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Detection of Dengue Virus Transovarial Transmission in Dengue Hemorrhagic Fever Endemic Areas

Malik Saepudin[1]✉, Heru Subaris Kasjono[2], Martini[3]
[1]Department of Environmental Health of Ministry of Health Polytechnic Pontianak, West Kalimantan, Indonesia.
[2]Department of Environmental Health of Ministry of Health Polytechnic Yogyakarta. Indonesia.
[3]Department of tropical disease epidemiology - Faculty of Public Health, Diponegoro University

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Abstract

Dengue virus is a group of RNA viruses that are highly pathogenic in humans and spread quickly through the bites of Aedes aegypti and Aedes albopictus mosquitoes, especially in tropical countries. More than half a billion out of 100 countries worldwide are at serious risk of dengue virus infection. Vector surveillance activities with Ovitrap and detection of dengue virus types in Aedes aegypti and Aedes albopictus have never been carried out in Pontianak City. It is important in early alert systems at transmission foci. The purpose of this study was to prove the transovarial transmission of dengue virus in Aedes aegypti and Aedes albopictus mosquitoes with a transovarial transmission index (TTI) in endemic areas in Pontianak City, West Kalimantan. The method used in this research is descriptive observational, viral examination method with immunocytochemistry streptavidin-biotin peroxidase complex (ISBPC) and Polymerase Chain Reaction Transcription Reaction (PCR) aimed at proving the presence of transovarial transmission of dengue virus in the same period. The conclusion in this study is that there is evidence of transovarial transmission of dengue virus in Aedes mosquitoes in endemic areas by 29.3% in Sungai Jawi Dalam sub-district, West Pontianak sub-district, and 39.6% in Batu Layang sub-district, North Pontianak sub-district, mosquito density from the results of the Ovitrap Index measurement (OI) in Batu Layang Village is denser, namely 41.3%, compared to Sungai Jawi Village, which is 38.22% and has succeeded in identifying the type of dengue virus, namely the Dengue virus strain, in the two research locations.

Introduction

Dengue Hemorrhagic Fever is a health problem in Indonesia. All regions of Indonesia are at risk of contracting dengue disease because both the virus that causes it and the mosquitoes that transmit it are widespread in residential areas and public facilities throughout Indonesia. Based on the report from the Ministry of Health of the Republic of Indonesia, DHF has become an endemic problem in 33 provinces and 436 districts or cities, 605 sub-districts, and 1800 villages or urban villages. From 2014 to 2015, it reached 41.25/100,000 population, with a case fatality rate of 0.7%.

DHF in West Kalimantan Province in 2009, the mortality rate of DHF CFR ranked second in Indonesia. Although, the number of sufferers was only 979 cases compared to West Java with 35,453 and DKI Jakarta with 27,964. But the mortality rate reached CFR: 3,38%, after Jambi with a CFR of 3.67%. West Kalimantan Province ranks 2nd in the Kalimantan Islands region, after East Kalimantan with 5,762 cases of DHF. An increase in dengue cases occurs every year. In 2017 there were 5,049 dengue fever cases with 68 deaths. The highest mortality rate was in Pontianak with CFR; 7% and declared KLB.

✉ Correspondence Address:
Department of Environmental Health of Ministry of Health Polytechnic Pontianak, West Kalimantan, Indonesia.
Email : malik_saepudin@yahoo.co.id

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Previous research by Sucipto (2012) in the Pontianak Kota sub-district showed a transovarial transmission of 76.6%. This study was conducted one month before being declared a DHF outbreak. Another study in Malalayang and Banjar Negara Districts showed the transovarial transmission of the Dengue virus in Aedes mosquitoes. But the results were lower than in Pontianak City, namely 6.1%-17.1% and 9.42% (Sorisi et al., 2014). Transovarial transmission of the dengue virus in an area is an important etiological phenomenon. It is responsible for outbreaks due to the maintenance of the virus during the disease inter-epidemic period.

