

LIMITED POTENTIAL FOR TRANSMISSION OF LIVE DENGUE VIRUS VACCINE CANDIDATES BY *AEDES AEGYPTI* AND *AEDES ALBOPICTUS*

MICHAEL R. SARDELIS, ROBERT EDELMAN, TERRY A. KLEIN, BRUCE L. INNIS,
J. ROBERT PUTNAK, JAMES W. JONES, AND MICHAEL J. TURELL

Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland; Center for Vaccine Development, University of Maryland at Baltimore, Baltimore, Maryland; Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, District of Columbia

Abstract. To evaluate the transmission risk of four live dengue (DEN) vaccine candidates developed by the U. S. Army (DEN-1, 45AZ5 PDK 20; DEN-2, S16803 PDK 50; DEN-3, CH53489 PDK 20; and DEN-4, 341750 PDK 20), we tested 3,010 *Aedes aegypti* and 1,576 *Aedes albopictus* mosquitoes blood-fed on 21 volunteers who had been administered one of the four vaccine candidates or the licensed yellow fever (YF) vaccine (17D). We used an indirect immunofluorescence assay (IFA) to detect DEN or YF viral antigen in the heads of mosquitoes. Corresponding to the lack of a detectable viremia among volunteers inoculated 8–13 days previously with live DEN-1 or DEN-2 vaccine candidates, only six mosquitoes developed disseminated infections after feeding on these volunteers. These six mosquitoes included 4 of 247 *Ae. albopictus* fed on volunteers inoculated with the DEN-1 vaccine candidate and 2 of 528 *Ae. aegypti* fed on volunteers inoculated with the DEN-2 vaccine candidate. Infection was confirmed in each of these IFA-positive mosquitoes by isolating infectious virus from the mosquito's body in Vero-cell culture. None of the 1,252 or the 969 mosquitoes fed on DEN-3 or DEN-4 recipients, respectively, were infected. Overall, dissemination rates in *Ae. albopictus* and *Ae. aegypti* were low. Dissemination rates were 0.5%, 0.3%, < 0.1%, and < 0.1% for the DEN-1 through DEN-4 vaccine candidates, respectively. Because of the observed low dissemination rates, it is unlikely that these vaccine viruses would be transmitted under natural conditions.

INTRODUCTION

Dengue (DEN) fever and dengue hemorrhagic fever (DHF) are serious health threats in many tropical and subtropical regions of the world. To prevent or minimize this threat, there have been extensive efforts to develop vaccines against the four serotypes of DEN virus using live-attenuated DEN viruses.^{1–7} Although live viral vaccines typically offer many advantages over inactivated products (e.g., single inoculation, more efficient induction of mucosal immunity, and longer duration of immunity), there is the potential problem of reversion to virulence. This is particularly true for arboviral vaccines, such as for DEN, where the virus has the potential to revert to virulence in the invertebrate vector as well as in the immunized host.

In a study of an earlier live DEN-2 vaccine, not only was virus isolated from the volunteers, but mosquitoes were infected by feeding on viremic DEN vaccinees.⁸ Moreover, mosquitoes became infected after feeding on a blood meal preparation (droplets) containing other live DEN vaccine candidate viruses.^{9–11} The transient circulation of infectious virus in recipients of live vaccine raises concern about the potential for the virus infecting vector mosquitoes and of its reverting back to a virulent form. This sequence of events might introduce virulent DEN virus into areas currently free from DEN virus transmission.

To determine the risk of infecting mosquitoes with each of the four dengue virus vaccine candidates and introducing dengue into a nonendemic area, we allowed *Aedes aegypti* and *Aedes albopictus* to feed on volunteers, who had each been administered monovalent vaccine candidates. These vaccine candidates of the four DEN serotypes are in an early stage of clinical testing by the Walter Reed Army Institute of Research (WRAIR).

