BUNYAVIRUS ISOLATIONS FROM YUKON MOSQUITOES 1972–81[1]

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ABSTRACT. Snowshoe hare (SSH) virus was isolated from 6 of 10,188 unengorged female mosquitoes of 4 species, including 3 of 7017 *Aedes communis* and 3 of 2088 *Ae. nigripes* collected throughout the boreal forest of the Yukon Territory between 3 June and 22 July 1981. During 10 summers from 1972 to 1981, 48 SSH and 4 Northway virus strains have been isolated from 125,442 mosquitoes of 7 species collected in the western Canadian Arctic. Plaque reduction neutralization tests revealed close antigenic relationships between strains of SSH virus isolated during 8 of 10 years.

INTRODUCTION

Surveillance of bunyavirus infections among mosquitoes throughout portions of the western Canadian Arctic which are accessible by automobile, has been maintained regularly during each summer since 1972 (McLean et al. 1975, 1981). Long-term investigations were initiated following isolation of 2 bunyaviruses, snowshoe hare (SSH) virus within the California (CAL) serogroup and Northway (NOR) virus within the Bunyamwera serogroup, from mosquitoes in eastcentral Alaska during 1970 (Ritter and Feltz 1974), and SSH virus in the southern Yukon Territory during 1971 (McLean et al. 1972). The present report confirms the importance of *Aedes communis* as major vector of SSH virus and suggests for the first time the vector potential of *Ae. nigripes*. These findings provide a 10 year data base of bunyavirus infection rates among mosquitoes of the Yukon Territory and Mackenzie Valley, Northwest Territories.

METHODS

Unengorged adult female mosquitoes were collected by hand aspirators at 8 locations throughout the boreal forest of the Yukon Territory between latitudes 61 and 67°N from 3 June to 22 July 1981.

Mosquitoes were placed in cylindrical cages 8 cm diam × 11 cm length, containing pledgets moistened with 10% sucrose, and these were sealed in plastic bags. They were held in styrofoam containers at 4°C during shipment by air to Vancouver, when they were transferred, sealed in ampoules and stored at −70°C to await assay for virus during the succeeding 3 months. Pools comprising usually 50 to 60 mosquitoes of the same species were macerated, extracted with 2 ml diluent comprising 20% fetal calf serum in Eagle's minimal essential medium, and assayed for virus content by intracranial injection of newborn mice as described previously (McLean et al. 1972). Virus isolates were typed both by intracranial suckling mouse neutralization tests using undiluted typing sera, and by plaque reduction neutralization tests (Hunt and Calisher 1979) in continuous baby hamster kidney tissue culture monolayers under Agarose overlay in Linbro FB16–24TC plastic plates (McLean 1982). Typing sera were prepared in rabbits by single intravenous injections with Yukon topotypes of SSH and NOR viruses (McLean et al. 1975, 1979).

RESULTS

Between 3 June and 22 July 1981, 10,188 unengorged adult female mosquitoes with 4 species collected at 8 sites

[1] Supported by National Research Council Canada, Contract OSU79–00019.

[2] Presented at the American Mosquito Control Association Annual Meeting, Sacramento, CA, 20 April 1982.

throughout the Yukon Territory were assayed for virus content in 201 pools. Snowshoe hare virus at titers of 2.5 to 3.5 $\log_{10}$ mouse LD_{50} per pool was isolated from 3 lots of *Ae. communis* (De Geer) collected at 2 locations and from 3 lots of *Ae. nigripes* (Zetterstedt) collected at the same 2 locations in southern and northern portions of the Yukon Territory (Table 1). Virus isolation rates (per cent) at Marsh Lake (61°N, 134°W) ranged from 1:61 (1.6) *Ae. nigripes* and 1:739 (0.1) *Ae. communis* during early June to 1:174 (0.6) *Ae. communis* during late July. Along the Dempster Highway at Km. 222, virus isolation rates were 1:280 (0.4) *Ae. nigripes* and 1:1973 (0.05) *Ae. communis* on 26 June. All 6 isolates reacted typically as SSH strains both in mouse and in plaque reduction neutralization tests.

