

TRANSOVARIAL TRANSMISSION OF CALIFORNIA ENCEPHALITIS VIRUS IN THE MOSQUITO *Aedes dorsalis* AT BLUE LAKE, UTAH¹

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ABSTRACT. Mosquito larvae, collected 14 May to 7 August 1975 at Blue Lake, near Wendover, Utah, were reared to adults and assayed for arboviruses. The most common species was

Aedes dorsalis from which 2 California encephalitis subtype viruses were isolated. This finding is discussed in relation to the isolation of other California Group viruses.

California Group viruses, isolated from field-collected *Aedes* spp. eggs, larvae and/or adults reared from larvae include LaCrosse (LAC), Keystone (KEY), Snowshoe hare (SSH), and Jamestown Canyon (JC) viruses (Pantuwatana et al. 1974, Berry et al. 1974, Watts et al. 1974, Balfour et al. 1975, LeDuc et al. 1975, McLean et al. 1975, McLintock et al. 1976, and Berry et al. 1977). At Blue Lake, 17 miles south of Wendover, Utah, in 1965, adult *Ae. dorsalis* were collected which yielded numerous California Group viruses (Smart et al. 1972). Presented here are isolations of California encephalitis (CE) subtype virus of the California Group from *Ae. dorsalis* adults collected as larvae at Blue Lake on 4 June 1975.

METHODS. Larvae and water samples, collected from receding pools in saltgrass (*Distichlis stricta*) adjacent to Blue Lake, were transported in pint ice cream cartons to Dugway where the lids were replaced with pint cartons that had open bottoms and cheese cloth covers (Harmston and Lawson 1967). Larvae were maintained in the water in which they were collected and although no nutrients were added, distilled water was periodically added to compensate for that lost by evaporation. Emerging adults were removed from the

upper cartons, anesthetized with carbon dioxide, then frozen and stored in a mechanical freezer at -65° C. Using the guide of Nielsen and Rees (1961), mosquitoes were identified on a CDC chill table (Sudia et al. 1965) and pooled by species, sex, and date of larval collection. Mosquitoes were assayed for viruses in suckling mice by the methods described by Crane et al. (1970). Isolates were identified by neutralization in suckling mice and by plaque reduction on Vero cell cultures (Lindsay et al. 1976).

RESULTS. On 24 April 1975 at Blue Lake, 25 adult *Culiseta inornata* were obtained in 7 CDC miniature light traps with dry ice attractant but no mosquito larvae were found. However, larvae were collected on 14 May, 4 June, 15 July, and 7 August; 97% of the emerging adults were *Ae. dorsalis* but a few *Ae. campestris*, *Ae. niphadopsis* and *Cs. inornata* were also present (Table 1). A total of 654 male and 762 female adults emerged, the majority within 4 weeks, but the August collection took 6 weeks. No viruses were obtained from the May 1975 collection (Table 1). A decade earlier, 27–28 April 1965, no viruses were isolated from adults reared from larvae collected at Blue Lake. Fifteen pools were assayed, of which 6 contained 509 unidentified males, 7 contained 414 female *Ae. dorsalis*, 1 contained 15 *Ae. campestris* females and 1 contained 5 *Ae. niphadopsis* females.

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Table 1. Adult mosquitoes reared from larvae collected at Blue Lake, Utah in 1975.

Date collected	<i>Aedes dorsalis</i>		<i>Aedes campestris</i>		<i>Aedes niphadopsis</i>		<i>Culiseta inornata</i>		Totals	
	(male)	(female)	(female)	(male)	(male)	(female)	(male)	(female)	(male)	(female)
14 May	1/101 ^a	1/72	1/3	1/2	1/2	1/9	1/2	1/3	3/105	4/87
4 June	+3/207	+3/189							+3/207	+3/189
15 July ^b	2/164	3/225			1/20	1/9			3/184	4/234
7 Aug.	2/158	3/252							2/158	3/252
Totals	+8/530	+10/738	1/3	1/2	1/2	1/9	2/22	2/12	+11/654	+14/762

^a Number of pools/number of mosquitoes assayed.^b July specimens thawed prior to processing due to a refrigeration failure.

+ 2 isolates of California encephalitis subtype virus, 1 from a pool of 97 males and 1 from a pool of 73 females.

In 1975 the only isolates were from *Ae. dorsalis* collected as larvae on 4 June (Table 1). The 2 isolates, Pool Numbers 753040 and 753041, were specifically identified as the CE subtype virus of the California Group (Table 2); Pool Number 753040 contained 97 male and Pool Number 753041 contained 73 female *Ae. dorsalis*.

DISCUSSION. The isolation of California Group viruses from *Aedes* spp. eggs, larvae, or adults reared from field-collected larvae and eggs, suggests that the virus may overwinter in the immature stages. California Group viruses isolated from developmental stages include LAC virus from *Ae. triseriatus* collected in tree holes in Wisconsin (Pantuwatana et al. 1974 and Watts et al. 1974), in Ohio (Berry et al. 1974) and in Minnesota (Balfour et al. 1975); KEY virus from *Ae. atlanticus* collected in the swamp waters of Maryland (LeDuc et al. 1975); SSH virus from *Aedes* spp. larvae collected from ice-covered ditches in the Yukon Territory (McLean et al. 1975) and from developmental stages of *Ae. implicatus* collected in Saskatchewan (McIntock et al. 1976); and JC virus from *Ae. triseriatus* in Ohio (Berry et al. 1977). The only isolations from eggs or larvae collected before adult mosquitoes were prevalent were in April and May by Watts et al. (1974), and Balfour et al. (1975) and May by McLean et al. (1975). Thus, overwintering in *Aedes* spp. eggs is strongly supported. Although Berry et al. (1974) made their isolations from larvae collected on 7 June, they believed that the virus most likely overwintered in mosquito eggs; no adults were observed when they collected larvae but emergence can occur in late May.

