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**Original Article**

# *Detection of Dengue Virus in Female Aedes aegypti Mosquito using Reverse Transcription-polymerase Chain Reaction (RT-PCR) in West Jakarta*

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***ABSTRACT***

*Dengue hemorrhagic fever (DHF) is an acute disease caused by dengue virus infection which is a clinical manifestation of bleeding, transmitted through the bite of a female mosquito Aedes aegypti. The incidence of dengue fever is still a health problem in developing countries, including Indonesia. There are several ways to detect the presence of dengue virus, namely by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). The purpose of this study was to detect Dengue virus in adult female Aedes aegypti mosquitoes using RT-PCR. The type of research is a descriptive survey to determine the number of dengue virus transmission in Aedes aegypti mosquitoes in Kembangan Village, West Jakarta, especially in RW02 RT 1-10. There are 62 Aedes aegypties found in 100 houses that were selected by simple random sampling. Totally 23 female Aedes aegypties were identified from a total of 62. Moreover, the result showed that all Aedes aegypti mosquitoes caught in were negative for dengue virus. There are several reasons for the limitation of RT-PCR that caused zero findings, including potential false negatives, sensitivity, low quality control measures. Moreover, the small sample size and seasonal reason also played a role in impacting the zero result.*

***Keywords:*** *Aedes aegypti, Dengue, RT-PCR.*

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## INTRODUCTION

The dengue virus, which is a member of the Flaviridae family and the genus Flavivirus, is what causes dengue hemorrhagic fever (DHF), an infectious disease that spreads to people when the Aedes aegypti mosquito bites them[1]. DHF is still one of the biggest health problems in Asia and the world. DHF can appear throughout the year and attack all ages, both children and the elderly[2].

Transmission of the dengue virus occurs through the bite of the Aedes aegypti mosquito, which will bite people if the mosquito is infected with the dengue virus and transmit through bites to people who are not infected with the dengue virus[3]. However, there are also dengue cases that arise when there are no previous dengue cases. This is thought to be due to the transovarial transmission of the dengue virus in dengue hemorrhagic fever vectors[4].

The detection of the dengue virus in female Aedes aegypti mosquitoes using reverse transcription-polymerase chain reaction (RT-PCR) is crucial for understanding the transmission dynamics of dengue fever. RT-PCR has been widely used for the detection and serotyping of dengue virus in mosquitoes[5]. While virus isolation and RT-PCR are highly sensitive and definitive for diagnosing dengue fever, they are time-consuming and require trained staff and sophisticated equipment[6]. Studies have utilized rapid diagnostic RT-PCR for the detection and typing of dengue viruses in adult female Aedes aegypti mosquitoes in

field populations[7]. Additionally, the effectiveness of dengue vector control has been assessed through the analysis of dengue serotypes using RT-PCR[8]. Furthermore, RT-PCR has been used for the detection of dengue virus in Aedes aegypti mosquitoes in various geographical locations, such as Ternate City in Indonesia and Taman Connaught in Cheras[9,10]. The importance of direct detection of dengue virus using RT-PCR has been emphasized in different regions, including Sokaraja in Indonesia[11]. Moreover, the specificity of virus detection using conventional RT-PCR followed by sequencing has been highlighted in the context of proactive preparedness for orthobunyaviruses in India[12].

In Kembangan District, West Jakarta has 6 Kelurahan, and IR figures for August 2022 were recorded in each Kelurahan, namely South Kembangan which is 378.3/100,000 residents, South Meruya which is 196/100,000 residents, North Kembangan which is 194.7/100,000 residents, Joglo which is 186.6/100,000 residents, North Meruya which is 140.2/100,000 residents, and in Srengseng sub-districts which is 115.3/100,000 residents [13]. In September 2022, the incidence of dengue fever was recorded at 136 cases in North Kembangan Village, South Kembangan Village with 133 cases, Joglo Village with 97 cases, North Meruya Village with 81 cases, South Meruya Village with 80 cases, and in Srengseng Village, there were 66 cases [13].

Efforts to overcome dengue cases have been implemented through various programs, the most effective is to break the chain of larvae breeding, such as, mosquito larvae examination, fogging implementation, 3M plus movement (draining, closing and utilizing/recycling) and socialization about dengue fever to the community [14].

Based on the background of problems that occur in the field, the purpose of this study is to detect the *Dengue Virus* in Female *Aedes aegypti* Mosquitoes using Polymerase Chain Reaction (PCR) in Kembangan District, North Kembangan Village, West Jakarta.

## METHOD

The type of research used in this study is descriptive survey using molecular laboratory test conducted by catching female Aedes aegypti mosquitoes from perch in North Kembangan Village. This research will be carried out in June-Agust 2023 in Kembangan District, North Kembangan Village, West Jakarta. The sample selection technique in this study was carried out by Random sampling technique. The samples used in this study were 100 houses located in RW 02 RT 01-10, then traps were installed in each house. Virus detection dengue using RT-PCR.

