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Isna Hikmawati *

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Research Article

Persistence of Dengue Virus (DENV-1, 2, 3,4) Transovarial-Transgenerational with Realtime Polymerase Chain Reaction (qPCR) in Ae. Aegypti and Ae. Albopictus (Diptera: Culicidae)

Isna Hikmawati [1]*, Hendro Wahjono [2], Martini Martini [2], Edi Darmana [2], Soeharyo Hadisaputro [2], Kisdjamiatun Retna Mustika Djati [2], Sugeng Juwono Mardihusodo [3]

[1]Faculty of Health Sciences, Universitas Muhammadiyah Purwokerto.

[2]Faculty of Medical, University Diponegoro, Semarang.

[3]Faculy of Medical, University Gajah Mada, Yogyakarta.

***Corresponding Author:** Isna Hikmawati, Faculty of Health Sciences, Universitas Muhammadiyah Purwokerto.

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Abstract

Ae. aegypti and Ae. albopictus have an important role in DHF transmission because they can simultaneously transmit the dengue virus vertically / transovarially or horizontally. This phenomenon indicates the persistence of the dengue virus by vectors. The aim of this research was to prove the persistence of the transovarial-transgenerational dengue virus (DENV-1,2,3,4) with real time polymerase chain reaction (qPCR) in Ae. aegypti and Ae. albopictus (Diptera: Culicidae). Quasi experimental design with intervention infects DENV 1-2-3-4 serotypes in Ae. aegypti and Ae. albopictus intratoracally. Research population Ae. aegypti and Ae. albopictus laboratory colony females. Dengue virus detection uses real-time polymerase chain reaction (qPCR). Transovarial detection by qPCR indicates detection of dengue virus in Ae. albopictus DENV-1 to progeny 1 (F1), DENV-2 and DENV-3 to F2, DENV-4 to F3. Next to Ae. aegypti DENV-1 to 1st progeny (F1), DENV-2 to F2, DENV-3 to F4 and DENV-4 to F3. there was no difference in MIR value (p value: 0.356) for the four serotypes in Ae. albopictus and Ae. aegypti. DENV-3 is the most persistent serotype in Ae. aegypti with 83.3% MIR and DENV-4 were the most persistent serotypes in Ae.albopictus with 100% MIR. The need to improve vector control models that focus not only on the main vector, but also other co-vectors.

Key words: persistence; transovarial; intratoracal; ae. Aegypti; ae. albopictus

1.Introduction

Dengue Hemorrhagic Fever (DHF) is an infectious disease caused by the dengue virus (DENV). DENV is a single-stranded RNA virus consisting of four different serotypes namely DENV-1, DENV-2, DENV-3 and DENV-4 belonging to the Flavivirus Genus, Family Flaviviridae. The disease is characterized by biphasic fever, leukopenia, lymphadenopathy, myalgia or arthralgia and a rash. [1] The results of research by Bhatt, S. et al. Estimate that 70-500 million people are infected with the dengue virus every year in more than 100 countries around the world, with the Cartography approach, it is predicted that DHF will continue to spread, especially in tropical areas with an estimated 390 million / year and 96 million cases. indicates clinical or sub-clinical severity.2 The Worldh Health Organization (WHO) reports that DHF cases in endemic countries have reached 4,975,807 cases with 68,977 (1.4%) deaths for approximately 40 years. DHF is a big problem in Southeast Asia, because during that period there were 67,295 deaths from a total of 68,977 deaths worldwide. This means that an average of 1682 deaths occurs / year due to dengue fever. [1] DHF mostly occurs in tropical and sub-tropical areas, with the main vector being the mosquito Aedes aegypti and its co vector Aedes albopictus. [3] DHF vectors and diseases are concentrated in tropical and sub-tropical areas, the spread of vectors and the increase in the movement of the mosquito population causes the virus to become endemic in temperate regions. [4] The results of the study by Hu, et al showed that there were differences in the incidence of dengue based on geographic area characteristics and the incidence was more in the tropics. [5] Globalization, trade, urbanization, travel, demographic changes, inadequate domestic water supplies and warming temperatures are all associated with the spread of Ae. aegypti and Ae. albopictus. [6]

Indonesia is one of the tropical countries with the distribution of the four serotypes DENV-1, 2, 3, 4. [7] Infection by one serotype will produce antibodies against the serotype concerned, while the antibodies formed against other serotypes cannot provide adequate protection against these other serotypes. Therefore, a person can be infected with 3 or even 4

serotypes during his lifetime.[8] An increase in a person's antibody and good knowledge about dengue can reduce the risk of the second infection.

