

ISOLATION OF LACROSSE VIRUS (CALIFORNIA ENCEPHALITIS GROUP) FROM FIELD COLLECTED *Aedes triseriatus*(SAY) LARVAE IN OHIO
(DIPTERA: CULICIDAE)¹

R. L. BERRY,² B. J. LALONDE,³ H. W. STEGMILLER,³ M. A. PARSONS² AND G. T. BEAR²

ABSTRACT. Two isolations of LaCrosse virus (California encephalitis group) were made from *Aedes triseriatus*(Say) larvae collected on June 7,

1973, from tree holes in Gambier, Knox County, Ohio. The larvae are believed to have hatched from overwintering eggs.

INTRODUCTION. Isolation of California encephalitis virus (CEV), LaCrosse strain from field collected larvae of *Aedes triseriatus*(Say) and passage of the virus from infected female mosquitoes through eggs to subsequent generations has recently been reported from Wisconsin by Watts *et al.* (1973) and Pantuwatana *et al.* (1974). The following is a report of 2 isolations of LaCrosse virus from field collected *Aedes triseriatus* larvae in Ohio.

METHODS. Larvae of *Aedes triseriatus* were collected from tree holes in the village of Gambier, Knox County, Ohio, on June 7, 1973. At the time of collection most larvae were 2nd and 3rd instars, but there were also some 1st and 4th instars and some pupae. They were pooled into samples that included larvae from several tree holes from a specific area rather than from individual tree holes. They were placed in plastic-lined ice cream cartons and stored in the laboratory in a refrigerator at 1 to 4° C until September 7, 1973, and were not provided with food except for the detritus from the tree holes. When they were removed from the refrigerator, it was noted that the pupae and many of the larvae had died. Those remaining were all 3rd and 4th instars.

The larvae were transferred through a wash of distilled water to petri dishes containing a layer of clean filter paper moistened with tap water. The petri dishes were resting on the surface of a mechanical chill table. Seven pools were made, containing 28, 18, 32, 20, 50, 15, and 15 larvae. They were stored in a Revco™ freezer at -70° C until processed for virus isolation. The larvae were ground and centrifuged prior to inoculation into suckling mice (SM) using essentially the same methods described by Sudia and Chamberlain (1967) for mosquitoes. The supernatant fluids were drawn off and stored at -70° C.

Each larval pool was inoculated into a litter of 6 SM, 1 to 3 days old. The mice were inoculated intracerebrally with 0.02 ml of the supernatant. The litters were observed for 14 days. Reisolation attempts were made with each suspected positive pool by inoculation of the original larval suspension into another litter of SM. Brains from sick or dead mice were passed into an additional litter of SM.

The two virus isolates were identified by complement fixation (CF) test using crude antigens of the passage SM brains suspended in borate saline (pH 9) and immune mouse ascites fluid (U. S. Dept. Health, Education and Welfare, 1962; Sever, 1962). Agar-gel diffusion was used to identify the strain of CEV isolated Murphy and Coleman, 1967). Infected brains from passage mice were reacted against California BFS-283, LaCrosse, Keystone, Snowshoe Hare, and Trivittatus strain immune mouse ascites fluids.

¹ Supported in part by Public Health Service Grant CC 00342, from the Center for Disease Control, Atlanta, Georgia.

² Vector Borne Disease Unit, Communicable Disease Division, Ohio Department of Health, 1571 Perry St., P.O. Box 2568, Columbus, Ohio 43216.

³ Virology Unit, Laboratory Division.

TABLE 1. Suckling mouse deaths during isolation of LaCrosse virus from field collected *Aedes triseriatus* larvae.

Pool no.	Inoculation	Days after inoculation							Suckling mice no. deaths/ no. inoculated
		1	2	3	4	5	6	12	
279 (50 larvae)	Original*	0	0	0	0	4	2	..	6/6
	1st reisolation*	0	0	0	0	1	2	1	4/6
	2nd reisolation*	0	0	0	0	0	0	0	0/6
	Passage†	0	0	6	6/6
281 (15 larvae)	Original*	0	0	0	0	2	1	0	3/6
	1st reisolation*	0	0	1	0	0	2	0	3/6
	2nd reisolation*	0	0	0	0	0	0	0	0/6
	Passage†	0	0	6	6/6

* Inoculations of supernates from triturated mosquito larvae.

† Inoculations of supernates from infected SM brain.

RESULTS. Two pools (279 with 50 larvae and 281 with 15 larvae) contained virus. The results of the original inoculation, reisolation, and passage are shown in Table 1.

