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MULTIPLICATION OF CALIFORNIA ENCEPHALITIS VIRUS IN YUKON MOSQUITOES[1]

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Arising from the isolation of the Grey Sage strain of California encephalitis (CE) virus from a pool of *Aedes canadensis* and *A. vexans* mosquitoes collected near Penticton, B.C. (49° 30'N, 120°W) during June 1969 (McLean, 1970) plus detection of CE neutralizing antibody in sera of marmots (*Marmota flaviventris*) at Penticton (McLean *et al.,* 1970) and snowshoe hares (*Lepus americanus*) from Penticton north to Dawson Creek (56°N, 120°W) (McLean *et al.,* 1971, Newhouse *et al.,* 1963), attempts were undertaken during summer 1971 to define the northern limit of prevalence of CE virus in British Columbia and immediately northward in the Yukon Territory. Isolation of CE virus strains from *Aedes communis* and *A. stimulans* mosquitoes collected near Rochester, Alberta (54°N, 113°W) from 1965 through 1968 (Hoff *et al.,* 1969), and from *Aedes* spp. mosquitoes collected in east central Alaska (approximately 63° N, 145°W) during 1970 (Feltz *et al.,* 1971) further suggested the possibility of endemic prevalence of CE virus in the Yukon. Isolation of 12 strains of CE virus, antigenically identical with the Grey Sage strain by mouse neutralization tests, from *Aedes canadensis* collected near Whitehorse (61°N, 135°W) during June and July 1971 (McLean *et al.,* 1972) together with the demonstration of CE neutralizing antibody in sera from 76 of 298 (25 percent) *Lepus americanus* confirmed the existence of a natural cycle of infection of CE virus in the Yukon. Both the Grey Sage strain, and isolates from Alaska and Alberta show close antigenic relationships to the Montana snowshoe hare strain of C virus.

Aedes vexans and *A. canadensis* collected wild in Ontario, and also domes-

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tically reared *A. aegypti* have supported the multiplication of the Ontario R2929 isolate of CE virus which is antigenically close to the Montana snowshoe hare strain, both after intrathoracic injection of each species, and also after feeding of virus to *A. vexans* (Chernesky, 1968). The present report describes the multiplication of a 1971 Yukon isolate and the Grey Sage (1969) isolate of CE virus following intrathoracic injection of *A. canadensis* and *A. aegypti* mosquitoes.

MATERIALS AND METHODS

MOSQUITOES. Adult female *A. canadensis* were collected by hand aspirator near Whitehorse, Y.T., held at 10° C, and air-freighted once weekly to Vancouver during midsummer 1971. After intrathoracic injection with 0.003 ml virus suspension in 0.15 M sodium chloride, mosquitoes were held in cardboard cages at 27±1° C and approximately 80 percent humidity. Adult female *A. aegypti* mosquitoes were bred in Vancouver and obtained through the courtesy of Dr. R. H. Wright, British Columbia Research Council. Following intrathoracic injection, these mosquitoes were maintained constantly at 28° C.

VIRUSES. The Marsh Lake 23 strain (McLean *et al.*, 1972, in press) of CE virus which was isolated from *A. canadensis* collected at Marsh Lake near Whitehorse, Y.T. on 28 June 1971 was used in its second suckling mouse brain passage. The Grey Sage strain of CE virus which was recovered from *A. canadensis* and *A. vexans* mosquitoes collected near Penticton, B.C. in June 1969 was used in its fifth suckling mouse brain passage. All titrations for virus infectivity were performed by intracerebal injection of groups comprising three mice each aged 3 to 4 weeks with 0.03 ml aliquots of serial tenfold dilutions of test materials. Infected mice contracted fatal encephalitis 4 to 6 days following inoculation. All infectivity titers were calculated by the method of Reed and Muench (1938) and expressed as $\log_{10}$ mouse LD_{50} per mosquito (log).

RESULTS

Multiplication of Marsh Lake 23 virus was demonstrated in both species of mosquito following intrathoracic injection (Table 1). Infectivity increments of 30 to 500-fold were detected in *A. canadensis* 5 days after injection, but somewhat reduced titers were observed at 7 to 9 days. A 100-fold increment of infectivity was detected in *A. aegypti* 2 days after injection of 3.0 log, but at 7 to 9 days a mean titer of 4.0 log per mosquito was detected both after injection of 3.0 and also 3.8 log of stock virus.

Following intrathoracic injection of both *A. canadensis* and *A. aegypti* with as little as 1.7 to 1.8 log of Grey Sage virus, infectivity was detected in all mosquitoes tested 2 to 3 days subsequently, at mean titers of 4.3 to 4.7 log per mosquito (Table 1). Virus proliferation regularly followed

TABLE 1.—Multiplication of two strains of California encephalitis virus in two species.