[9] The Crude Fatality Rate (CFR) of DHF in Indonesia tends to fluctuate from year to year. 2014 (0.9), 2015 (0.83), 2016 (0.78), 2017 (0.72), 2018 (0.65), 2019 (0.94), these data describe an increase in DHF during five years, because for four consecutive years the CFR has decreased. [10] The results of Hikmawati and Pattima's research in Banyumas Regency, as one of the dengue endemic areas in Central Java, showed that the 2016 Case Fatality Rate (CFR) was 8.69, with high CFR that year, Banyumas was declared an outbreak of DHF. [11] Eradication of DHF through vector control has not been able to cut the transmission from mosquitoes to mosquitoes because there is still empirical evidence of transovarial transmission. Transovarial transmission is a vertical transmission mechanism in the body of a mosquito, namely the virus is transmitted by female mosquitoes to their eggs. [12] Transovarial transmission is one way for the existence of the dengue virus to maintain its presence in nature, thereby increasing the tendency for dengue cases to occur in the same location repeatedly. Transovarial transmission is one form of the role of a competent vector in maintaining viral serotypes during interepidemic events.

Controlling the vector of DHF has actually been carried out by various methods including chemical, biological, physical, environmental engineering and others. However, this has not yet obtained effective results, because there is still evidence of transovarial transmission. The results of the transovarial study of the dengue virus in Malaysia reported that transovarial transmission occurred in urban and sub-urban communities both in Ae. aegypti and Ae. albopictus. The results of these studies indicate that in nature, mosquitoes can function as natural reservoirs. [12] Transovarial studies in the Amazon showed a transovarial infection rate of 46% and the detection of serotypes identified included DENV-1 and DENV-4 serotypes. [13] The results of the DENV infection study in Bangkok concluded that transovarial transmission increased during the summer, or 4 months before the incidence of dengue increased in humans. The results of this study found the transovarial infection of the dengue virus was 47.9% by DENV-4, 13.4% by DENV-3, 5% by DENV- 1, 3.4% by DENV-2 and overall as much as 30.3. % contains all of these serotypes. [14] Indonesia has several dengue endemic areas, so geographically it is a good place for breeding Ae. aegypti and Ae albopictus. The results of research in endemic areas found 50% Container Index between 50-100%. [15] Research by Lidiasari, et al from Menado City shows the Transovarial Transmission Index (ITT) Ae. aegypti ranged from 39.1% -70%. 16 The results of research on the distribution of serotypes in Indonesia found the frequency of DENV-1 was 9.6%, DENV-2 was 55%, DENV-3 was 29% and DENV-4 was 0.4%. Based on the wide distribution of the dengue virus serotypes, DENV-2 and DENV- 3 are the serotypes with the most extensive distribution. The results of this study indicate a difference in the proportion of virus serotype variations with different endemic areas. [17]

Ae. aegypti and Ae albopictus have an important role in DHF transmission because they can simultaneously transmit the dengue virus vertically / transovarially or horizontally. This is in line with the results of a study by Mourya, et al, who found horizontal transmission of DENV- 2 serotype by infected mosquitoes through vertical transmission. The results of these studies conclude there is a vertical transmission before horizontal transmission. [18] This phenomenon indicates the persistence of the dengue virus by vectors. Viral persistence is covert viral infection with an equilibrium level between the virus and the host immune system resulting in a long duration of infection. [19] The results of the persistence study by Ahmad et al showed transovarial persistence in the Ae. aegypti in the DENV-2 serotype persists through the fifth generation.

[20] The presence of the virus in mosquitoes when there are few cases or no outbreaks, indicates the ability of the vector to maintain the virus in the interepidemic period. The results of Joshi's research on the Ae. aegypti found the persistence of DENV-3 serotype until the seventh generation. The results of this study found a difference in mortality of larvae and the average number of eggs that hatched between mosquitoes infected with DENV-3 serotype and those not infected with DENV-3 serotype. [21] Transovarial occurrence indicates that mosquitoes survive in nature. In surviving conditions, the mosquito will undergo a metamorphosis process and a gonotropic cycle. Dengue virus detection in the next progeny gonotropic cycle indicates the persistence of transovarial- transgenerational dengue virus. This certainly has an impact on increasing the spread of dengue disease. The aim of this study was to prove the persistence of the transovarial-transgenerational dengue virus (DENV- 1,2,3,4) with real time polymerase chain reaction (rt-PCR) in Ae. aegypti and Ae. albopictus (Diptera: Culicidae).