MATERIALS AND METHODS

Mosquitoes. Mosquito species used in this study included *Ae. aegypti* (New Orleans), derived from mosquitoes collected in New Orleans, Louisiana, in 1991 and *Ae. albopictus* (Oahu), derived from mosquitoes collected on Oahu, Hawaii, in 1971. These species were selected because *Ae. aegypti* is the principle vector of dengue virus throughout most of the natural distribution of dengue,¹² and *Ae. albopictus* is implicated as a possible vector of dengue virus in many Pacific and far eastern regions.^{13–16} In addition, the introduction of *Ae. albopictus* into the Americas may pose an increased threat for dengue virus transmission in the United States.¹⁷

Preliminary studies with a membrane-feeding technique indicated that the New Orleans strain of *Ae. aegypti* was susceptible to infection with both the DEN-1 and DEN-2 vaccine candidates (Turell M, unpublished data). The Oahu strain of *Ae. albopictus* is susceptible to all four dengue serotypes.^{9,18,19}

All mosquitoes were reared at 27°C under a 16:8 hr (light:dark) photoperiod. Larvae were given a diet of ground catfish chow, and adults were held in 3.8-liter cardboard containers and provided a water-soaked cotton pad to maintain humidity and an apple slice as a source of carbohydrates. Adults were grouped by age to ensure that the mosquitoes were 4–6 days old on the day they were permitted to feed on the volunteers. Two to 3 days before blood feeding on vaccinees, approximately 50 female mosquitoes each were transferred from the 3.8-liter cartons to 0.5-liter cartons with netting over the open end and provided water and apple slices as described above. The apple slices were removed from the cartons approximately 4 hr before the volunteers were exposed to the mosquitoes.

Volunteers and serological and virus assays. Volunteers

TABLE 1

Evidence of infection in vaccinees and flavivirus-specific fluorescence in the heads of female *Aedes aegypti* and *Aedes albopictus* 12–16 days after feeding on volunteers that received one of four candidate dengue vaccines or yellow fever vaccine

Vaccine	Infection in vaccinees (No.)	No. heads fluorescent positive/No. examined (% positive)		
		<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	Combined
DEN-1 (45AZ5 PDK20)	Yes (4)	0/513	4/247 (1.7)	4/760 (0.5)
DEN-2 (S16803 PDK50)	Yes (3)	2/528 (0.4)	0/244	2/772 (0.3)
DEN-3 (CH53489 PDK20)	Yes (3)*	0/503	0/291	0/794
	No (2)	0/307	0/161	0/468
DEN-4 (341750 PDK20)	Yes (2)	0/133	0/28	0/161
	No (3)	0/504	0/304	0/808
YF (YF-VAX®)†	Yes (4)	0/522	0/301	0/823

Yes = either IgM positive (>20 units rise by ELISA on Day 30) or PRNT₅₀ antibody positive (≥40 reciprocal titer against homologous serotype strain on Days 30 or 60). No = no detectable antibody conversion.

* Two of these volunteers had detectable viremias by delayed plaque method on Days 11–14 after inoculation.

† Connaught Labs, Swiftwater, Pennsylvania.

for mosquito feeding were part of a clinical trial to evaluate safety and immunogenicity of four candidate dengue vaccines: DEN-1, 45AZ5 PDK 20; DEN-2, S16803 PDK 50; DEN-3, CH53489 PDK 20; and DEN-4, 341750 PDK 20 (WRAIR, BB-IND #7074). Four volunteer recipients each received the DEN-1 or DEN-2 vaccine candidates, whereas five recipients each received the DEN-3 or DEN-4 candidates. Also included in the trial were volunteers who received the licensed yellow fever (YF) vaccine (Strain 17D, YF-VAX®, Connaught Labs, Swiftwater, PA). All volunteers were seronegative for flaviviruses before their inclusion in the study. Each participant provided voluntary consent according to guidelines for human experimentation established by the U.S. Army Medical Research and Development Command and the Institutional Review Board, University of Maryland, Baltimore, Maryland. Volunteers were inoculated with 0.5 mL of freshly reconstituted vaccine candidate containing about 10⁵ plaque-forming units (PFU). Blood for serology and/or virus isolation was collected on Day 0 (before vaccination), daily during Days 8–14 (just before mosquito feeding) and on Days 30, 60, and 90 after vaccination. The sera were tested for plaque-neutralizing antibody (Days 0, 30, 60, and 90),²⁰ IgM antibody by enzyme-linked immunosorbent assay (Days 0 and 30),²¹ and delayed plaque assay on LLC-MK₂ cells (Days 8–14).²² Twenty-one of the 22 vaccinees volunteered to be bitten by mosquitoes. The 21 volunteers for the mosquito feedings were divided randomly in two groups, Group A with 10 volunteers and Group B with 11 volunteers. Mosquitoes were allowed to feed on Group A volunteers on Days 8, 10, and 12 after vaccination, and on Group B volunteers on Days 9, 11, and 13 after vaccination. Based on prior studies, the expected period of viremia was Days 8–14.⁶

