

Transovarial Transmission of Dengue Virus in *Aedes aegypti* and *Aedes albopictus* in Relation to Dengue Outbreak in an Urban Area in Malaysia

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Abstract

The transovarial transmission of dengue virus in field populations of *Aedes aegypti* and *Ae. albopictus* was monitored in an urbanized residential area in Malaysia. The mosquito larval populations were monitored using standard ovitraps and the larvae retrieved were subjected to dengue virus isolation by culturing in C6/36 cells and detected by peroxidase anti peroxidase staining. Data on serologically-confirmed dengue cases in the study area were obtained. *Ae. albopictus* was the dominant mosquito vector in the study area as indicated by ovitrap survey. Similarly, transovarial dengue virus was detected in *Ae. albopictus* in seven instances, compared to three in *Ae. aegypti* larvae. The minimum infection rate (MIR) for *Ae. albopictus* and *Ae. aegypti* larvae ranged between 2.35–14.30 and 5.77–40.00 respectively. When dengue occurrences were analysed together with transovarial transmission of dengue virus in *Ae. albopictus*, it was found that transovarial dengue virus often occurred prior to the reporting of human cases. The intervals between detecting the transovarial dengue virus and the occurrence of first clinical case/human case ranged from 7 to 41 days. However, occurrence of many viraemic asymptomatic and mildly symptomatic individuals before detection of clinical cases put limitations in forestalling the potential outbreak. Transovarial dengue infection in *Ae. aegypti* larvae appeared to maintain or enhance the epidemics.

Keywords: Dengue, *Aedes aegypti*, *Aedes albopictus*, transovarial transmission, Malaysia.

Introduction

Dengue is a mosquito-borne viral disease which affects millions in the tropical and subtropical regions of the world. In the absence of an effective vaccine, mosquito control is the only known option to interrupt the transmission of the disease. The cyclical nature of dengue epidemics and how the virus is maintained during inter-epidemic periods has led to present studies to evaluate the importance of transovarial transmission in dengue virus maintenance in nature. Transovarial transmission of all four dengue serotypes in mosquitoes has also been

demonstrated experimentally. The mosquito species in which the dengue virus has been transovarially transmitted experimentally are *Ae. albopictus*^[1,2,3], *Ae. aegypti*^[2,4], *Ae. mediovittatus*^[5], *Ae. alcasidi*, *Ae. cooki*, *Ae. herbrideus*, *Ae. katherinensis*, *Ae. malayensis*, *Ae. polynesiensis*, *Ae. pseudoscutellaris* and *Ae. tongae tabu*^[6]. More recently, 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^[7,8,9,10,11]. The study was conducted during August 1996 – December 1997^[12].

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Materials and Methods

Study area

The study area was an urbanized residential area (TSG) about 13 km from the Capital, Kuala Lumpur. The premises comprised terraced brick double-storey houses located in an area of about 1000 hectares. Two rows comprising about 100 houses each were selected for the study. Dengue was reported from time to time in this area and conventional control via fogging of chemical adulticides was conducted whenever a case was reported.

Ovitrap surveillance

The mosquito larval population was monitored using a standardized ovitrap. The ovitrap consisted of a 300 ml plastic container with straight, slightly tapered sides. The opening measured 7.8 cm in diameter, the base diameter was 6.5 cm and the container was 9.0 cm in height. The outer wall of the container was coated with a layer of black oil paint. The oviposition paddle was made from hardboard with measures 10 cm x 2.5 cm x 0.3 cm and water was added to a level of 5.5 cm^[13]. Each ovitrap was placed indoor and outdoor in 15 randomly selected houses in each row of about 100 houses scattered over each study site. The ovitraps were collected after 5 days and replaced with fresh ovitrap and paddle. The collected ovitraps were brought back to the laboratory and poured into plastic bowls filled with fresh water and allowed to further develop in the laboratory for another 4–5 days. A small piece of fresh beef liver was added into each bowl as larval food. The hatched larvae were subsequently counted and identified at 3rd instar. The numbers of larvae were recorded individually for each positive ovitrap*. Larvae were then pooled and

*Processing of ovitraps placed outdoors and indoors separately would have helped determine the ecology and occurrence of the dengue vectors.

either homogenized for inoculation into C6/36 cells or dried onto filter paper, transferred into cryotubes and stored at –70 °C until used.