2. Methods

Subject and research design

The research design used a quasi-experimental, The Equivalent Materials Design. [22] Intervention was by infecting DENV 1-2-3-4 serotypes in Ae. aegypti and Ae. albopictus intratoracally. The population in this study was the 115th progeny laboratory colony female mosquito (F115) in Ae. aegypti and the 45th progeny (F45) in Ae. albopictus, which had previously been tested by Reserve Transcription-Polymerase Chain Reaction (RT-PCR) on its parent.

Sampling and epidemiological data collection Mosquitoes.

The mosquitoes used in the present study were obtained from the laboratory colony maintained at the Laboratorium Parasitologi, Fakutas Kedokteran UGM (Jogjakarta, Indonesia). This colony originated from the mosquitoes collected in Jogjakarta City, and has been maintained for 5 years. The sample in this study used an unpaired numerical analytical research formula. [23] The intervention was carried out 2 times/serotype, one intervention against 35 mosquitoes, so that in the implementation of this study, the total sample was 280 Ae. aegypti and 280 Ae. albopictus.

Dengue virus.

The dengue-1234 virus used in the study was obtained from parasitology laboratory, UGM medical faculty, viruses originally from Namru (Jakarta, Indonesia). It was originally isolated from a febrile patient with dengue fever. Before intrathoracic injection, the viral supernatant was examined by Reverse Transcription Polymerase Chain Reaction (RT- PCR), with the aim of being intrathoracic according to the type of serotype used.

Intrathoracic procedures

A total of 35 Ae. aegypty is put in a test tube filled with ice cubes, after fainting, take one by one, place it under a microscope and a set of mosquito intrathoracal tools, then inject ± 2 µ of the DEN-1 virus supernatant each. The following day the same procedure was carried out on 35 individuals. Furthermore, the same procedure was carried out for the DENV-2,3,4 serotype. To Ae. albopictus performed the same procedure as in Ae. aegypti.

Maintenance of mosquitoes after intrathoracic

Two to three days after intrathoracal, the mosquitoes were put in a cage measuring 20 cm3 to be fed blood by means of membrane feeding. Each one is coded between Ae. aegypti and Ae. albopictus and each serotype. Membrane feeding is carried out for approximately 7-9 hours. The day after the feeding membrane, the cage is given a cup with filter paper and 1/3 of the cup water then input the male mosquito with a ratio of 1: 1 and incubated at 25 ± 4 ° C and a relative humidity of 80 ± 5%. The mosquitoes are allowed to lay eggs and replace wet cotton with sugar water every other day. After seen laying eggs, save the eggs for about half a month. The eggs produced from the gonotropic cycle are then hatched to become mosquitoes as F1.

Incubate infectious eggs

Put the F1 eggs that are on the filter paper into plastic cups. Add water, approximately approaching ½ cup height, leave for a few days. After seeing the larvae, pour the water in several cups, in a plastic tray. Feed it with chicken liver every day. After you see the pupa, move the pupa into a cup filled with water, put it in the mosquito cage, let it become a mosquito. After you look like a mosquito, enter a cotton swab filled with sugar water in a

small tube, then place it in the mosquito cage, changing the sugar water every 2 days. After 4-5 days of age, take 80 male and female mosquitoes for membrane feeding.[24] Make a place to lay eggs by inserting a cup filled with wet cotton and placing the filter paper on top of the wet cotton. The eggs produced were recorded as progeny 2 (F2). Perform the same procedure for each progeny. Store 10 female mosquitoes per progeny in ependop tubes labeled according to the type of mosquito and the type of serotype. Then put in the sample box and stored at -800C for dengue virus detection with Realtime Polymerase Chain Reaction (qPCR).