The spread of DHF in Pontianak City is evenly distributed in all sub-districts so that it is declared a DHF endemic city which every year there is an increase in cases of potential outbreaks. The 2017 data survey showed that the highest is in two urban villages, namely Batulayang Village, Batu Layang Health Center, North Pontianak Subdistrict. There are 40 cases of DHF and Sungai Jawi Village, Komyos Sudarso Health Center Working Area, West Pontianak District, with 93. It is known that the transmission of the dengue virus is from the bite of the Aedes aegypti mosquito, which initially bites people infected with the dengue virus and transmits it through bites to people who are not infected with the dengue virus. But there are also cases of DHF that appear when there are no previous cases of DHF. It is thought to be due to the transovarial transmission of the dengue virus in dengue hemorrhagic fever vectors. This study aims to determine transovarial transmission in dengue-endemic areas and detect the virus. This research is also an active observation in the context of implementing an early warning system (SKD) or an early warning system (EWS) in transmission foci, where dengue cases occur almost every year to prevent outbreaks (Fuadzy et al., 2020; Achmadi, 2012)

Method

This study used a descriptive design, carried out in July 2018 for four months, with the type of descriptive research with a cross-sectional study design, namely to prove the existence of transovarial Virden transmission in dengue-endemic villages, consisting of 2 outputs, namely dengue-endemic villages. Determination of population The sample was carried out by purposive sampling of Aedes aegypti and Aedes albopictus mosquitoes from 200 houses from 2 research locations, namely Batu Layang Village and Sungai Jawi Dalam Village (Saepudin, 2011). The research samples were Aedes aegypti and Aedes albopictus eggs taken from two locations, then colonized into adult mosquitoes with an average age of 7 days, not yet sucking blood, full of 10% sugar solution.

Laboratory research starts from the pre-adult stage to become an adult mosquito. Each village took 1000 mosquitoes that met the criteria for the test mosquitoes. The total number is 2,000. The method used to identify dengue virus infection in mosquitoes. Examination of dengue virus by immunocytochemical methods streptavidin-biotin peroxidase complex (ISBPC) and Reverse-Transcription Polymerase Chain Reaction (RT-PCR). The materials and tools used in this RT-PCR test are as follows:

a.Object glass, Cover slip, Phosphate Buffer Saline (PBS), absolute methanol, H2O2 (hydrogen peroxide), primary antibody (DSSC7 monoclanate antibody).

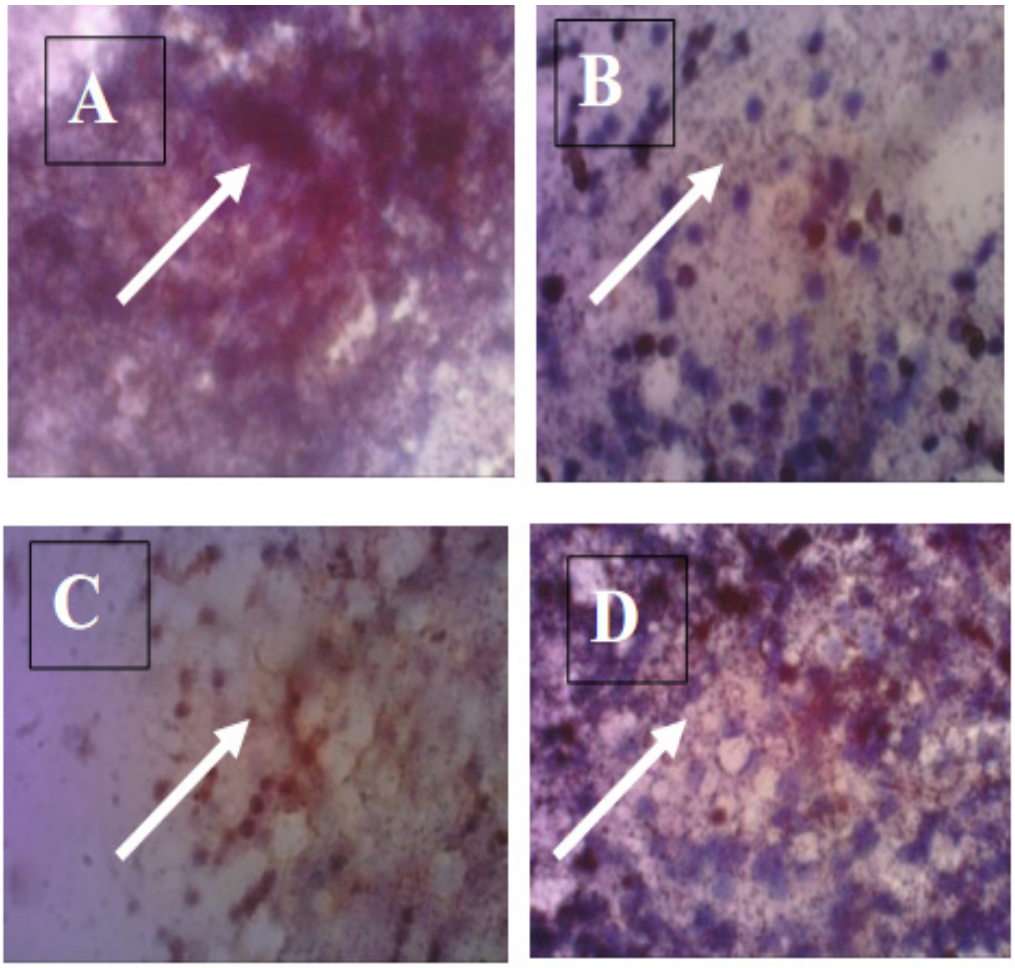
b. Starr Trek Detection Kit (Biocare medical) which contains five ready-to-use reagents: (i) Background sniper (cat. No. BS966L10) as a protein blocking solution containing non-immune serum; (ii) Trekki Universal Link (cat. No. STU700L10) containing a secondary antibody labeled Biotin; (iii) TrekAvidin-HRP label (cat. No. STHRP700L10), which contains streptavidin peroxidase Conjugate labeled with the enzyme horseradish peroxidase (HRP), (iv) Betazoid Diaminobenzidine retracloride (DAB) chromogen (cat. No. BDB900G5), and (v)) Betazoid DAB Substrate Buffer (paint NO. DS900L10), Mayer Hematoxylin paint (counterstain), alcohol, entellon, aluminum foil, tissue and immersion oil, the female mosquito Aedes aegypti (egg, larva, pupa, and adult stages).