Virus was reisolated from the original suspensions. The second reisolation attempts of both pools were negative. Passage inoculations, using brain material from mice killed following the original inoculation resulted in the death of all SM on day 3. The isolates were identified as California group virus by CF test. The agar-gel diffusion test showed a reaction between the virus isolates and BFS-283 and LaCrosse strain (Fig. 1), but the reaction between the virus isolates (Fig. 1, A, B) and LaCrosse antibody (Fig. 1, 2) was slightly more intense than the reaction between them and BFS-283 antibody (Fig. 1, 1).

DISCUSSION. Since our knowledge of the earlier isolation of LaCrosse virus from larvae was limited at the time of these isolations, we considered the possibility of contamination of the specimens sometime during processing. The cartons in which the larvae were kept were closed throughout the three months of storage, and the

refrigerator had never been used for storage of mosquito or virus specimens, so the virus recovered is believed to have originated from the larvae themselves or from the tree hole contents in the cartons. The larvae were ground separately from other mosquito pools being processed. The larval pools were inoculated along with other mosquito pools in the regular mouse colony, but there was no reason to suspect mechanical error. The negative results of the 2nd reisolation attempts of the original larval pools possibly may be the result of 4 freezings and thawings. The dead mice were stored in plastic bags along with other, separately bagged mice, at -50 to -70° C. The passage room had not been used for any other virus work for 88 days. However, the 2 virus isolations were prepared for passage along with 29 other suspected virus isolates from adult mosquitoes. Of the 31 suspected virus isolates prepared, 12 were positive. The only other LaCrosse virus isolate was from an adult mosquito pool which was inoculated 1 day before the larval pools were inoculated and was located on a separate rack on the opposite side of the passage room. All the other positives were Trivit-

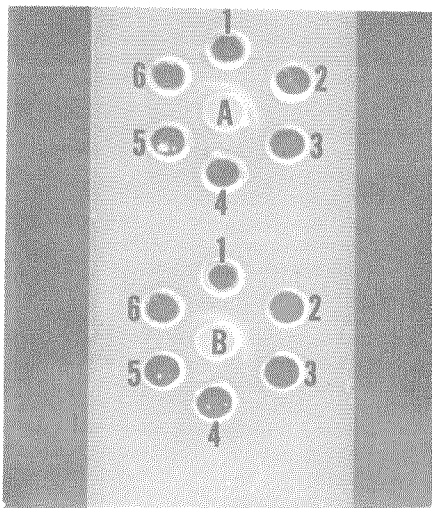


FIG. 1. Agar gel diffusion test of CEV isolates from *A. triseriatus* larvae showing reaction between crude suckling mouse brain (SMB) antigen, and BFS-283 and LaCrosse antibody. A, B=CEV isolates in SMB; 1-5=antibodies to CEV strains; 6=control: A, SMB 279; B, SMB 281: 1, BFS-283; 2, LaCrosse; 3, Trivittatus; 4, Keystone; 5, Snowshoe Hare; 6, normal mouse ascites fluid.

tatus virus (California encephalitis group) or Flanders viruses. All controls were negative. Thus we believe that these isolations did not result from accidental contamination.

Other evidence supporting the validity of these isolations includes: 1. These larvae were collected in a study area where LaCrosse virus is endemic. We made 10 isolations of LaCrosse virus from adult *Aedes triseriatus* from this area during the years 1971-72. No other California-group viruses have been isolated from mosquitoes collected in this area (Ohio Department of Health, unpublished data). 2. We have demonstrated transovarial transmission in the laboratory using LaCrosse virus and *A. triseriatus*, both obtained from the Gambier study area in 1971 (Ohio Department of Health,

unpublished data). 3. Our findings support similar reports from Wisconsin by Watts *et al.* (1973) and Pantuwatana *et al.* (1974).

The reaction found between BFS-283 and LaCrosse antibody and the larval isolates represents a close antigenic relationship of the two virus strains. This is primarily of academic interest to us, since BFS-283 is not known to occur in Ohio. According to Sudia *et al.* (1971), it occurs in the western United States.

