Mortality Rate and Gonotropic Cycle Serotipe 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 gonotropic 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 gonotropic 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 gonotropic cycle.

Keywords: *Gonotropic 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 mosquitoes[1]. Transovarial is the transmission of dengue virus vertically from female mosquitoes to its eggs [2]. 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 sub-clinical severity. This disease has infected 70-500 million people/year in more than 100 countries around the world [3]. CDC (Centers 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-tropics[4]. 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 regions[8]. The results of the research by Hikmawati, I and Pattima, S showed a CFR of 8.69 [9]. The results showed that the Transovarial Transmission Index (TTI) ranged from 39.1%-70%[10]. Transovarial proportion with four types of serotype by 30,3% [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 gonotropic 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 aegypty* 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 ± 2 μ 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 cm^3 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 gonotropic egg (G1). The mosquitoes are then given blood back in a membrane feeding to lay eggs as gonotropic 2. The eggs produced from the gonotropic 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 gonotropic 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.

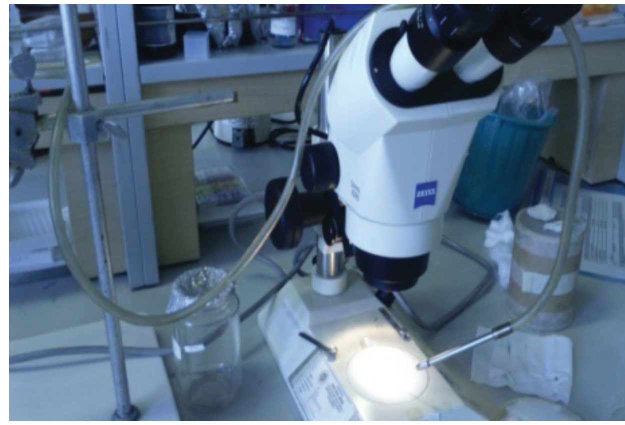

Fig. 1: Set of Intratoracal

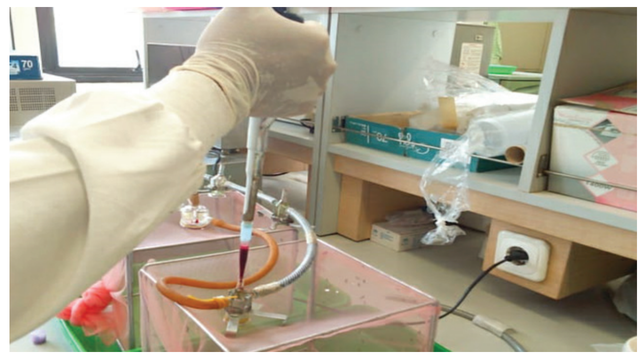

Fig. 2: Membrane feeding process

Results

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

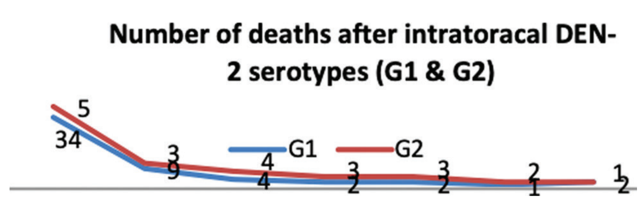

Fig. 4 Deaths after intratoracal

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

Table 1: Number of eggs and gonotropic cycle length

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

[a]one 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.

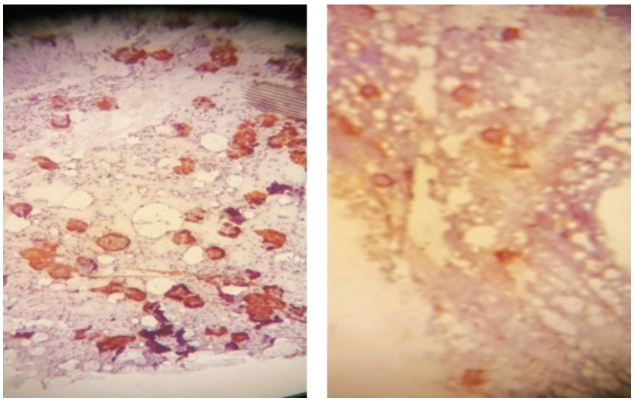

Fig. 5: Transovarial detection with (Immuno Histo Chemistry) Aedes aegypti mosquitoes

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 gonotropic cycle than in the second gonotropic cycle. This is because in the gonotropic 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 survival [14]. 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 year [15]. 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 slums [16].

