LACROSSE VIRUS ACTIVITY IN ILLINOIS DETECTED BY OVITRAPS

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ABSTRACT: LaCrosse (LAC) virus (California encephalitis group) was isolated from adult *Aedes triseriatus* mosquitoes that were collected as eggs from ovitraps placed in Peoria and Cook Counties, Illinois during June–September 1979. Because LAC virus is transmitted transovarially, this technique is very useful in identifying areas of LAC transmission without searching for natural or artificial oviposition sites or collecting adults attracted to human volunteers.

INTRODUCTION

In 1976, the Illinois Department of Public Health (IDPH) initiated an Arbovirus Surveillance Program in response to the 1975 epidemic of St. Louis encephalitis in Illinois (Clark et al. 1977). Once the program was established and with increased awareness of arboviral encephalitis, it became apparent that California encephalitis (CE) was a significant cause of human morbidity in the state. Since 1966, excluding the period from 1969 to 1971, human CE cases have been diagnosed annually in Illinois residents (Clark et al. 1980). The etiologic agent of these infections has been established as LaCrosse (LAC) virus (Clark et al. 1982a).

About 50% of the cases have occurred in Peoria County where field studies have been conducted since 1976. From 1976 through 1978, isolations of LAC virus were made from the treehole mosquito, *Aedes triseriatus* (Say), a recognized vector of LAC virus (Pantuwatana et al. 1972, Thompson et al. 1972, Watts et al. 1972) collected in the adult and larval stage. Results of these studies verified the occurrence in northcentral Illinois of transovarial transmission of LAC virus, a phenomenon first reported for LAC virus by Watts et al. (1973).

In the midst of considerable evidence of LAC virus transmission (Clark et al. 1982b) we initiated a study in 1979 to further define the spatial distribution of LAC virus in highly endemic Peoria County and in populous Cook County. The latter county accounted for only 3 of the CE cases identified in the state. We planned to accomplish our objective by using an ovitrap that exploited the treehole breeding habits of *Ae. triseriatus* complex mosquitoes and which also aided in detection of transovarial transmission of LAC virus.

METHODS AND MATERIALS

The ovitrap used was patterned after that of Novak and Peloquin (1981) and suggested to us by Grimstad (personal communication). The trap consisted of a 355 ml (12 fl oz) aluminum beverage can with top removed. Cans were rolled in a 50% solution of black Rustoleum® paint[3] which darkened the inner and outer surfaces of the can. Cans were nailed approximately 0.5 m above the ground to larger trees, preferably near shrubs or low-hanging vegetation. A 0.2 × 5.1 × 15.2 cm (1/16 × 2 × 6 in) strip of water-

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[3] Mention of product names does not constitute an endorsement by the Illinois Department of Public Health.

soaked balsa wood was inserted and tap water was added until it reached the overflow hole, about 5 cm (2 in) from the top. A small amount of leaf litter and debris was added to each trap.

Forty-five traps, in groups of 2 or 3, were originally set on 7 June at 16 locations in Peoria County. Sites were widely distributed in wooded areas, some of which were near the residence of prior human LAC cases. A description of this area was previously reported (Clark et al. 1982b). The balsa strips were collected on 5 July, 1 August, 4 September and 3 October. In Cook County, two 10-trap transects were established in each of 5 forest preserves in southern and southwestern parts of the county on 25 June. Strips were collected on 27 July, 23 August and 25 September. At the end of each monthly interval, sticks were removed and placed in individual plastic bags containing a moist cotton ball and labelled with tree location and date of collection. Fresh, water-soaked strips replaced those that were removed.

The egg-laden strips were returned to the laboratory, rinsed with tap water to eliminate extraneous material, and stored in clear plastic bags under a light regime of 17 hr light: 7 hr dark. Holding temperature in the insectary ranged from 20 to 23°C. Eggs were maintained for approximately 3 months until large-scale hatching and rearing was initiated.