c.Materials Preparation: (i) Peroxidase blocking solution: one part 30% hydrogen peroxide plus nine parts absolute methanol,

(ii) DSSE10 monoclonal antibody 1:10:100 l DSSE10 antibody plus 900µl fresh PBS, (iii) DAB chromogram substrate: 1 l Betazoid DAB chromogen was diluted with 600 l Betazoid DAB Substrate Buffer, immediately before use.

Results and Discussions

This research has received an ethical permit from the Pontianak Health Polytechnic Research Ethics Committee with certificate number: 019 / KEPT-PK.PKP / VI / 2018. The study was conducted in an endemic area of DHF with a case study in Pontianak City. The measurement results show that the average Ovitrap Index is mostly outside the house, 41.3% in Batu Layang Village, compared to 38.22% in Sungai Jawi Village. The mosquitoes used were Aedes aegypti and Aedes albopictus mosquitoes with an average age of 7 days, full of 10% sugar water solution. Each glass slide contains 12 head squash preparations. Specifically, the positive and negative control mosquitoes were taken from the mosquitoes of the Parasitology Laboratory of the Faculty of Medicine, UGM. Following the immunocytochemical streptavidin-biotin peroxidase complex (ISBPC) method, which was compiled and standardized by Umniyati (Sorisi et al., 2014).



Picture. 1 Micrograph photo of head squash preparation

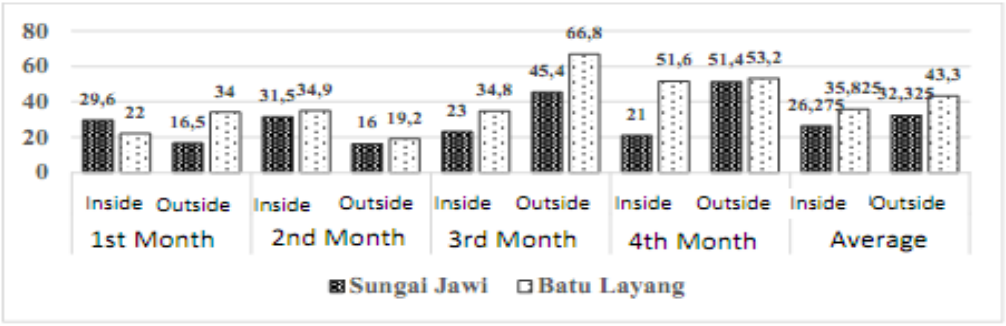
Picture. 1 Micrograph photo of head squash preparation with a magnification of 100x10, which shows positive DEN antigen in the form of brownish hexagonal granules that spread to mosquito brain tissue from Batu Layang sub-district (C) and Sungai Jawi sub-district (D). Figure A is a negative control of non-Aedes aegypti mosquito preparations and Figure B is positive control antigens from mosquitoes infected with Dengue virus with an incubation period of 7 days.

