

PENELITIAN | RESEARCH

## Detection of Transovarial Transmission on *Aedes* sp. in Gombang Kebumen Central Java

*Deteksi Penularan Secara Transovarial pada Nyamuk Aedes sp. di Gombang Kebumen Jawa Tengah*

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**Abstrak.** Kecamatan Gombang merupakan salah satu daerah endemis demam berdarah dengue di Kabupaten Kebumen, Jawa Tengah. Tingginya kasus DBD di daerah tersebut memunculkan pertanyaan apakah telah terjadi penularan virus dengue secara transovarial dari nyamuk ke telurnya. Penularan secara transovarial akan membahayakan karena nyamuk generasi berikutnya akan langsung menjadi vektor penular yang mengandung virus dengue. Tujuan dari penelitian ini untuk mendeteksi penularan virus dengue secara transovarial pada nyamuk *Aedes* sp. di Kecamatan Gombang. Penelitian ini merupakan studi deskriptif di tiga desa yakni Desa Gombang, Semanding dan Kalitengah, Kecamatan Gombang. Total 300 rumah dipilih dalam penelitian ini, 100 rumah untuk setiap desa, Sebanyak 600 ovitrap dipasang di luar dan di dalam setiap rumah terpilih selama 6 hari. Ovitrap index (OI) dihitung setelah ovitrap diambil dari lapangan. Deteksi penularan secara transovarial dilakukan dengan rearing nyamuk sampai menjadi generasi F1 (Fillial 1), dan kemudian diidentifikasi dengan Uji RT-PCR. Penelitian ini menunjukkan bahwa OI di luar rumah lebih tinggi dibandingkan di dalam rumah. Semua sampel nyamuk menunjukkan hasil yang negatif virus dengue yang mengindikasikan tidak terjadi penularan secara transovarial di wilayah tersebut. Penularan di darah penelitian mungkin terjadi melalui mekanisme penularan horizontal. Walaupun tidak ada penularan secara transovarial, kewaspadaan terhadap penularan dengue harus terus dilakukan dengan melakukan pemberantasan sarang nyamuk seperti kegiatan 3M plus secara rutin.

**Kata Kunci:** penularan, transovarial, dengue, virus, RT-PCR

**Abstract.** Gombang Subdistrict is one of dengue-endemic area in Kebumen Regency, Central Java. The high number of dengue cases in this area raises the question of whether there has been transovarial transmission occurred from the *Aedes* sp. to their eggs. Transovarial transmission could be dangerous because the next generation of mosquitoes can directly become competent vectors as transmitters of the dengue virus (DENV). The purpose of this study is to detect dengue virus transovarial transmission in *Aedes* sp. in Gombang Subdistrict. This is a descriptive research in Gombang, Semanding, and Kali Tengah villages, Gombang Sub District. A total of 300 houses, 100 houses from each village were selected in this study. There were 600 Oviposition traps (ovitrap) were installed both inside and outside of houses for 6 days. Ovitrap index (OI) was calculated by its Ovitrap Index (OI). Detection of transovarial transmission was carried by rearing field mosquitoes to Filial 1 then identified by RT-PCR assay. This study showed that OI in the three villages was higher in outdoor compared to indoor positions. All tested samples were negative DENV, indicated that there were no transovarial transmission occurred at the study sites. Transmission in these study areas might still through horizontal mechanism transmission by mosquito bites. Although there is no transovarial transmission, awareness of dengue transmission must be continued by eradicating of mosquito nests such as 3M plus activities on a regular basis.

