

DETECTION OF DENGUE TRANSOVARIAL VIRUS FOR AIDES AEGYPTI MOSQUITOES IN ENDEMIC AREAS OF DENGUE HEMORRHAGIC FEVER

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Article Info: Received 30 August 2019; Accepted 23 September. 2019

DOI: <https://doi.org/10.32553/ijmbs.v3i9.565>

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Conflict of interest: No conflict of interest.

Abstract

Background: The incidence rate (IR) of Dengue Hemorrhagic Fever per 100,000 population in Central Java has increased dramatically in five years. Banyumas Regency is an endemic area of dengue hemorrhagic fever in Central Java Province. The cause of dengue hemorrhagic fever is four dengue viruses known as DEN-1, DEN-2, DEN-3, and DEN-4. The purpose of this research is to detect transovarial dengue virus in *Aedes aegypti* mosquito in Banyumas Regency.

Methods: The study was conducted by installing ovitrap in Mersi Village, East Purwokerto District, and in Pliken Village, Kembaran District, Banyumas Regency. The study was conducted by installing ovitrap in Mersi Village (9 samples) and Pliken Village (3 samples). The eggs obtained are hatched into larvae in the laboratory. The 3rd and 4th instar larvae were examined for the presence of dengue virus using the Elisa method.

Result: The results showed that in Mersi Village, there were two positive samples, and in Pliken Village, there was one positive sample containing dengue virus. The presence of the dengue virus found in the hatched *Aedes aegypti* larvae (F1) indicates that there has been a transovarial dengue virus from the parent mosquito in its offspring.

Conclusion: The study concluded that in Banyumas District, a dengue virus transovarial had occurred in *Aedes aegypti* mosquitoes. The government and the community should increase efforts to eradicate mosquito nests (dengue hemorrhagic fever) to reduce the vector population of dengue hemorrhagic fever so that cases of dengue hemorrhagic fever can be eliminated.

Keyword: Transovarial dengue virus, *Aedes aegypti*, Dengue Hemorrhagic Fever

Introduction:

The number of cases of dengue hemorrhagic fever is always fluctuating every year all over Indonesia.¹ The incidence rate of dengue hemorrhagic fever per 100,000 population in Central Java in the last five years was as follows: in 2011 amounted to 15.27 in 2012 amounted to 19.29 in 2013 amounted to 45.52 in 2014 amounted to 32.95 and in 2015 amounted to 34.87 in 2016 amounted to 43.4.² Incidence of dengue hemorrhagic fever in Banyumas district can still be found. Based on data from Banyumas District Health Office from 2010-2015, cases of dengue hemorrhagic fever are always found. In 2013 cases experienced an increase recorded 543 cases (4 people died) with ir 32.14 / 100,000 and CFR 0.74%. In 2014, dengue hemorrhagic fever cases decreased by 209 cases (4 people died) with ir 12.89 / 100,000 and CFR 1.91%. Cases of dengue hemorrhagic fever have increased again in 2015, which is 231 cases (1

person died) until October 2015 with ir 14.14 / 100,000 and CFR 0.43%.³

The case of dengue hemorrhagic fever in Banyumas district illustrates that there are still many problems associated with handling dengue fever, so that dengue fever cases are always high every year. Control of dengue fever can be done by eliminating the cause of the disease (dengue virus), isolation of patients, preventing mosquito bites, and vector control. Efforts to avoid dengue hemorrhagic fever are more focused on breaking the vector development chain.⁴

It is known that transmission of dengue virus from the bite of the *Aedes aegypti* mosquito which initially bit the person 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 dengue hemorrhagic fever that appear when there were no dengue fever cases before. This is

presumably due to the transovarial transmission of dengue virus in the dengue vector.⁵

Previous studies have shown that the transovarial transmission of dengue virus in *Ae.aegypti* and *Ae.albopictus* mosquitos occurred in 21 mosquitoes positive for dengue antigens from 223 mosquitoes examined. Based on the results of these studies, it is proven that there is the potential of *Aedes aegypti* and *Aedes albopictus* transmits dengue virus transovarially in Banjarnegara Regency. The detection method of the Transovarial Transmission Index (ITT) used is the SBPC (Streptavidin-Biotin-Peroxidase-Complex) immune-cytochemical method with monoclonal antibodies DSSE10 1: 5 as primary antibodies that show positive reactions to dengue antigens on head squash preparations.⁶

METHOD

The type of research used is exploratory research. The study was conducted by installing ovitrap in Mersi Village, Purwokerto Timur District, and Pliken Village, Kembaran District, Banyumas Regency. The study was conducted by installing ovitrap in Mersi Village (9 samples) and Pliken Village (3 samples). The eggs obtained are hatched into larvae in the laboratory. The 3rd and 4th instar larvae were examined for the presence of dengue virus using the Elisa method.