Picture 1. shows the positive (+) infection level, 400x magnification shows brownish-colored sand grains scattered between brain tissue, but almost no cells that show brown color in the cytoplasm. On the positive (++) the sand grains were more spread out, and 1-10 cells showed a brown color in the cytoplasm per field of view at 400x magnification. In positive (+++), the distribution of sand grains is getting wide. The 10-100 cells found show a brown color in the cytoplasm so that the infection appeared at 100x magnification. The description of positive (+++), (++), and (+) infection levels can be found in the preparations from the Sugai Jawi Dalam village. While in Batu Layang, only positive (+) infection rates. The results of dengue virus detection in Aedes aegypti mosquitoes from eggs can be seen in Table 1 below:

Table 1. Results of Microscopic Examination of Positive and Negative Head Squash Preparations in Aedes aegypti Mosquitoes

Villages	Numbers			ITT (%)
	Samples	(+)	(-)	
Sungai Jawi Dalam	1000	293	607	29,3
Batu layang	1000	396	604	39,6
Total	**2000**	**789**	**1.211**	**60,55**

Tabel 1 shows the TTI on Sungai Jawi Dalam is higher, namely 29,3% than Batu Layang with 39,6%. Based on table 1 and Picture 3, the highest TTI on dengue endemic areas is on Batulayang village namely 43,33% outside and 32,32% inside.

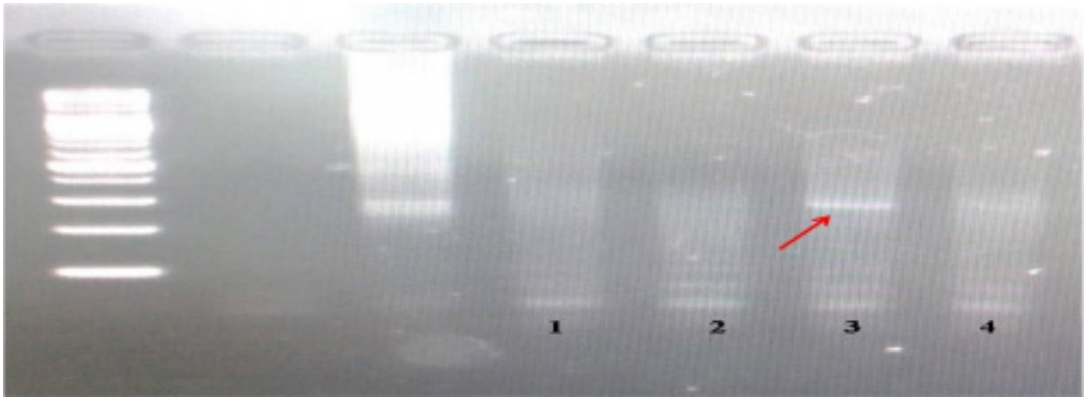


Picture 2. Transovarial Transmission Index in Pontianak

Table 2 Number of Positive Samples Based on the Gender of *Aedes* mosquitoes

Villages	Samples (+)	Female		Male	
		n	%	n	%
Sungai Jawi Dalam	293	268	91	25	9
Batu layang	396	359	90	37	10
Total	**789**	727	91	62	9

From table 2, in each research location in Sungai Jawi Dalam and Batu Layang Villages, male Aedes mosquitoes were positive for Dengue Virus, namely 9% and 10%. The results of the examination of the Aedes aegypti mosquito using the Reverse-Transcription Polymerase Chain Reaction (RT-PCR) method found a strain of the dengue virus. More details can be seen in Figure 3 below.



Picture 3, Left to Right: DNA marking, negative control, positive control, sample number 1,2, 3 and 4

Examination of the type of dengue virus in Aedes aegypti mosquitoes is by the RT-PCR method. Based on the observations and documentation of the electrophoresis results with Gel Doc, as shown in Figure 3, shows that in sample number 3 it is positive for Dengue strain 3.

Field research was carried out by collecting eggs of Aedes aegypti and Aedes albopictus carried out four times for four months with ovitrap. Then the positive ovistrip eggs were colonized in the parasitology laboratory of the Faculty of Medicine, Gadjah Mada University. The results of mosquito colonization in the laboratory showed the total population of each mosquito cage. The Aedes aegypti was almost the same as Aedes albopictus. The results of the Ovitrap Index (OI) calculation are higher outside the home (41.3%) than inside the house (38.22%). It indicates the number of vector mosquitoes in Batu Layang Village is denser. The results of this study are in line with the results of research in several urban villages in Jakarta. OI is higher outside (36.4%) than inside the house (33.5%) because Aedes albopictus prefers to lay eggs outside than inside the house. The density of vector mosquitoes also increases contact with the host (humans) in the vicinity. George et al. (2015) stated that Aedes aegypti and Aedes albopictus mosquitoes like resting areas and indoor and outdoor activities, specifically Aedes albopictus in gardens, so they have less contact with humans, also concluded that ovitrap is quite effective as a one way to monitor the density of Aedes aegypti.