The eggs were conditioned for hatching by placing the moistened stick in cans covered by water-soaked paper towels and a thin sheet of clear plastic. After 24 hr, the cans were filled with a freshly prepared 0.05% liver powder suspension. Larvae that had hatched 24–48 hr later were placed in clear, plastic rearing trays and fed a 0.5% liver powder suspension until pupation. Pupae were then separated and placed in tap water in emergence cages. A cotton ball soaked in honey and one soaked in water were provided for emerging adults. After all adult mosquitoes had emerged, they were taken into a −18°C walk-in freezer, quickly killed, placed in pools of approximately 50 mosquitoes each by sex and trap site, and stored at −90°C. All mosquitoes were identified with the aid of a key (Siverly 1972) under a stereomicroscope (14–60X).

Mosquito pools were ground in a chilled mortar and pestle with sterile alundum and a buffered diluent (20% fetal bovine serum-heat-inactivated at 56°C for 30 min, 80% Medium 199, and antibiotics). They were centrifuged for 30 min at 15,000 rpm and the supernate was decanted. The suspension (0.2 ml) was inoculated into 30 ml plastic bottles containing continuous African green monkey kidney (Vero) cell cultures. The cultures were incubated for 1 hr at 37°C and overlaid with a maintenance medium (1% nutrient agar and 2% fetal calf serum in Medium 199). Cultures were incubated for 4 days and then stained with a 1:60,000 dilution of neutral red for 2 hr at 37°C. Inoculated cell cultures were then observed over a period of 14 days for plaque formation. These methods followed a protocol provided by the Arbovirus Reference Branch, Vector-Borne Viral Diseases Division (VBVDD), Centers for Disease Control (CDC) in Fort Collins, Colorado. Pools producing plaques were reinoculated into Vero cell cultures, fluids were harvested and virus isolates were identified by complement-fixation (CF) tests with mouse hyperimmune ascitic fluid prepared against LAC, trivittatus (TVT), Jamestown Canyon (JC) viruses, and a grouping fluid against Bunyamwera serogroup viruses and normal suckling mouse brain. A representative sample of virus isolates was subtyped in serum dilution-plaque reduction neutralization tests at the VBVDD at CDC.

RESULTS

Except for a minimal amount of woodland mammal and human vandalism, trap survival was good. In some areas, mammals had removed, broken and chewed the balsa strips. Although larvae were often present, no pupae were observed in

the traps at the time the strips were removed.

Once mass-rearing was begun in the laboratory, it was seldom necessary to rehatch eggs on the strips since approximately 95% of the eggs hatched after the first flooding. Larval mortality was not a problem and good adult emergence was obtained. All specimens were identified as *Ae. triseriatus* complex mosquitoes.

From Peoria County, 14,994 adult *Ae. triseriatus* complex mosquitoes in 342 pools ($\bar{x}$=43 mosquitoes) were tested (Table 1). Sixteen (4.7%) of the pools

Table 1. LaCrosse virus positive *Aedes triseriatus* mosquitoes collected as eggs in ovitraps, Peoria County, Illinois, 1979.

Month eggs were laid	No. tested	No. of pools	No. of pools positive (%)
June	1,062	32	0
July	4,839	105	3(2.9)
August	5,798	129	13(10.1)
September	3,295	76	0
Total	14,994	342	16(4.7)

yielded isolates of LAC virus. The percentage of positive pools increased dramatically from July (2.9%) to August (10.1%) followed by a precipitous drop in September when no isolates were made. Isolates were from 4 of 16 sites (Table 2) and were widely distributed across the county (Fig. 1). No site yielded virus during more than a single monthly collecting interval. Nine (56%) of the isolates from Peoria County were from pools of male *Ae. triseriatus* while 7 (44%) were from females.

