VERTICAL MAINTENANCE OF DENGUE-1 VIRUS IN SEQUENTIAL GENERATIONS OF *AEDES ALBOPICTUS*[1]

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ABSTRACT. Vertical transmission of dengue-1 virus was demonstrated over 3 consecutive generations of *Aedes albopictus* by tracing descendants of individual, vertically infected females. This study is the first to examine efficiency of vertical transmission of a flavivirus by *vertically infected* mosquitoes. Vertically infected *Ae. albopictus* females were more efficient vertical transmitters than females infected by inoculation (i.e., horizontally). Three of 4 vertically infected females examined transmitted virus to their offspring, whereas ≤ 0.7% of females infected by inoculation were capable of vertical transmission.

Host genetic factors appear to influence establishment and persistence of *Aedes albopictus* (Skuse) lineages displaying efficient transovarial transmission of San Angelo virus (California encephalitis group)(Shroyer 1986). It has also been demonstrated that mosquitoes infected *horizontally* (i.e., by inoculation, or *per os*) with San Angelo virus may display a much lower efficiency of transovarial transmission than mosquitoes infected *vertically* (i.e., by transovarial transmission)(Shroyer 1986). This difference raised the question of whether the same disparity was evident with vertically transmitted flaviviruses, studied previously only by means of horizontally infected female mosquitoes. The present study evaluated this possibility for vertical transmission of dengue-1 (DEN-1) virus by *Ae. albopictus.*

A long-colonized strain (OAHU) of *Ae. albopictus*, established from mosquitoes collected in 1971 on Oahu, Hawaii, was used. Mosquitoes were reared and maintained at 25°C as described previously (Shroyer 1986), except for the method of collecting eggs from individual females. In this study females were isolated in 9-dram polystyrene vials containing a 6.0- × 3.5-cm strip of brown, desk blotter paper as an oviposition substrate. Vial caps were screened with nylon organdy for air exchange and to permit addition of water (by hypodermic syringe) to the paper strip.

The strain of DEN-1 virus used was originally obtained from human serum from Fiji. It had been passed once in *Ae. albopictus* and twice in *Toxorhynchites amboinensis* (Doleschall) mosquitoes before use in this study. In this study the original, parental generation *Ae. albopictus* were infected by intrathoracic inoculation (Rosen and Gubler 1974) to assure that virtually all females were infected.

Mosquitoes were assayed for the presence of DEN-1 virus by examining head squashes of individual mosquitoes with an indirect fluorescent antibody technique (IFAT) (Kuberski and Rosen 1977). Sensitivity of the head squash assay for detecting all vertically infected mosquitoes was tested by conducting infectivity tests on bodies of F_1 mosquitoes whose heads contained no detectable DEN-1 antigen by IFAT. When these bodies were triturated (in pools of 100 or less) and inoculated into *Tx. amboinensis* mosquitoes, subsequent assay by IFAT revealed that no DEN-1 positive *Ae. albopictus* had been missed with the head squash procedure.

Uninfected *Ae. albopictus* females were allowed to feed on an anesthetized, uninfected guinea pig. Eggs produced by fully engorged females in this gonotrophic cycle were collected and discarded, and the primiparous females were then inoculated with approximately $10^{5.5}$ mosquito infectious doses_{50} (50% endpoint) of DEN-1 virus. Based on earlier studies, the preinfection gonotrophic cycle was expected to increase slightly the efficiency of vertical transmission in these inoculated mosquitoes (Rosen et al. 1983). Seven days after inoculation, infected females were allowed to engorge on uninfected mice to initiate a second gonotrophic cycle. Eggs from the postinfection cycle were collected *en masse* from 279 females.

A sample of the F_1 eggs was hatched and reared at 25°C. Adults were allowed to mate, and the females were then allowed to engorge on uninfected mice. Engorged F_1 females (n = 475) were isolated in individual cages for oviposition. After oviposition, the head of each female was assayed by IFAT to detect DEN-1 infection.

Two of the F_1 females were found to have been infected by the vertical route. The overall filial infection rate of the F_1 females was 0.4%, a result typical of numerous experiments conducted ear-

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lier using inoculated or orally infected parental females (Rosen et al. 1983). Fortunately, viable eggs had been obtained from each of the vertically infected females, which made it possible to evaluate their efficiencies of vertical transmission to the next generation.