Detection of dengue virus

Larvae of *Ae. aegypti* and *Ae. albopictus* were pooled separately with 25 larvae per pool. Pools of larvae were ground in chilled eppendorf tubes with 1.5 ml of a growth medium (Eagle's minimum essential medium, MEM), supplemented with 5% fetal bovine serum (FBS), 0.2 mM of non-essential amino acids and antibiotics. The mosquito suspensions were then centrifuged at 14 000 rpm for 15 minutes at 4 °C and the mosquitoes supernatants were used for virus isolation in culture tubes with C6/36 cells monolayer^[14]. The presence of dengue virus was detected by peroxidase anti peroxidase (PAP) staining^[15].

Dengue case data

Data on serologically confirmed dengue cases in the study area were obtained from the Vector Borne Disease Control Programme of the Ministry of Health, Malaysia.

Results

The Figure shows the fluctuation of the *Aedes* larval populations with respect to time scale. The dominant vector species was *Ae. albopictus*. A total of 19 434 *Ae. albopictus* larvae and 3759 *Ae. aegypti* larvae were collected during the period of study. From these, about 777 pools of *Ae. albopictus* and 150 pools of *Ae. aegypti* were available for dengue virus isolation and detection. The occurrence of transovarial dengue virus was detected in 7 instances and 3 instances in *Ae. albopictus* and *Ae. aegypti* respectively. The



minimum infection rate (MIR) for *Ae. albopictus* and *Ae. aegypti* larvae ranged from 2.35–14.30 and 5.77–40.00 respectively. Apparently, the larval population has had little relationship with the occurrence of dengue. However, transovarial dengue virus appeared to be the initiator of the dengue cases in human patients. The trend of transovarial dengue transmission in *Ae. albopictus* and occurrence of dengue cases could be divided into five periods (Figure). In the first period, lasting from 22 August 1996 to 14 November 1996, transovarial dengue virus was detected 41 days prior to the first human case, while in the subsequent periods of 23 November 1996 to 11 March 1997; 12 March 1997 to 24 July 1997 and 25 July 1997 to 1 September 1997, the interval of transovarial virus detection and first human case reported was 7, 13 and 25 days respectively (Table). In the last period from 2 September 1997 onwards

until the end of December 1997, no transovarial virus was detected and its transmission to the human population also stopped. It appeared that transovarial dengue virus in *Ae. aegypti* assisted in maintaining and enhancing the outbreak which may overlap into the next period (Figure).

Discussion

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 inter-epidemic periods. Despite this well-known phenomenon, no studies have been conducted to examine the temporal effect of

Figure. *Aedes* larval population, dengue cases and transovarial transmission of dengue virus in TSR, Selangor, Malaysia, August 1996–December 1997