**Keywords:** transovarial, transmission, dengue, virus, RT-PCR

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## INTRODUCTION

Dengue virus (DENV) transmission usually occurs horizontally, through the bite of a mosquito that contained the virus. However, it also could transmit vertically from parent to their eggs through the transovarial route during fertilization.<sup>1</sup> This will cause F1 (Filial1) or offspring from mosquitoes which contain dengue virus will immediately become a potential vector to spread DENV. The existence of transovarial transmission in *Aedes* sp. will cause DENV circulated continuously in a certain area.<sup>1,2</sup> This type of transmission also play role to the maintenance of the virus over time during low epidemic periods.<sup>3</sup> The importance of transovarial transmission to DENV incidence is still a debate, previous studies believe that transovarial phenomena are very important to maintain the presence of DENV in a population.<sup>4</sup> Using mathematical models suggested that the transovarial transmission plays an important role in dengue spread when the reproduction number is near one.<sup>5</sup>

Several studies have confirmed that transovarial transmission in *Aedes* sp. occurred in various countries, including Indonesia.<sup>6-8</sup> Transovarial detection assay in *Aedes* sp. in Indonesia mainly using immunohistochemistry (IHC) method with staining techniques on mosquito headsquashes with the principle of streptavidin-biotin Peroxidase Complex (SBPC). However, this method has a limitation because the results are very dependent on the subjectivity of the researchers because they only distinguish the brown color of the granule as a positive and pale (pale blue) as a negative result. This microscopic analysis method is less valid when compared with the Polymerase Chain Reaction (PCR) test.<sup>9</sup> Therefore, in this study, we detect transovarial transmission in Gombang Subdistrict by RT-PCR.

Gombang Subdistrict is one of dengue-endemic area in Kebumen Regency. The number of dengue reported cases increased from 129 cases (Incidence Rate 10.92) in 2014 to 215 cases (IR 18.1 per 100,000 population) in 2015. The highest number of dengue cases occurred mainly at Gombang II Health Center particularly in Gombang Village, Semanding, and Kalitengah. The number of cases that occurred in the three villages during 2016 was 27, 32 and 24 cases. The presence of high dengue cases in this area raises the question of whether there has been transovarial transmission occurred from the *Aedes* sp. mosquito to the eggs. Results of this study will provide important information for

local health officer in order to plan effective prevention and control of DENV transmission.

## MATERIALS AND METHODS

### Study Area and Design Study

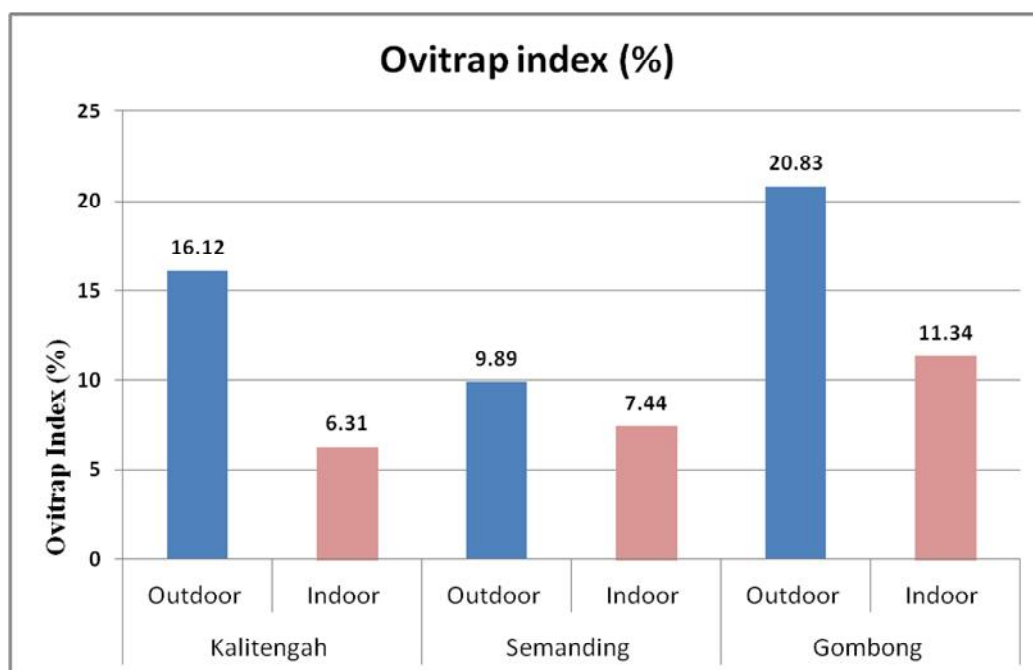
This is descriptive research with cross-sectional design. This research located in Semanding Village, Gombang and Kalitengah, Gombang Sub District, Kebumen Regency, Central Java Indonesia. Kebumen Regency is located at the coordinates of 7 ° 27 ' - 7 ° 50' South Latitude and 109 ° 22 ' - 109 ° 50' East Longitude. Rearing, DNA extraction, and RT-PCR assay were carried out at the Research and Development Center of Class 1 Banjarnegara.