This study also showed transovarial transmission in Batu Layang Village 39.6% and Sungai Jawi Dalam 29.3%. Transovarial transmission of Aedes aegypti and Aedes albopictus mosquitoes play a role in increasing and maintaining the dengue epidemic in dengue-endemic areas. According to Matangkasombut et al. (2020), female mosquitoes experience a viral infection of their ovarian tissue and are maintained until the next generation genetically and persistently. VirDen will be transmitted transovarially and increase the frequency to filial (F)-7 then persist in the next generation. The same thing was conveyed by Rahayu et al. (2019), stating a transovarial transmission of dengue virus in Aedes aegypti eggs in Malaysia up to the 5th generation of female mosquitoes, but absent from the 6th and 7th generations. The vertical transmission mechanism of arbovirus in the mosquito's body is by female mosquitoes in their eggs (transovarial). It will later become mosquitoes. This infection rate exceeds 80% (da Cruz et al., 2015).

It indicates that transovarial transmission potentially affects the maintenance of dengue endemicity, with the Aedes aegypti mosquito as the dengue virus reservoir over time. It proves that in villages with dengue-endemic, there are always cases of DHF every year. Transovarial transmission like this also occurred in several villages of Yogyakarta. Namely, the transovarial transmission rate with an infection rate of 38.5-70.2%, also in several DHF endemic districts in Central Java and Sampit, East Kotawaringin Regency, Central Kalimantan. The possibility continues to spread to other areas in Indonesia transovarial transmission of VirDen will naturally occur. The tests in several urban villages of Yogyakarta were also found similar things. (Rosa and Salmah, 2015).

The same study conducted in Puerto Iguazú, Misi, Argentina showed that 11.6% of positive mosquitoes were lower than TTI in Pontianak (Espinosa et al., 2014). In Malalayang and Banjar Negara Districts, there was a transovarial transmission of Dengue virus in Aedes mosquitoes, but the results were lower than in Pontianak, namely 6.1%-17.1% and 9.42% (Sorisi et al., 2014). Compared with previous research by Sucipto (2012) in the Pontianak Kota sub-district, it shows a much higher number, namely TTI of 76.6% one month before being declared a DHF outbreak. The results of previous studies and naturally 60% of transovarial transmission occurred in the 1st generation. The studies stated the Dengue virus transovarial transmission as a vital etiological phenomenon responsible for outbreaks. It is due to the maintenance of the virus during the inter-epidemic period of the disease (Hikmawati et al,. 2020: Ferreira-De-Lima & Lima-Camara, 2018). This study provides the natural transovarial infection evidence by the Dengue-3 virus in Aedes aegypti. Dengue virus-3 is the primary viral strain that is the most virulent (Sunardi et al., 2018). In line with

the results of Utama et al. (2019), infection with any of the four serotypes can lead to subclinical, life-threatening diseases. DENV 1-4 serotypes circulated during the study period, with the highest overall level from January to March, among the strains the most dominant being the Dengue Virus 3 starin, as researched by Halsey et al. (2012) and Soo et al. (2016), showed that severe cases occurred in DHF patients infected with VirDen-3. In contrast, the results of a study in Mato Grosso provided evidence of natural transovarial infection by the Dengue Virus-4 in Aedes aegypti. This type of infection may have served as a virus maintenance mechanism during the interepidemic period in Cuiabá, where dengue outbreaks are reported annually. These results emphasize the need for efficient vector population control measures to prevent arbovirus outbreaks in the state (da Cruz et al., 2015).

The difference in type and malignancy in each region is due to variations in topography and socio-demographic seasons. They cause the extrinsic cycle time to fluctuate. Cases that occurred in the study area are tropical climates and population mobility. As well as the optimal season for the extrinsic cycle of dengue strain-3. It is indicated by the data on BDB mortality with the highest number in the two research areas, namely Batu Layang Village, Case Fatality Rate (CFR) of 7.14%, and Jawi Dalam River Output of 4.44% (Dinas Kesehatan Kota Pontianak, 2017). Research using RT-PCR examination is very important. Besides identifying four dengue virus serotypes in larval samples, it can also contribute to the development of early detection systems for virus circulation and predictive models of outbreaks and epidemics of this disease (Da Costa et al., 2017; Da Cruz et al., 2015).

