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Mortality Rate and Gonotrophic Cycle Serotype Denv-2 Transovarial Transmission through Intratoracal

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Abstract

Transovarial transmission is an important role of *Aedes aegypti* as an intermediate host. This role is one of the factors dengue virus can survive in nature during interepidemic. DENV-2 transovarial transmission is an important problem because there has been evidence of horizontal transmission of DENV-2 serotypes by vertically infected mosquitoes. The study aimed to determine the mortality rate and gonotrophic cycle of serotype denv-2 transovarial transmission through intratoracal in *Aedes aegypti* mosquitoes. The research method used a quasi-experimental design with the intervention of intratoracal DENV-2 serotype virus infection. The population in this study was *Aedes aegypti* mosquito female laboratory colony. The results showed a mortality rate of 87.2%, there were differences in the number of gonotrophic cycle eggs ($p < 0.0001$) with mean different: 13.5. and infection rate of DENV-2 transovarial transmission is 30-40%. An active role for the community is needed in eradicating mosquito breeding places in each gonotrophic cycle.

Keywords: Gonotrophic Cycle, DENV-2, Transovarial, Intratoracal.

Introduction

DENV-2 transovarial transmission is an important problem because there has been evidence of horizontal transmission of DENV-2 serotypes by vertically infected mosquitoes¹. Transovarial is the transmission of dengue virus vertically from female mosquitoes to its eggs². Dengue as a vector-borne disease is currently still a major disease problem especially in tropical and sub-tropical regions. The results of the research with cartographic approach estimate that there are 390 million / year of infection due to DHF and 96 million indicate clinical or β -clinical severity. This disease has infected 70-500 million people³ in more than 100 countries around the world³. CDC (Center for Diseases Control and Prevention) reported that a third of the world's population is at high risk of developing dengue fever, especially in the tropics and sub-tropics⁴. Transovarial transmission is an important role of *Aedes aegypti* as an intermediate host. This role is one of

the factors dengue virus can survive in nature during interepidemic. *Aedes aegypti* is the main vector and the population in nature is more (92.9%) than the co-vector *Aedes albopictus* (6.8%) with an infection rate of 0.81%⁵. The proportion of transovarial events in Kenya between 2013-2014 shows the proportion of 8-11%⁶. Results of research in India on third and fourth instar larvae showed transovarial infection rates of 35%⁷.

DHF is still a problem in Indonesia due to the presence of all four DENV-1, 2, 3, 4 serotypes and a high incidence rate in some regions⁸. The results of the research by Hikmawati, I and Pattima, S showed a CFR of 8.69⁹. The results showed that the Transovarial Transmission Index (TTI) ranged from 39.1%-70%¹⁰. Transovarial proportion with four types of serotype by 12.3%¹¹. The results of transovarial in Indonesia showed the positive rates of *Aedes aegypti* higher than *Aedes albopictus*¹². The results of serotype distribution studies in Indonesia found that the overall proportion of DENV-1 was 9.6%, DENV-2 was 55%, DENV-3 was 29% and DENV-4 was 0.4%¹³. This study aims to determine the mortality rate and gonotrophic cycle of serotype denv-2 transovarial transmission through intratoracal in *Aedes aegypti* mosquitoes.

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Method

Research Design: The design of the study used quasi-experimental.

Sampling and Epidemiological Data Collection

Mosquitoes: The population was female *Aedes aegypti* mosquitoes free of dengue virus from the laboratory colony maintained at the Laboratory of Parasitology, Gajah Mada University. The number of samples were 86 female mosquitoes.

Virus: The dengue-2 virus used in the study was obtained from a parasitology laboratory, Gajah Mada University.

Intratoracal Procedure: Put 86 *Aedes aegypti* mosquitoes into a test tube, then put it in a beaker glass that has been filled with ice cubes, after fainting, take one at a time, put it under a microscope and a set of mosquito intratoracal devices, then an injection of $\pm 2 \mu$ supernatant of the DENV-2 virus each tail (Fig. 1).

