ISOLATIONS OF SNOWSHOE HARE VIRUS FROM YUKON MOSQUITOES, 1983[1]

DONALD M. McLEAN AND SHARON A. LESTER

Division of Medical Microbiology, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

ABSTRACT. Snowshoe hare (SSH) virus was isolated from one pool of 54 unengorged adult female *Aedes nigripes* mosquitoes, among a total of 4,466 mosquitoes of 4 species collected throughout the Yukon Territory, Canada during the summer of 1983. Another SSH virus strain was isolated from 1 of 22 pools of *Aedes* sp. larvae collected near Whitehorse during April 1983. During 12 summers from 1972 to 1983, 54 SSH strains and 4 Northway (NOR) virus strains were isolated from 136,854 adult mosquitoes of 7 species. An additional 4 SSH strains have been recovered from 453 pools of *Aedes* sp. larvae collected during the springs of 5 years.

INTRODUCTION

Throughout 12 summers between 1972 and 1983, mosquitoes collected along transportation corridors of the Yukon Territory and the Mackenzie District, Northwest Territories, Canada have been assayed for bunyaviruses in order to determine the geographic distribution and mosquito infection rates of 2 mosquito-borne viruses, snowshoe hare (SSH) (California serogroup) and Northway (NOR) (Bunyamwera serogroup). These investigations were stimulated by the isolation of these 2 bunyaviruses from Alaskan mosquitoes in 1970 (Ritter and Feltz 1974) and SSH virus from Yukon mosquitoes in 1971 (McLean et al. 1972). This paper reports additional SSH virus isolations from both larval and adult mosquitoes collected during 1983 and provides a concluding summary of bunyavirus isolation rates during 12 summers.

METHODS

Unengorged adult female mosquitoes were collected by hand aspirators at 9 locations throughout the boreal forest of the Yukon Territory between latitudes 61° and 67°N from 29 April to 25 July 1983. Mosquito larvae were collected from grassy roadside ponds at 2 southern Yukon locations (61°N) on 29 April. Adult and larval mosquitoes in suitable containers were held alive at 4°C in styrofoam boxes during shipment by air to the Vancouver laboratory. There they were transferred to sealed vials and stored at −70°C to await identification and assay for virus content. Pools of 1 to 50 adult mosquitoes of the same species, and pools of about 100 *Aedes* sp. larvae, were assayed by intracranial injection of newborn mice as described previously (McLean et al. 1972, 1975). Virus isolates were typed by neutralization tests performed both in suckling mice and in monolayer cultures of baby hamster kidney (BHK) cells by plaque reduction (Hunt and Calisher 1979, McLean 1982b).

RESULTS

Between 29 April and 26 July 1983, 4,466 unengorged adult female mosquitoes were collected at 9 principal roadside locations throughout the boreal forest of the Yukon Territory. They comprised 175 *Culiseta inornata* (Williston), 2,247 *Aedes communis* (DeGeer), 524 *Ae. hexodontus* (Dyar) and 1,520 *Ae. nigripes* (Zetterstedt). Snowshoe hare virus was isolated from one mosquito pool of 54 *Ae. nigripes* that was collected on 15 July 1983 at Dempster Highway km 66 (Table 1).

Throughout 12 consecutive summers between 1972 and 1983, 54 SSH strains and 4 NOR strains were isolated from 136,854 (0.042%) adult mosquitoes belonging to 7 species (Table 2). These included 5 SSH and 3 NOR strains from 6,661 *Cs. inornata* (0.12%), 32 SSH strains from 86,400 *Ae. communis* (0.037%), 5 SSH plus 1 NOR strain from 25,490 *Ae. hexodontus* (0.024%), 7 SSH strains from 10,567 *Ae. nigripes* (0.066%) and 5 SSH strains from 7,736 mosquitoes of 3 other species (McLean 1983). Overall virus isolation rates (0.03 to 0.082%) within the boreal forest (BF) throughout the Yukon Territory and the southern Mackenzie District NWT, exceeded the overall rate of 0.009% for the open woodlawn (OW) of the northern Mackenzie District NWT, mainly around Inuvik (69°N, 135°W). Although virus isolation rates within each region fluctuated from year to year, both the total isolation rate (0.082%) and the numbers of summers in which viruses were recovered from mosquitoes, were higher for Marsh Lake (61°N, 134°W) than for other regions of the Yukon Territory and Northwest Territories.