	Aedes canadensis				*Aedes aegypti*			
	Dose	Days after injection			Dose	Days after injection		
Virus	injected	2–3	5	7–9	injected	2–3	5	7–9
Marsh	4.2	..	*5.7(1/1)	3.7(6/6)	3.8	..	4.0(1/1)	4.0(2/2)
Lake	2.2	..	5.0(1/1)	..	3.0	5.0(1/1)	..	4.0(7/2)
Grey	3.7	4.7(1/1)	..	..	2.8	4.8(3/3)	..	..
Sage	3.0	..	3.5(8/8)	3.7(2/2)	1.8	4.3(3/3)	..	4.3(1/1)
	1.7	4.7(3/3)	..	..	1.2	3.5(3/3)	..	..

* Geometric mean titers expressed as $\log_{10}$ mouse LD_{50} of CE virus per mosquito.

Figures in parentheses indicate the proportion of mosquitoes infected relative to the total number tested.

injection of larger amounts of virus, and infectivity was detected 7 to 9 days subsequently.

The eclipse phase during multiplication of Grey Sage virus in *A. aegypti* was determined by assay of mosquitoes for virus content frequently during the initial 6 hours after injection with 2.8 log of Grey Sage virus. Infectivity titers ranging from 3.2 to 3.7 log were detected in mosquitoes tested 1.5, 2.5, 3.0 and 6.0 hours subsequently, but no infectivity was detected at 2.75 hours. Following this early eclipse phase, peak titers of 4.0 to 4.3 log were attained 1 and 2 days after injection, but titers declined to 3.3 log at 4 days, and persisted at a mean of 3.0 log for 17 to 21 days.

The 50 percent infective dose of Grey Sage virus for *A. aegypti* was determined by assay of the proportion of mosquitoes infective for mice 2 days after injection of serial tenfold dilutions of stock virus. The infectivity titer of stock virus was assayed in mice immediately after injection of mosquitoes. Although occasional mosquitoes became infected by injection of as small a virus dose as 0.001 mouse LD_{50}, minimal virus dose which induced infection in 50 percent of mosquitoes was 0.03 mouse LD_{50} (Table 2).

DISCUSSION

Multiplication of a Yukon 1971 isolate and a British Columbia 1969 isolate of California encephalitis virus both in Yukon collections of *A. canadensis* and domestically reared *A. aegypti* confirms and extends earlier observations on the proliferation of an Ontario 1963 isolate of CE virus in Ontario collections of *A. canadensis* and the same strain of domestically reared *A. aegypti* after intrathoracic injection of comparable virus doses (Chernesky, 1968). In both series of experiments, peak titers of comparable magnitude were attained 2 to 3 days after injection. In order to circumvent the gut barrier in *A. aegypti*, where imbibing of 4.5 log of the Ontario CE strain failed to induce infection (Chernesky, 1968), virus was introduced into all mosquitoes in the current series by intrathoracic injection. The minimal 50 percent infective dose of 0.03 mouse LD_{50} of CE virus for *A. aegypti* was comparable to 0.1 mouse LD_{50} for dengue 4 virus in this same mosquito strain (McLean, 1972, in press), and to 0.01 mouse LD_{50} for Murray Valley encephalitis virus in another strain of *A. aegypti* (McLean, 1957). This dose was somewhat lower than 0.7 mouse LD_{50} of St. Louis encephalitis virus which induced infection in *Culex quinquefasciatus* and *C. pipiens* (Sudia, 1959).

Recovery of 12 strains of CE virus from *A. canadensis* collected in the Yukon Territory at latitude 61° N during summer 1971 indicates the importance of this mosquito species as a natural vector of the California encephalitis group of arboviruses in subarctic regions of western Canada, and demonstrates a western extension of its previously described role as a vector of CE virus in upstate New York (Vianna *et al.*, 1971). These Yukon 1971 isolates confirm the endemic prevalence of CE virus throughout the western portion of North America from California (Sather and Hammon, 1967; Sudia *et al.*, 1971) through Montana (Newhouse *et al.*, 1963), Alberta (Morgante and Shemanchuk, 1967; Hoff *et al.*, 1969), and British Columbia (McLean *et al.*, 1970, 1971) to Alaska (Feltz *et al.*, 1971).

TABLE 2.—Determination of 50% infective dose of Grey Sage strain of CE virus for *A. aegypti*.

Mosquito inoculum (mouse LD_{50} injected)	10	1	0.1	0.01	0.001	0.0001
Proportion of mosquitoes infected after two days	6/6	6/6	7/10	1/8	2/9	0/4

SUMMARY

Yukon 1971 and British Columbia 1969 isolates of California encephalitis virus multiplied readily after intrathoracic injection of *Aedes canadensis* mosquitoes collected in Yukon Territory and laboratory reared *Aedes aegypti*. Peak titers of 4.7 to 5.0 $\log_{10}$ mouse LD_{50} per mosquito were attained 2 to 5 days after injection of 1.7 to 3.0 log CE virus. The 50 percent infective dose of the British Columbia 1969 isolate (Grey Sage strain) for *A. aegypti* mosquitoes by injection was 0.03 mouse LD_{50}.

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