The pedigree in Fig. 1 follows the lineages started from the 2 vertically infected F_1 mothers. Each F_2 family was reared separately, siblings were allowed to mate with one another, and females were provided an uninfected blood meal before being isolated for oviposition. After oviposition of F_3 eggs, head squashes of all surviving males and females were assayed for presence of virus as before. Both infected F_1 females transmitted DEN-1 virus to their offspring by the vertical route. Filial infection rate of each F_2 family was also substantially higher than that seen in the preceding generation, although still less than 5%.

In one F_2 family shown in Fig. 1, only one infected male mosquito was observed; consequently, it was impossible to evaluate vertical transmission to the F_3 in this lineage. However, the second F_2 family contained 2 infected females whose progeny were available for further study. One of these F_3 families contained 2 vertically infected females for a filial infection rate of 3.4%. There was no vertical transmission to the remaining F_3 family; however, this family contained only 11 mosquitoes. No attempt was made to propagate the F_4 generation.

Vertical transmission rates represent the proportion of females that vertically transmit to *at least* one offspring, while the filial infection rate

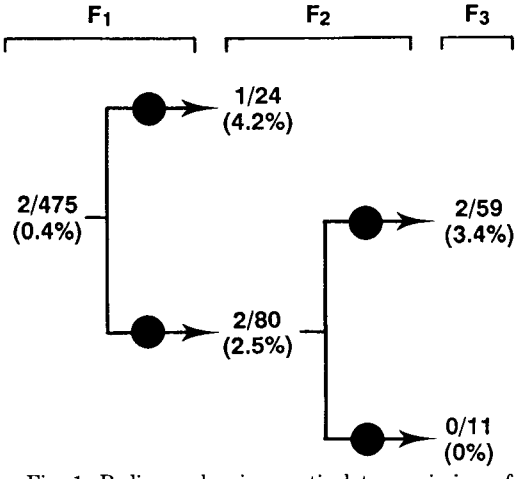

Fig. 1. Pedigree showing vertical transmission of dengue-1 virus over 3 generations of *Aedes albopictus*. Solid circles represent vertically infected females. Infection rates of offspring are shown opposite the arrowheads, given as number infected over family size and as percentage infected (in parentheses).

provides the infection rate within each family. In this study, the vertical transmission rate from F_1 to F_2 generations increased 100-fold from $\leq$ 0.7% ($\leq$ 2/279 mothers) to 100%, dramatically illustrating the fundamental differences between horizontally and vertically infected *Ae. albopictus* females. The data on filial infection rates also suggested qualitative differences in the mechanism of vertical transmission, although to a lesser degree. The data shown are of necessity restricted to only a few vertically infected females and families in each generation, notwithstanding the large number of mosquitoes used to initiate the experiment. While these filial infection rates are lower than those observed in similar studies using San Angelo virus (Shroyer 1986), they argue against the presumption that vertical transmission of dengue viruses by *Ae. albopictus* is too inefficient for it to play a role in the natural history of these viruses. These data also reinforce the argument (Shroyer 1986) that crude filial infection rates based on pooled progenies of horizontally infected female mosquitoes do not necessarily predict the efficiency of vertical transmission as it might occur in nature.

In view of the known capability of male *Ae. albopictus* to infect their female mates venereally with dengue viruses in the laboratory (Rosen 1987) and the repeated isolation of dengue viruses from males of other sylvan mosquito species (Cornet et al. 1979, Cordellier et al. 1983, Cornet et al. 1984, Khin and Than 1983), the hypothesis that endemic maintenance of dengue viruses may be dependent on vertical transmission now warrants even greater attention than before. These data also suggest that the mechanism for vertical transmission of flaviviruses may not necessarily differ fundamentally from that of bunyaviruses, as earlier experimental data (Rosen et al. 1983) seemed to suggest.

Vertical transmission of flaviviruses by horizontally infected female mosquitoes is probably not, strictly speaking, transovarial in nature; offspring are apparently infected during fertilization and/or oviposition (Rosen 1988, Rosen et al. 1989). One possible explanation for the enhanced efficiency of vertical transmission of DEN-1 virus seen in this study is that vertically infected females may be capable of infecting offspring by a genuinely transovarial mechanism.

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