Larval *Aedes* sp. mosquitoes were collected from grassy roadside ponds at 2 southern Yukon locations on 29 April 1983. Snowshoe

[1] Supported by Health and Welfare Canada Project No. 6610-1306-54X.

hare virus was isolated from 1 of 5 larval pools collected at Marsh Lake, but no isolations were achieved from 17 larval pools collected near Kusawa Lake (61°N, 136°W) (Table 1), Overall, 4 SSH strains were isolated from 453 larval pools collected during the springs of 5 years before, or at the time of, emergence of adult mosquitoes, including 1 of 84 during 1974, 2 of 218 in 1975, none of 75 in 1976, none of 54 in 1982 and 1 of 22 in 1983.

DISCUSSION

Isolation of SSH virus from adult mosquitoes collected throughout the boreal forest of the Yukon Territory during 8 of 10 summers and from larvae collected during 3 of 5 years, including one year when adult mosquitoes failed to yield virus, demonstrates clearly the prevalence of SSH virus in this western portion of subarctic Canada. At Marsh Lake in the southern Yukon, where mosquitoes were collected during 10 summers, SSH virus was recovered from adults during 6 summers and from larvae during springtime of an additional 2 years when virus was not recovered from adult mosquitoes. This proves that transovarial transfer of SSH virus occurs and strongly suggests that this mechanism may be important in overwintering of SSH virus in subarctic Canada. Evi-

Table 1. Snowshoe hare virus isolation from Yukon mosquitoes, 1983.

Location	Week no.	Date	Adult mosquito species[1] Cs. in.	Ae. com.	Ae. hex.	Ae. nig.	Total	Larva[2]
Marsh Lake	17	29 Apr	0/150[3]				0/150	1/5
(61°N 134°W)	22	1 June		0/99		0/79	0/178	
	23	8 June		0/31		0/21	0/52	
	24	15 June		0/107		0/47	0/154	
	27	8 July		0/59	0/29	0/51	0/139	
	29	19 July		0/50	0/37	0/35	0/122	
	30	24 July		0/32	0/17	0/9	0/58	
Jackson Lake (61°N 135°W)	22	1 June	0/22	0/4		0/24	0/50	
Kusawa Lake	17	29 Apr						0/17
(61°N 136°W)	22	2 June	0/2	0/77		0/26	0/105	
	23	7 June		0/2			0/2	
	24	14 June		0/27		0/10	0/37	
	27	6 July		0/50	0/19	0/33	0/102	
	28	10 July		0/43	0/22	0/20	0/85	
	29	20 July		0/98	0/60	0/43	0/201	
	30	26 July		0/46	0/59	0/24	0/129	
Haines Junction	22	7 June		0/85		0/41	0/126	
(61°N 137°W)	24	14 June		0/6			0/6	
	27	6 July		0/44	0/27	0/41	0/112	
	28	10 July		0/24	0/14	0/27	0/65	
	29	20 July		0/79	0/35	0/44	0/158	
	30	25 July		0/65	0/47	0/37	0/149	
Carmacks (62°N 136°W)	28	15 July		0/69	0/57	0/46	0/172	
Watson Lake (60°N 129°W)	25	19 June		0/597	0/3	0/376	0/976	
Hunker Ck. (64°N 139°W)	26	26 June		0/142	0/56	0/72	0/270	
Dempster Hwy Km 26–72	26	26 June	0/1	0/129		0/130	0/260	
(65°N 139°W)	28	15 July		0/122	0/42	1/120	1/284	
Dempster Hwy Km 194–342 (67°N 138°W)	26	27 June		0/160		0/164	0/324	
TOTAL			0/175	0/2247	0/524	1/1520	1/4466	1/22

[1] Cs. in. = *Culiseta inornata*, Ae. com. = *Aedes communis*, Ae. nig. = *Aedes nigripes* and Ae. hex. = *Aedes hexodontus*.
[2] *Aedes* sp. larvae were tested in pools of approximately 100.
[3] Ratio of the number of SSH virus isolates (per number of adult mosquitoes or number of larval pools tested).

Table 2. Bunyavirus isolations from mosquitoes collected throughout the Yukon and the Mackenzie District NWT, 1972–83.