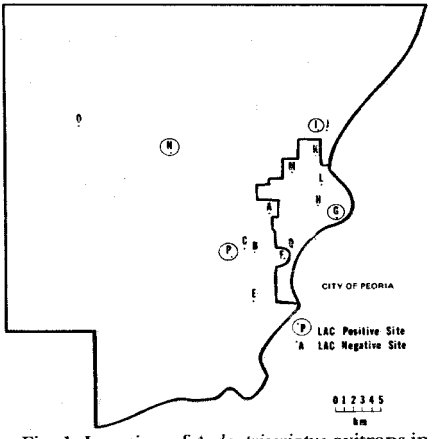

Fig. 1. Location of *Aedes triseriatus* ovitraps in Peoria County, Illinois, 1979.

Minimum field infection rate (MFIR) of adults reared from field collected eggs at site G (July) was 23/1,000 for males and 19/1,000 for both sexes. The MFIR of August eggs at site I was 22/1,000 for females and 17/1,000 for males; at site N was 8/1,000 for females and 5/1,000 for both sexes; and at site P was 7/1,000 for males and 3/1,000 for both sexes. The combined MFIR for both sexes was 20, 5, and 3 per 1,000 tested at sites I, N, and P. respectively. The seasonal combined sex MFIR for Peoria County was 1.1/1,000.

The positive sites were widely distributed. Site G was in a steep, wooded area which overlooked the Illinois River (the eastern boundary of the county) and was

Table 2. Ovitrap sites producing *Aedes triseriatus* mosquitoes, infected with LaCrosse virus, Peoria County, Illinois, 1979.

Site	Months eggs were laid			
	June	July	August	September
G	23/2(0)*	158/4(3)	583/12(0)	263/6(0)
I	201/4(0)	955/20(0)	462/10(9)	55/2(0)
N	246/5(0)	**	426/10(2)	1336/27(0)
P	5/1(0)	277/6(0)	580/12(2)	272/6(0)
Total	475/12(0)	1390/30(3)	2051/44(13)	1926/41(0)

* No. tested/No. pools (No. LaCrosse positive).

** Numerous eggs collected and hatched but label on rearing tray was lost.

adjacent to a country club and large private residences. Site I was in a children's camp approximately 11 km (7 mi) north of site G. Within the camp, approximately 3 km (1.7 mi) east of the river, nearby site J did not yield any LAC virus. Site N was in the edge of state park northwest of Peoria and adjacent to an open area with a row of recently constructed houses. Site P was in a wooded park in the village of Norwood (population approximately 700) west of the metropolitan area.

Virus positive pools ranged in size from 2 to 67 mosquitoes. An average of 83 mosquitoes was tested per ovitrap per month in Peoria County.

There were 29,172 *Ae. triseriatus* hatched and reared from Cook County ovitraps (Table 3, Fig. 2). These were tested in 763 pools ($\bar{x}$=38 mosquitoes) and resulted in isolation of 11 strains of LAC virus. The isolates originated from eggs laid in September at Yankee Woods Forest Preserve and were from 5 (45%) pools of male and 6 (55%) pools of female mosquitoes. Positive pools contained 32 to 67 mosquitoes. The number of isolates made from individual balsa strips ranged from 1 to 8. The MFIR in one 10-trap transect was 8/1,000 for both sexes and in a second transect was 4/1,000 for female and 2/1,000 for both sexes. This site was typical for the Cook County sites and provided riding trails and picnic areas for recreational activities. When transects were combined, the overall MFIR at Yankee Woods was 6/1,000. The seasonal combined six MFIRs for Cook County was 0.4/1,000. In Cook County an average of 100 mosquitoes was tested per ovitrap per month.

A sample of 5 virus isolates was tested and all were identified as LAC virus. Those from Peoria County were strains 79G530 (site P, collected as eggs on 4 Sept 79), 79G538 (site I, 4 Sept 79), 79G588 (site N, 4 Sept 79), and 79G663 (site G, 1 Aug 79). The strain from Yankee Woods in Cook County was 79G1618 and originated in eggs collected on 25 September.

Table 3. LaCrosse virus positive *Aedes triseriatus* mosquitoes collected as eggs in ovitraps in Cook County, Illinois, 1979.

