VERTICAL TRANSMISSION OF DENGUE 1 VIRUS BY *HAEMAGOGUS EQUINUS* MOSQUITOES

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ABSTRACT. Vertical transmission of dengue type 1 virus was demonstrated by a strain of *Haemagogus equinus* mosquitoes from Panama. Parental females were infected by intrathoracic inoculation and egg production was stimulated by feeding on mice. Dengue 1 virus was detected in fourth instar larval progeny obtained from two installment hatches of eggs. Minimum filial infection rates ranged up to 1:495.

The cyclic nature of dengue virus epidemics and how these viruses are maintained in areas where epidemics have occurred previously pose unresolved questions. Hereditary passage of dengue viruses in vector mosquitoes is a possible mechanism for the natural maintenance of these viruses. Laboratory studies have demonstrated vertical passage of all 4 dengue serotypes by females of *Aedes albopictus* (Skuse), *Ae. aegypti* (Linn.) (Rosen et al. 1983), by 8 species of the *Ae. scutellaris* group (Freier and Rosen 1987) and by *Ae. mediovittatus* (Coq.) (Freier and Rosen 1988). Field isolations of dengue virus have recovered type 2 virus from *Ae. aegypti* larvae in Burma (Khin and Than 1983) and type 4 virus from larvae of the same species in Trinidad (Hull et al. 1984). Dengue type 2 virus was also isolated in Africa from a pool containing a mixture of *Ae. furcifer* (Edwards) and *Ae. taylori* Edwards male mosquitoes (Cornet et al. 1984). These field observations further support the contention that vertical transmission may be a natural survival mechanism for dengue viruses.

The goal of our experiments was to determine if dengue type 1 virus could be transmitted vertically by the sylvan mosquito, *Haemagogus equinus* Theobald. This forest canopy species from Central and South America also breeds in man-made containers, inhabits peridomestic environments and is attracted to human hosts. *Haemagogus equinus* has also been incriminated as a vector of yellow fever (YF) virus (Trapido and Galindo 1956), and vertical transmission of YF virus by this species was demonstrated by Dutary and LeDuc (1981).

A strain of *Hg. equinus* from Panama, colonized in 1975, was used in our tests. *Aedes aegypti* mosquitoes from Villalba, Puerto Rico, colonized in 1980, were used to assay F_1 progeny from vertical transmission experiments.

Mosquitoes were reared at a temperature of 28°C and a RH of 85% with a photoperiod of 16 h light and 8 h dark. Installment hatching was used to obtain larvae from *Hg. equinus* eggs. This entailed drying eggs for 1 wk, flooding eggs, drying them again for 1 wk, and then reflooding. This cycle was repeated 2–3 times. Larvae were fed a suspension of rabbit chow and adults were given 10% sucrose as a carbohydrate source.

A dengue type 1 virus strain, originally isolated in 1975 from human serum in Suva, Fiji, was used in all experiments. The strain had undergone 3 mosquito passages in *Toxorhynchites amboinensis* (Doleschall) and had never been passed in either mice or tissue culture. Stock pools of virus were stored in 1-ml aliquots at −70°C.

Cage-mated *Hg. equinus* female mosquitoes were inoculated intrathoracically with 0.17 µl of dengue type 1 virus about 5–7 days after emergence. Inoculated mosquitoes were held at 28°C for 7 days. On the 7th day after infection, and 1 wk thereafter, female mosquitoes were provided with an uninfected mouse as a blood source to stimulate egg production. Infection of parental female *Hg. equinus* was confirmed by head squash assay 14 or more days after inoculation.

Eggs were collected *en masse* on moistened strips of dark brown blotter paper. After removal from oviposition jars, ovistrips were stored at 28°C in 85% RH for 1 wk. Installment hatching was carried out at weekly intervals. Two or 3 floodings were performed for each F_1 egg collection. Larvae from each installment were maintained separately. Immature stages were divided into pools of 50 fourth instar larvae or pupae and triturated in a Ten Broeck tissue grinder with 1.0-ml phosphate-buffered saline (pH 7.4) containing 0.5% gelatin, 30% heat-inactivated fetal calf serum (56°C for 30 min) and antibiotics (200 units/ml penicillin and 200 µg/ml streptomycin). Larval suspensions were clarified by centrifugation at 1,000 × g for 30 min at 4°C.