The larvae positive for LaCrosse virus most likely represented individuals which had overwintered in the egg stage. Observations made in 1971 in the Gambier area showed that adults did not emerge until late May or early June. No adults were observed trying to bite while the samples were being taken, although a few pupae were collected. Our findings provide additional evidence that transovarial transmission of LaCrosse virus occurs in nature. These findings have meaningful implications regarding the control of LaCrosse virus. Watts *et al.* (1972) stated that control of California encephalitis (CE) can possibly be accomplished by filling tree holes, a more environmentally acceptable method of control than spraying insecticides. It now seems likely that filling tree holes is a most important means of controlling CE, since, even though insecticides might kill all the infected adults on the wing, infected adults could be emerging from tree holes soon after the insecticide application. It is doubtful that aerial or ground applications of insecticides would control larval populations in tree holes. Indeed, eliminating tree holes may be the only way that LaCrosse virus could be eradicated in an area, provided *A. triseriatus* is the only vector present. Because of the limited volume of the breeding site, *A. triseriatus* populations are rarely very large. Thus, filling even one tree hole in the vicinity of a home with children could provide significant benefits.

References Cited

Murphy, F. A. and P. H. Coleman. 1967. Cali-

- fornia group arboviruses: Immunodiffusion studies. *J. Immunol.* 99:276-284.
- Pantuwatana, S., W. H. Thompson, D. M. Watts, T. M. Yuill and R. P. Hanson. 1974. Isolation of LaCrosse virus from field collected *Aedes triseriatus* larvae. *Am. J. Trop. Med. Hyg.* 23(2):246-250.
- Sever, J. L. 1962. Application of a microtechnique to viral serological investigations. *J. Immunol.* 88:320-329.
- Sudia, W. D. and R. W. Chamberlain. 1967. Collection and processing of medically important arthropods for arbovirus isolation. Public Health Service, Center for Disease Control, Atlanta, Georgia. 29 pp.
- Sudia, W. D., V. F. Newhouse, C. H. Calisher and R. W. Chamberlain. 1971. California group arboviruses: Isolations from mosquitoes in North America. *Mosq. News* 31(4):576-600.
- U. S. Department of Health, Education, and Welfare, Public Health Service. 1962. Diagnostic complement fixation method (LBCF), CDC Laboratory Branch Training Manual.
- Watts, D. M., C. D. Morris, R. E. Wright, G. R. DeFoliart and R. P. Hanson. 1972. Transmission of LaCrosse virus (California encephalitis group) by the mosquito *Aedes triseriatus*. *J. Med. Entomol.* 9(2):125-127.
- Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill and W. H. Thompson. 1973. Transovarial transmission of LaCrosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. *Science* 182(4117):1140-1141.

EXPERIMENTAL HYBRIDIZATION BETWEEN *Aedes sollicitans* (WALKER) AND *Aedes mitchellae* (DYAR)

GEORGE F. O'MEARA, JUDY W. KNIGHT AND DAVID G. EVANS

Florida Medical Entomology Laboratory, Florida Division of Health,
P.O. Box 520, Vero Beach, Florida 32960

ABSTRACT. *Aedes sollicitans* and *Aedes mitchellae* were hybridized using induced mating techniques. Viable, fertile, F₁ hybrids were obtained from reciprocal crosses between the two species. These F₁ hybrids were successfully backcrossed to both parental species. In general, each backcross progeny had a normal sex ratio with minimal mortality in developmental stages. However, the hatch rate was less than 50% in most of the

backcross progenies. Most of the non-hatching eggs did not contain embryos. The larval siphons of the F₁ hybrids were more like those of *A. sollicitans* than *A. mitchellae*. Hybrid females could be readily distinguished from parental types. No evidence was found for natural hybridization between field populations of *A. sollicitans* and *A. mitchellae*.

Hybridization studies with highly eurygamous mosquitoes must use some type of induced mating technique (McDaniel and Horsfall, 1957). This procedure can sometimes overcome the premating mechanisms that serve to isolate closely related species. The elimination of premating barriers has frequently uncovered additional isolating mechanisms (Davidson *et al.*, 1967; McClelland, 1967; Kitzmiller *et al.*, 1967), including hybrid inviability, hybrid sterility, and hybrid breakdown. Although most hybridization experiments have encountered at least one of these isolating mechanisms, there have been notable exceptions. For example, in the

hybridization of *Aedes sollicitans* and *Aedes nigromaculis* by Fukuda and Woodward (1974), the F₁, F₂ and backcross progeny were fully viable and fertile.

In the present study, we examined the genetic affinity and potential for gene flow between *A. sollicitans* and *A. mitchellae*. Both *A. mitchellae* and *A. nigromaculis* have morphological similarities with *A. sollicitans*. There is a close relationship between larval, as well as adult, characteristics. All three species are highly eurygamous and they can be mated in the laboratory only by induced copulation.

MATERIALS AND METHODS. Field collections of *Aedes mitchellae* were made with