denvir-3 and also Denvir-4 (Poole-Smith et al., 2015).

Further research in Kupang City should prove whether the Dengue virus can transmit biologically by reaching the mosquito proboscis and be released to humans when it is sucking human blood. If the virus cannot reach the proboscis and cannot release to humans, thus this vector is not competent as a 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, evidenced by proboscis 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. The incidence is always high every year and the cases are found even in the dry season when the mosquito density is low. Transovarial infection in *Aedes* sp makes mosquitoes can be infected with 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 2013 only found Denvir-1 that can be transmitted transovarially, but it changed based on the discovery in this research. It found out that Denvir-2 also could be transmitted transovarially in *Aedes aegypti* and Denvir-3 in *Aedes albopictus*. Other 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 has proven that the dengue virus can be transmitted by transovarial transmissions, such as in Brazil, Trinidad, Tobago, Peru, Bolivia, Argentina, Costa Rica, Mexico, India, Myanmar, Thailand, Malaysia, Philippines, Singapore, and Indonesia (Ferreira-de-Lima & Lima-Camara, 2018). Transovarial transmission makes mosquitoes infected with the Dengue virus do not have to 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 the 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. A study in Singapore also has found that infecting dengue by different serotypes and genotypes may have a vital role in disease severity among dengue patients (Yung et al., 2015).

Infection by the Dengue virus of any serotype will affect body to produce active immunity against specific serotype. The body immunity generated from primary dengue virus infection is lifelong (long life immunity), but it cannot prevent secondary infection by different virus serotypes. The chances of getting a more severe dengue infection are more significant if other serotypes infect people. Considering Dengue virus's transovarial transmission in the *Aedes* sp mosquito, the 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 DHF transmission peak (Thongrungrat, 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 by brushing the inside part of the reservoir and also replacing water with a new water; tightly closing the water reservoir; burying 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 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 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.

Conclusions

This research found a transovarial infection not only in *Aedes aegypti* mosquitoes but also in *Aedes albopictus*. It 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*. It is an improvement 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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