This research has not yet concluded a relationship between the severity of infection between patients with a specific dengue virus. But in the future, further studies can be carried out to ensure the relationship between the agent and the host. As large-scale cross-sectional studies conducted in Latin America are very important, longitudinal studies relating the temporal sequence of serotype-specific dengue infection and clinical development of manifestations are needed to confirm some of the new findings of this study. In addition, future studies concentrating on clinical differences in serotypes (eg, genotype and lineage-specific serotypes) will further elucidate the role of interindividual serotypes and DENV morbidity (Halsey et al., 2012).

This infection rate was also depicted in this study through microscopic images of head squash preparations showing positive (+) infection rate found in Sungai Jawi Dalam sub-district was lower than Batulayang output (+++). It shows that mosquitoes with a heavy infection rate when infecting the host will more easily cause symptoms of DHF because the amount of virus that enters is more. Some virologists say that the cause of the disease is the virulence of the virus. The theory of viral virulence says that for the emergence of dengue fever does not need two infections Just once is enough if the virus is virulent. The problem with proving this theory is that there are no laboratory markers for virulence. Until now there is no material, such as monoclonal antibodies, can be used to show virulent or non-virulent Dengue Virus. A Dengue infection will appear with clinical symptoms influenced by host immunity, the amount of virus, and the strain of the virus. However, the transovarial transmission may be a vital mechanism in the spread of DHF. Using RT-PCR, it was possible to identify four DENV serotypes in larval samples (Da Costa et al., 2017; Espinosa et al., 2014).

This study also found that 10% of male Aedes aegypti were infected with the virus transovarially in Batu Layang Village and 9% in Sungai Jawi Dalam village. It is in line with the study in Kenya, which detected dengue virus in immature mosquitoes, both male and female sexes. It provided evidence of transovarial transmission of this arbovirus in local mosquitoes. This phenomenon may be driving the maintenance of the underlying virus, highly contributing to its periodic reappearance among humans in Kenya (Heath et al., 2020). This research is vital information that the increase in cases throughout the year occurred in both regions due to the presence of the Dengue virus found in male and female mosquitoes.

Fridolina Mau (2014) emphasized that non-infective female mosquitoes mate with infective male mosquitoes, causing infection

with female mosquitoes. Transmission of the dengue virus through the mating behavior of Aedes mosquitoes (transveneral transmission) is part of the vertical transmission of mosquitoes that is still rarely studied (Da Costa et al., 2017). Recent laboratory studies have proven that male Aedes aegypti mosquitoes infected with DENV-3 intrathoracic with an incubation period of 5 days and 14 days can transmit DENV-3 to non-infectious female Aedes aegypti mosquitoes. The study also stated that the natural polygamous behavior of male mosquitoes also plays a vital role in the dengue virus spread. So a male mosquito infected with DENV-3 can mate with a certain number of non-infectious female mosquitoes. As a result, the infectious female mosquito will produce infected fertile eggs. Knowledge of mosquito mating behavior is related to some factors. Such as optimal physiology of mosquitoes, physiological mechanisms that regulate mosquito mating, and appropriate environmental conditions. Other factors need to be studied further. This study provides vital information in developing a more effective dengue control strategy in vector mating mechanisms.

The discovery of the Transovarial Transmission Index (TTI) value, the identification of dengue virus serotype 3, and the discovery of dengue virus in male mosquitoes in Batu Layang and Sungai Jawi villages in Pontianak City shows how high the potential for transovarial transmission of dengue virus is. The large number of Aedes aegypti and Aedes albopictus mosquitoes determines the potential for transovarial transmission of the dengue virus. The presence of housing density conditions in the urban environment can trigger optimal temperature and humidity levels in the Research Area, thus supporting the occurrence of transovarial transmission cycles throughout the year, having the potential for outbreaks in endemic areas of Pontianak City. It is in line with the in-depth study of Amazonian urban areas showing that transovarial transmission is a vital mechanism for the maintenance and spread of disease in the Amazon (Da Costa et al., 2017)

