Table 4. Larvicidal and pupicidal activity of monomolecular film using the Dema injection valve system.

	Arosurf® MSF[a]
Mortality (24 hr)[b] (larvae and pupae)	A) 100%
	B) 90%
Control (water only)	C) 0%
	D) 0%

[a] 11.5 ml/min a.i. 5.1 l/min water, final dilution 1:443.

[b] Test organism consisted of *Cx. quinquefasciatus*, pupae and 4th instar larvae in a ratio of approximately 1:1.

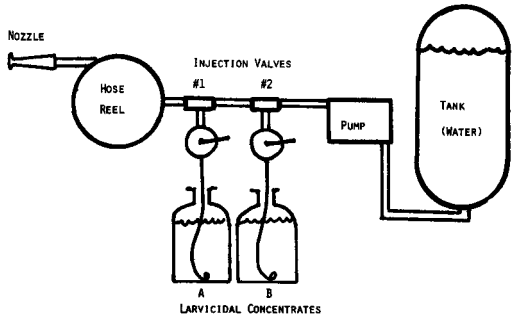

Fig. 2. Dema injection valve system.

containing the concentrate and refrigerated, if necessary. It also permits the application of water-borne Arosurf MSF without high speed agitation.

These tests with the Dema injection system indicate that a spray truck can carry two or more larvicidal concentrates and switch from one to another as the conditions and requirements change (Fig 2). The use of the injection system can achieve proper dosage rates of the test formulation that will kill mosquito larvae.

References Cited

Roberts, D. W. 1969. Some effects of *Metarhizium anisopliae* and its toxins on mosquito larvae, pp. 243–246. In: P. A. van der Laan (ed.). Insect pathology and microbial control. North Holland, Amsterdam.

Levy, R. and T. W. Miller, Jr. 1977. Experimental release of *Romanomermis culicivorax* (Mermithidae: Nematoda) to control mosquities breeding in southwest Florida. Mosq. News 37:483–486.

Levy, R., C. M. Powell, B. C. Hertlein, W. D. Garrett and T. W. Miller, Jr. 1982. Additional studies on the use of monomolecular surface film Arosurf®66–E2 for operational control of mosquito larvae and pupae. J. Fla. Anti-Mosq. Assoc. 53(2):100–106.

ISOLATION OF CALIFORNIA ENCEPHALITIS SEROTYPE FROM MOSQUITOES COLLECTED IN MANITOBA, CANADA

H. ARTSOB[1], L. P. SPENCE[1], C. H. CALISHER[2], L. H. SEKLA[3] AND R. A. BRUST[4]

Three California (CAL) serogroup viruses, snowshoe hare, Jamestown Canyon and trivittatus (Artsob 1983), have been isolated from Canada, and CAL serogroup virus activity has been demonstrated serologically in all 10 Canadian provinces as well as the Yukon and Northwest Territories. This communication reports the isolation and identification in Canada of a fourth CAL serogroup member.

A pool of 8 *Culiseta inornata* (Williston) collected on July 16, 1979 from Selkirk/Oak Hammock, Manitoba yielded an isolate, Mn 296–300, which was identified in complement fixation tests as a CAL serogroup virus. Neutralization tests employing a Tissue Culture Infective $Dose_{50}$ method (Artsob et al. 1983) and enzyme-linked immunosorbent assay (ELISA) typing (Artsob et al. 1984) of the isolate were undertaken using hyperimmune mouse ascitic fluid. The ELISA typing method has been shown to successfully differentiate CAL serogroup members isolated in North America.

Isolate Mn 296–300 was shown to be closely related or identical to California encephalitis (CE) serotype (Table 1). This identification was confirmed using a serum dilution, plaque neutralization test (Lindsey et al. 1976) with single dose hamster sera (Karabatsos and Mathews 1980) (Table 2).

This marks the first identification of CE serotype in Canada. The serotype was first iso-

[1] National Arbovirus Reference Service, Department of Microbiology, University of Toronto, Toronto, Ontario, M5G 1L5, Canada.

[2] Vector-Borne Viral Diseases Division, Centers for Disease Control, Post Office Box 2087, Fort Collins, Colorado, 80522–2087, U.S.A.

[3] Cadham Provincial Laboratory, Box 8450, Winnipeg, Manitoba, R3C 3Y1, Canada.

[4] Department of Entomology, University of Manitoba, Winnipeg, Manitoba, R3T 2N2, Canada.

Table 1. Typing of isolate Mn 296–300 using hyperimmune mouse ascitic fluids.

