Survival of Arboviruses in *Aedes albonotatus*, A Peridomestic Bahaman Mosquito* (34516)

CRAIG H. LLEWELLYN, ANDREW SPIELMAN, AND THOMAS E. FROTHINGHAM
(Introduced by T. H. Weller)

Department of Tropical Public Health, Harvard School of Public Health, Boston, Massachusetts

Aedes albonotatus, a common, domestic mosquito of the Bahamas, has been collected from human bait (1), but nothing is known of its potential as an arbovirus vector. This report compares the survival and transmission, in the laboratory, of chikungunya and dengue 2 viruses in *A. albonotatus* and in *A. aegypti*, the latter a known vector of both agents (2).

Materials and Methods. Viruses. Chikungunya virus (Gibbs 63–263), in its third mouse-brain passage since isolation from human sera in India, was provided by Dr. Jordi Casals, Yale Arbovirus Research Unit. Stock virus was prepared in the brains of suckling mice. Infectivity was assayed by testing 0.5-ml aliquots, or 10-fold dilutions thereof, for plaque-forming units (PFU) in conventional agar-overlaid cultures of embryonic chick cells. Plaques appeared in 2 days and did not become more numerous after day 3, at which time they were counted.

A Jamaican strain of dengue 2 virus (1203), in its fifth tissue culture passage since isolation from human sera, was provided by the Virus Dept., Walter Reed Army Institute of Research. A stock suspension was prepared in primary cell cultures of green monkey kidneys. Dengue virus was assayed in the same type of cell culture by an interference method (3) as follows: 0.1-ml aliquots, or 10-fold dilutions thereof, were inoculated into cultures (four cultures per dilution) and observed daily for cytopathic effect (CPE). On the eighth day postinoculation, cultures lacking CPE were further studied. Two cultures from each dilution were challenged [Challenge Virus Resistance test (CVR)] with approximately 100 TCD_{50} of poliovirus Type II. In each test, one or two cultures at each dilution remained unchallenged and were observed for 8 additional days for dengue-induced CPE. Appropriate controls comprising normal cells, challenge virus, known dengue virus, and challenge virus titrations were included. Cultures were slanted at 34° and examined daily for CPE. Cultures in which little or no CPE occurred by 24 hr after the challenge virus controls had developed 4+ CPE were considered to be CVR positive (CVR +). Cultures showing either dengue-induced CPE or interference, as indicated by a positive CVR test, were considered to contain dengue virus. Infectivity titer was calculated by the method of Reed and Muench and expressed as tissue culture infective doses 50% ($TCID_{50}$).

Cell cultures. Cell suspensions used for plaque-assay were prepared from trypsinized 10-day-old chick embryos. Five ml of growth medium containing 25×10^6 cells were pipetted into 5-cm plastic tissue culture Petri dishes. This medium contained 9 parts Earle's salt solution (4), with 0.5 g lactalbumin hydrolyzate and one part inactivated (56°, 30 min) horse serum per 100 ml. The suspension was overlayed with Earle's solution containing 1 g Difco Bacto Agar, 0.5 g gelatin, 0.1 g Difco yeast extract and 0.003 g neutral red per 100 ml. Penicillin (100 units) and streptomycin (100 μg) were included per 100 ml of all solutions, and cultures were incubated at 36° in humidified air containing 5% CO_2.

Cell cultures used in the CVR test were obtained from Microbiological Associates and

* Supported in part by Grants AI00046, AI00177, and AI01023 from the United States Public Health Service.

TABLE I. Infectivity of Suckling Mice for *A. aegypti* at Various Times after Inoculation with Chikungunya Virus.