The RT-PCR process for dengue virus detection in this study was carried out in two stages. The first is the conversion of viral RNA into cDNA as well as the amplification of the Dengue virus in general. The second stage is an amplification of viral cDNA with specific primers for all four serotypes of the dengue virus.

RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) has been widely used for the detection of dengue virus in Aedes aegypti mosquitoes. Several studies have employed RT-PCR to identify and characterize the presence of dengue virus in field-caught adult Aedes aegypti mosquitoes [15,16]. This method has been effective in detecting the virus in mosquitoes, including transovarial transmission, where the virus is passed on to the next generation [17]. Additionally, RT-PCR has been used to confirm the presence of dengue virus in mosquito samples, contributing to the understanding of the epidemiological persistence of dengue in various regions [18].

However, despite its widespread use, RT-PCR has limitations when it comes to detecting dengue virus in mosquitoes. One of the challenges is that while RT-PCR can provide evidence of the presence of the virus, it does not confirm active infection or circulation of the virus among the mosquito population [19.] Furthermore, the use of RT-PCR for routine surveillance of dengue virus in mosquitoes presents challenges for public health professionals. Additionally, the limitations of RT-PCR for dengue virus detection in mosquitoes are evident in the context of extreme temperatures that may prevent the mosquito from surviving long enough to allow dengue virus transmission, thus affecting the accuracy of RT-PCR results [20]. In conclusion, while RT-PCR has been a valuable tool for detecting dengue virus in Aedes aegypti mosquitoes, it is important to consider its limitations, particularly in confirming active infection and in the context of environmental factors that may affect the accuracy of results.

This research was conducted after obtaining ethics from the Health Research Ethics Committee, Faculty of Public Health Diponegoro University with registration number: 19/EA/KEPK/-FKM/2024.

## RESULTS

Based on the results of the study showed that mosquitoes caught from as many as 100 houses in RW 02 RT 01-10 were carried out in the morning and evening to get Aedes aegypti mosquitoes. The number of house mosquitoes per location is as follows (Table 1). Table 1 shows the of the many houses inspected in RW 02 RT 01-10, the most Aedes aegypti mosquitoes were caught in RW 02 RT 01 where the catch in the morning was 12 Aedes aegypti mosquitoes and in the afternoon 4 Aedes aegypti mosquitoes were caught. The total from the capture in RT 01 was 16 Aedes aegypti mosquitoes. Then followed by RT 07 as many as 8 Aedes aegypti mosquitoes caught in the morning and as many as 5 Aedes aegypti mosquitoes caught in the afternoon. The total number of mosquitoes caught in RT 07 was 13 Aedes aegypti mosquitoes.

**Table 1. Catching of Aedes aegypti mosquitoes in North Kembangan Village, Kembangan District, West Jakarta**

| Location | Houses Inspected | Catch Morning | Catch Afternoon | Total |
|---|---|---|---|---|
| Rt 1 | 10 | 12 | 4 | 16 |
| Rt 2 | 10 | 4 | 2 | 6 |
| Rt 3 | 10 | 6 | 2 | 8 |
| Rt 4 | 10 | 3 | 0 | 3 |
| Rt 5 | 10 | 2 | 0 | 2 |
| Rt 6 | 10 | 5 | 2 | 7 |
| Rt 7 | 10 | 8 | 5 | 13 |
| Rt 8 | 10 | 2 | 0 | 2 |
| Rt 9 | 10 | 0 | 0 | 0 |
| Rt 10 | 10 | 3 | 2 | 5 |

Based on table 2 in the below, the results of the study showed that the results of dengue virus identification showed the number of female Aedes aegypti mosquitoes caught in North Kembangan Village in each house examined as many as 100 houses per RT, it was found that in RT 01 out of 10 houses that had been inspected, 5 female Aedes argypti mosquitoes were obtained in the morning and 1 female Aedes aegypti mosquito in the afternoon. RT 03 of the 10 houses that have been inspected found 3 female Aedes aegypti mosquitoes in the morning, RT 04 and RT 05 obtained as many as 2 female Aedes aegypti mosquitoes in the morning, RT 06 as many as 5 female Aedes argypti mosquitoes in the morning and 1 female Aedes aegypti mosquito in the afternoon, RT 07 obtained 3 Aedes aegypti mosquitoes in the morning and RT 10 obtained 1 Aedes aegypti mosquito in the afternoon. From the results of examinations that have been carried out using RT PCR detection, it is obtained that all mosquitoes caught and have been examined all show negative results of the dengue virus.

