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Dengue virus transovarial transmission by *Aedes aegypti*

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ABSTRACT

Dengue is a disease that is caused by dengue virus and transmitted to humans through the bite of infected *Aedes* mosquitoes, especially *Aedes aegypti*. The disease is hyper-endemic in Southeast Asia, where a more severe form, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), is a major public health concern. The purpose of the present study was to find evidence of dengue virus transovarial transmision in local vectors in Jakarta. Fifteen Aedes larvae were collected in 2009 from two areas in Tebet subdistrict in South Jakarta, namely one area with the highest and one with the lowest DHF prevalence. All mosquitoes were reared inside two cages in the laboratory, eight mosquitoes in one cage and seven mosquitoes in another cage and given only sucrose solution as their food. The results showed that 20% of the mosquitoes were positive for dengue virus. Dengue virus detection with an immunohistochemical method demonstrated the occurrence of transovarial transmission in local DHF vectors in Tebet subdistrict. Transovarial dengue infection in *Ae.aegypti* larvae appeared to maintain or enhance epidemics. Further research is needed to investigate the relation of dengue virus transovarial transmission with DHF endemicity in Jakarta.

Keywords: Dengue hemorrhagic fever, Aedes mosquitoes, transovarial transmission

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INTRODUCTION

Dengue is an acute disease in humans caused by antigenically distinct serotypes of dengue virus (DEN) and transmitted by the bite of infected mosquitoes, especially *Ae. aegypti* as a prime vector and *Ae. albopictus* as a covector. The disease is endemic in most tropical and subtropical areas of the world, with an estimated annual occurrence of 100 million cases of dengue fever (DF) and 250,000 cases of dengue hemorrhagic fever (DHF).(1) Dengue viruses are plus-sense, ssRNA viruses belonging to the genus *Flavivirus*. It is believed that they once were mosquito viruses that became adapted to lower primates and humans.(3) There are four serotypes of dengue virus, DEN-1, DEN-2, DEN-3, and DEN-4. DEN-3 virus is the dominant serotype in Indonesia, capable of causing fatal disease, especially in children.[2] Demographic and societal changes such as population growth, urbanization, and modern transportation greatly contributed to the increased incidence and geographic spread of dengue activity. The World Health Organization (WHO) classifies dengue as a major international public health concern because of the expanding geographic distribution of the virus and its mosquito vector, the increased frequency of epidemics, the cocirculation of multiple viral serotypes, and the emergence of DHF in new areas.[4] To date, DHF prevention still mostly depends on vector control because there is no effective treatment for DHF and no vaccine for DHF prevention. Control of *Aedes* mosquitoes is the most important measure in DHF prevention, because these mosquitoes tend to act as a reservoir or amplifier host for dengue viruses and the virus can be transmitted horizontally as well as vertically (transovarial transmission). Most remarkably, the virus can be maintained in nature by transovarial transmission in mosquitoes and can replicate in potentially vulnerable tissues of mosquito ovaries and embryos without deleterious effects.[5]

Transovarial transmission of dengue virus is a crucial etiological phenomenon responsible for persistence of virus during inter-epidemic periods.[6] The occurrence of dengue virus transovarial transmission in *Aedes* mosquitoes was demonstrated in a study conducted in 2005 by Rohani et al., who found infected larvae from 16 different locations in Malaysia, with higher virus infection rates (VIR) in *Ae. aegypti* than in *Ae. albopictus*.[7] There is increasing evidence for dengue transovarial transmission, which tends to potentially become a factor that can initiate DHF outbreaks in endemic areas.[4] For this reason, it was considered imperative to conduct a research study on the ability of local vectors in Jakarta to transmit dengue virus transovarially, with the goal of supporting dengue virus and *Aedes* mosquito control measures for prevention of DHF outbreaks in Jakarta.

METHODS

Research design

This preliminary study of transovarial transmission of dengue virus in *Aedes* mosquitoes as local vectors was conducted as a survey and used a cross-sectional design.

Mosquito collection

Ae. aegypti larvae were collected randomly from two different areas in Tebet subdistrict, South Jakarta, in the year 2009, consisting of 8 larvae from an area with the highest DHF prevalence (designated RW 01) and 7 larvae from an area with the lowest DHF prevalence (RW 02). Tebet subdistrict was selected as the research location because it was endemic for DHF in 2009. Fifteen *Aedes* mosquitoes were grown from the collected larvae and reared inside two cages in the laboratory for about 1 week to allow replication of any dengue virus present. The mosquitoes were only given sugar water as food and no blood, to rule out cross-infection by dengue virus in the blood meal.