Region	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	TOTAL	
Marsh Lake YT 61°N, 134°W	4	2	0	0			3[2]	0	4	3	1	0	17	
(BF)	3682	2057	2324	1887			3361	14	1460	1761	3184	853	20583	(0.082)
Other southern Yukon 60–63°N	0	1	1	0			5[3]		1	0	4	0	12	
(BF)	1971	2223	3232	3957			4765		1336	190	2987	2475	23136	(0.052)
Northern Yukon 64–67°N	2	1	3	0			7	0	2	3	0	1	19	
(BF)	4664	1998	1257	4637			4762	7677	3189	8237	700	1138	38257	(0.049)
Southern Mackenzie 60–62°N					7	0							7	
(BF)					11586	11074							22660	(0.030)
Northern Mackenzie 63–69°N			0		2[1]	0	1	0	0		0		3	
(OW)			3078		12161	6692	7417	1306	1449		115		32218	(0.009)
TOTAL	6	4	4	0	9	0	16	0	7	6	5	1	58	
	10317	6278	9891	10481	23747	17766	20305	8997	7432	10188	6986	4466	136854	(0.042)[4]

Ratio: number of virus isolates/number of mosquitoes processed (percentage rate in parentheses). All isolations were SSH virus except as indicated below:

[1] Inuvik 1976: 1 SSH, 1 NOR.

[2] Marsh Lake 1978: 2 SSH, 1 NOR.

[3] South of Whitehorse 1978: 3 SSH, 2 NOR.

[4] Total isolates: 54 SSH, 4 NOR.

BF: Boreal forest. OW: Open woodland.

dence derived from field observations on other California serogroup bunyaviruses in California (Turell and Le Duc 1983), Illinois (Clark et al. 1983), Iowa (Rowley et al. 1983), Maryland (Le Duc et al. 1975), Ohio (Berry et al. 1974), Wisconsin (Watts et al. 1974) and Utah (Crane et al. 1977), implicates transovarial transfer as an important mechanism of overwintering of these viruses in the North Temperate Zone. Recent isolations of dengue-2 virus from *Aedes aegypti* (Linn.) larvae collected from domestic water tanks in Rangoon, Burma (Khin and Than 1983) provide the first evidence from the field that this tropical flavivirus, in addition to bunyaviruses of cooler regions, is transmitted transovarially. This is substantiated by laboratory evidence that all 4 dengue serotypes are readily transmitted transovarially by colonized *Aedes albopictus* (Skuse) (Rosen et al. 1983), but only rarely was a single serotype, dengue-1 transmitted by colonized *Ae. aegypti*. However transovarial transmission is relatively unimportant for the maintenance of tropical flaviviruses due to the abundance of adult mosquito vectors throughout the year.

Results obtained during 1983 conclude a 12-year study of bunyavirus infection rates among mosquitoes of subarctic and arctic portions of western Canada. Patterns of infection among the 4 principal mosquito species paralleled those of the 10-year data base between 1972 and 1981 (McLean 1982a). They illustrate clearly the dominance of *Ae. communis* (Wood et al. 1979) as the natural vector species in the summer. This species has transmitted SSH virus following incubation at 4°C and 13°C in the laboratory after oral feeding or intrathoracic injection (McLean et al. 1983). Field collections of *Ae. communis* in the Canadian Provinces of Alberta and Manitoba (National Research Council Canada 1982) have also yielded SSH virus. In the Yukon Territory, *Ae. nigripes* appears to serve as a secondary vector species throughout summer, whilst *Cs. inornata* may transmit SSH virus naturally during spring, and late summertime transmission to mammals may be principally by *Ae. hexodontus*.

Serological evidence of SSH virus infection has been documented in 11 non-fatal human cases of viral meningitis or encephalitis which occurred in the eastern Canadian Provinces of Ontario, Quebec and Nova Scotia (Artsob 1983). No clinically manifested human infections with SSH virus were documented in the Yukon Territory between 1972 and 1983, possibly due to the sparse population and lack of serological surveillance. However, subclinical infections with a California serogroup virus were recorded among human residents of east-central Alaska during 1970 where SSH virus was isolated from mosquitoes (Ritter and Feltz 1974), and human subclinical infections have been detected in Canadian residents of Alberta, British Columbia and Ontario (National Research Council Canada 1982). In each of these Canadian provinces plus the Yukon and Northwest Territories, serological evidence of SSH virus infection has been demonstrated in snowshoe hares (*Lepus americanus*) and also in arctic ground squirrels (*Citellus undulatus*) from

the Yukon (McLean et al. 1975), thus suggesting their role as natural vertebrate reservoirs.