RESULTS

Houses for ovitrap placement include endemic areas (Mersi Village) and non-endemic areas (Pliken Village). Ovitrap installation in Kelurahan Mersi was grouped into three zones, namely zone A (1 house with dengue fever/cluster), zone B (4 closest homes from the patient's house) and zone C (4 houses within ≥100 meters from the patient's house). The installation of ovitrap in Pliken Village was carried out randomly by ten homes in RT 02 / RW 07.

In each respondent's house, five ovitraps were installed inside the house. Ovitrap is observed every three days, and if it is positive for *Aedes aegypti* mosquito eggs, the ovitrap is immediately taken to be drained and aerated until dry (not dried in direct sunlight so that the mosquito eggs are not damaged). Subsequently, the ovitrap was sent to the microbiology laboratory at the Banjarnegara Research and Development Center for examination for the presence or absence of dengue virus. The procedure is that the eggs are first hatched into 3 and

4 instar larvae, then the larvae are tested positive or negative for dengue virus.

Dengue virus examination on *Aedes aegypti* larvae (instar 3 and 4) was carried out by the microbiology laboratory at the Banjarnegara Research and Development Center (Table 1). The examination was carried out using the ELISA (Enzyme-Linked Immunosorbent Assay) method. Larvae samples from each sampling zone (zones A, B, C and D) were further divided into three small sample groups in a test tube, each containing 10 *Aedes aegypti* larvae (Samples A1, A2, A3; B1, B2, B3; C1, C2, C3; D1, D2, D3), then after that the presence or absence of dengue virus was examined in each of these samples. The results of the examination are as follows:

Table 1: Dengue Virus Examination Results on *Aedes aegypti* Larvae by ELISA Method

No	Sample Code Larvae	Examination Results
1	A1	Positive
2	A2	Negative
3	A3	Negative
4	B1	Negative
5	B2	Negative
6	B3	Negative
7	C1	Negative
8	C2	Negative
9	C3	Positive
10	D1	Negative
11	D2	Negative
12	D3	Positive

Remarks:

Code A: sample from the sufferer's home zone sufferers of dengue hemorrhagic fever (Mersi District)

Code B: sample from the closest home zone sufferers of dengue hemorrhagic fever

Code C: sample from the zone of the house is ≥ 100m from the house of sufferers of dengue hemorrhagic fever

Code D: sample from non-endemic areas (Pliken village)

DISCUSSION

Temperature conditions at the location research allow mosquitoes to develop due to the influence of

temperature also makes the development of mosquitoes in the larval phase can be faster.⁷ The effect of sunlight makes the temperature at the location ideal for mosquito larvae to accelerate the process of changing skin or molting, so that in research there is within one week has become a pupa and even has become an adult mosquito.⁹

Air temperature affects the development of viruses in the mosquito's body. The *Aedes sp* mosquito can survive at low temperatures, but the metabolism decreases and even stops when the temperature is below the critical temperature. At temperatures higher than 35°C, mosquitoes also experience changes in the sense of slower physiological processes. This is due to protein denaturation in the mosquito's body and the possibility of disturbed balance in the metabolic process and the emergence of toxic metabolic remnants in the body that will reduce the effectiveness of mosquitoes.⁸

Moisture measurements were made at the respondent's home, carried out near the installation of ovitrap. The average humidity measurement results from the respondent's house ranged from 70-75%. The humidity in the research location supports mosquito breeding. Mosquitoes generally like humidity above 60%, because at humidity <60% the age of mosquitoes will be shorter so that the virus growth cycle is not formed. At high humidity, mosquitoes become more active and bite more often so that it will speed up the process of mosquito breeding.¹⁰

Mosquitoes are insects that breathe using the tracheal system, spiracles as a place for air to get in, spiracles open when insects fly or do their activities. While the resting time of the spiracle will be closed, this system is included to maintain the mosquito's body moisture and evaporation so that it is not easily dehydrated because mosquitoes are very dependent on environmental humidity.¹² At low humidity high evaporation occurs in the mosquito's body, because the mechanism of regulation of evaporation does not occur, the effect of mosquitoes will be loss of fluid large enough to experience a liquid dryness.¹¹

A sampling of mosquitoes at the study site is easier to do with the egg phase compared to the larval and adult stages. That is because there are several factors, among others, when the initial sampling is done during the dry season conditions, puddles in water reservoirs outside the house do not exist (dry), and the air temperature at the beginning of sampling

tends to be cold (23°C). In the presence of these factors, the researchers decided to take an egg phase sample using ovitrap.