### Ovitrap Installation and Rearing

Ovitrap installation was conducted in 100 houses in Semanding, Gombang, and Kalitengah villages (Total 300 houses).<sup>10</sup> House selection determined by cluster sampling (cluster per RW "rukun warga"). Ovitrap were installed for 6 days in both outdoor and indoor of the house with a total of 600 ovitraps. After 6 days of installation, the ovitraps were taken and then the egg was counted. To conduct a transovarial assay, mosquito eggs from the area of study must be bred to become adult mosquitoes. Ovitrap strips were placed on a tray filled with water, then about 1-3 days will hatch into larvae. The ovitrap index was calculated based on the percentage of ovitrap positive eggs per total number of observed ovitrap

### RNA Extraction and Polymerase Chain Reaction (PCR)

For RNA extraction, 100 adult mosquitoes from Filial 1 were taken from each village. Every 10 mosquitoes were collected in 1 tube for RNA extraction as one pool (total 10 pool mosquitoes). Ten adult mosquitoes then added by 100µl PBS, crushed and 200µl PBS was added (the total volume was 300µl). The sample tubes then centrifuged for 2 minutes at 8000 rpm. 200 µl of supernatant was taken for RNA extraction process. RNA extraction of mosquito samples was carried out using High Pure Viral Nucleic Acid Kit and following the procedure provided in the kit. 200 µl binding buffer which was supplemented with 50 µl Poly (A) for RNA binding and 50 µl Proteinase K for protein digestion was added to 200 µl of the supernatant samples. The tube then incubated for 10 minutes at 72 ° C, then added with 100 µl binding buffer.



**Figure 1.** Ovitrap Index in three villages in Gombong, Kebumen

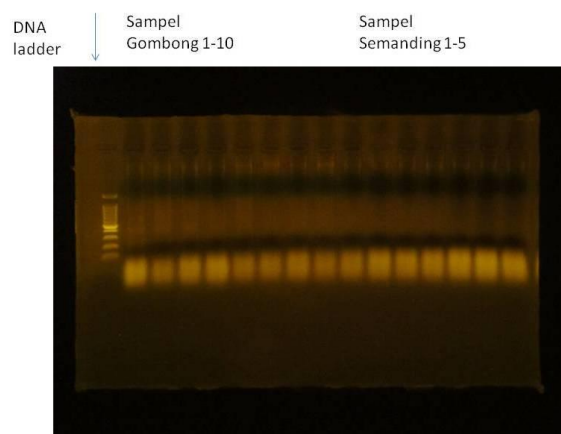
The sample centrifuged for 1 minute at a speed of 8000 x g. A 500 µl inhibitor removal buffer was added and centrifuged again for 1 minute at 8000 x g. 450 µl of wash buffer was added and centrifuged again for 1 minute at a speed of 8000 x g. The washing process was carried out twice. After centrifugation for 1 min at 8000 x g, then the tube columns were centrifuged again for 10 seconds with a maximum speed (13000 x g). 50 µl of elution buffer was added to the filter tube then centrifuged for 1 min at a speed of 8000 x g. RNA was stored at -80 ° C.<sup>11</sup> For the positive control, extraction of 1, 2, 3 and 4 dengue virus supernatants which grown on C636 cells was conducted. PCR assay was performed using Thermo scientific Verso 1-Step RT PCR Hot Start Kit and primer by Yong et al (2007). Amplifications were visualized on 2% agarose gel with ethidium bromide.