Days after mouse infected by inoculation	PFU in individual mosquitoes			Mosquitoes transmitting virus to mice		
	Days after feeding on inoculated mice			Days after feeding on inoculated mice		
	0	10	15	5	10	15
1	3×10^1	—	—	0/4	—	—
2	1×10^1	—	—	0/4	0/2	0/3
3	4×10^2	4×10^6	7×10^6	0/3	1/2	0/2
4	3×10^3	6×10^5	8×10^6	0/3	2/2	2/3
5	6×10^4	8×10^4	2×10^7	0/3	2/2	3/3

maintained with Leibovitz L-15 media (purchased from the same source) supplemented with 2% bovine albumin and 300 mg L-glutamine per liter. Cultures were incubated in a slanted position at 34°.

Mosquitoes. A. albonotatus and *A. aegypti* from colonies originally established on Grand Bahama Island in 1965 were kept in gauze-covered glass chimneys at 24°, 75% relative humidity, and 16 hr of light per day. Water, raisins, and sugar cubes were provided. Isolated infected mosquitoes, similarly fed, were held in plastic tubes (from WHO Insecticide Susceptibility Test Kits) closed at one end with netting and at the other by a sliding partition.

Female mosquitoes were infected in two ways: (1) 5-day-old *A. aegypti* and 8-day-old *A. albonotatus* fed on 6-day-old suckling white mice (Harvard colony) inoculated intracerebrally 4 days previously with 0.02 ml of chikungunya virus stock (6×10^7 PFU/0.5 ml). (2) Restrained 5-day-old mosquitoes were inoculated via the thorax with approximately 0.001 ml of a 10^{-3} dilution of chikungunya or dengue 2 virus (5).

Transmissibility of chikungunya virus was assessed by exposing 2–3-day-old white mice to individual infected mosquitoes. Mice from which mosquitoes took a blood meal were observed for 10 days, and transmission of virus was assumed if death occurred between the second and sixth day after exposure. In order to determine the quantity of virus ingested, fully engorged mosquitoes were individually triturated in 1 ml of growth media in a chilled mortar. Suspensions were centrifuged for 15 min (1,500 rpm, 5°) and the infectivity of the supernatant fluid was assayed.

Results. Infection by feeding on mice. It was established that *A. aegypti* could be infected by feeding on mice inoculated with chikungunya virus 4 days earlier (Table I) and that 10 days later such mosquitoes could transmit the virus to other suckling mice. These results differ slightly from those of Gilotra and Shah (6), who observed a shorter incubation period in the mouse and a longer one in *A. aegypti.* In comparing *A. albonotatus* with *A. aegypti* in this regard, three experiments were performed; the results are combined and summarized in Table II. Chikungunya virus was found in all mosquitoes that were tested immediately after feeding. Virus was present in all females tested on days 2-5, but none transmitted the infection to mice. After day 5, most *A. aegypti* transmitted infection, and each contained more virus than did those assayed on days 2–5. *A. albonotatus* did not infect mice nor did they contain detectable virus.

Infection by intrathoracic injection. A. aegypti and *A. albonotatus* females were injected with suspensions of virus or with diluent alone. All females that were inoculated with dengue virus contained virus immediately following inoculation, while females inoculated with diluent did not (Table III). Virus was present in virtually all *A. aegypti* sampled. On the other hand, less than ¼ of *A. albonotatus* were CVR+ on day 10 and none were

TABLE II. Persistence of Chikungunya Virus in *A. aegypti* and *A. albonotatus* and Rate of Transmission to Infant Mice.

Days after feeding on viremic mice	*A. aegypti*		*A. albonotatus*	
	Number transmitting infection	Amount of virus (PFU) in triturated mosquitoes[a]	Number transmitting infection	Amount of virus (PFU) in triturated mosquitoes[a]
0	—	$11 \times 10^2 - 3 \times 10^{4}$[b]	—	$8 \times 10^2 - 7 \times 10^{3}$[b]
2	0/2	$31 \times 10^0 - 5 \times 10^1$	0/3	$16 \times 10^0 - 9 \times 10^1$
5	0/7	$15 \times 10^1 - 6 \times 10^2$	0/3	$0 - 2 \times 10^0$
10	13/16	$26 \times 10^4 - 8 \times 10^6$	0/18	0
15	4/5	$10 \times 10^4 - 3 \times 10^5$	0/4	0
17	3/4	$29 \times 10^4 - 6 \times 10^5$	0/4	0
22	2/3	$10 \times 10^4 - 33 \times 10^4$	0/3	0

[a] Virus content of each mosquito was determined after testing for transmission. The range of PFU detected is given.