Maintenance of Mosquitoes after Intratoracal and Detection of Transovarial: Two-three days after the intratoracal, the mosquito was inserted in a 20 cm³ cage (Fig. 2) to feed the healthy human blood by means of membrane feeding. Mosquitoes are left to lay their eggs and are given cotton every day with sugar water and daily deaths are recorded. After being seen laying eggs, take the resulting eggs and count them with a microscope as one gonotrophic egg (G1). The mosquitoes are then given blood back in a membrane feeding to lay eggs as gonotrophic 2. The eggs produced from the gonotrophic cycle are then hatched until they become mosquitoes as farian 1 (F1). Detection of Transovarial Transmission by Immuno Histo Chemistry (IHC) test.

- A. **Statistical Methods:** Bivariate analysis using independent t test with p value <0.05 to see differences in eggs produced in the gonotrophic cycle.
- B. **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.



Fig. 1: Set of Intratoracal



Fig. 2: Membrane feeding process

Results

Mortality Rate after Intratoracal: 86 mosquitoes are infectious with intratoracal, death occurred in gonotrophic cycles one and two 75. The description of the death of *Aedes aegypti* after the intratoracal supernatant DENV-2 as in Fig. 4.

Number of deaths after intratoracal DEN-2 serotypes (G1 & G2)

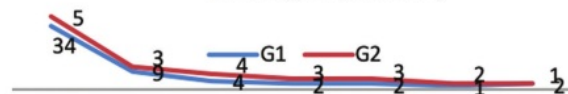


Fig. 4 Deaths after intratoracal

Gonotrophic Cycles: Gonotrophic cycles 1 and 2 in the *Aedes aegypti* mosquito as the following Table 1.

Table 1: Number of eggs and gonotrophic cycle length

	Mean Different	Standar Deviasi	P value ^a
Number of Eggs	13,5	20,1	0,0001
Gonotrophic Cycle length	6,7	1,1	0,0001

^aone sample T test

Transovarial Transmission of *Aedes Aegypti* Mosquito: The result of transovarial detection of *Aedes aegypti* mosquitoes after intratoracal, the IHC method found an infection rate of 30-40%. As shown in Fig. 5.

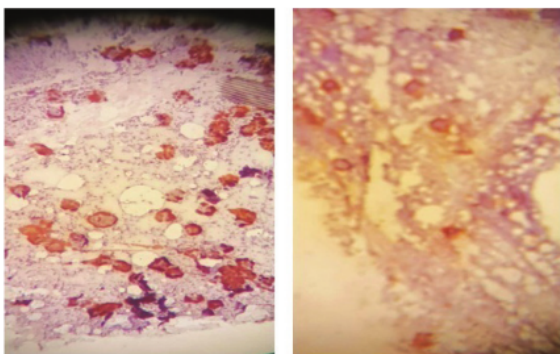


Fig. 5: Transovarial detection with (Immunohistochemistry) *Aedes aegypti* mosquitoes

F1 Metamorphosis: Metamorphosis of egg-larvae (3-19 days), larva-pupa (7-20 days), pupa-mosquito (2-23 days).

Discussion

The results showed the mortality rate of 87.2% meant that the intrathoracal infection act only gave a life expectancy of 12.8%. Higher mortality in one gonotrophic cycle than in the second gonotrophic cycle. This is because in the gonotrophic cycle, the content of the supernatant virus is still high, causing a more severe infection which ultimately results in higher mortality. Blood biting/sucking behavior is a parameter underlying the capacity of mosquito vectors in addition to other factors such as population density, extrinsic incubation period and survival¹⁴. The results of Fouque's study showed that the survival of female *Aedes aegypti* in French Guiana was on average 0.913. Thus, transmission and endemic patterns of dengue fever are predicted throughout the year¹⁵. The results of other studies show in urban areas the female mosquito population is lower and this indicates a lower risk of dengue transmission compared to rural areas and slums¹⁶.

The results of this study indicate that the mean of different gonotrophic cycles is longer (6.7 days) than the normal gonotrophic cycle (3-4 days), this is due to one of the low laboratory temperature factors (20°C-21°C) compared to the optimum average temperature for mosquito development (25°C - 27°C). As it is known that air temperature will affect the metabolic process. At low temperatures the metabolism is slow, affecting

egg development. At high temperatures can reduce the size of the larvae so that at the adult level the size of the mosquito becomes small due to high metabolic rate and requires more food intake and more eggs¹⁴. Previous research results showed that the duration of the gonotrophic cycle in Wonosobo District averaged 4 days (3 - 5 days) and the average biological cycle length was 10 days (9 - 11 days)¹⁷. The results of previous studies showed that the estimated length of the gonotrophic cycle was different between the rainy and dry seasons, during the rainy season the correlation coefficient was 4 days, at 26.7 ± 1.22 °C, and 3 days in the dry season at $29.8 \pm 1, 47$ °C. Minimum estimated time to ripen eggs after administration of blood in the 3.5-day rainy season and 3.25 days in the dry season¹⁸. Research results in Puerto Rico gonotrophic cycles between 3-7 days¹⁹.