Detection of dengue virus

After identification of the fifteen *Aedes* mosquitoes by light microscopy, the presence of dengue virus in each mosquito was determined by means of head squash preparations. Any dengue virus present in the tissues was detected by monoclonal dengue antibodies using the immunocytochemical streptavidin-biotin-peroxidase complex (ISBPC) assay. The head squash preparation, after fixation in cold methanol (-20°) for 2-3 minutes, was first incubated with peroxidase blocking solution to quench endogenous peroxidase activity. The specimen was then incubated with serum blocking solution to

Table 1. Numbers of *Aedes* mosquitoes from areas with highest and lowest DHF prevalence in Tebet subdistrict

Location	Number of *Aedes* mosquitoes	Dengue positive
Area of highest DHF prevalence (RW 01)*	8	2
Area of lowest DHF prevalence (RW 02)*	7	1

*RW = *Rukun warga*

suppress non-specific binding of subsequent reagents which would interfere with the results, followed by incubation with primary antibody. The specimen was then washed in phosphate buffer solution, and sequentially incubated with biotin-labelled secondary antibody and peroxidase-streptavidin conjugate to bind the biotin residue. The presence of peroxidase was visualized by substrate-chromogen solution. Peroxidase catalyzes its substrate, hydrogen peroxide, and changes the chromogen substrate, diaminobenzidine tetrachloride (DAB), into a brown precipitate at the antigen site. Positive results were characterized by a brown coloring of the tissues, the color intensity depending on the total amount of chromogens reacting with peroxidase. Dengue-negative tissue specimens were blue in color. The study also included dengue virus-positive and negative head squash specimens as controls.[8]

Statistical analysis

The chi-square test was done to compare the proportion of dengue virus in the two areas, RW 01 and RW 02, which had the highest and the lowest prevalence of DHF in 2009, repectively. A p value less than 0.05 was considered as statistically significant. Statistical analysis was performed with SPSS 15.0 software.

RESULTS

There were two (25%) positive results among the eight head squash specimens from the area with the highest DHF prevalence in Tebet subdistrict, South Jakarta. In contrast, there was only one (14.28%) positive result among the seven head squash specimens from the area with the lowest DHF prevalence. Overall, 20% of the mosquitoes were positive for dengue virus. Table 1 lists the number of larvae collected in the two study areas. Table 2 presents the dengue virus distribution of the two areas. There was no significant difference in positive results between the two areas ($p > 0.05$). Figure 1 shows a dengue-positive *Ae. aegypti* head squash, where the brownish precipitation (black arrow) indicates the location of dengue antigen in the infected mosquito.

Table 2. Dengue virus distribution of two areas in Tebet subdistrict

Location	Dengue virus		p-value*
	Positive (n/%)	Negative (n/%)	
Area of highest DHF prevalence (RW 01)**	2 (25)	6 (75)	0.95
Area of lowest DHF prevalence (RW 02)**	1 (14.28)	6 (85.72)	

*Chi-square test; **RW = *Rukun warga*

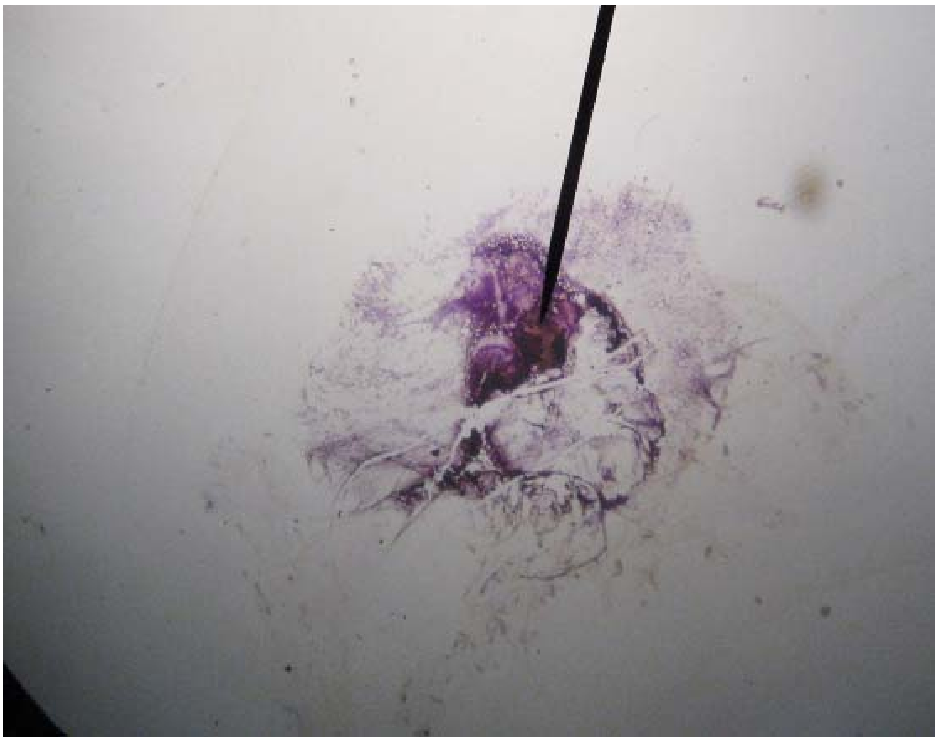

Figure 1. *Ae. aegypti* head squash preparation with positive streptavidin-biotin peroxidase complex staining (40x magnification)