Mosquito blood-feeding on volunteers. Volunteers were exposed to mosquitoes by resting a forearm or calf on top of the netting-covered end of two mosquito containers, one with *Ae. aegypti* and one with *Ae. albopictus*, for approximately 15 min. After the feeding period, unengorged mosquitoes were removed from the containers, killed, and discarded. Engorged mosquitoes were held at 31°C for 12–16 Days. At this time, mosquitoes were anesthetized by chilling (–20°C); counted; placed in cryovials by species, volunteer number, and day of blood-feeding; and stored at –70°C until assayed by immunofluorescence (IFA) assay.

Immunofluorescence assay. Dengue and YF viral antigen

in the heads of mosquitoes was detected by using an indirect immunofluorescence assay (IFA) as previously described.²³ Immediately after head removal, all mosquito carcasses were returned to the appropriately labeled cryovial and placed at –70°C for possible assay for virus at a later date. A mouse anti-flavivirus reference antiserum²⁴ provided by the Department of Virology, Armed Forces Research Institute of the Medical Sciences, Bangkok, Thailand, and a fluorescein-conjugated, sheep anti-mouse IgG (Sigma, St. Louis, MO) were used in all head squash assays. Mosquito head-tissue smears were examined with a Zeiss (Wetzlar, Germany) microscope with an HBO Osram 60W mercury vapor bulb, 450–490 exciter and LP520 barrier filters, and a T510 chromatic beam splitter.

Virus isolation. To confirm the infection status of IFA head-positive mosquitoes, the legs and bodies of mosquitoes from head-positive groups were triturated separately in 1 mL of diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Earle's salts plus antibiotics and NaHCO₃), clarified by centrifugation, and assayed for virus on Vero-cell monolayers. An additional 10–27 IFA-negative mosquitoes from each of the groups containing a positive mosquito (same species, patient, and date of feed) were assayed. After 6–7 days of incubation, the cells were stained with neutral red and plaques were enumerated the following day.

RESULTS

Viremia, confirmed as DEN-3, was detected in two volunteers inoculated with the DEN-3 vaccine candidate on Days 11, 12, 13, and 14 after inoculation. No virus was recovered from any of the other 20 volunteers. In accord with the lack of detectable viremias, only six mosquitoes developed disseminated infections after feeding on volunteers inoculated 8–13 days previously with live DEN-1 or DEN-2 vaccine candidates (Table 1). Infected mosquitoes included 4/247 *Ae. albopictus* fed on four volunteers inoculated with the DEN-1 vaccine candidate and 2/528 *Ae. aegypti* fed on three volunteers inoculated with the DEN-2 vaccine candidate. Although no viremia was detected in these seven volunteers by plaque assay, all were infected based on the presence of anti-DEN antibodies in their convalescent sera (Table 1).

None of 3,054 *Aedes* that fed on 14 volunteers inoculated with the DEN-3 or DEN-4 vaccine candidates or the licensed

TABLE 2

Virus isolations and body and leg titers for groups of mosquitoes containing the bodies of mosquitoes that had heads positive for dengue antigen by immunofluorescence assay (IFA)

Vaccine* (No. volunteers)	Mosquito species	No. tested	No. of		Viral titer†	
			IFA pos. heads	Iso-lates	Body	Leg
DEN-1 (11)	<i>Aedes albopictus</i>	14	1	1	3.0	<2
DEN-1 (18)	<i>Aedes albopictus</i>	45	3	3	4.6	2.5
					4.7	NT
					4.2	<2
DEN-2 (8)	<i>Aedes aegypti</i>	28	2	2	5.4	3.7
					5.3	3.4

* For candidate dengue vaccines: DEN-1 = 45AZ5 PDK20, and DEN-2 = S16803 PDK50.

† Log₁₀ PFU/specimen. NT = not tested, <2 = undetectable.