Throughout 10 arctic summers 1972 through 1981, bunyaviruses including 48 SSH and 4 NOR strains were isolated from 125,442 adult mosquitoes of 7 species which were collected throughout the Yukon Territory and the Mackenzie Valley, N.W.T. (Table 2). *Aedes communis* was the species collected in greatest numbers, and yielded 31 SSH virus isolates. However the overall virus isolation rate (0.04) was lower than rates for 4 of 5 other species of *Aedes* (0.05 to 0.07) and for *Culiseta inornata* (Williston) (0.1). The lowest virus isolation rate (0.02) was obtained for *Ae. hexodontus* (Dyar). Although *Ae. communis* was collected throughout June and July, *Cs. inornata* was collected principally during early June, when mean and maximum temperatures are lower than during July when *Ae. hexodontus* become abundant.

Representative SSH isolates from 8 of the 10 years of this study, together with the SSH prototype (Burgdorfer et al. 1961), were examined by plaque reduction neutralization tests in BHK tissue cultures, using 40 to 200 plaque forming units (PFU) virus. Topotypes were used in their first passage in suckling mouse brains. Antisera were prepared in rabbits against 1974 and 1976 Yukon topotypes. Antibody titers of both sera revealed fivefold or smaller differences in tests against homologous and heterologous strains from each year plus the prototype SSH strain (Table 3). Thus the Yukon SSH topotypes for each year within the decade were antigenically similar.

Similarly in plaque reduction neutralization tests employing single-injection rabbit antisera to the NOR prototype and a 1978 Yukon topotype (McLean et al. 1979), antibody titers against the sole NOR isolate during 1976 and all 3 NOR isolates during 1978 showed fivefold or smaller differences from homologous titers.

DISCUSSION

Isolation of SSH virus from 2 species of *Aedes* mosquitoes at 2 locations within the boreal forest at northern and southern locations in the Yukon Territory during summer 1981, demonstrates for the first time the possible vector role of an additional species, *Ae. nigripes*. These observations confirm previous virological evidence (McLean et al. 1975, 1981) of the persistence of SSH virus at these foci throughout the past decade, with *Ae. communis* as the principal summertime vector species. During early summer in the Yukon Territory, *Cs. inornata* may be an important SSH vector; whilst during late summer in the Canadian Arctic, both in the boreal forest (McLean et al. 1979) and the tundra (Wagner et al. 1975), *Ae. hexodontus* may be a significant SSH vector. Principal vertebrate reservoirs include snowshoe hares (*Lepus americanus*) and arctic ground squirrels (*Citellus undulatus*) (McLean et al. 1975).

Detection of 2 bunyavirus serotypes only, together with the virtual antigenic homogeneity of isolates within their respective serotype, particularly for SSH virus isolates throughout the past decade in the Canadian Arctic, parallels observations in woodland portions of Manitoba and Saskatchewan. In both provinces, the sole CAL serogroup agent isolated from mosquitoes was SSH virus (Iversen et al. 1973, Wagner et al. 1979), whilst the

Table 1. Snowshoe hare virus isolations from Yukon mosquitoes 1981.

Locality	Week no.	Date	Mosquito species[1] *Aedes communis*	*Aedes hexodontus*	*Aedes nigripes*	*Culiseta inornata*	Total
Marsh Lake	22	3 June	0/159		1/61 (2.5)	0/2	1/222
(61°N 134°W)	23	8 June	1/739 (2.5)[2]		1/148	0/5	1/892
	24	19 June	0/261		0/83	0/5	0/349
	28	15 July	0/29	0/14			0/43
	29	20 July	1/174 (3.5)	0/31			1/205
	30	28 July	0/20	0/18	0/12		0/50
Fish Lake Road	22	3 June	0/11			0/1	0/12
(61°N 135°W)	24	14 June	0/101		0/33	0/1	0/135
Carmacks	28	14 July	0/13	0/19	0/11		0/43
(62°N 136°W)							
Klondike Hwy.	29	22 July	0/163	0/47			0/210
Km 680 (64°N 139°W)							
Clinton Creek	29	22 July	0/67	0/7			0/74
(64°N 141°W)							
Dempster Hwy							
Km 222 (66°N 138°W)	25	26 June	1/1973 (3.5)	0/197	2/560 (2.5)	0/1	3/2731
Km 236 (66°N 137°W)	25	26 June	0/814	0/189	0/283	0/4	0/1290
Km 371 (67°N 137°W)	25	25 June	0/2493	0/51	0/897	0/1	0/3932
Total			3/7017	0/1063	3/2088	0/20	6/10,188
Percent infected			0.04		0.14		0.06