The results of this study indicate that the mean of different gonotropic cycles is longer (6.7 days) than the normal gonotropic cycle (3-4 days), this is due to one of the low laboratory temperature factors (20^0c-21^0c) compared to the optimum average temperature for mosquito development (25ºC - 27ºC). 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 eggs [14]. Previous research results showed that the duration of the gonotropic 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 gonotropic 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 ± 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 season [18]. Research results in Puerto Rico gonotropic cycles between 3-7 days [19].

Besides affecting the gonotropic 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 mosquito 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 temperatures [20]. 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 imflamation 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 63 human blood meals per day [22]. The results of the study concluded that the DENV-2 serotype affects mosquito behavior for motivation to feed (fist feed) compared to avidity (second feed) [23].

The results of this study indicate that vector infecsius 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 generation [24]. 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 °C [25].

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 days[27]. 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 serotype[28]. 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 gonotropic cycle (p <0.0001) with mean different: 13.5. The F1 vector infecsius 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 gonotropic cycles. An active role for the community is needed in eradicating mosquito breeding places in each gonotropic cycle.

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Conflict of interest: NIL

Ethical clearance: Done by Research committee

References

1. Mourya DT1, Gokhale, Basu A, Barde PV, Sapkal GN, Padbidri VS GM. Horizontal and vertical transmission of dengue virus type 2 in highly and lowly susceptible strains of Aedes aegypti mosquitoes. Acta Virol. 2001;45(2):67–71.
2. Halstead SB. Dengue. Geofrei, Pasfol and Stephen L H, editor. London: imperial College Press; 2008.
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
4. Mubbashir H, Munir S, Kashif R, Nawaz HB, Abdul B BK. Characterization of dengue virus in Aedes aegypti and Aedes albopictus spp. of mosquitoes: A study in Khyber Pakhtunkhwa, Pakistan. Mol Biol Res Commun. 2018;7(2):77–82.
5. Couret J, Dotson E BM. Temperature, larval diet, and density effects on development rate and survival of Aedes aegypti (Diptera: Culicidae). PLoS One. 2014;9(2).
6. Lutomiah J, Barrera R, Makio A, Mutisya 2, Koka H, Owaka S, Koskei E, Nyunja A, Eyase F, Coldren R SR. Dengue Outbreak in Mombasa City, Kenya, 2013-2014: Entomologic Investigations. PLoS Negl Trop Dis. 2016;10(10).
7. Joshi V, Mourya DT, Sharma RC. Persistence Of Dengue-3 Virus Through Ttranovarial Ttransmission Passage In Successive Generations Of Aedes Aegypti Mosquitoes. Am J Trop Med Hyg. 2002;67(2):158–61.
8. Wang SF, Wang WH, Chang K, Chen YH, Tseng SP, Yen CH, Wu DC CY. Severe Dengue Fever Outbreak in Taiwan. Am J Trop Med Hyg. 2016;94(1):193–7.
9. Hikmawati, I, Pattima S. Cross Sectional Study: The Relationship Between Comorbidities and

Hematocrit with the Hospitalization of Patients of Dengue Hemorrhagic Fever(DHF). Adv Sci Lett. 2018;14(1):112–5.