This study concludes that the dengue virus maintains its life in optimal numbers through the transovarial transmission to a genetically superior mosquito population, vital in dengue transmission. The phenomenon needs special attention. Because these two outputs, apart from endemic outputs, are also the areas with the highest cases and deaths in 2017. Therefore, continuous vector control efforts are needed until the F-8 generation (4 months) to stop the virus circulation by transovarial transmission. The most effective control is carried out on the source and their habitat by engineering the environment as a breeding place using a modified ovitrap plus tool called "Rekaitidiri". It can kill adult female larvae and mosquitoes, thus, effectively reducing the density index of larvae and adult Aedes aegypti mosquitoes (Saepudin et al., 2017; Saepudin et al., 2019). Detection of transovarial transmission of dengue virus in vector mosquitoes can be a vital part of the epidemiological survey of dengue fever and is used in the development of an early warning system to anticipate the spread of dengue virus transmission to humans and the emergence of new cases of dengue fever that previously had no cases of dengue.

Conclusion

This study finds evidence of transovarial transmission of the dengue virus in Aedes mosquitoes in Pontianak City. Previous research in 2012 showed the value of the dengue virus transovarial index (TTI) was 76.6%. Meanwhile, in this study, in the same area, there was a decrease of 29.3% in the Sungai Jawi Dalam sub-district, West Pontianak sub-district, and 39.6% in Batu Layang sub-district, North Pontianak district. Successfully identified the type of Dengue Virus strain 3.

References

Achmadi, U.F., 2012. *Manajemen Demam Berdarah Berbasis Wilayah, Buletin Jendela Epidemiologi Puslitbang Ekologi Dan Statistik Kesehatan*. Jakarta: Kementerian Kesehatan RI.

Da-Costa, C.F., dos-Passos, R.A., Lima, J.B.P., Roque, R.A., Sampaio, V.d-S., Campolina, T.B., Secundino, N.F.C., & Pimenta, P.F.P., 2017. Transovarial Transmission of DENV in Aedes Aegypti in the Amazon Basin: A Local Model of Xenomonitoring. *Parasites and*

Vectors 10(1), pp.1–9.

Da-Cruz, L.C.d-T.A., Serra, O.P., Leal-Santos, F.A., Ribeiro, A.L.M., Slhessarenko, R.D., dos-Santos, M.A., 2015. Natural Transovarial Transmission of Dengue Virus 4 in Aedes Aegypti from Cuiabá, State of Mato Grosso, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 48(1), pp.18–25.

Dinas Kesehatan Kota Pontianak., 2017. *Profil Kesehatan Kota Pontianak Tahun 2017*.

Espinosa, M., Giamperetti, S., Abril, M., & Seijo, A., 2014. Transmisión Vertical de Virus Dengue En Aedes Aegypti, Capturados En Puerto Iguazú, Misiones, Argentina. *Revista do Instituto de Medicina Tropical de Sao Paulo*, 56(2), pp.165–67.

Ferreira-De-Lima., Henrique, V., & Lima-Camara, T.N., 2018. Natural Vertical Transmission of Dengue Virus in Aedes Aegypti and Aedes Albopictus: A Systematic Review. *Parasites and Vectors*, 11(1), pp.1–8.

Fuadzy, H., Widawati, M., Astuti, E.P., Prasetyowati, H., Hendri, J., Nurindra, R.W., & Hodijah, D.N., 2020. Risk Factors Associated with Dengue Incidence in Bandung, Indonesia: A Household Based Case-Control Study. *Health Science Journal of Indonesia*, 11(1), pp.45–51.

George, L., Lenhart, A., Toledo, J., Lazaro, A., Han, W.W., Velayudhan, R., Ranzinger, S.R., & Horstick, O., 2015. Community-Effectiveness of Temephos for Dengue Vector Control: A Systematic Literature Review. *PLoS Neglected Tropical Diseases*, 9(9).

Halsey, E.S., Marks, M.A., Gotuzzo, E., Fiestas, V., Suarez, L., Vargas, J., Aguayo, N., Madrid, C., Vimos, C., Kochel, T.J., & Laguna-Torres, V.A., 2012. Correlation of Serotype-Specific Dengue Virus Infection with Clinical Manifestations. *PLoS Neglected Tropical Diseases*, 6(5).

