VERTICAL TRANSMISSION OF ST. LOUIS ENCEPHALITIS VIRUS TO AUTOGENOUSLY DEVELOPED EGGS OF *AEDES ATROPALPUS* MOSQUITOES

ELIZABETH G. PELZ AND JEROME E. FREIER[1]

Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University Medical School, 60 College Street, New Haven, CT 06501

ABSTRACT. Vertical transmission of St. Louis encephalitis virus was demonstrated by a strain of *Aedes atropalpus* mosquitoes from Garrett County, MD. Five-day-old parental females containing fully formed autogenous eggs were infected by intrathoracic inoculation. St. Louis encephalitis virus was detected in batches of fertilized eggs and fourth instar larvae. Estimated filial infection rates of approximately 1% were observed for both eggs and larvae.

INTRODUCTION

The northern rockpool mosquito, *Aedes atropalpus* (Coq)., has become a well-established container-breeding species in several midwestern and eastern states. In Indiana, Ohio (Restifo and Lanzaro 1980), Kentucky (Covell and Brownnell 1979), and New York (White and White 1980), this species has been described as an effective colonizer of discarded tires and is often the dominant species in tire yards (Beier et al. 1983). *Aedes atropalpus* is sympatric with *Aedes triseriatus* (Say), the natural vector of La Crosse encephalitis (LAC) virus and has also been shown to be susceptible to oral infection with LAC virus (Freier and Beier 1984). The same study also showed that parenterally infected *Ae. atropalpus* females could transmit LAC virus through autogenously and anautogenously developed eggs.

A study by Hardy et al. (1984) showed that parenterally infected *Ae. atropalpus* females could transmit St. Louis encephalitis (SLE) virus to their progeny. However, in their experiments, most of the progeny tested developed from anautogenous eggs, since infected parental mosquitoes were fed on normal chickens. Tests of autogenously developed eggs were not done, presumably because it was believed that infection of progeny occurred simultaneously with egg development.

The purpose of our study was to determine whether SLE virus could be transmitted through the autogenously developed eggs. If this occurs, then vertical transmission could maintain this virus in endemic foci.

MATERIALS AND METHODS

Virus: The SLE virus strain used in this study was isolated from a pool of overwintering female *Culex pipiens* Linn. mosquitoes collected from ammunition bunkers at Fort Washington National Historic Park, MD, on March 3, 1978. The virus had been passed only in mosquitoes, mainly *Toxorhynchites amboinensis* (Doleschall). Stock pools of virus were prepared from infected mosquitoes triturated in fetal calf serum, clarified by centrifugation, and stored at −70°C.

Mosquitoes: The mosquito strain used in this study was derived from a 1973 collection at Swallow Falls State Park, Garrett County, MD. A strain of *Ae. aegypti* (Linn.) originally colonized in 1980 from Villalba, Puerto Rico, was used in viral assays.

Aedes atropalpus eggs were hatched by flooding in an enamel pan with 2 liters of deionized water and 3 ml of a nutrient suspension containing 75 g of finely ground rabbit chow per liter of deionized water. Approximately 9 ml of nutrient suspension was added every other day until the beginning of pupation, which began about 6 days after hatching. Larvae were maintained at 28°C with a RH of 85% and a photoperiod of 16 h of light and 8 h of darkness.

In experiments requiring uninseminated females, pupae were picked, identified by sex on the basis of relative size, and the pupae of each sex were placed into separate cages. In experiments using mated females, both sexes were placed in pupal cups for emergence in regular 17-liter colony cages. Adults were held for 5 days following emergence to ensure that mating occurred. A sample of females from each group was tested for insemination prior to inoculation. The frequency of insemination was determined by examination of the spermathecae. Adult mosquitoes were provisioned with 10% sucrose and maintained at 28°C as previously described.

[1] Present address: Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, P.O. Box 2087, Fort Collins, CO 80522.