Transovarial transmission detection with qPCR

Initial handling of the sample by means of: 10 intrathoracic mosquitoes stored at -800C, separating the thorax and the head. Then input it into the microtube. Add 200 µl of PBS. Crush with stick holder until crushed. Centrifuge at 5000 rpm for 5 minutes. The premer is used as where Table. 1 of the following:

Assay	*Premer*	*Sequences*
DENV-1	DENV1_F	CAA TGG ATG ACA ACA GAA GAY ATG
	DENV1_R	TCC ATC CAT GGG TTT TCC TCT AT
DENV-2	DENV2_F	GCA GAA ACA CAA CAT GGA ACR ATA GT
	DENV2_R	TGA TGT AGC TGT CTC CRA ATG G
DENV-3	DENV3_F	ATG GAA TGT GTG GGA GGT GG
	DENV3_R	GGC TTT CTA TCC ART AGC CCA TG
DENV-4	DENV4_F	GCA GAT CTC TGG AAA AAT GAA CCA
	DENV4_R	GAG AAT CTC TTC ACC AAC CCY TG

Table 1: Premer Dengue Virus Detection by qPCR

Furthermore, Viral RNA Isolation by centrifuge method. Furthermore, the qPCR process by preparing the One Step qPCR examination procedure. Quantinova R, SYBr (R) Green RT-PCR Kit Cat No. 208152 with the following composition: (2x SYBr Green RT-PCR Master Mix 11 µl, QN SYBr Green RT-Mix 0.2 µl, Premer Revers (R) 1 µl, Premer Forward (F) 1 µl, RNA Template 2 µl, RNase- Free Water 4.8 µl. Premer Forward (F) and Premer Revers (R) are adjusted according to the type of serotype, then input into the Q-PCR optical flat tube 8- cap Strips for 0.2 ml tube (Biorad), put into the tool , then the program is carried out in the Q-PCR device as follows: 10 minutes 500C Reverse Transcription Reaction, 1 minute 900C Polymerase Activation and DNA Denaturation, 10 minutes 950C Denaturation, 30 seconds 600C Annealing, Denaturation and Annealing performed 39 times, Melt-Curve analysis 5 seconds 0.5 0C. Allow it to finish ± 1.5 hours. When finished, then the system automatically displays the results on the computer monitor of the tool.

Statistical methods

Data were analyzed using SPSS software (version). The Minimum Infection Rate (MIR) is taken from the number of positive microcentrifugation tubes divided by the number of mosquitoes examined X1000. Bivariate analysis using independent t test with p value <0.05 to see the difference in MIR between Ae. aegypti and Ae. albopictus in all four serotypes.

Ethical consideration

The study was approved by the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Gadjah Mada University and Dr. Sardjito General Hospital by Number: Ref: KE/FK/0176/EC/2018 and data were confidentially preserved according to the revised Helsinki decelerations of biomedical ethics.

3. Result

Transovarial Transmition

Dengue virus detection for each progeny in Ae. aegypti and Ae. albopictus as shown in Table. 2 below:

Vector	Progeny	Serotype			
		DENV-1	DENV-2	DENV-3	DENV-4
Ae. albopictus	F1	+	+	+	+
	F2	-	+	+	+
	F3	-	-	-	+
Ae. aegypti	F1	+	+	+	+
	F2	-	+	+	+
	F3	-	-	+	+
	F4	-	-	+	-

Table 2: Dengue Virus Detection Ae. Albopictus and Ae. aegypti

Table.2 shows DENV-4 on Ae. albopictus is the most persistent serotype because the virus is detected until the 3rd progeny, whereas in Ae. aegypti serotype DENV-3 was the most persistent because it was detected by the virus until the 4th progeny.

Minimum Infection Rate (MIR)

Vector	Progeny	Mean	Sd	MIR (^{0}/00)				95% CI	*p value*
					DENV- 2	DENV- 3	DENV- 4		
Ae. albopictus	F1-F3	66.7	27.2	33,3	66,7	66,7	100	23.3-110.0	0.356
Ae. aegypti	F1-F4	54,1	25.0	33,3	33,3	83,3	66,7	14.3-93.9	

Table 3: shows there is no difference in MIR values (p value: 0.356), meaning that the four serotypes have persistence in Ae. albopictus and Ae. aegypti.

Realtime Polymerase Chain Reaction (qPCR)

Transovarial detection qPCR results Ae. albopictus in DENV-1 to progeny 1 (F1) as a Figure.1, DENV-2 to F2 as a Figure. 2 and Figure.3, DENV-3 to F2 as a Figure. 4 and Figure.5, while DENV-4 to F3 as a Figure. 6, Figure.7 and Figure.8. Next up Ae. aegypti in DENV-1 to progeny 1 (F1) as a Figure.9, DENV-2 to F2 as a Figure.10 and Figure.