## RESULTS

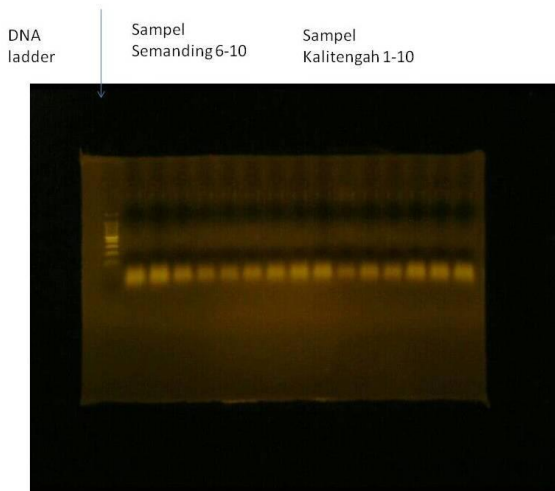
Based on Ovitrap Index in three villages, the highest ovitrap index observed in Gombong Village in the outdoor position (20.83%), and also in indoor position (11.34%), compared the other two villages. While the lowest OI for outdoor positions was in Semanding Village (9.89%), and the lowest indoor position was in Kalitengah Village (6.31%). From these results, OI in the three villages in the area of study was

higher in outdoor positions compared to indoor. Detail of OI in the area of study could be seen in Figure 1.

Filial 1 of mosquito from the area of study then processed into RNA extraction. The RT-PCR assay begins with the RNA extract process, and then one-step RT-PCR was performed and the results were seen by electrophoresis. The results of electrophoresis tests from the three villages can be seen in Figures 2 and 3.



**Figure 2.** Electrophoresis results from RT-PCR assay from Gombong and Semanding Villages. No band detected in samples indicated negative results of DENV detection.



**Figure 3.** Electrophoresis results from RT-PCR assay from Semanding and Kalitengah villages. No band detected in samples indicated negative results of DENV detection

The results of the test with RT-PCR as the gold standard method for virus testing showed negative results in all tested samples.

## DISCUSSION

In this study, we found that Ovitrap Index in the three villages in the area of study was higher in outdoor positions compared to indoor. This indicated that *Aedes* sp. female mosquitoes prefer to lay eggs outside the house than inside. The choice of a place to lay eggs of *Aedes* sp. mosquito is an important step because they want to ensure that the eggs placed in a conducive environment to develop into larvae and hatch into the next generation of mosquitoes.<sup>12</sup> Usually, female mosquitoes will lay their eggs in a place which could increase their survival and fewer competitors or predators also had good access to food resources.<sup>13</sup> Several factors which correlated to the choice of the place where the female mosquito puts its eggs are the presence of other specific larvae or pupae, sun exposure and the size of the container.<sup>14</sup>

Result of this study in accordance with previous studies which found *Aedes* sp. laid their eggs more outdoors than indoors. Although female mosquitoes entered the house to suck blood and rest, however mature female mosquitoes find a more suitable environment for laying their eggs outside of the house but still around the house.<sup>15</sup> The preference of breeding site can be determined from the mosquito species. *Aedes aegypti* mosquitoes usually prefer to lay eggs indoor while the *Aedes albopictus* mosquito prefers to lay eggs outdoors.<sup>16</sup> However, unfortunately, this cannot be

confirmed in this study because no eggs were identified. The implication of the information from the results of this study is the awareness of used goods such as used tires, buckets, bottles or cans that are left around the house which are left unchecked. These items are very potential for *Aedes* sp. mosquito breeding site because *Aedes* sp. mosquitoes prefer to lay eggs around the house than inside the house.