**Table 2. Results of dengue virus identification in *Aedes aegypti***

| Location | Female Mosquitoes Catch Morning | Female Mosquitoes Catch Afternoon | Detection Results *RT-PCR* |
|---|---|---|---|
| Rt 1 | 5 | 1 | Negative |
| Rt 2 | 0 | 0 | - |
| Rt 3 | 3 | 0 | Negative |
| Rt 4 | 2 | 0 | Negative |
| Rt 5 | 2 | 0 | Negative |
| Rt 6 | 5 | 1 | Negative |
| Rt 7 | 3 | 0 | Negative |
| Rt 8 | 0 | 0 | - |
| Rt 9 | 0 | 0 | - |
| Rt 10 | 0 | 1 | Negative |

## DISCUSSION

From the results of catching mosquitoes that have been done, it can be seen that the mosquitoes obtained are few (table 1). This is because at the time of capture was and dry season and the temperature at the location is $25^0$C in the morning and in the afternoon the ambient temperature still ranges $31^0$C. temperature is one of the environmental factors that can affect the breeding of Aedes aegypti mosquitoes. The average optimum temperature for mosquito breeding is 25-$30^0$C and the average mosquito breeding takes 12 days[21]. If the optimum temperature is above the average, the mosquito life cycle may be short, on average only 7 days[22]. If at extreme temperatures that are around 10°C or more than 40°C, then the development of mosquitoes stops, so mosquitoes die. Tolerance to temperature depends on mosquito species[23].

Aedes aegypti mosquitoes today are a living place for various microorganisms that play a vital role in growth, lambing, immune system, and mosquito efficiency as a spreader of diseases such as dengue. The composition of

microbes in the body of adult Aedes aegypti mosquitoes is strongly influenced by environmental factors, especially in the environment of mosquito larvae that are in the water[24].

The detection of the dengue virus was obtained from the results of capture in Kembangan Village Rw 02 Rt 1–10, and mosquitoes caught only a few. According to data from the Kembangan Health Center, this is possible because, when conducting research during the long dry season, which spans September–October 2023, temperature and humidity do not support the growth and breeding of mosquitoes. Similar studies have been conducted before, where climatic conditions favourable to the development of Aedes aegypti mosquitoes make the area they studied vulnerable to the entry and endemicity of dengue disease[25,26]. However, the results of the examination of dengue virus infection in Aedes aegypti mosquitoes using the reverse transcriptase-polymerase chain reaction assay in, southern Iran, did not indicate any dengue virus infection in this species[27].

By using the RT-PCR method to detect Dengue virus in adult mosquitoes, it can predict outbreaks with a period of up to 6 weeks before the first case appears in humans[28]. In addition, PCR-based screening makes it possible to map an area with varying levels of endemicity. PCR testing can be a practical tool in vector control, which helps to identify high-risk areas. All of this also enhances the capabilities of epidemiological surveillance systems in anticipating outbreaks and detecting viruses that may go undetected.

Studies have detected the presence of dengue virus serotypes, such as DENV4, in adult female mosquitoes reared from wild-captured eggs, indicating transovarial transmission in local A. aegypti populations[29]. Furthermore, research has aimed to detect dengue virus transovarial transmission in A. aegypti collected from dengue haemorrhagic fever patients' residences, highlighting the importance of understanding the potential for vertical transmission of the virus[30].

Additionally, investigations have utilized immunohistochemistry to examine the transovarial transmission of dengue virus in Aedes spp., providing insights into the mechanisms and prevalence of this mode of transmission[31]. Moreover, the transovarial infection of dengue virus in both Aedes aegypti and Aedes albopictus has been studied, emphasizing the significance of understanding the potential involvement of multiple mosquito species in the vertical transmission of the virus[32]. These studies collectively contribute to our understanding of the transovarial transmission of dengue virus in Aedes aegypti mosquitoes, shedding light on the potential mechanisms, prevalence, and implications of this mode of virus transmission.

Environmental factors, particularly temperature, play a crucial role in the breeding and population dynamics of mosquitoes. Studies have consistently highlighted the influence of temperature on mosquito survival, development, and population dynamics [33–35]. For instance, the temperature at breeding and resting locations has been identified as a significant factor in the organismal development and abundance of mosquitoes [36]. Additionally, temperature, along with other environmental factors, has been shown to influence the selection of breeding sites for various mosquito species [37,38]. Furthermore, the impact of temperature on the metabolism, growth, development, and population of mosquitoes has been emphasized, with temperature variations affecting the water temperature in breeding sites according to seasonal and circadian weather patterns [39]. Moreover, temperature has been found to provide favorable breeding conditions for mosquitoes, contributing to their infestation and proliferation [40].

Comparatively, studies have also investigated the impact of transovarial transmission on the spread of dengue virus in various endemic areas. Furthermore, the detection of transovarial transmission in Aedes aegypti in endemic areas has been associated with the role of this mechanism in the spread of dengue hemorrhagic fever [41]. Additionally, the prevalence of dengue virus transovarial transmission in Grogol Sub-district has been linked to the maintenance and improvement of the dengue epidemic [42.]

The findings of this study cannot be generalized to the broader population and different time settings. Moreover, this study is limited to descriptive so there is no more advanced analysis used. Future research is recommended to use a larger sample size, multiple seasons timing, and examine the multivariate analysis.

## CONCLUSION

Based on the results of the study, the results of identification of the Dengue virus by RT-PCR and the number of samples used in 10 RTs in North Kembangan Village that had been examined by female Aedes aegypti mosquitoes were all negative results on 23 samples of Aedes aegypti mosquitoes.


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## CONFLICTS OF INTEREST

All authors declared no conflict of interest in this study.

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