Forest Preserve	No. tested	No. of pools	No. of pools positive (%)
Little Red School House (LRSH)	6,165	156	0
Cherry Hill (CH)	10,206	230	0
Yankee Woods (YW)	3,804	114	11(9.6)
Jurgenson Woods (JW)	3,181	101	0
Sauk Trail North (STN)	5,816	152	0
Total	29,172	753	11(1.5)

DISCUSSION

We believe this was the first successful large-scale effort using ovitraps for collection of *Ae. triseriatus* complex eggs in which LAC virus was isolated. This represents another application of the basic *Ae. triseriatus* ovitrap prepared by Loor and DeFoliart (1969). Jakob and Bevier (1969) and Furlow and Young (1970) have detected this mosquito in southeastern U.S.

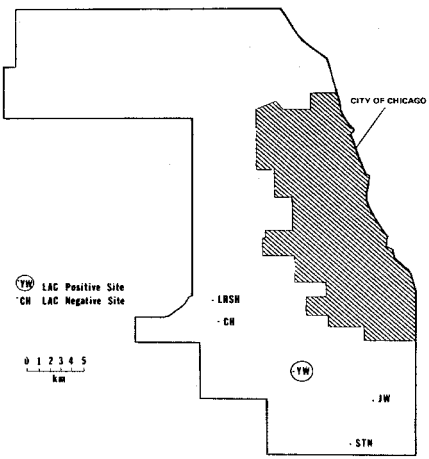

Fig. 2. Location of *Aedes triseriatus* ovitraps in Cook County, Illinois, 1979.

using similar ovitraps prepared from pint glass jars that were painted black. Gauld et al. (1974) utilized the Loor and DeFoliart ovitrap, replaced the black muslin sleeve with oak bark, and brought larvae that developed in the field into the laboratory for identification.

Balfour et al. (1975) collected *Ae. triseriatus* eggs in ovitraps and from treehole debris and transported them on filter paper to the laboratory. They tested 18, 382 eggs in 1972 and 1973 and did not recover virus but did isolate 1 strain of LAC virus from 150 eggs tested in 4 pools in 1974. Berry et al. (1977) reported transovarial transmission of JC virus of the California encephalitis group from laboratory-reared adult *Ae. triseriatus*. These mosquitoes were collected as eggs in a large ovitrap consisting of a green, 1 gallon metal can containing a piece of muslin cloth.

The modification of the trap made by Novak and Peloquin (1981) utilized light-colored balsa wood which enabled persons with minimal training and experience to recognize attached mosquito eggs in the field. The egg-laden strips can be easily stored for several months prior to mass rearing efforts during winter months. Animal vandalism might be prevented by placing a screen over the top of the trap (Mortenson, Rotramel and Prine 1978) or by using a wire clip to retain the balsa strip.

We were aware of the inherent difficulty in separating *Ae. triseriatus* from its sibling species, *Ae. hendersoni* Cockerell, in the adult stage. Recognizing this, our primary goal was to define areas where LAC virus was present. These areas should correspond to localities with active virus transmission. *Aedes hendersoni* is not regarded as a good vector for LAC virus (Watts et al. 1975) and does not preferentially oviposit near the ground (Sinsko and Grimstad 1977). Therefore, even though a few *Ae. hendersoni* may have been collected and tested, we did not feel compelled to separate them.

The higher individual MFIRs found in Peoria County may reflect a greater intensity of LAC virus transmission there than in Cook County. The highest combined MFIR (20/1,000) found in the study was from site I in Peoria County. This was greater than the 1/110 (9/1,000) found in larvae from 2 treeholes in Wisconsin by Watts et al. (1974). They also reported a MFIR of 1/41 (24/1,000) from male mosquitoes originating from the same treeholes. Beaty and Thompson (1975) found 0.6% (6/1,000) of 1,698 individually tested adults reared from larvae collected in 4 Wisconsin study areas had LAC virus before seasonal emergence of adults. Of 1,280 *Ae. triseriatus* processed throughout the season, 1.2% (12/1,000) contained virus. Their figures represent the true larval field infection rate.