Nine of 12 identified isolates from adult *Ae. dorsalis* collected at Blue Lake in 1965 were CE subtype virus (Sudia et al. 1971, and Smart et al. 1972). Therefore, it is not surprising that this virus was obtained, although indirectly, from *Ae. dorsalis* larvae. As indicated by Nielsen and Rees (1961), *Ae. dorsalis* is a multi-brooded species which may be on the wing in Utah as early as March. It is possible that the infected larvae collected on 4 June may have come

Table 2. Results of serum-dilution plaque-reduction neutralization tests in Vero cells: Blue Late Isolate Numbers 753040, 753041, and 7 North American subtype viruses of the California group.

Virus*	Antibody to:						
	CE	SAN	SSH	LAC	JC	KEY	TVT
753040	80 ^b	<40	40	<80	<160	<40	<80
753041	320	40	40	<80	<160	<40	<80
CE	160	80	40	80	80	40	40
SAN	10	160	<10	40	40	10	80
SSH	20	20	320	160	160	10	40
LAC	10	10	40	320	<10	10	20
JC	20	<10	20	160	1280	20	80
KEY	<10	10	10	<10	20	320	20
TVT	10	10	10	10	20	20	320

*Viruses: CE—California encephalitis, SAN—San Angelo, SSH—Snowshoe hare, LAC—LaCrosse, JC—Jamestown Canyon, KEY—Keystone, TVT—Trivittatus.

^bTiters given as reciprocals.

from eggs laid by *Ae. dorsalis* females that had taken an infective blood meal earlier in the spring. In 1965 Smart et al. (1972) isolated 2 California Group viruses from 7,196 *Ae. dorsalis* adults collected at Blue Lake in May. On 24 April 1975 mosquito larvae were not found and the only adults collected were 25 *Cs. inornata*. No viruses were isolated from adults reared from mosquito larvae collected 27–28 April 1965 and 14 May 1975. However, the isolation of California Group viruses from developmental stages of other *Aedes* spp. suggests that CE virus could overwinter in *Ae. dorsalis* eggs at Blue Lake. The CE subtype isolation extends the list of known California Group viruses recovered from developmental stages of *Aedes* species. This is the first report of transovarial transmission in *Ae. dorsalis*, or in any aedine mosquito in the western United States.

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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 virus. *J. Infect. Dis.* 131:712–716.
- Berry, R.L., B.J. LaLonde, H.W. Stegmiller, M.A. Parsons and G.T. Bear. 1974. Isolation of LaCrosse virus (California encephalitis group) from field-collected *Aedes triseriatus* (Say) larvae in Ohio (Diptera: Culicidae). *Mosquito News* 34:454–457.
- Berry, R.L., B.J. LaLonde, C.H. Calisher, M.A. Parsons and G.T. Bear. 1977. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. *Mosquito News*, 37: 494–496.
- Crane, G.T., R.E. Elbel, D.E. Klimstra and K.L. Smart. 1970. Arbovirus isolations from mos-

- quitoes collected in central Utah in 1967. Amer. J. Trop. Med. Hyg. 19:540-543.
- Harmston, F.C. and F.A. Lawson. 1967. Mosquitoes of Colorado. U.S. Pub. Health Ser., Nat. Commun. Dis. Cen., 140 pp.
- LeDuc, J.W., W. Suyemoto, B.F. Eldridge, P.K. Russell and A.R. Barr. 1975. Ecology of California encephalitis viruses on the Del Mar Va Peninsula. II. Demonstration of transovarial transmission. Amer. J. Trop. Med. Hyg. 24:124-126.
- Lindsay, H.S., C.H. Calisher and J.H. Mathews. 1976. Serum-dilution neutralization test for California group virus identification and serology. J. Clin. Micro. 4:503-510.
- McLean, D.M., S.K.A. Bergman. A.P. Gould, P.N. Grass, M.A. Miller and E.E. Spratt. 1975. California encephalitis virus prevalence throughout the Yukon Territory, 1971-1974. Amer. J. Trop. Med. Hyg. 24:676-684.
- McIntock, J., P.S. Curry, R.J. Wagner, M.K. Leung and J.O. Iverson. 1976. Isolation of snowshoe hare virus from *Aedes implicatus* larvae in Saskatchewan. Mosquito News 36:233-237.
- Nielsen, L.T. and D.M. Rees. 1961. An identification guide to the mosquitoes of Utah. Univ. Utah Biol. Ser. 12:1-58.
- 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. Amer. J. Trop. Med. Hyg. 23:246-250.
- Smart, K.L., R.E. Elbel, R.F.N. Woo, E.R. Kern, G.T. Crane, G.L. Bales and D.W. Hill. 1972. California and Western encephalitis viruses from Bonneville Basin, Utah in 1965. Mosquito News 32:382-389.
- Sudia, W.D., R.W. Chamberlain and M.I. Collier, 1965. CDC entomological chill table, a refrigerated unit for use in processing mosquitoes for arbovirus isolation studies. Mosquito News 25:385-389.
- Sudia, W.D., V.F. Newhouse, C.H. Calisher and R.W. Chamberlain. 1971. California group arboviruses: isolations from mosquitoes in North America. Mosquito News 31:576-600.
- Watts, D.M., W.H. Thompson, T.M. Yuill, G.R. DeFoliart and R.P. Hanson. 1974. Overwintering of LaCrosse virus in *Aedes triseriatus*. Amer. J. Trop. Med. Hyg. 23:694-700.

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