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# Vertical Transmission of Dengue Virus in *Aedes aegypti* Collected in Surabaya, Indonesia, during 2008–2011

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Dengue fever is a mosquito-borne disease of major global public health concern. It is endemic to tropical and subtropical countries, especially in the urban and suburban areas (1). Since 2004, Indonesia has witnessed the largest incidence of dengue fever in Southeast Asia (2). Surabaya, the second largest city in Indonesia and the capital of the East Java province, has had an annual incidence of approximately 2,000–3,000 dengue cases and 10 associated deaths, over the past 10 years

(Surabaya Health Office, unpublished data).

Infective female *Aedes* mosquitoes can transmit 4 different dengue viruses (designated DENV1–4) to humans. Mosquitoes generally acquire the virus while feeding on the viremic blood of an infected human. After an extrinsic incubation period of 8–12 days, the infected mosquitoes can transmit the virus for the rest of their lives (3). This horizontal transmission between mosquito vectors and human hosts is the primary mechanism for the maintenance of the virus in urban areas.

Vertical transmission via the transovarial route in female *Aedes* mosquitoes also occurs in nature (4,5). This mechanism is particularly important for maintenance of the virus, because mosquito eggs are capable of surviving in the environment even under adverse conditions for long periods, sometimes for more than a year (6). Vertical transmission in *Aedes aegypti* L., the principal vector for DENV1–4, has been proven experimentally (5,7–9) and in the field (10–14). However, the extent to which vertical transmission affects the percentage of infected *Ae. aegypti* population during the dry and rainy seasons is unclear. In the present study, we examined the field-collected adult males, females, and larvae, as well as adults that had emerged from the field-collected larvae, for the presence of viral RNA; the minimum infection rates (MIRs) of the viruses were determined and comparisons made between the findings obtained in the dry and rainy seasons in Surabaya.

A total of 717 *Ae. aegypti* adults and 2,785 *Ae. aegypti* larvae were collected from homes in Surabaya that had recently housed patients with dengue fever during the dry and rainy seasons of 2008–2011 (Fig. 1 and Table 1). Adult mosquitoes were captured indoors, freeze-killed at −30°C, morphologically identified, sorted by species and gender, pooled (primarily 30 individuals/pool), and stored at −80°C prior to homogenization. Larvae were found in potential breeding sites of the *Aedes* sp., both indoors and outdoors. The larvae were reared to adulthoods under standard laboratory conditions (i.e., 28 ± 2°C; relative humidity, 75–85%). The emerged adults were maintained for 3–4 days, sorted for species and gender, pooled (primarily 30 individuals/pool), and then stored at −80°C. For virus isolation, each pool of adult males, females, or larvae was homogenized in 1 mL of Eagle's minimal essential medium. The homogenate was clarified and filtered through a 0.22-$\mu$m membrane filter (Sartorius Biotech GmbH, Goettingen, Germany) before inoculation into C6/36 cells. The cells were incubated at 28°C, and culture supernatants were collected after a maximum of 5 blind passages. The samples showing cytopathic effects (CPEs) on C6/36 cell cultures were examined for the presence of viral RNA using

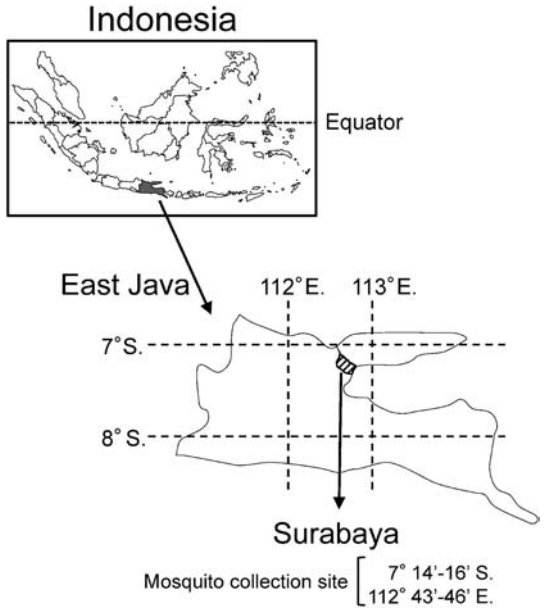


Fig. 1. The location of *Aedes aegypti* collection site. The map in box shows Indonesia archipelago with the equator and the gray area indicates East Java province. The striped area indicates a location of Surabaya. The longitude and latitude ranges of the sample collection site are indicated in the map.