Human LAC cases have been epidemiologically traced to site I (children's camp) with its high MFIR and site P (Norwood). Although no human cases have been linked directly with site G, numerous cases have occurred along the bluff extending north toward site I (Clark et al. 1982a). No cases have been identified near site N.

Had traps been placed randomly in both counties, the seasonal MFIR for all sites in Peoria County (1.1/1,000), which was almost 3 times greater than Cook County (0.4/1,000), might be interpreted as an accurate reflection of comparative LAC virus activity. This interpretation is further confounded by multiple virus isolations from eggs on individual strips that may have been deposited by a single infected female. Nevertheless, these values are consistent with the current distribution of recognized human cases, i.e. many from Peoria County and very few from Cook County (Clark et al. 1982a).

Use of this inexpensive ovitrap for detecting the presence of *Ae. triseriatus* complex mosquitoes is superior to the laborious and time-consuming task of locating naturally-occurring treeholes with larvae or collecting female mosquitoes attracted to human volunteers. This tool has the potential for greatly expanding the capability of arbovirus surveillance programs

to define areas of high LAC virus transmission. Miller et al. (1977) found that 98% of the transovarially infected *Ae. triseriatus* transmitted LAC virus to their progeny and 71% of the offspring were infected. This would indicate a high degree of stability in localities where the virus is being maintained. Identification of areas of high transmission levels and thus risk, would contribute to the ultimate goal which is prevention of unnecessary morbidity and mortality in children.

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References Cited

Balfour, H. H., Jr., C. K. Edelman, F. E. Cook, W. I. Barton, A. W. Buzicky, R. A. Siem and H. Bauer. 1975. Isolates of California encephalitis (LaCrosse) virus from field-collected eggs and larvae of *Aedes triseriatus:* Identification of the overwintering site of California encephalitis. J. Infect. Dis. 131:712–716.

Beaty, B. J. and W. H. Thompson. 1975. Emergence of LaCrosse virus from endemic foci, fluorescent antibody studies of overwintered *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 24:685–691.

Berry, R. L., B. J. L. Weigert, C. H. Calisher, M. A. Parsons and G. T. Bear. 1977. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. Mosq. News 37:494–496.

Clark, G. G., H. L. Pretula, T. Jakubowski and M. A. Hurd. 1977. Arbovirus surveillance in Illinois, 1976. Mosq. News 37:389–395.

Clark, G. G., R. J. Martin, H. L. Pretula, C. W. Langkop and H. H. Rohrer. 1980. California group virus infections in Illinois. Illinois Med. J. 157:91–96.

Clark, G. G., H. L. Pretula, C. W. Langkop, R. J. Martin, and C. H. Calisher, 1982a. LaCrosse (California serogroup) encephalitis viral infections in Illinois. Am. J. Trop. Med. Hyg. (in press)

Clark, G. G., H. L. Pretula, W. H. Rohrer, R. N. Harroff and T. Jakubowski. 1982b. Persistence of LaCrosse virus (California serogroup) in north-central Illinois. Am. J. Trop. Med. Hyg. (in press).

Furlow, B. M. and W. W. Young. 1970. Larval surveys compared to ovitrap surveys for detecting *Aedes aegypti* and *Aedes triseriatus*. Mosq. News 30:468–470.

Gauld, L. W., R. P. Hanson, W. H. Thompson and S. K. Sinha. 1974. Observations on a natural cycle of LaCrosse virus (California group) in southwestern Wisconsin. Am. J. Trop. Med. Hyg. 23:983–992.

Jakob, W. L. and G. A. Bevier. 1969. Application of ovitraps in the U.S. *Aedes aegypti* eradication program. Mosq. News 29:55–62.

Loor, K. A. and G. R. DeFoliart. 1969. An oviposition trap for detecting the presence of *Aedes triseriatus* (Say). Mosq. News 20:487–488.

Miller, B. R., G. R. DeFoliart and T. M. Yuill. 1977. Vertical transmission of LaCrosse virus (California encephalitis group): Transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 14:437–440.