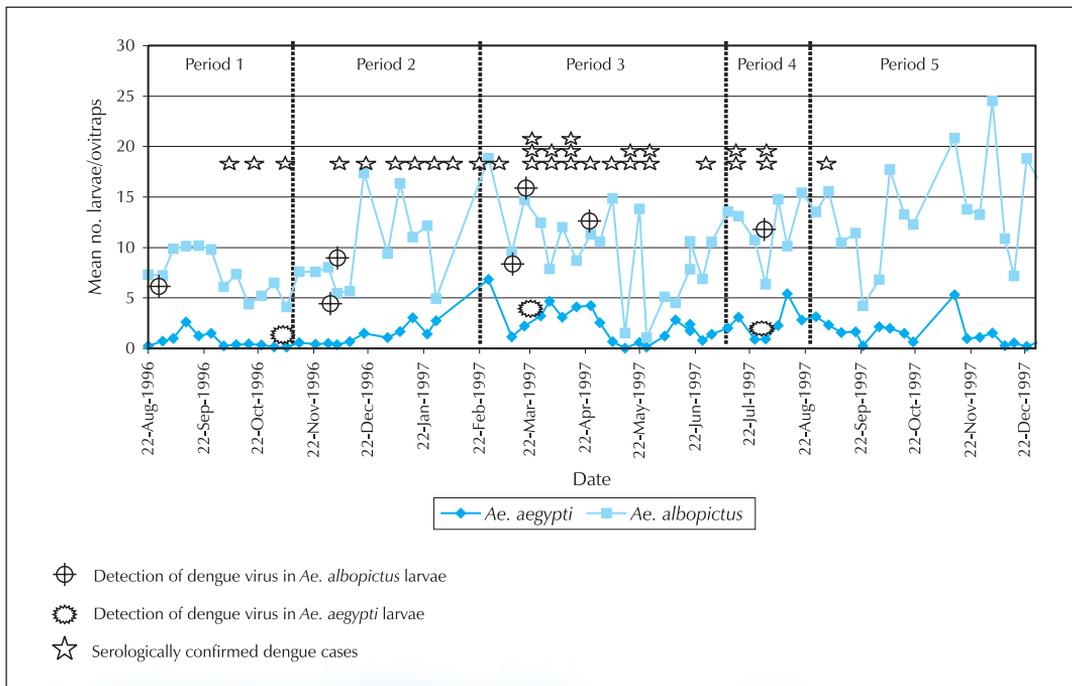


Table. Intervals between detection of transovarial dengue virus in larvae of *Ae. albopictus* and report of first human dengue cases

Date of detection of dengue infection in mosquito larvae	Date of first human dengue cases	Intervals (days)
22 August 1996	2 October 1996	41
23 November 1996	30 November 1996	7
12 March 1997	1 April 1997	15
25 August 1997	19 August 1997	25

transovarial transmission in precipitating an outbreak and the possibility of using the surveillance of viral infection in immatures for early warning and action to curb or forestall dengue outbreaks.

Early and advance warning of dengue outbreak is extremely important for the control of the disease. Currently, vector control measures are generally initiated after the occurrence of viral infection in the human population. An outbreak will be in progress once the virus has been introduced and started circulating in a human population. By then, vector control is often insufficient in preventing or suppressing the epidemic. Hence, modern dengue control should stress 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. Chow^[16] showed that by detecting dengue virus in adult mosquitoes using RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction), it was possible to predict an outbreak six weeks in advance of the occurrence of the first human case in Singapore. However, detection of dengue virus in mosquito adults is often hampered by the fact that the method of collection of sufficient number of adult mosquitoes is slow, tedious and not cost-effective. Hence, if the detection of transovarial dengue virus can be utilized similarly, this will

simplify the procedure as larvae can be collected in large numbers simply by using ovitraps.

Data and observation from this study confirmed that transovarial dengue virus actually played an important role in initiating and maintaining the outbreak in human populations. Present limited available data indicated that in *Ae. albopictus*, the intervals between transovarial dengue virus detection and first human cases ranged from 7 to 41 days (Table 1). The infectivity of transovarial dengue virus in the emerged adult mosquitoes had been reported earlier^[17]. Hence, based on presently available data, it is reasonable to postulate that transovarial dengue virus is infectious in the adult stage of the mosquito when the mosquito develops from the immature stages. The virus is probably transmissible to humans bitten by the emerged adults. However, it is difficult to predict the time interval between detection of virus from emerging adults and appearance of clinical cases, as there may be many viraemic symptomatic or mildly symptomatic cases. Therefore, considering the complexities of dengue epidemiology, this information puts limitations in forestalling the potential outbreak.

Isolation and detection of dengue virus using cell culture and PAP staining was often slow, tedious and costly. Other alternative



detection methods need to be utilized. Recently, RT-PCR was found to be effective in detecting dengue infection in mosquitoes^[11]. With the recent development of a RT-PCR kit for detecting dengue virus in mosquitoes, rapid detection of dengue infection can be carried out effectively without the use of cell culture.

The ecology and mechanisms of the involvement of transovarial dengue virus in disease outbreak remain unknown due to scarcity of data. It is therefore pertinent to initiate more studies on these aspects and also

examine the possibility of utilizing such observation in forestalling an outbreak to ensure early warning and more effective control of dengue.

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