Experimental design: Inseminated and uninseminated female *Ae. atropalpus* mosquitoes were infected by intrathoracic inoculation with SLE virus (Rosen and Gubler 1974) 5–7 days after emergence and held at 28°C. Three days after infection, an oviposition cup lined with brown blotter paper was placed inside the cage and the eggs were collected for a 24-h period every 3 days. Fertilized eggs from inseminated females were held in the insectary for 3 days to permit the completion of embryonic development.

About half of the eggs on an ovistrip were then divided into pools of approximately 100 eggs each and placed in the mortar of a Ten Broeck tissue grinder. The remaining eggs were hatched, and the resulting larvae were reared to the fourth instar. Eggs from uninseminated females were processed immediately, since rapid desiccation prevented storage of these eggs. In all experiments, progeny eggs were triturated in 1.0 ml of mosquito diluent consisting of phosphate-buffered saline solution (pH 7.4), 0.5% gelatin, 30% heat-inactivated (56°C for 30 min) fetal calf serum, 200 units/ml of penicillin and 200 μg/ml of streptomycin.

To disrupt the fibers of the oviposition substrate and to ensure a thorough disruption of egg chorions, pooled eggs were first sonified at 70–80 watts for 30 sec (Sonifier Cell Disruptor Model W185 Heat Systems—Ultrasonics, Inc.) and then triturated for another 30 sec. Care was taken to insure that pools remained at 4°C. The egg suspension triturates were centrifuged at 1,000 × g for 25 min at 4°C to remove debris. The undiluted clarified suspension from each pool was tested for the presence of virus by inoculating 15–20 male *Ae. aegypti* mosquitoes (Rosen 1984).

The fourth instar F_1 larvae were divided into pools of 10 and triturated in Ten Broeck tissue grinders with 1.0 ml of the mosquito diluent described previously. Larval suspensions were clarified as above. Each undiluted suspension was inoculated into 15–20 male *Ae. aegypti* mosquitoes. All inoculated mosquitoes were held at 28°C for 14 days, before being assayed for SLE virus.

Virus assay: Mosquitoes were tested for the presence of SLE virus by the head squash technique (Kuberski and Rosen 1977). The indirect fluorescent antibody test was done using anti-SLE mouse hyperimmune ascitic fluid and fluorescein isothiocyanate-conjugated goat anti-mouse serum. Specimens were examined with an Olympus epifluorescence microscope at 200× magnification. A reference slide with positive and negative controls was used for comparison with the test slides. Each head squash was examined by 2 investigators, and the results were compared. At least 3 specimens were examined before a pool was considered negative. A pool was deemed positive when fluorescence was observed for at least 1 of 5 specimens examined. The infection of parental females was confirmed also by the head squash technique. Filial infection rates for F_1 progeny were generally estimated by the method of Le (1981), except for those instances when all test pools of progeny were positive and then a minimum filial infection rate was calculated.

RESULTS

The results of experiments to determine whether *Ae. atropalpus* could vertically transmit SLE virus are shown in Table 1. All progeny tested were derived from eggs that developed autogenously. These eggs were fully formed at the time of infection of parent females. Sper-

Table 1. Vertical transmission of SLE virus to F_1 progeny of inseminated female *Aedes atropalpus* mosquitoes.

Exp. no.	Days after infection	Total no. progeny	Stage tested	Pools pos./total	Estimated filial infection rate in percent
1	6	500	Eggs	5/5	1.0[a]
	9	2,000	Eggs	15/20	1.4
	12	1,200	Eggs	6/12	0.7
2	6	1,000	Eggs	0/10	—
	9	900	Eggs	9/9	1.0[a]
	12	1,500	Eggs	10/15	1.1
	14	1,000	Eggs	3/10	0.4
	6	600	Larvae	0/60	—
	9	1,048	Larvae	9/104	0.9
	12	90	Larvae	2/9	2.5
3	7	1,000	Eggs	10/10	1.0[a]
	10	300	Eggs	2/3	1.1
	7	530	Larvae	5/53	1.0
	10	60	Larvae	0/6	—

[a] Minimum filial infection rate in percent.