One of the obstacles when sampling is that the population of *Aedes aegypti* is at a low level. That is because, at the beginning of sampling (in August 2018), the air temperature was low (23°C). The existence of low temperatures is the impact of the dry season and cold wind coming from the Australian continent. The action taken by researchers in the face of the influence of freezing temperatures is to install ovitrap repeatedly (up to 4 times) and hope that the heat soon rises. At the time of the 1st to 3rd sampling, researchers had not yet obtained enough mosquito eggs for the research activities. At the time of the 4th sampling (13 October 2018) the air temperature had risen (27-29°C) and the number of *Aedes aegypti* mosquito populations had also increased, and the positive impact was that researchers could obtain the number of mosquito eggs that were eligible for the study.

Dengue virus examination using the ELISA method is done by pounding all parts of the mosquito's body with the addition of 10ml PBS and comparison of each buffer load. Positive results from reading with ELISA reader with a wavelength of 450 nm (as contained in the appendix) show that positive results with AV are more than 0.388.

The results of the transovarial examination showed that there had been a transovarial transmission at the study site (Mersi Village and Pliken Village). A large number of *Aedes aegypti* mosquitoes does not determine the potential transfer of dengue virus transovarial. This is caused by the dengue virus, including infections that are very dependent on the temperature and humidity of the air. Relatively low or relatively high temperatures and low humidity can reduce the viability of dengue viruses that live in the body of mosquitoes and also reduce the viability of mosquitoes themselves.

The transovarial mechanism of dengue virus can be explained as follows:

1. The infection penetrates the egg cell wall, namely by the capsid (protein coat that protects nucleic acid) in the virus, where the capsid facilitates the process of attaching to the egg and can penetrate into an egg. After sticking, the virus drills on the egg cell wall to form a hole.

2. After the hole is created, the viral capsid inserts nucleic acids (DNA and RNA) into the egg. So, the virus capsid remains outside the egg; if it is empty, the capsid is released and no longer functions.

3. The virus destroys the DNA of the egg, so the egg cannot control its own engine. Then the virus takes control of the life of these cells to replicate themselves repeatedly in large numbers in infected cells, and can then infect the cells around them.

4. Viral replication and multiplication in the organ where the infection occurs can be related to the blood vessels causing the spread of the virus throughout the body.

Mosquitoes that are infected by a virus through the process of biting the sufferer, the virus will replicate itself and spread throughout the mosquito's body and affect all organs including the brain, esophagus, ovaries and other bodily fluids. This is called viremia, which means that all organs have a viral infection, the nature of the virus that causes viremia is what causes transovarial or vertical transmission from infected mosquitoes to offspring through eggs produced.¹³

Mosquitoes that hatch from infected eggs will contain the same virus as its parent. This mechanism causes the presence of dengue virus to be maintained in the environment. The virus is in the mosquito's body throughout its life and at 30°C in the shape of the *Aedes aegypti* mosquito takes 8-10 days to complete the incubation period from the stomach to the salivary glands of the mosquito.

Infection in the mosquito's body can result in behavioral changes that lead to an increase in vector competence, namely the ability of mosquitoes to be less reliable in sucking blood, repeatedly thrusting the probes, but failing to suck blood, so mosquitoes move from one person to another. As a result, the risk of transmission of the virus becomes even more significant.

This research has been able to find the presence of dengue virus in *Aedes aegypti* larvae derived from egg culture. The results of this study support previous research that the transovarial infection of dengue virus plays a role in maintaining the virus during inter-epidemic periods in the environment. The discovery of transovarial transmission in *Aedes aegypti* larvae in Mersi, and Pliken villages deserve attention. The transovarial transmission of dengue virus in *Aedes aegypti* larvae is an essential part in the epidemiological survey of dengue hemorrhagic fever

disease and can be considered in the early alert system in analyzing the spread of dengue hemorrhagic fever in humans.

CONCLUSION

Sampling was done through the installation of ovitrap to obtain *Aedes aegypti* mosquito eggs in Mersi Village, East Purwokerto Sub-district and Pliken Village, Kembaran District, Banyumas Regency. *Aedes aegypti* eggs collected were hatched into mosquito larvae in the Banjarnegara Research and Development Center laboratory, then examined for the presence of dengue virus in the *Aedes aegypti* larvae. Laboratory examination results show that from 12 samples tested, and there were three positive larvae samples containing dengue virus, namely two samples in Mersi Village and 1 sample in Pliken Village.

DECLARATION

Funding: No funding sources

Ethical approval: Ethical clearance was obtained from the Semarang Ministry of Health Polytechnic.

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