Table 1. Dengue virus detection in *Aedes aegypti* mosquitoes collected in Surabaya, 2008–2011

| Time of collection | Season | Mosquitoes collected[1)] | Gender | Total no. mosquitoes | No. pools assayed | No. positive pools | DENV serotype detected | MIR[2)] |
|---|---|---|---|---|---|---|---|---|
| July–November 2008 | dry | Wild adults | Female | 127 | 5 | 2 | DENV2 | 15.7 |
| | | | Male | 144 | 5 | 0 | — | — |
| January–February 2009 | rainy | Wild adults | Female | 88 | 4 | 2 | DENV1 | 22.7 |
| | | | Male | 62 | 3 | 1 | DENV1 | 16.1 |
| | | Reared adults | Female | 350 | 7 | 4 | DENV1 | 11.4 |
| | | | Male | 350 | 25 | 2 | DENV1 | 5.7 |
| August–November 2010 | dry | Wild adults | Female | 94 | 4 | 3 | DENV1 | 31.9 |
| | | | Male | 59 | 3 | 0 | — | — |
| | | Reared adults | Female | 250 | 25 | 2 | DENV1 | 8.0 |
| | | | Male | 250 | 25 | 0 | — | — |
| January–April 2011 | rainy | Wild adults | Female | 65 | 6 | 2 | DENV1 | 30.8 |
| | | | Male | 78 | 6 | 2 | DENV1 | 25.6 |
| | | Reared adults | Female | 453 | 37 | 10 | DENV1 | 22.1 |
| | | | Male | 582 | 25 | 5 | DENV1 | 8.6 |
| | | Larvae | — | 550 | 28 | 3 | DENV1 | 5.5 |

[1)]: Larvae were not collected in 2008.

[2)]: Calculated as follows: (number of pools positive for dengue virus/total number of mosquitoes tested) ×1000.

PCR-based amplification with type-specific primers (15).

Overall, 38 of the 208 pools displayed CPEs and proved RT-PCR positive for the DENV2 genes in 2008 and the DENV1 genes in 2009–2011 (Table 1). The MIRs in wild-caught and laboratory-reared adults ranged from 5.7 to 31.9 in the pools that were positive for viral RNA. These values were consistent with the MIRs reported from other countries (i.e., MIR of 10–40) (12–14,16). In 2008, when only wild adults were collected in the dry season, DENV genes were only detected in female mosquitoes (MIR of 15.7). The absence of DENV genes in males suggests that, in the dry season, natural vertical transmission cannot occur frequently. To investigate this further, we collected and reared larvae and adults in our laboratory between 2009 and 2011. In the laboratory-reared adults, the MIRs ranged from 5.7 to 22.1 during the rainy season in 2009 and 2011; whereas, in the dry season of 2010, no virus-positive pools were detected in the males, and the MIR for the females was only 8.0. These results indicate that vertical transmission was possible in the dry season, but it was unlikely to be as frequent as in the rainy season. Finally, we attempted to detect viral RNA directly from the larvae collected in 2011; a low MIR value of 5.5 was obtained from the larvae. However, the fact that 3 pools tested positive for viral RNA supports a conclusion that natural vertical transmission in Surabaya occurred during the 2011 rainy season.

The MIRs obtained for the wild-caught adults were higher than those obtained for the laboratory-reared adults of each gender; exceptions occurred in 2008, when laboratory-reared adults were unavailable for comparison because larval collections were not performed, and in 2010, when the males did not produce virus-positive pools. Importantly, relatively high MIRs were observed in the wild-caught males collected during the rainy seasons. Since the difference observed in the MIR values between the wild-caught females and wild-caught males implies the extent of horizontal transmission, vertical transmission may have contributed to a significant percentage of infected females in the rainy season. On the other hand, the relatively low MIRs observed in the wild-caught males, laboratory-reared males, and females in the dry season suggest a higher rate of horizontal transmission of the virus in the females.

In this study, the detection of DENV2 in 2008 and DENV1 between 2009 and 2011 was consistent with our patient survey data, which showed that in Surabaya, a DENV type shift occurred between October and November 2008 (17). We observed that the nucleotide sequences of the entire envelope-coding region of the virus (obtained from mosquito samples) were identical to those obtained from patients whose houses were used for collection of the corresponding mosquitoes. This suggests that DENV was transmitted between mosquitoes and humans in a limited space, in accordance with the known behavioral characteristics of *Ae. aegypti*.

In conclusion, this study indicates that vertical transmission of DENV plays a role in viral maintenance in nature and potential transmission to humans, particularly in the rainy seasons.

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**Conflict of interest** None to declare.

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