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Each undiluted supernatant suspension was then tested for the presence of virus by inoculating 0.17 μl into 10–15 male or female *Ae. aegypti*. Assay mosquitoes were held for 14 days at 28°C.

Mosquitoes were tested for the presence of dengue type 1 virus by the head squash technique (Kuberski and Rosen 1977). The indirect fluorescent antibody test was used with anti-dengue type 1 mouse hyperimmune ascitic fluid and fluorescein isothiocyanate-conjugated anti-mouse goat serum. Specimens were examined with a Leitz transmission fluorescent microscope at 200× magnification. At least 3 specimens were viewed before a pool was considered negative. A pool was considered positive when fluorescence was observed for at least one of 5 specimens examined.

Vertical transmission of dengue virus was observed for the Panamanian strain of *Hg. equinus* (Table 1). Of the 2,656 F_1 larvae and pupae tested for the presence of virus, 4 of 92 pools contained dengue type 1 virus. Virus was recovered in our second experiment in which larger numbers of progeny were available for testing. In experiment 2, dengue type 1 virus was recovered from F_1 progeny obtained from both first and second egg installment hatches. The minimum filial infection rate (MFIR) was 1:495 for the first hatching and 1:750 for the second; the overall MFIR was 1:664. Also, in experiment 2, 22 pools of 1,055 pupae derived from the second egg hatch were assayed for dengue virus and all were negative. Examination of parental female mosquitoes held 14 days after inoculation showed that all were infected with dengue 1 virus.

The frequency of vertical transmission for *Hg. equinus* (1:622) in our study was higher than that reported by Rosen et al. (1983) for *Ae. aegypti* VILLALBA (1:1,543) but less than that reported by them for *Ae. albopictus* (1:200). Their experiments were conducted with parental females infected with the same strain of dengue type 1 virus used in our study. Although not directly comparable, the MFIR results obtained in our study were higher than the 1:1,727 MFIR obtained for vertical transmission of YF virus in parenterally infected females of the same strain of *Hg. equinus* PANAMA that we used (Dutary and LeDuc 1981).

Infected larvae were obtained after the second egg immersion. Recovery of virus from installment-hatched eggs is an important factor for virus maintenance. This finding was also observed by Beaty et al. (1980) with the AMPHUR strain of *Ae. aegypti* infected by intrathoracic inoculation with YF virus. Vertical transmission combined with installment hatching is more likely to ensure longer survival of a virus.

Although our data are preliminary, the potential for *Hg. equinus*, and possibly other sylvatic mosquito species that have drought-resistant eggs, to become maintenance hosts for dengue viruses must be considered. These species might serve as reservoirs for the survival of dengue viruses during periods of adverse climatic conditions or when susceptible vertebrates are few in number. Vertical transmission would enhance such a possibility by allowing virus persistence during unusually dry periods. In addition, the peridomestic behavior of *Hg. equinus* (Chadee et al. 1981) might permit this species to act as a vector of dengue virus in rural communities located near forests. One could also hypothesize that a sylvatic cycle of dengue virus activity involving *Hg. equinus* and other *Haemagogus* species might be perpetuated through horizontal transmission among monkeys present in the forest canopy. Such a zoonotic system of viral maintenance was proposed for Southeast Asia by Rudnick (1978). *Haemagogus equinus* feeding on cebus monkeys and marmosets has been reported by Waddell and Taylor (1945, 1947), and *Hg. capricornii* Lutz was found by Bugher et al. (1944) to be responsible for YF virus transmission among marsupials and monkeys in a forested region of eastern Colombia. New World monkeys, such as *Alouatta* and *Cebus*, are reported to be susceptible to infection with dengue virus as determined by virus isolation and antibody production (Rosen 1958).

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Table 1. Minimum filial infection rates (MFIR) for *Haemagogus equinus* F_1 progeny obtained from the first ovarian cycle of females infected parenterally with dengue type 1 virus.

Exp. no.	Egg hatch	No. progeny	No. pools pos/ total	MFIR
1	1st	0	—	—
	2nd	152 L, P[a]	0/4	—
	3rd	15 L	0/1	—
2	1st	989 L, P	2/21	1:495
	2nd	1500 L, P	2/30	1:750

[a] L = larvae; P = pupae.

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