This longitudinal study demonstrates the maintenance of SSH virus throughout the Yukon Territory by a natural cycle involving *Ae. communis* as the principal vector species, together with *Ae. nigripes*, throughout summer, with *Cs. inornata* and *Ae. hexodontus* as additional vectors during spring and late summer respectively. Although snowshoe hares and ground squirrels may serve as vertebrate reservoirs during summer, permitting amplification of virus within vectors, transovarial transfer provides an important mechanism for overwintering. Human infections may be acquired tangentially to this natural cycle of SSH infection.

Literature Cited

Artsob, H. 1983. Distribution of California serogroup viruses and virus infections in Canada, pp. 277–290. *In:* C. H. Calisher and W. H. Thompson (eds.) California serogroup viruses. Alan R. Liss, Inc., New York.

Berry, R. L., B. J. LaLonde, H. W. Stegmiller, M. A. Parsons and G. T. Bear. 1974. Isolation of California encephalitis virus (La Crosse) from field collected *Aedes triseriatus* (Say) larvae in Ohio (Diptera: Culicidae). Mosq. News 34:454–457.

Clarke, G. G., H. L. Pretula, W. H. Rohrer, R. N. Harroff and T. Jakubowski. 1983. Persistence of La Crosse (California encephalitis serogroup) in North-Central Illinois. Am. J. Trop. Med. Hyg. 32:175–184.

Crane, G. T., R. E. Elbel and C. H. Calisher. 1977. Transovarial transmission of California encephalitis virus in the mosquito *Aedes dorsalis* at Blue Lake, Utah. Mosq. News 37:479–482.

Hunt, A. R. and C. H. Calisher. 1979. Relationships of Bunyamwera serogroup viruses by neutralization. Am. J. Trop. Med. Hyg. 28:740–749.

Khin, M. M. and K. A. Than. 1983. Transovarial transmission of dengue 2 virus by *Aedes aegypti* in nature. Am. J. Trop. Med. Hyg. 32:590–594.

Le Duc, 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. Am. J. Trop. Med. Hyg. 24:124–126.

McLean, D. M. 1982a. Bunyavirus isolations from Yukon mosquitoes 1972–81. Mosq. News 42:324–329.

McLean, D. M. 1982b. Immunological investigations of human virus diseases. pp. 2. Vol. 5. *In:* R. C. Nairn (series ed.) Practical methods in clinical immunology. Churchill Livingstone, Edinburgh.

McLean, D. M. 1983. Yukon isolates of snowshoe hare virus, 1972–1982, pp. 247–256. *In:* C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. Alan R. Liss, Inc., New York.

McLean, D. M., E. J. Goddard, E. A. Graham, G. J. Hardy and K. W. Purvin-Good 1972. California encephalitis virus isolations from Yukon mosquitoes, 1971. Am. J. Epidemiol. 95:347–355.

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. Am. J. Trop. Med. Hyg. 24:676–684.

National Research Council Canada 1982. Biting Flies in Canada: health effects and economic consequences. National Research Council Canada Publ. No. 19248.

Ritter, D. G. and E. T. Feltz. 1974. On the natural occurrence of California encephalitis virus and other arboviruses in Alaska. Can. J. Microbiol. 20:1359–1366.

Rosen, L., D. A. Shroyer, R. B. Tesh, J. E. Freier and J. C. Lien. 1983. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Am. J. Trop. Med. Hyg. 32: 1108–1119.

Rowley, W. A., Y. W. Wong, D. C. Dorsey, W. J. Hausler, Jr. and R. W. Currier. 1983. California serogroup viruses in Iowa, pp. 237–246. *In:* C. H. Calisher and W. H. Thompson (eds.). California serogroup viruses. Alan R. Liss Inc., New York.

Turell, M. J. and J. W. Le Duc. 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.). California serogroup viruses. Alan R. Liss, Inc., New York.

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

Wood, D. M., P. T. Dang and R. A. Ellis. 1979. The insects and arachnids of Canada. Part 6. The mosquitoes of Canada Diptera: Culicidae. Agriculture Canada Publ. No. 1686.