Table 2. Vertical transmission of SLE virus to F_1 eggs of uninseminated female *Aedes atropalpus* mosquitoes.

Exp. no.	Days after infection	Total no. eggs	Pools pos./total	% estimated filial infection rate
4	7	500	0/5	—
	10	1,500	0/15	—
	13	1,500	0/15	—
5	7	500	5/5	1.0[a]
	10	1,000	0/10	—
	14	1,000	3/10	0.4
6	7	500	0/5	—
	10	500	0/5	—

[a] Minimum filial infection rate in percent.

mathecal examinations of 10 parent females in each experiment showed that all females examined were inseminated. In experiment 1, SLE virus was recovered from F_1 progeny as early as 6 days after the infection of their mothers.

Experiments 2 and 3 were conducted to compare filial infection rates among eggs and larvae. In experiment 2, no virus was recovered from either eggs or larvae when F_1 eggs were collected 6 days after infection; however, SLE virus was recovered from both eggs and larvae when eggs were collected 9 or more days after infection. In experiment 3, SLE virus was recovered from eggs and larvae when F_1 eggs were obtained 7 days after infection. The estimated filial infection rates were similar for both eggs and larvae.

The results of experiments to determine whether uninseminated *Ae. atropalpus* females could vertically transmit SLE virus to eggs are shown in Table 2. Virus was recovered from F_1 eggs of virgin females only in experiment 5. In this experiment, the infection rate of eggs collected 14 days postinoculation was less than half that of those collected 7 days postinfection.

DISCUSSION

This study confirms the finding of Hardy et al. (1984) that female *Ae. atropalpus* mosquitoes are capable of transmitting SLE virus vertically to eggs and larvae. Following infection of parental females by intrathoracic inoculation, SLE virus was recovered from fully formed eggs that developed autogenously and from fourth instar larvae that were reared from a sample of these eggs. Estimated filial infection rates were usually about 1% in both eggs and larvae. The filial infection rates that we observed are in the same range as those observed by Hardy et al. (1984) for the New Cooke and Bass Rock strains of *Ae. atropalpus* infected with a Glenn County, CA, strain of SLE virus.

Vertically transmitted SLE virus was recovered in F_1 progeny eggs as early as 6 days after the mothers were infected. This corresponds with the time required to reach maximum viral titer in mosquitoes infected by the parenteral route (Rosen 1984). Although eggs were collected at different times, no consistent differences in filial infection rate were observed between eggs harvested early and those collected later.

Filial infection rates from our experiments represented minimal estimates because eggs were collected en masse and the percentage of females that transmitted SLE virus to their progeny could not be determined. Since individual females vertically transmit virus at higher rates than others (Freier and Rosen 1988), the pooling of progeny probably resulted in an underestimation of filial infection rates observed in this study.

Although all our mosquitoes were infected parenterally, experimental vertical transmission of another flavivirus, dengue type 1, has shown that comparable filial infection rates are observed whether parent females are infected parenterally or orally (Rosen et al. 1983). We also did not test F_1 pupae or adults and thus do not know if SLE virus is readily transmitted transstadially beyond fourth instar larvae.

Our results are compatible with the mechanism of vertical transmission for SLE virus suggested by Francy et al. (1981) and supported by Rosen (1987). According to this hypothesis, virus present in the genital chamber enters the fully formed egg at the time of fertilization. Most of our experiments with infected virgin females failed to show vertical transmission of SLE virus. Although we recovered virus from uninseminated eggs in one experiment, these eggs were not surface sterilized, and it is not known whether virus was present in the eggs or as a surface contaminant.