11. DENV-3 to F4 as a Figure. 12, Figure.13, Figure.14, Figure.15 and DENV-4 to F3 as a Figure. 16, Figure. 17, Figure.18. as shown below:

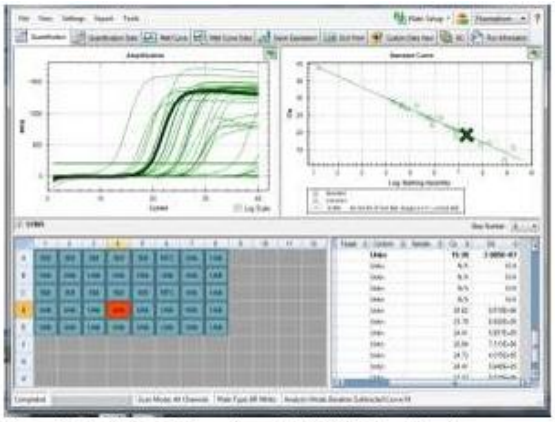

Figure 1: Real time PCR results on F1 DENV-1 Ae. Albopictus

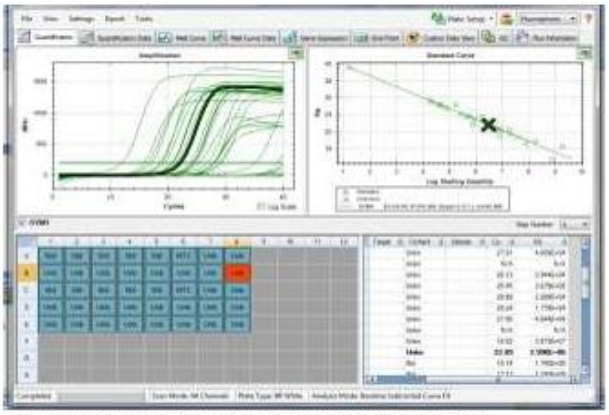

Figure 2: Real time PCR results on F1 DENV-2 Ae. Albopictus

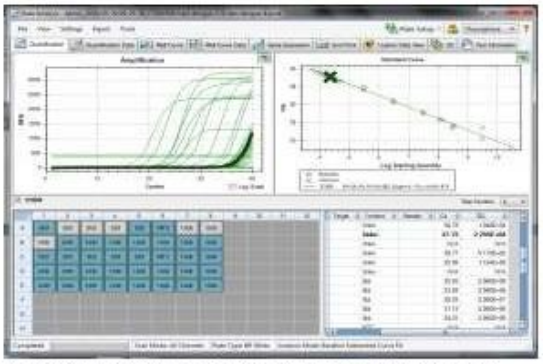

Figure 3: Real time PCR results on F2 DENV-2 Ae. Albopictus

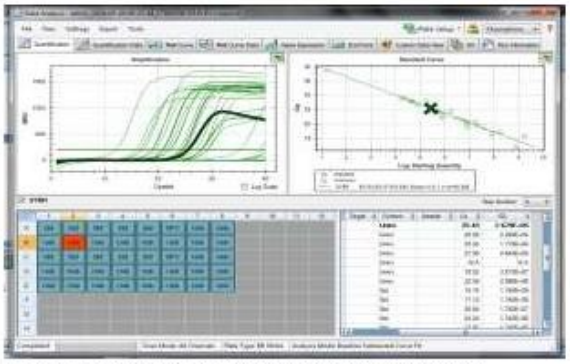

Figure 4: Real time PCR results on F1 DENV-3 Ae. Albopictus

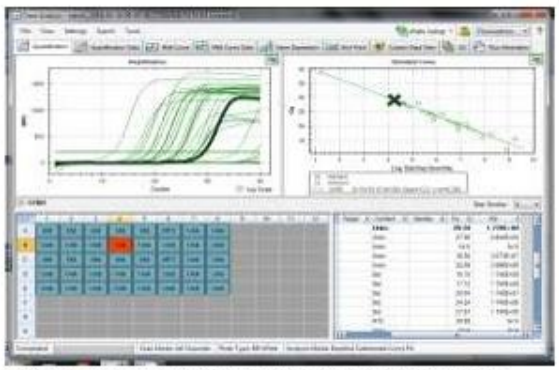

Figure 5: Real time PCR results on F2 DENV-3 Ae. Albopictus

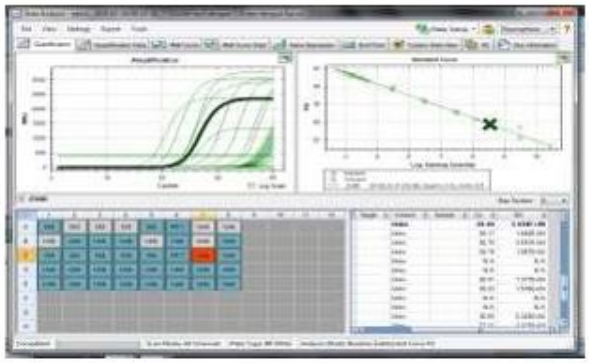

Figure 6: Real time PCR results on F1 DENV-4 Ae. Albopictus

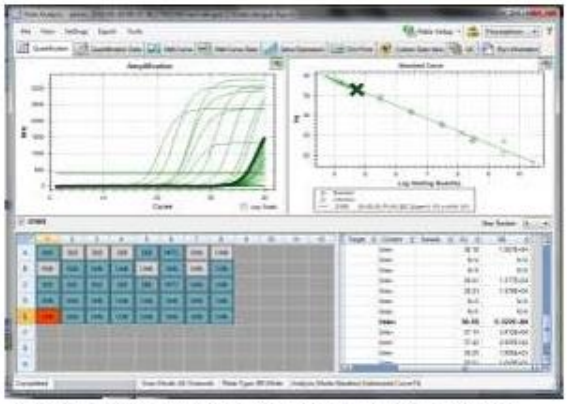

Figure 7: Real time PCR results on F2 DENV-4 Ae. Albopictus

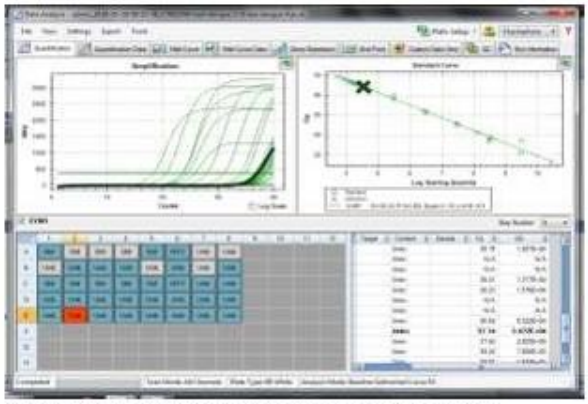

Figure 8: Real time PCR results on F3 DENV-4 Ae. Albopictus

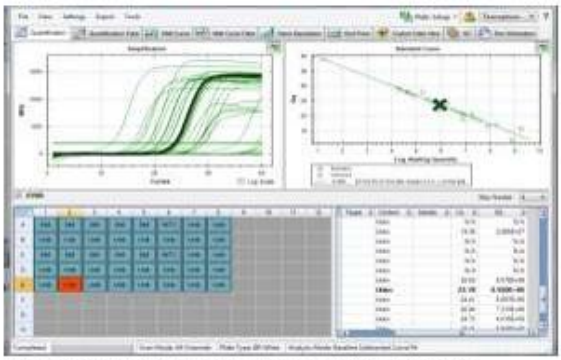

Figure 9: Real time PCR results on F1 DENV-1 Ae. aegypti

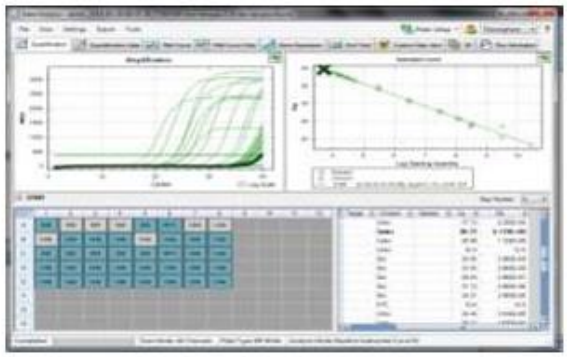

Figure 10: Real time PCR results on F1 DENV-2 Ae. aegypti

4. Discussion

The results showed the success of artificial transovarial transmission (artificial inoculation) by the intrathoracic method (Table 2). The success of transovarial transmission by artificial inoculation was seen from the results of rt-PCR for each progeny. The detection of viruses in several progeny indicates the role of mosquitoes as vectors of DHF. In the mosquito's body, the virus replicates very quickly. The results of this study indicate that the dengue virus is an organism capable of attacking cells in the body of mosquitoes. The entry of the virus into the mosquito's body indicates the success of the virus in overcoming various existing barriers. This success is influenced by many factors, including: the anatomical, physiological and molecular characteristics of the mosquito itself. During the extrinsic incubation period, the virus that enters the mosquito's midgut and enters the hemolymph can infect the body's cells, trachea, hemocytes, ovaries, nervous tissue, and reach the salivary glands.