Mortenson, E. W., G. L. Rotramel and J. E. Prine. 1978. The use of ovitraps to evaluate *Aedes sierrensis* (Ludlow) populations. Calif. Vector Views 25:29–32.

Novak, R. J. and J. J. Peloquin. 1981. A substrate modification for the oviposition trap used for detecting the presence of *Aedes triseriatus*. Mosq. News 41:180–181.

Pantuwatana, S., W. H. Thompson, D. M. Watts and R. P. Hanson. 1972. Experimental infection of chipmunks and squirrels with LaCrosse and Trivittatus viruses and biological transmission of LaCrosse virus by *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 21:476–481.

Sinsko, M. J. and P. R. Grimstad. 1977. Habitat separation by differential vertical oviposition of two treehole *Aedes* in Indiana. Environ. Entomol. 6:485–487.

Siverly, R. E. 1972. Mosquitoes of Indiana. Ind. St. Bd. Hlth., Indianapolis, 126 pp.

Thompson, W. H., R. O. Anslow, R. P. Hanson and G. R. DeFoliart. 1972. LaCrosse virus isolations from mosquitoes in Wisconsin, 1964–68. Am. J. Trop. Med. Hyg. 21:90–96.

Watts, D. M., P. R. Grimstad, G. R. DeFoliart and T. M. Yuill. 1975. *Aedes hendersoni:* Failure of laboratory infected mosquitoes to transmit LaCrosse virus (California encephalitis group). J. Med. Entomol. 12:451–453.

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. 9:125–127.

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 182:1140–1141.

Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart and R. P. Hanson. 1974. Overwintering of LaCrosse virus in *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 23:694–700.

REPELLENT ACTIVITY OF A PROPRIETARY BATH OIL (SKIN-SO-SOFT®)[1]

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ABSTRACT. A proprietary bath oil (Skin-So-Soft®) containing mineral oil, isopropyl palmitate, diisopropyl adipate, fragrance, dioctyl sodium sulfosuccinate and benzophenone was tested on the forearm for repellency to the yellow fever mosquito, *Aedes aegypti*. The median effective dosage (ED50) and effective half-life ($t_{1/2}$) of the product were estimated at 0.09 mg/cm^2 and 1.6 hours, respectively.

Recently we have learned through person-to-person channels that a commercial product, Skin-So-Soft® bath oil (Avon Products, Inc., New York), is widely used as a mosquito repellent. According to the label this product contains mineral oil, isopropyl palmitate, diisopropyl adipate, fragrance, dioctyl sodium sulfosuccinate and benzophenone-11.

Schreck and Kline (1981) tested Skin-So-Soft bath oil for repellency against several species of *Culicoides* (Diptera, Ceratopogonidae). They reported that Skin-So-Soft bath oil did not repel these insects but did trap them in the film formed by the material on the skin and prevent them from biting.

In July 1979 we initiated a series of tests of Skin-So-Soft bath oil against the yellow fever mosquito, *Aedes aegypti* (Linn.). These tests were terminated in October 1979 for reasons of "economy," but during the period of testing we were able to establish that Skin-So-Soft bath oil is a true repellent for *Ae. aegypti* and to obtain some indication of its relative persistence on the skin.

MATERIALS AND METHODS

TEST INSECTS. The University of California at San Francisco (UCSF) strain of *Ae. aegypti* was used in the tests. These were reared and maintained as described previously (Rutledge et al. 1978).

TEST MATERIAL. The material tested was a commercial sample of Skin-So-Soft bath oil.

TEST METHODS. The median effective dosage (ED50) of Skin-So-Soft bath oil for *Ae. aegypti* was determined by a modification of the method of Buescher et al. (1982a). Five circular test areas (29 mm diam) were outlined on the flexor region of the forearm with a plastic template and a fine-tipped felt pen. The 5 test areas were then treated at random with 0.0625,

[1] Use of trade names in this report does not imply official approval or indorsement of the items mentioned. Opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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