YF vaccine was positive by IFA. This included 357 *Aedes* mosquitoes fed on volunteers when they were viremic with DEN-3. However, only half (five of 10) of the volunteers inoculated with DEN-3 or DEN-4 became infected with these viruses, based on the presence of anti-DEN antibodies in their convalescent sera (Table 1). All four YF vaccine recipients seroconverted.

To confirm the presence of infectious dengue virus in the mosquitoes with IFA-positive heads, the carcasses of mosquitoes in vials that contained at least one mosquito that had an IFA-positive head were tested for infectious virus by plaque assay. Virus was isolated from the bodies of six of these mosquitoes (Table 2). For each of these pools, the number of bodies from which virus was isolated equaled the number of mosquitoes in that vial which had had an IFA-positive head.

DISCUSSION

We detected infection with dengue viruses in 6 of 3,763 *Aedes* mosquitoes fed on volunteers administered live DEN virus vaccine candidates. This included 4 (0.5%) of 760 mosquitoes fed on recipients of DEN-1 and 2 (0.3%) of 772 mosquitoes fed on recipients of DEN-2. Infection was confirmed in each of these IFA-positive mosquitoes by isolation of virus from the mosquito's body. These low infection rates were consistent with the low viremia levels in the volunteers. Although no viremia was detected in the four volunteers receiving the DEN-1 or the four volunteers receiving DEN-2 vaccine candidates, all of these volunteers seroconverted indicating that the virus did replicate in each of them. Because there was an interval of up to 3 hr between the blood draw to determine viremia and subsequent mosquito feeding, it is possible that these volunteers had a viremia during mosquito feeding that was not detected 3 hr earlier.

We used both *Ae. albopictus* and *Ae. aegypti* mosquitoes to determine the risk of infecting mosquitoes by allowing them to feed on inoculated volunteers. Both of these species are highly susceptible to dengue infection, but vary in their susceptibility to each of the four DEN serotypes.^{18,25} By using both species, we increased the likelihood of detecting transmission of DEN virus from one of the volunteers to a potential vector mosquito.

Our study indicated that the low viremias developed by

people vaccinated with these candidate vaccines were still sufficient to infect a small percentage of mosquitoes feeding during expected peak viremias. Similar results are reported for *Culex tarsalis* (western equine encephalitis virus),²⁶ *Culex torrentium* (Okelbo virus),²⁷ and for *Ae. albopictus* (chikungunya virus)²⁸ in which mosquitoes became infected when fed on hosts with undetectable viremias. However, mosquito infection rates with live-attenuated DEN virus vaccines were extremely low in our study. Each year, hundreds of infections with DEN virus are reported in the United States in individuals who have traveled to DEN endemic areas.²⁹ Despite relatively high viremia levels in these individuals and the high risk of viral transmission to mosquitoes biting such individuals,³⁰ there have been relatively few cases of DEN transmitted in the United States.³¹ The mosquito infection rate observed in our study was low, only 1/610 mosquitoes, even though we maintained our mosquitoes at 31°C after exposure to DEN virus. Previous studies indicate that environmental temperature is a critical factor in DEN transmission and that temperatures ≥ 31°C are needed for *Ae. aegypti* to transmit this virus efficiently.³⁰ Because the daily survival rate for *Ae. aegypti* under natural conditions where they are subject to predation and climate is about 80% per day,^{32,33} only about one in 10 survive the 10-day extrinsic incubation period. Thus, if only 1 in 600 mosquitoes becomes infected, it would take nearly 6,000 mosquitoes feeding on vaccinated people to have 600 mosquitoes (including the 1 infected) still alive 10 days later. In contrast, we would expect 60–80% of *Ae. aegypti* feeding on a person with a natural dengue viremia to become infected.^{30,34} Thus, the risk for initiating a DEN outbreak from the use of these vaccines is minimal when compared to the risk from importation of natural infections.

Acknowledgments: We thank Chris Pollard and Katie Rawlings for their technical assistance, and Katheryn Kenyon, Peter Summers, and Monica O'Guinn for their constructive criticism of the manuscript.

Financial support: Funding for the clinical study was provided from a grant made by the National Vaccine Program Office, Centers for Disease Control and Prevention, Atlanta, GA.