[25] The mechanism for virus entry begins by infecting the midgut epithelium and replicating before passing through the basal lamina into the hemolymph and spreading throughout the mosquito's body. In order for it to be transmitted to the next host, the virus must infect the salivary glands. The genetic diversity of the virus is reduced if there are anatomical barriers such as midgut infection, salivary gland infection. [26] The results of research by Lawadan, et al. Concluded that not every generation of transovarial transmission of dengue virus was detected in Ae. aegypti generation (F2-F3), especially in pale form morphology. The detection of the next generation of dengue virus (F2-F3) on dark form morphology shows that the persistence of transovarial transmission in several generations of mosquitoes is an important mechanism in the maintenance of dengue virus in the interepidemic period. The results of these studies indicate that some infected female mosquitoes do not suck blood and infertility occurs and the number of eggs produced is reduced. The transovarial occurrence in dark form morphology indicates that these vectors can function as a reservoir for the dengue virus in nature. This is one of the factors that support the prediction

of dengue fever outbreaks in certain areas. [27] The results of this research indicate an equally important role between Ae. aegypti as the main vector and Ae. albopictus as co-vector in transovarial transmission. In this transmission, the virus enters the egg during fertilization through the oviduct during embryogenesis, as a result, the infected egg produces infectious larvae. Transovarial becomes an important problem in vector control efforts because of Ae. aegypti can be a vector of other viruses besides the dengue virus, one of which is the chikungunya virus (CHIKV). This is in line with the results of research on Reunion Island which concluded Ae. Aegypti can be a vector for dengue and chikungunya viruses. [28] For this reason, an important effort that can be done is through vector control at all stages of the mosquito stage. Research on larvicides conducted by Joshi et al, shows that >50% of the total districts used as research sites, larvicide intervention at the breeding spots for mosquitoes that are positive for dengue virus can reduce 90-100% of dengue fever incidence. [29]

The results of qPCR analysis showed fluctuations in viral load values that were different for each progeny (Figure.1- Figure.18). This phenomenon indicates that there are differences in viral strains in the ability to bind to and infect target cells. This is related to the ability to produce progenic viruses and different gene products and provide different aspects. The results of previous studies concluded that the severity of dengue infection correlates with high viral load viremia, secondary infection and type of serotype. [30] The results of this study are in line with previous studies which found fluctuations in different viral replication rates in the two Ae vector progeny. aegypti and Ae. albopictus. The results showed that the replication strain DEN2-FJ10 was greater in Ae. aegypti than in Ae. albopictus 5 days after infection while DEN2-FJ11 replication was greater in Ae. albopictus than in Ae. aegypti 7 days after infection. [31] The reproduction of viruses in different organs during the embryogenesis process or in the final stages of a mosquito's life may vary due to tissue tropism, viral offspring, and host genetics. [21] The value of viral load for the generation of progeny in mosquitoes Ae.aegypti and Ae. albopictus shows DENV transmission without blood sucking from the infected host. After DENV breeds in the mosquito / Extrinsic Incubation Period (EIP), the female mosquito must survive before the next transmission occurs. The results of previous studies show that EIP DENV in Ae. aegypti about 12-16 days. This means that mosquitoes must survive longer than 12 days before they can transmit the virus to an uninfected person. [32] Transovarial DENV transmission by both the main vector (Ae. Aegypti) and the co-vector (Ae. Albopictus) is an important role phenomenon in the interepidemic period. Reactive and nonneutralizing or subneutralizing antibodies that occur after primary DENV or flaviviral infection can bind to heterologous DENV to increase infection, resulting in viral load and disease virulence.1 This concept is known as Antibody-Dependent Enhancement (ADE) from DENV infection. In a prospective study in Bangkok, Thailand, taking serum specimens from school children with secondary dengue fever. Furthermore, its ability to increase DENV-2 replication in human monocytes in vitro was tested. The results of these studies indicate that the specific titer of non-neutralized dengue virus antibodies can increase and is associated with an increased risk of severe dengue disease. A prospective study with a cohort design concluded that ADE of dengue disease occurs in human subjects with pre-condensing anti-DENV antibody infection in a certain concentration range. [33] Another study concluded that viral load was significantly higher in IgM antibody negative patients. In contrast, circulating NS1 levels were found to be unaffected by the presence of IgM. Thus, IgM antibody in serum can be a modulator affecting the level of viremia of the patient. [34]