Heath, C.J., Grossi-Soyster, E.N., Ndenga, B.A., Mutuku, F.M., Sahoo, M.K., Ngugi, H.N., Mbakaya, J.O., Siema, P., Kitron, U., Zahiri, N., Hortion, J., Waggoner, J.J., King, C.H., Pinsky, B.A., & LaBeaud, A.D., 2020. Evidence of Transovarial Transmission of Chikungunya and Dengue Viruses in Fieldcaught Mosquitoes in Kenya. *PLoS Neglected Tropical Diseases*, 14(6), pp.1–17.

Hikmawati, I., Wahjono, H., Martini., Hadisaputro, S., Darmana, E., Djati, K.R.M., Mardihusodo, S.J., & Umniyati, S.R., 2020. Rapid Detection of Dengue Virus Transovarial Transmition from Nature and Artificial Inoculation. *Journal of Critical Reviews*, 7(1), pp.41–47.

Matangkasombut, P., Manopwisedjaroen, K., Pitabut, N., Thaloengsok, S., Suraamornkul, S., Yingtaweesak, T., Duong, V., Sakuntabhai, A., Paul, R., & Singhasivanon, P., 2020. Dengue Viremia Kinetics in Asymptomatic and Symptomatic Infection. *International Journal of Infectious Diseases*, 101, pp.90–97.

Rahayu, A., Saraswati, U., Supriyati, E., Kumalawati, D.A., Hermantara, R., Rovik, A., Daniwijaya, E.W., Fitriana, I., Setyawan, S., Ahmad, R.A., Wardana, D.S., Indriani, C., Utarini, A., Tantowijoyo, W., & Arguni, E., 2019. Prevalence and Distribution of Dengue Virus in Aedes Aegypti in Yogyakarta City before Deployment of Wolbachia Infected Aedes Aegypti. *International Journal of Environmental Research and Public Health*, 16(10), pp.1–12.

Rosa, E., & Salmah, S., 2015. Detection of Transovarial Dengue Virus with RT-PCR in Aedes Albopictus (Skuse) Larvae Inhabiting Phytotelmata in Endemic DHF Areas in West Sumatra , Indonesia. *American Journal of Infectious Diseases and Mircobiology*, 3(1), pp.14–17.

Saepudin, M., 2011. *Metodologi Kesehatan Masyarakat*. Jakarta: Trans Info Media (TIM).

Saepudin, M., Hadisaputro, S., Suwondo, A., & Suhartono., 2017. The Effect of Rekattidiri Ovitrap towards Aedes Aegypti Larval Density. *International Journal of Public Health Science (IJPHS)*, 6(1), pp.80–86.

Saepudin, M., Hadisaputro, S., & Umniyati, S.R., 2019. The Effect of Sticky Autocidal Mosquito Trap (SAMT) on the Index of Transovarial Transmission of Dengue Viruses. *Indian Journal of Public Health Research & Development*, 10(12), pp.1812–17.

Soo, K.M., Khalid, B., Ching, S.M., & Chee, H.Y., 2016. Meta-Analysis of Dengue Severity during Infection by Different Dengue Virus Serotypes in Primary and Secondary Infections. *PLoS ONE*, 11(5), pp.4–14.

Sorisi, A.M.H., Umniyati, S.R., Satoto, T.B.T., 2014. Transovarial Transmission Index of Dengue Virus on Aedes Aegypti and Aedes Albopictus Mosquitoes in Malalayang District in Manado, North Sulawesi, Indonesia. *Tropical Medicine Journal*, 1(2), pp.87–95.

Sucipto, C.D., 2012. Dengue Transovaria Virus Transmission Detection in the Aedes Aegypti Mosquito. *Journal Of Health Science*, XII, pp.121.

Sunardi, P., Kusnanto, H., Satoto, T.B.T., & Lazuardi, L., 2018. Prevalence of Dengue

Virus Transovarial Transmission and DHF Incidence Rate in Grogol Sub-District of Sukoharjo District. *Jurnal Medicoeticolegal dan Manajemen Rumah Sakit*, 7(2), pp.102–7.

Utama, I.M.S., Lukman, N., Sukmawati, D.D., Alisjahbana, B., Alam, A., Murniati, D., Utama, I.M.G.D.L., Puspitasari, D., Kosasih, H., Laksono, I., Karyana, M., Karyanti, M.R.K., Hapsari, M.M.D.E.A.H., Meutia, N., Liang, C.J., Wulan, W.N., Lau, C.Y., & Parwati, K.T.M., 2019. Dengue Viral Infection in Indonesia: Epidemiology, Diagnostic Challenges, and Mutations from an Observational Cohort Study. *Plos Neglected Tropical Diseases, 2019*, pp.1–19.