Besides affecting the gonotrophic cycle, temperature can also affect the number of eggs produced. The results of this study show the mean different number of eggs produced by 13.5 fewer than normal production between 100-150 each mosquito¹⁵ without infection. Research conducted by Gloria et al on the effect of temperature on dengue infection shows Extrinsic Incubation Temperature (EIT) and DENV-2 genotype have a direct effect on infection rates and EIT affects infection rates differently in each mosquito population, this shows the effect of environmental interactions on genotypes. These results indicate that the magnitude of the DENV epidemic does not only depend on viruses and mosquito genotypes but also adjustments to interact with local temperatures²⁰. The results of Ritadi's research, et al concluded that the temperature and duration of storage affected the percentage of hatching of *Aedes aegypti* eggs in the laboratory ($p = 0.046$)²¹. The low egg production in this research is likely due to the infection of the DENV-2 virus that enters the mosquito's body after an intratoracal, so that the mosquito undergoes an inflammation process. An important part to develop and amplify the dengue virus is cells from the salivary and ovarian glands, because for viruses infected by the virus the part shows morphological and histological changes in shape compared to healthy mosquitoes or not exposed to the virus (accentic). Both parts of this mosquito organ look much bigger (swell). The number of eggs produced is influenced by the amount of human blood sucked by female *Aedes aegypti* mosquitoes to ripen their eggs, blood is used for protein intake that is needed to produce eggs. The results of research in Thailand and Puerto Rico estimated that the average *Aedes aegypti* took 0.76 and 0.63 human blood meals per day²². The results

of the study concluded that the DENV-2 serotype affects mosquito behavior for motivation to feed (first feed) compared to avidity (second feed)²³.

The results of this study indicate that vector infescius requires a longer metamorphosis time (12 days) than non-infectious (8 days) from the stage of the egg to adulthood. Siraj's research, et al. Shows that an increase in temperature can accelerate the epidemic of dengue fever virus, due to the rapid metamorphosis of each generation so that more infections each generation²⁴. The proportion of hatched eggs is positively correlated with the immersion temperature. Survival was 30% at 13.2 °C and above 90% at 20 °C, while the development time at low temperatures was 49.4 d at 13.2 °C and 17.7 d at 20 °C²⁵.

Transovarial transmission in this study showed an infection rate of 30-40% meaning that in 100 *Aedes aegypti* mosquitoes there were 30-40 infected by DENV-2 through intratoracal. The results of this research clarify the role of *Aedes aegypti* mosquitoes through transovarials. Research results in Amazon City showed a transovarial infection rate of 46%²⁶. Population fecundity in mosquitoes infected with DENV-2 was significantly higher than DENV-4, after administering membrane feeding with non-infectious blood for 7 days²⁷. Transovarial phenomena show persistence / maintenance of viral serotypes through intermediate hosts before being transmitted to humans as horizontal transmissions. The results of the study in Colombia showed the presence of dengue virus in larvae in rural areas, all serotypes (DENV-1, DENV-2, DENV-3, DENV 4) were detected in all regions and DENV-1 as the most dominant serotype²⁸. Limitations in this study were not detected in the transovarial transmission of DENV-2 transovarial serotypes via intratoracal, only in the infection rate through IHC test.

Conclusions

The results showed a mortality rate of 87.2%, there was a difference in the number of eggs per gonotrophic cycle ($p < 0.0001$) with mean different: 13.5. The F1 vector infescius metamorphosis takes longer (minimum 12 days) than non-infectious (minimum 8 days) from egg stage to adult and 30-40% infection rate of transovarial transmission of DENV-2 serotype. Vector control through eradication of mosquito breeding sites must be improved considering that the *Aedes aegypti* mosquito as the main vector of dengue fever has several gonotrophic cycles. An active role for the community is

needed in eradicating mosquito breeding places in each gonotrophic cycle.

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Conflict of interest: NIL

Ethical clearance: Done by Research committee

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