Authors' addresses: Michael R. Sardelis, James W. Jones, and Michael J. Turell, Vector Assessment Branch, Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702-5011. Terry A. Klein, HHC, 18th MEDCOM, Unit 15281, APO AP 96205-0054. Robert Edelman, Center for Vaccine Development, University of Maryland at Baltimore, 685 West Baltimore Street, Baltimore, Maryland 21201-1509. Bruce L. Innis and J. Robert Putnak, Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Reprint requests: Michael J. Turell, Vector Assessment Branch, Virology Division, USAMRIID, 1425 Porter Street, Fort Detrick, Maryland 21702-5011.

REFERENCES

- McKee KT Jr, Bancroft WH, Eckels KH, Redfield KT, Summers PL, Russell PK, 1987. Lack of attenuation of a candidate dengue-1 vaccine (45AZ5) in human volunteers. *Am J Trop Med Hyg* 36: 435–442.
- Bancroft WH, Top FH Jr, Eckels KH, Anderson JH, McCown JM, Russell PK, 1981. Dengue-2 vaccine: virological, im-

- munological, and clinical responses of six yellow fever-immune recipients. *Infect Immun* 31: 698–703.
3. Bhamarapravanti N, Yoksan S, Chayaniyayothin T, Angsubhankorn S, Bunyaratvej A, 1987. Immunization with a live attenuated dengue-2-virus candidate vaccine (16681-PDK 53): clinical, immunological and biological responses in adult volunteers. *Bull WHO* 65: 189–195.
 4. Innis BL, Eckels KH, Kraiselburd E, Meadors GF, Dubois DR, Burke DS, Bancroft WH, 1988. Virulence of a live dengue 3 vaccine suggests a possible new marker of dengue virus attenuation. *J Infect Dis* 158: 876–880.
 5. Nyven MJ, Dubois DR, Larsen LK, Summers PL, Kraiselburd EG, Gubler DJ, Eckels KH, 1990. Preparation of an attenuated dengue 4 (341750 CARIB) virus vaccine. I. Pre-clinical studies. *Am J Trop Med Hyg* 43: 212–218.
 6. Edelman R, Tacket CO, Wassermann SS, Vaughn DW, Eckels KH, Dubois DR, Summers PL, Hoke CH, 1994. A live-attenuated dengue-1 vaccine candidate (45A25) passaged in primary dog kidney cell culture is attenuated & immunogenic for humans. *J Infect Dis* 170: 1448–1455.
 7. Hoke CH Jr, Malinoski FJ, Eckels KH, Scott RMcN, Dubois DR, Summers PL, Simms T, Burrous J, Hasty SE, Bancroft WH, 1990. Preparation of an attenuated dengue 4 (341750 Carib) virus vaccine. II. Safety and immunogenicity in humans. *Am J Trop Med Hyg* 43: 219–226.
 8. Bancroft WH, Scott RMcN, Brandt WE, McCown JM, Eckels KH, Hayes DE, Gould DJ, Russel PK, 1982. Dengue-2 vaccine: infection of *Aedes aegypti* mosquitoes by feeding on viremic recipients. *Am J Trop Med Hyg* 31: 1229–1231.
 9. Schoepp RJ, Beaty BJ, Eckels KH, 1990. Dengue 3 virus infection of *Aedes albopictus* and *Aedes aegypti*: comparison of parent and progeny candidate vaccine viruses. *Am J Trop Med Hyg* 42: 89–96.
 10. Miller BR, Beaty BJ, Aitken THG, Eckels KH, Russell PK, 1982. Dengue-2 vaccine: oral infection, transmission, and lack of evidence for reversion in the mosquito, *Aedes aegypti*. *Am J Trop Med Hyg* 31: 1232–1237.
 11. Khin MN, Jirakanjanakit N, Yokson S, Bhamarapravati N, 1994. Infection, dissemination, transmission, and biological attributes of dengue-2 PDK53 candidate vaccine virus after oral infection in *Aedes aegypti*. *Am J Trop Med Hyg* 51: 864–869.
 12. Monath TP, 1986. Flaviviruses. Fields BW, Knipa DN, Chanock RM, Malnick JL, Roizman B, Shope RE, eds. *Virology*. New York: Raven Press, 955–1004.
 13. Sabin AB, 1952. Research on dengue during World War II. *Am J Trop Med Hyg* 1: 30–50.
 14. Jumali S, Gubler DJ, Nalim S, Eram S, Sulianti Saroso J, 1979. Epidemic dengue hemorrhagic fever in rural Indonesia. III. Entomological studies. *Am J Trop Med Hyg* 28: 717–724.