10. Lidiasani P. Mosesa, Angle Sorisi VDP. Deteksi transmisi transovarial virus dengue pada Aedes aegypti dengan teknik imunositokimia di Kota Manado. J e-Biomedik (eBm),. 2016;4(1):116–21.
11. Thongrungkiat S, Maneekan P, Wasinpiyamongkol L. Prospective field study of transovarial dengue-virus transmission by two different forms of Aedes aegypti in an urban area of Bangkok , Thailand. J Vector Ecol. 2011;36(1):147–52.
12. Sorisi AMH, Umniyati SR, Satoto TB. Transovarial Transmission Index of Dengue Virus on Aedes aegypti and Aedes albopictus Mosquitoes in Malalayang District in Manado , North Sulawesi , Indonesia. TMJ. 2012;01(02):87–95.
13. Prasetyowati H, Puji Astuti E. Serotipe Virus Dengue di Tiga Kabupaten / Kota Dengan Tingkat Endemisitas DBD Berbeda di Propinsi Jawa Barat Dengue Virus Serotypes in Three Districts / Municipalities with Different Endemicity Level of Dengue in West Java. Aspirator. 2010;2(2):120–4.
14. SB H. Dengue-virus mosquito interactions. In: Dengue. 2008. p. 273–291.
15. Fouque F, Carinci R, Gaborit P, Issaly J, Bicout DJ SP. Aedes aegypti survival and dengue transmission patterns in French Guiana. J Vector Ecol. 2006;31(2):390–9.
16. David MR, Lourenço-de-oliveira R, Freitas RM De. Container productivity , daily survival rates and dispersal of Aedes aegypti mosquitoes in a high income dengue epidemic neighbourhood of Rio de Janeiro : presumed influence of differential urban structure on mosquito biology. Mem Inst Oswaldo Cruz , Rio Janeiro. 2009;104(September):927–32.
17. Farid Faradilah. Kajian Siklus Biologik dan Siklus Gonotropik Nyamuk Aedes aegypti di Daerah Endemis Demam Berdarah Dengue (DBD) Di Kecamatan Wonosobo. Universitas Diponegoro Semarang; 2013.
18. Garcia-Rejon CMB-BU-GC-TCTDM-WMT-CCNE. Blood Feeding Status, Gonotrophic Cycle and Survivorship of Aedes (Stegomyia) aegypti (L.) (Diptera: Culicidae) Caught in Churches from Merida, Yucatan, Mexico. Neotrop Entomol. 2017;46(6):622–30.
19. Morrison AC1, Costero A, Edman JD, Clark GG ST. Increased fecundity of Aedes aegypti fed human blood before release in a mark-recapture study in Puerto Rico. J Am Mosq Control Assoc. 1999;15(2):98–104.
20. Gloria-Soria A, Armstrong PM, Powell JR TP. Infection rate of Aedes aegypti mosquitoes with dengue virus depends on the interaction between temperature and mosquito genotype. Proc Biol Sci. 2017;11(284):1864.
21. Alfiah RS, Alfiah S. Pengaruh Suhu Penyimpanan Terhadap Presentase Tetas Telur. Vektora. 2014;6(1):9–12.
22. Scott TW1, Amerasinghe PH, Morrison AC, Lorenz LH, Clark GG, Strickman D, Kittayapong P EJ. Longitudinal studies of Aedes aegypti (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. J Med Entomol. 2000;37(1):89–101.
23. Maciel-de-freitas R, Sylvestre G, Gandini M, Koella JC. The Influence of Dengue Virus Serotype-2 Infection on Aedes aegypti (Diptera:Culicidae) Motivation and Avidity to Blood Feed. PLoS One. 2013;8(6):6–10.
24. Siraj AS, Oidtman RJ, Huber JH, Kraemer MUG, Brady OJ, Johansson MA, et al. Temperature modulates dengue virus epidemic growth rates through its effects on reproduction numbers and generation intervals. PLoS Negl Trop Dis. 2017;11(7):1–19.
25. De Majo MS1, Montini P FS. Egg Hatching and Survival of Immature Stages of Aedes aegypti (Diptera: Culicidae) Under Natural Temperature Conditions During the Cold Season in Buenos Aires, Argentina. J Med Entomol. 2017;54(1):106-13.
26. Da Costa CF, Dos Passos RA, Lima JBP, Roque RA, de Souza Sampaio V, Campolina TB, Secundino NFC PP. Transovarial transmission of DENV in Aedes aegypti in the Amazon basin: a local model of xenomonitoring. Parasit Vectors. 2017;10(1):249.
27. Muturi EJ, Buckner E BJ. Superinfection interference between dengue-2 and dengue-4 viruses in Aedes aegypti mosquitoes. Trop Med Int Heal. 2017;22(4):399–406.
28. Velandia-Romero ML1, Olano VA, Coronel-Ruiz C, Cabezas L, Calderón-Peláez MA, Castellanos JE MM. Dengue virus detection in Aedes aegypti larvae and pupae collected in rural areas of Anapoima, Cundinamarca, Colombia. Biomedica. 2017;37(0):193–200.