California serogroup virus typed	Neutralizing antibody titer to:				Elisa O.D. reading[1] to:						
	SSH[2]	CE[2]	JC[2]	TVT[2]	SSH	LAC[2]	CE	SA[2]	JC	KEY[2]	TVT
Mn296–300	5,120[3]	2,560	—[4]	—	0.56	0.67	1.45	1.06	0.31	0.08	0
SSH	*81,940*	2,560	40	—	*1.63*	0.22	0.14	0.23	0.05	0.10	0.05
LAC	N.T.[5]	N.T.	N.T.	N.T.	1.05	*1.61*	0.61	0.94	0.16	0.47	0.04
CE	5,120	*2,560*	—	—	0.50	0.36	*1.27*	0.66	0.31	0.23	0.03
SA	N.T.	N.T.	N.T.	N.T.	0.89	0.96	0.90	*1.49*	0.62	0.60	0.05
JC	40	—	*320*	—	0.09	0.15	0.10	0.11	*1.11*	0.30	0.03
KEY	N.T.	N.T.	N.T.	N.T.	0.05	0.09	0.21	0.07	0.36	*1.01*	0.08
TVT	160	40	—	*640*	0	0	0	0	0	0	*0.86*

[1] Exact conditions of ELISA test performed are described elsewhere (Artsob et al. 1984). Optical density (OD) readings represent an average of duplicate samples from which vero control background was subtracted.

[2] SSH = snowshoe hare Burgdorfer; CE = California encephalitis BFS283; JC = Jamestown Canyon 61V-2235 TVT = trivittatus 7941; LAC = La Crosse Original; SA = San Angelo 20230; KEY = Keystone B64-5587.05.

[3] Reciprocal of antibody dilution.

[4] – = <40.

[5] N.T. = not tested.

Table 2. Typing of isolate Mn 296-300 using hamster immune sera.

California serogroup virus	Neutralizing antibody titer with serum to:[1]					
	SSH[2]	LAC[2]	JC[2]	KEY[2]	TVT[2]	CE[2]
Mn 296-300	160[3]	160	—	80	80	640
Homologous titer	1280	1280	160	320	1280	1280

[1] Neutralization test was undertaken as described by Linsay et al. (1976).

[2] Footnote as in Table 1.

[3] Reciprocal of antibody dilution.

[4] – = <40.

lated in California in 1943 and has since been isolated in Utah, New Mexico and Texas (Calisher 1983). Principal vectors include *Aedes melanimon* in California and *Ae. dorsalis* (Meigen) in Utah, but isolates have been obtained from *Ae. nigromaculis* (Ludlow), *Ae. vexans* (Meigen), *Anopheles freeborni* Aitken, *Culex tarsalis* Coq., *Cs. inornata* and *Psorophora signipennis* (Coq.) (Turell and LeDuc 1983). CE virus has been known to cause illness in humans (Hammon and Reeves 1952).

Studies at the National Arbovirus Reference Service in Toronto were supported by National Health Grant No. 6606–2095–54.

References Cited

Artsob, H. 1983. Distribution of California serogroup viruses and virus infections in Canada, pp. 277–291. *In:* C. H. Calisher and W. H. Thompson (Eds.). International symposium on California serogroup viruses. Alan R. Liss, New York.

Artsob, H., L. Spence and C. Th'ng. 1984. Enzyme-linked immunosorbent assay typing of California serogroup viruses isolated in Canada. J. Clin. Microbiol. 20:276–280.

Artsob, H., L. Spence, G. Surgeoner, C. Th'ng, V. Lampotang, L. Grant and J. McCreadie. 1983. Studies on a focus of California group virus activity in southern Ontario. Mosq. News 43:449–455.

Calisher, C. H. 1983. Taxonomy, classification and geographic distribution of California serogroup bunyaviruses, pp. 1–16. *In:* C. H. Calisher and W. H. Thompson, (Eds.). International symposium on California serogroup viruses. Alan R. Liss, New York.

Hammon, W. McD. and W. C. Reeves. 1952. California encephalitis virus, a newly described agent. Calif. Med. 77:303–309.

Karabatsos, N. and J. H. Mathews. 1980. Serological reactions of fractionated hamster immunoglobulins with California group viruses. Am. J. Trop. Med. Hyg. 29:1420–1427.

Lindsey, H. A., C. H. Calisher and J. H. Mathews. 1976. Serum dilution neutralization test for California group virus identification and serology. J. Clin. Microbiol. 4:503–510.

Turell, M. J. and J. W. LeDuc. 1983. The role of mosquitoes in the natural history of California serogroup viruses, pp. 43–55. *In:* C. H. Calisher and W. H. Thompson (Eds.). International symposium on California serogroup viruses. Alan R. Liss, New York.