 15. Metselar DC, Grainger CR, Oei KG, Reynolds DG, Pudney M, Leake CJ, Tukei PM, D'Offay RM, Simpson DIH, 1980. An outbreak of type 2 dengue fever in the Seychelles, probably transmitted by *Aedes albopictus* (Skuse). *Bull WHO* 58: 937–943.
 16. Chan YC, Hox BC, Chan KL, 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 5. Observations in relation to dengue hemorrhagic fever. *Bull WHO* 44: 615–657.
 17. Rai KS, 1991. *Aedes albopictus* in the Americas. *Annu Rev Entomol* 36: 459–484.
 18. Gubler DJ, Rosen L, 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility with dengue viruses. *Am J Trop Med Hyg* 25: 318–325.
 19. Boromisa RD, Rai KS, Grimstad PR, 1987. Variation in the vector competence of geographic strains of *Aedes albopictus* for dengue 1 virus. *J Am Mosq Control Assoc* 3: 378–386.
 20. Russell PK, Nisalak A, Suckanavachana P, Vivona S, 1967. A plaque reduction test for dengue virus neutralizing antibodies. *J Immunol* 99: 285–290.
 21. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Putisri P, Hoke C, 1989. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 40: 418–427.
 22. Yuill TM, Sukhavachana P, Nisalak A, Russell P, 1968. Dengue-virus recovery by direct and delayed plaques in LLC-MK2 cells. *Am J Trop Med Hyg* 17: 441–448.
 23. Kuberski TT, Rosen L, 1977. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. *Am J Trop Med Hyg* 26: 533–537.
 24. Brandt WE, Buescher EL, Hetrick FM, 1967. Production and characterization of arbovirus antibody in mouse ascitic fluid. *Am J Trop Med Hyg* 16: 339–347.
 25. Gubler DJ, Nalim S, Tan R, Saipan H, Sulianti Sarso J, 1979. Variation in susceptibility to oral infection with dengue viruses among geographic strains of *Aedes aegypti*. *Am J Trop Med Hyg* 28: 1045–1052.
 26. Kramer LD, Hardy JL, Presser SB, 1983. Effect of temperature of extrinsic incubation on the vector competence of *Culex tarsalis* for western equine encephalomyelitis virus. *Am J Trop Med Hyg* 32: 1131–1139.
 27. Lundstrum JO, Niklasson B, Francly DB, 1990. Swedish *Culex torrentium* and *Cx. pipiens* (Diptera: Culicidae) as experimental vectors of Ockelbo virus. *J Med Entomol* 27: 561–563.
 28. Turell MJ, Malinoski FJ, 1992. Limited potential for mosquito transmission of a live, attenuated chikungunya virus vaccine. *Am J Trop Med Hyg* 47: 98–103.
 29. Rigau-Perez JG, Gubler DJ, Vorndam AV, Clark GG, 1997. Dengue: a literature review and case study of travelers from the United States, 1986–1994. *J Travel Med* 4: 65–71.
 30. Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A, 1987. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. *Am J Trop Med Hyg* 36: 143–152.
 31. Rawlings JA, Hendricks KA, Burgess CR, Campman RM, Clark GG, Tabony LJ, Patterson MA, 1998. Dengue surveillance in Texas, 1995. *Am J Trop Med Hyg* 59: 95–99.
 32. McDonald PT, 1977. Population characteristics of domestic *Aedes aegypti* (Diptera: Culicidae) in villages on the Kenya coast. I. Adult survivorship and population size. *J Med Entomol* 14: 42–48.
 33. Nyar JK, 1981. *Aedes aegypti* (L.) (Diptera: Culicidae): observations on dispersal, survival, insemination, ovarian development and oviposition characteristics of a Florida population. *J Fla Anti-Mosquito Assoc* 52: 24–40.
 34. Focks DA, Daniels E, Haile DG, Keesling JE, 1995. A simulation model of the epidemiology of urban dengue fever: literature analysis, model development, preliminary validation, and samples of simulation results. *Am J Trop Med Hyg* 53: 489–506.