The epidemiologic significance of *Ae. atropalpus* in the maintenance of SLE virus in nature needs further ecological and behavioral analysis. Avian hosts are considered to be the primary vertebrate reservoirs for SLE virus (McLean and Bowen 1980), and it is not known to what extent *Ae. atropalpus* feeds on birds. However, since *Ae. atropalpus* is autogenous, it is possible

that SLE virus could be maintained in an endemic focus solely by vertical transmission to autogenous progeny. Successful feeding on vertebrate hosts and resultant virus amplification might be unnecessary. This hypothesis needs to be evaluated by measuring serial virus transmission through several generations.

ACKNOWLEDGMENTS

The authors wish to thank R. E. Shope and R. B. Tesh for providing facilities. We also thank G. B. Craig, Jr., at the University of Notre Dame for providing the mosquito strain and L. Rosen at the University of Hawaii for providing the virus strain. The authors are also grateful to D. B. Francy, C. J. Mitchell and D. J. Gubler for reviewing this manuscript. This work was supported in part by NIH grant AI 17995 to L. Rosen.

REFERENCES CITED

Beier, J. C., M. Travis, C. Patricoski and J. Kranzfelder. 1983. Habitat segregation among larval mosquitoes in tire yards. J. Med. Entomol. 20:76–80.

Covell, C. V., Jr. and A. J. Brownnell. 1979. *Aedes atropalpus* in abandoned tires in Jefferson County, Kentucky. Mosq. News 39:142.

Francy, D. B., W. A. Rush, M. Montoya, D. F. Inglish and R. A. Bolin. 1981. Transovarial transmission of St. Louis encephalitis virus by *Culex pipiens* complex mosquitoes. Am. J. Trop. Med. Hyg. 30:699–705.

Freier, J. E. and J. C. Beier. 1984. Oral and transovarial transmission of La Crosse virus by *Aedes atropalpus*. Am. J. Trop. Med. Hyg. 33:708–714.

Freier, J. E. and L. Rosen. 1988. Vertical transmission of dengue viruses by *Aedes mediovittatus*. Am. J. Trop. Med. Hyg. 39:218–222.

Hardy, J. L., L. Rosen, W. C. Reeves, R. P. Scrivani and S. B. Presser. 1984. Experimental transovarial transmission of St. Louis encephalitis virus by *Culex* and *Aedes* mosquitoes. Am. J. Trop. Med. Hyg. 33:166–175.

Kuberski, T. T. and L. Rosen. 1977. A simple technique for the detection of dengue antigen in mosquitoes by immunofluorescence. Am. J. Trop. Med. Hyg. 26:533–537.

Le, C. T. 1981. A new estimator for infection rates using pools of variable size. Am. J. Epidemiol. 114:132–136.

McLean, R. G. and G. S. Bowen. 1980. Vertebrate hosts, pp. 381–450. *In:* T. P. Monath (ed.), St. Louis encephalitis. American Public Health Association, Washington, DC.

Restifo, R. A. and G. C. Lanzaro. 1980. The occurrence of *Aedes atropalpus* (Coquillett) breeding in tires in Ohio and Indiana. Mosq. News 40:292–294.

Rosen, L. and D. J. Gubler. 1974. The use of mosquitoes to detect and propagate dengue viruses. Am. J. Trop. Med. Hyg. 23:1153–1160.

Rosen, L., D. A. Shroyer, R. B. Tesh, J. E. Freier and J. C. Lien. 1983. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Am. J. Trop. Med. Hyg. 32:1108–1119.

Rosen, L. 1984. Use of mosquitoes to detect and propagate viruses, pp. 281–292. *In:* K. Maramorosch and H. Koprowski (eds.), Methods in virology, vol. 8. Academic Press, Orlando, FL.

Rosen, L. 1987. Sur le mecanisme de la transmission verticale du virus de la dengue chez les moustiques. C. R. Acad. Sci. (Ser. III) (Paris) 304:347–350.

White, D. J. and C. P. White. 1980. *Aedes atropalpus* breeding in artificial containers in Suffolk County, New York. Mosq. News 40:106–107.