[1] 10,188 mosquitoes of 4 species processed in 201 pools.

[2] Figures in parentheses indicate $\log_{10}$ mouse LD_{50} SSH virus per mosquito pool.

Table 2. Bunyavirus isolations from Yukon mosquitoes 1972–81.

Year	Mosquito species							Total
	Culiseta inornata	*Aedes communis*	*Aedes hexodontus*	*Aedes punctor*	*Aedes nigripes*	*Aedes canadensis*	*Aedes cinereus*	
1972	0/18	6/9,048S				0/1,251		6/10,317
1973	1/1,648S	1/2,932S				1/519S	1/1,179S	4/6,278
1974[1]	0/112	3/5,676S	0/3,133			1/970S		4/9,891
1975[1]	0/40	0/7,990	0/2,080			0/103	0/268	0/10,481
1976[2]	0/477	2/12,716S	5/5,096 (4S 1N)	2/2,808S	0/2,650			9/23,747
1977[2]	0/146	0/8,677	0/7,884	0/638	0/421			0/17,766
1978[2]	7/3,726 (4S 3N)	9/13,311S	0/1961		0/1,307			16/20,305
1979	0/3	0/7,915	0/1,079					0/8,997
1980[2]	0/217	7/5,772S	0/1,483					7/7,472
1981	0/20	3/7017S	0/1063		3/2,088S			6/10,188
Total	8/6,407	31/81,054	5/23,779	2/3,446	3/6,466	2/2,843	1/1,447	52/125,442
Rate[3]	0.1	0.04	0.02	0.06	0.05	0.07	0.07	0.04
Type	5S, 3N	31S	4S, 1N	2S	3S	2S	1S	48S, 4N

S = Snowshoe hare virus (California encephalitis group).
N = Northway virus (Bunyamwera group).
[1] = One larval SSH isolate during 1975 and 2 larval SSH isolates during 1974 are not included in the total.
[2] = Includes mosquitoes collected in the Mackenzie Valley, N.W.T. adjacent to the Yukon Territory.
[3] = Rate: $\frac{\text{number of virus isolations}}{\text{number of mosquitoes tested}} \times 100$

Table 3. Plaque reduction neutralization tests on Yukon SSH topotypes from each year 1972–81.

Topotype Antiserum	72-Y-144[1]	73-Y-347	74-Y-234	75/L-10	76-Y-316	78-Y-133	80-Y-123	81-Y-121	SSH[2]
74-Y-234	250[3]	100	250[4]	100	100	250	250	50	50
76-Y-316	100	100	100	50	250	100	250	100	50

[1] Topotypes are designated according to year in isolation, adult mosquito (Y) or larval (L) pool, and serial number of pool during that year.
[2] Prototype SSH (Burgdorfer et al. Am. J. Hyg. 344–349, 1961).
[3] Reciprocal of antiserum dilution which reduced plaque count by 90.%
[4] Homologous titers are underlined.