[b] Four mosquitoes were tested.

positive on day 15. Similarly, chikungunya virus was found at the time of inoculation in all females inoculated with this agent, while the mosquitoes inoculated with diluent were CVR—. After day 5, virtually all *A. aegypti* contained high titers of virus. However, relatively few *A. albonotatus* contained virus on day 10 and none did so at 15 days after inoculation (Table IV).

TABLE III. Proportion of *A. albonotatus* and *A. aegypti* CPE and CVR Test Positive for Dengue 2 Virus after Intrathoracic Inoculation.

Species of mosquito	Days after inoculation		
	0	10	15
A. aegypti	2/2	8/10	7/8
A. albonotatus	2/2	2/9	0/9

Discussion. It appears that chikungunya and dengue 2 viruses do not persist in *A. albonotatus* and that chikungunya is not transmitted by *A. albonotatus* during feeding. We have found that *A. albonotatus* females do not feed avidly on man. Furthermore, these females are autogenous and will, therefore, feed less frequently than might otherwise be the case. Taken together, these observations indicate that *A. albonotatus* may be a poor vector in nature for these viruses.

There is currently great interest in the elimination of *A. aegypti* from portions of its range. Spielman and Weyer (1) reported in 1965 that *A. albonotatus* and *A. aegypti* were both present on Grand Bahama Island and were found in similar breeding sites. However, the two mosquitoes did not occur together. This was subsequently confirmed during repeated surveys of the island. If studies, currently in progress, indicate that *A. albonotatus* displaces or excludes *A. aegypti*, deliberate introduction of *A. albonotatus* might be

TABLE IV. Presence of Chikungunya Virus after Inoculation of *A. aegypti* and *A. albonotatus*.

Mosquito type	Days after inoculation		
	0	10	15
A. aegypti	4/4,[a] 2×10^{2}[b]	9/10, $16 \times 10^3 - 13 \times 10^4$	8/10, $6 \times 10^4 - 3 \times 10^6$
A. albonotatus	4/4, 5×10^{2}[b]	3/10, $6 \times 10^0 - 4 \times 10^2$	0/12, 0

[a] Numerator is the number of mosquitoes positive for chikungunya virus. Denominator is the number tested.

[b] Titers expressed as PFU; data at day 0 are based on pooled samples; data for days 10 and 15 show range of titers of individual mosquitoes.

considered as a control method elsewhere. Such an introduction would be permissible only if *A. albonotatus* appeared to be less dangerous as an arbovirus vector than is *A. aegypti*.

Summary. Experimental infections with chikungunya and dengue 2 viruses did not persist in *Aedes albonotatus*. On the other hand, titers increased in *A. aegypti* and transmission to suckling mice occurred during feeding. These results and other considerations suggest that *A. albonotatus* is not a potentially dangerous vector for these arboviruses.

1. Spielman, A. and Weyer, A. E., Mosquito News **25**, 339 (1965).

2 Rao, T. R., Ind. J. Med. Res. **52**, 719 (1964).

3. Halstead, S. B., Sukhavachana, P., and Nisalak, A., Proc. Soc. Exp. Biol. Med. **115**, 1062 (1964).

4. Dulbecco, R. and Vogt, M., J. Exp. Med. **99**, 167 (1954).

5. Hurlbut, H. S. and Thomas, J. I., Virology **12**, 391 (1960).

6. Gilotra, S. K. and Shah, K. V., Amer. J. Epidemiol. **86**, 379 (1967).

.Received Sept. 9, 1969. P.S.E.B.M., 1970, Vol. 133.