The results of the RT-PCR assay which is the gold standard method for virus testing showed negative results in all test samples. This showed that the dengue virus did not detect in all mosquito samples at the study site. Negative results on the RT-PCR test that have been carried out it means that there has been no transovarial transmission from mosquitoes to the next generation during embryogenesis. Negative results are also similar to the research result in other areas such as Purwokerto and South Sulawesi which also negative for transovarial transmission of detection.<sup>17,18</sup> However, the transovarial transmission has been detected in other regions in Indonesia such as Yogyakarta and Kupang.<sup>6,19</sup> This should be an awareness of transovarial transmission which could happen in the future.

The role of transovarial transmission in the spread of DENV is still a debate. Some studies argue that transovarial transmission does not significantly influence the incidence of dengue. Grunnil and Boots (2015) argued that the existence of asymptomatic infections and population mobility were more influential in the spread of dengue.<sup>4</sup> The negative result of transovarial transmission in the area of the study indicated that DENV is still transmitted by horizontal transmission, by the bite of mosquitoes that have been infected with dengue virus transmitted to others. Endemicity status of dengue in the area of study is likely more due to population mobility. Several studies have linked population mobility as an important factor in the pattern of dengue transmission.<sup>20,21</sup> Dynamic population mobility has a stronger effect on the spread of dengue infection compared to its vector mobility, *Aedes* sp..<sup>22</sup> This is possible because of the limited flight distance of *Aedes* sp. mosquitoes (no more than 500 m.<sup>23</sup> Therefore, the spread of dengue is mostly clustered because of the limited flight distance of mosquitoes.<sup>24</sup>

This is also observed in the research location, where the occupancy of the population is very dense with the location of the house is very close. In fact, some of the relatives' houses are incorporated into a series of buildings. This characteristic could be seen in Semanding and Gombong Villages, while in Kali Tengah Village the distance between houses looks wider. This

can support the occurrence of dengue virus transmission among residents more easily. Gombong sub-district is the second highest population density after Kebumen District. Population density per km<sup>2</sup> according to the Central Bureau of Statistics is 2455 (total area of 19.48 km with a population in 2016 of 47,827 people. While the population growth rate in Gombong Subdistrict from 2010-2016 reached 2.19.<sup>25</sup>

Some studies stated that the characteristics of regions with high population density are one of the factors that support the transmission of dengue cases. Areas with densely populated populations can facilitate the transmission of the dengue virus through mosquito bites due to adjacent houses. The more densely populated in an area, the higher the risk for transmission of dengue disease.<sup>26</sup> The high-density population will also pose a risk of a large number of containers in the area that have the potential to breed mosquitoes. In addition, it is supported by the nature of the *Aedes* sp. mosquito which requires several bites to be able to obtain human blood protein for the maturation of its eggs. So mosquitoes tend to move to bite other humans around them to get enough food, this increases the risk of dengue transmission in dense dwellings.<sup>27</sup>

## CONCLUSION

Based on the RT-PCR test, no detection of transovarial transmission in *Aedes* sp. mosquitoes samples in Gombong Subdistrict. Based on the results of this study, although transmission was not detected transovarially, preparedness must still be done by minimizing mosquito breeding sites.

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## AUTHOR CONTRIBUTION

In this article, Siwi Pramatama M.W. role as the lead author, and Devi Octaviana and Arnika Dwi Asti as co-author. Contribution of all author can be seen below.

**Conception** : SP

**Data Curation** : DO

**Data Analysis** : DO

**Funding** : The Regional Planning Agency of Kebumen Regency

**Investigation** : SP, DO, ADA

**Methology** : SP

**Project Administration** : SP

**Resource** : DO

**Software** : ADA

**Supervision** : SP

**Writing – Original Draft Preparation** : SP

**Writing – Review & Editing** : DO, ADA

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