DISCUSSION

The present study showed that the precentage of positive results among the head squash specimens was 20%, consisting of 25% for the area with the highest DHF prevalence in 2009, and 14.28% for the area with the lowest DHF prevalence in 2009. The results showed that there was dengue virus transovarial transmission in local DHF vectors in Tebet subdistrict, South Jakarta. Although the percentage of positive results was higher in the area of highest DHF prevalence in 2009, there was no significant difference between the areas with the highest and the lowest DHF prevalence in 2009 in Tebet subdistrict (p>0.05). Similar results were obtained in the 2005 study of Rohani et al., who demonstrated the occurrence of transovarial transmission of dengue virus in *Ae. aegypti* and *Ae. albopictus* mosquitoes at 13% and 4%, respectively.(7)

There are many reports of transovarial transmission of dengue viruses.(9-11) In a recent study, Mourya et al. have shown that transovarially infected mosquitoes can orally transmit the virus. This study also found that when eggs obtained from infected females were hatched after several weeks of incubation at room temperature, the rate of vertical transmission increased. It has been suggested that at room temperature the virus has an opportunity to multiply and increase its copy number in the quiescent embryos. Occurrence of this phenomenon in nature may be advantageous for maintenance of this virus.(12) In their experimental study, Joshi et al. reported on the persistent transovarial infection of successive generations of *Ae. aegypti* mosquitoes with dengue-3 virus.(3) Several workers have also reported the transovarial transmission of the dengue virus in both *Ae. aegypti* and *Ae. albopictus* experimentally and/

or from field collected mosquito larvae.[11,13,14] It is important to point out that both of the principal dengue vectors have now been demonstrated to be capable of sustained transovarial maintenance of virus in the absence of any horizontal transmission from vertebrate blood meals. The transovarial transmission of the dengue virus in the *Aedes* vectors is now a well-documented phenomenon reported from many parts of the endemic areas in the world. This observation has further emphasized the importance of larval control since the immature stages may become the reservoir of the virus during the interepidemic periods.

There are three types of mechanisms of dengue transovarial transmission:[15] (i) uninfected female mosquitoes bite and suck the host's viremic blood, allowing the virus to replicate within the mosquitoes, the infected eggs giving rise to infected larvae; (ii) uninfected female mosquitoes have sexual activity with transovarially infected male mosquitoes, resulting in sexual transmission of infection to the female mosquitoes; and (iii) the ovarial tissues of female mosquitoes become infected and the virus is genetically transmitted to the next generation.

Transovarial transmission of the dengue virus potentially increases the probability of dengue outbreaks or at least contributes to persistence of DHF cases in endemic areas.[15] Infected female mosquitoes lay their eggs in microniches, where most of the eggs survive through the inter-epidemic season, to emerge as adult mosquitoes that may enter the human-mosquito-human cycle. The successive generations obtained from infected parental mosquitoes showed that the virus could persist in mosquitoes into succesive generations through transovarian passage.[4] Transovarially infected mosquitoes have the ability to transmit the dengue virus orally, through the salivary glands.[7] Multigenerational, transovarial passage and horizontal transmission of dengue from vertically infected progeny has been observed with *Ae. aegypti*.[3,12]

In Indonesia, on average dengue outbreaks take place every three to four years, while in some regions the outbreaks occur annually or biannually. Without proper intervention, DHF cases in Indonesia could amount to 125.000 cases in 2007, an increase of almost 11% compared to 2006.[16] According to Jakarta Health Official profile for the year 2009, about 2300 persons among the inhabitants suffered from DHF, of whom 65 died.[12] In Jakarta, there are 174 areas of dengue outbreaks in 10 subdistricts and it is highly probable that these figures will increase.[17]

Modern dengue control should focus on early detection of dengue virus infection in the mosquito vectors prior to its introduction into the human population, so that remedial control action can be taken immediately to curb the impending outbreak. Akbar et al. showed that detection of dengue virus in adult mosquitoes using reverse transciptase-polymerase chain reaction (RT-PCR) was successful in predicting an outbreak in Bandung, Indonesia.[18]

Our investigation has several limitations. The *Aedes* larvae were collected only from households that were willing to participate, thus the sample was not representative of current dengue infection in Tebet subdistrict. Time limitation was also a problem, because the dengue virus was probably not allowed enough time for replication, which may have resulted in false negative results. Further research studies are needed to provide more data about dengue transovarial transmission in local vectors in the Tebet area, to be followed up with studies in the whole Jakarta area, to support an early warning system on the development of dengue outbreaks, so that in the future the number of DHF cases might be reduced.

CONCLUSION

Transovarial transmission of dengue virus by available vector species in a dengue endemic area could explain the specific emergence of dengue in this region.

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