Bunyamwera serogroup agent was Cache Valley (Burton et al. 1973, Sekla et al. 1980). These findings contrast with the isolation of 2 CAL serogroup agents, SSH and Jamestown Canyon viruses, from mosquitoes in the boreal forest of Alberta (Iversen et al. 1969), together with a Bunyamwera serogroup isolate elsewhere in Alberta (Hall et al. 1968). Similarly, 2 CAL group agents, SSH and Trivittatus viruses, were isolated in mixed woodlands of southern Ontario, together with Cache Valley virus (Thorsen et al. 1979). However the abundance of SSH strains among the total mosquito isolates within the CAL serogroup in Canada differs from the paucity of SSH isolates among 4 serotypes of CAL serogroup agents isolated in woodlands of Wisconsin (Issel et al. 1972) and within the 2 CAL serogroup members isolated in upstate New York (Whitney et al. 1969). In these and other states surrounding the Great Lakes, La Crosse virus is the dominant serotype (Sudia et al. 1971).

Literature Cited

Burgdorfer, W., V. F. Newhouse and L. A. Thomas. 1961. Isolation of California encephalitis virus from the blood of a snowshoe hare (*Lepus americanus*) in western Montana. Am. J. Hyg. 73:344–349.

Burton, A. N., J. R. McLintock and D. B. Francy. 1973. Isolation of St. Louis encephalitis and Cache Valley viruses from Saskatchewan mosquitoes. Can. J. Public Health 64:368–373.

Hall, R. R., J. A. McKiel and J. H. Brown, 1968. Isolation of Turlock virus and a member of the Bunyamwera group, probably Cache Valley virus, from Alberta mosquitoes. Can. J. Public Health 59:159–160.

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

Issel, C. J., D. O. Trainer and W. H. Thompson. 1972. Serologic evidence of infections of white-tailed deer in Wisconsin with three California group arboviruses (La Crosse, Trivittatus, and Jamestown Canyon). Am. J. Trop. Med. Hyg. 21:985–988.

Iversen, J. O., R. P. Hanson, O. Papadopoulos, C. V. Morris and G. R. De Foliart. 1969.

Isolation of viruses of the California encephalitis virus group from boreal *Aedes* mosquitoes. Am. J. Trop. Med. Hyg. 18:735–742.

Iversen, J. O., R. J. Wagner, C. De Jong and J. R. McLintock. 1973. California encephalitis virus in Saskatchewan: isolation from boreal *Aedes* mosquitoes. Can. J. Public Health 64:590–594.

McLean, D. M. 1982. Immunological investigations of human virus diseases. Vol. 5, Practical Methods in clinical immunology, series editor R. C Nairn, Churchill Livingstone, Edinburgh.

McLean, D. M., P. N. Grass and B. D. Judd. 1979. Bunyavirus infection rates in Canadian Arctic mosquitoes, 1978. Mosq. News 39:364–367.

McLean, D. M., B. D. Judd and S. K. A. Shives. 1981. Snowshoe hare virus infections in Canadian Arctic mosquitoes during 1980. Mosq. News 41:287–290.

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.

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.

Sekla, L. H., W. Stackiw and R. A. Brust. 1980. Arbovirus isolations from mosquitoes in Manitoba. Mosq. News 40:377–380.

Sudia, W. D., V. F. Newhouse, C. H. Calisher and R. W. Chamberlain. 1971. California group arboviruses: isolations from mosquitoes in North America. Mosq. News 31:576–600.

Thorsen, J., H. Artsob, G. Surgeoner, B. Helson, L. Spence and R. Wright. 1979. Virus isolations from mosquitoes in Southern Ontario 1976 and 1977. *In* Arboviral encephalitides in Ontario with special reference to St. Louis encephalitis. Ministry of Health, Ontario, pp. 199–207.

Wagner, R. J., C. De Jong, M. K. Leung, J. McLintock and J. O. Iversen. 1975. Isolations of California encephalitis virus from tundra mosquitoes. Can. J. Microbiol. 21:574–576.

Wagner, R. J., M. K. Leung, J. J. R. McLintock and J. O. Iversen. 1979. On the natural occurrence of snowshoe hare virus in Manitoba. Mosq. News 39:238–243.

Whitney, E., H. Jamnback, R. G. Means, A. P. Roz and G. O. Rayner. 1969. California virus in New York State. Isolation and characterization of California encephalitis virus complex from *Aedes cinereus*. Am. J. Trop. Med. Hyg. 18:123–131.