The results showed MIR in both vectors ≥33,3o/oo (Table.3). This shows that both have the same chance of causing persistent dengue virus during interepidemic although there is no statistically significant difference in MIR in the four serotypes (DENV-1, 2, 3, 4). MIR is the most commonly used indicator to estimate mosquito infection rates. MIR is calculated as the ratio of the number of positive batches to the total number of mosquitoes tested, assuming that only one individual is infected in each positive batch. MIR measures the lower limit, so MIR applications are limited to low transmission situations. [35] MIR was originally adopted as an indicator of a potential arbovirus outbreak in the United States. [36] MIR in arboviral transmission is usually less than 0.1%, but infection in mosquitoes is usually much higher than 0.1%. There are several drawbacks to seeing the proportion of infections with MIR, especially in situations when the mosquito infection rate is high / the population is wide. To determine the domain justified use of MIR, the conditional probability (p> 1) means that more than one mosquito is infected, given the positive pool, can be determined based on the binomial model. 37 Dengue infection can occur in both male and female Aedes sp. Mosquitoes. This is as a study conducted by Dutta P, et al, who found dengue infection with RT-PCR analysis revealed DENV-2 serotypes were detected with MIR values in Ae. aegypti female and male recorded 10.87/ mile and 11.03 / mile, respectively. [38] The results of research in Thailand during one year of evaluation showed that the transovarial infection rate increased four months before the dengue fever outbreak occurred and MIR in the study was between 0-24.4 /mile. [14] Research on the Amazon found the MIR value ranges varied from 11.4 / mile to 24.1 / mile. Larvae tested with MIR averaged 17.7 / mile. Four serotypes were detected with two serotypes, namely DENV-1 and 4 as the most dominant serotypes found in each city and the number of cases of dengue fever reported varied during that period. [13] Previous research on MIR concluded that the MIR of mosquitoes infected with DENV gradually increased from zero in the rainy season to 48.22 / mile in the middle of the dry season. This increased MIR provides an early warning signal for the upcoming dengue epidemic in the next rainy season (outbreak period) so that intervention during the dry season (non-outbreak period) can be implemented to prevent the spread of dengue fever to new areas. [39] The results of this study are in line with research conducted by Thenmozhi, V et al in Kerala which provided information on the role of Ae. albopictus in transovarial transmission with MIR in males of 0.007 / mil and females 2.20 / mil. Although the MIR in the study was classified as low, these results indicate that Ae. albopictus plays an important role in the maintenance of dengue virus through vertical transmission in nature. [40] The persistence of the four serotypes in this study is a risk factor for cross-infection of the various serotypes present. This is similar to a study conducted by Cristiano, et al, who found a viral co-infection in Ae. aegypti by more than one serotype with MIR in the study was 13.8. [13] The results of the same study regarding cross infection in Ae. aegypti was conducted in the City of Belo Horizonte-MG, Brazil. The results showed that 37.4% of the samples were positive for DENV. 21.4% of DENV-2 positive samples tested individually. DENV-2 and DENV-1 co-infections were detected in 6.7% of the samples and DENV-2 and DENV-3 co- infections were detected in 6.1% of the analyzed samples. [41] The limitation in this study is that sampling for the qPCR test uses the polling method so that it allows sampling of each progeny with a sample of non- infective mosquitoes besides that it cannot calculate the Infection Rate (IR).

5. Conclusion

There was dengue virus persistence in all four serotypes (DENV-1, 2, 3, 4) in Ae. aegypti and Ae. albopictus. DENV-3 is the most persistent serotype in Ae. aegypti with 83.3% MIR and DENV-4 were the most persistent serotypes in Ae. albopictus with 100% MIR. There is no difference in MIR in the four serotypes either in Ae. aegypti and Ae. albopictus. It is necessary to improve the vector control model which not only focuses on the main vector, but also other co-vectors.

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Competing Interests

The authors declare that there is no conflict of interest exists in the submission of this paper.

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