ON THE NATURAL OCCURRENCE OF SNOWSHOE HARE VIRUS IN MANITOBA

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ABSTRACT. Snowshoe hare (SSH) virus of the California encephalitis group of arboviruses was isolated from a pool of 60 female *Aedes communis* collected in the forested region of west-central Manitoba during the summer of 1976. This is the first reported isolation of SSH virus from Manitoba. *Aedes* accounted for 99% of the 7,491 mosquitoes examined for arboviruses by suckling mouse inoculation. Seroconversion in 58% of 19 sentinel rabbits deployed in various vegetation types within the forested area is consistent with the hypothesis of widespread vernal cycling of SSH virus between univoltine *Aedes* and mammals in the boreal forest of Canada.

INTRODUCTION

Since the first isolation of snowshoe hare (SSH) virus in Canada by McKiel et al. (1966) in Ontario using "indicator" or sentinel domestic rabbits, the virus has been demonstrated in all of the western provinces with the exception of Manitoba (Morgante and Shemanchuk 1967, Iversen et al. 1969, McLean 1970, Iversen et al. 1973). In addition, SSH virus has been isolated in both the Yukon and Northwest Territories (McLean 1975, Wagner et al. 1975). A recent serologic survey showed the presence of antibodies to SSH virus in snowshoe hares sampled in Nova Scotia (Embil et al. 1978). The cited investigations demonstrated a correlation between virus activity, small wild mammals, and *Aedes* mosquitoes of the boreal forest (McLintock and Iversen 1975). The purpose of this paper is to report the isolation of the SSH virus of the California encephalitis (CE) group of the Bunyavirus supergroup (Theiler and Downs 1973) from mosquitoes in Manitoba and to present serological evidence that the virus is cycling in Manitoba in the manner hypothesized for the rest of the boreal forest of western Canada.

MATERIALS AND METHODS

Studies were conducted at 5 sites in west-central Manitoba. The sites were within the Canadian life zone of the mixed-wood forest on podzolic soils in the subarctic climatic region (Shelford 1963). The forests were principally a mixture of aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*), black (*Picea mariana*) and white spruce (*Picea glauca*), and jack pine (*Pinus banksiana*). Each study site had a characteristic predominant canopy species, viz., balsam poplar at Porcupine Hills Provincial Forest (52° 30'N, 101° 20'W), aspen poplar at Renwer adjacent to the northern edge of

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Duck Mountain Provincial Forest (52° 05'N, 100° 44'W), jack pine at Briggs Spur (52° 03'N, 100° 35'W), black and white spruce at Grandview adjacent to the southern edge of Duck Mountain Provincial Forest (51° 20'N, 100° 50'W), and mixed-wood forest at Ochre River adjacent to the northern edge of Riding Mountain National Park (51° 00'N, 99° 50'W). The understory of the forest was luxuriant in the relatively pristine sites at the first 4 sites, whereas it was heavily grazed by domestic animals near Ochre River, an area with some mixed farming activity.

Mosquitoes were collected by sweep netting the vegetation in each area or by aspiration of resting mosquitoes with a battery-operated aspirator. The collected mosquitoes were delivered to a wire mesh holding cage (a cylinder with a length of 50 cm and a diameter of 30 cm). These holding cages were kept in waxed cardboard boxes with a water-moistened sponge bottom to maintain a high relative humidity and maximize the survival of the mosquitoes until such time as they could be delivered to the Canada Agriculture Research Station in Saskatoon. Upon arrival at the laboratory, the collections were sorted with identification of the mosquitoes based on a revised key to adult aedine females of northern Canada (Vockeroth 1954). Identified mosquitoes were pooled by species and location of collection, and then the pools were stored as live specimens in a refrigerator until they could be processed for virus isolation.

Pools of fewer than 70 live mosquitoes were ground in 3 ml of diluent. The diluent consisted of brain heart infusion broth containing 0.75% bovine serum albumin (Fraction V) (Iversen et al. 1973). Antibiotics were added to this solution in amounts sufficient to bring the final concentration to 500 units of penicillin and 50 μg of streptomycin per ml. The suspensions were then centrifuged for 10 minutes at 3,000 rpm and the supernatant was collected and inoculated into 1–3 day old white mice via the intracerebral route. The suckling mice were observed for 10 days, and their health status was recorded daily. Brains of moribund mice were assayed for virus following the technics described by Iversen et al. (1969). Identification of the viral isolate was done at the second suckling mouse brain passage level by neutralization tests using hyperimmune sera for prototype strains of California group viruses. Three strains were provided by the National Arbovirus Reference Centre, Department of Medical Microbiology, University of Toronto, and they included snowshoe hare (SSH), LaCrosse (LAC), and Trivittatus (TVT) viruses. An isolate of Jamestown Canyon (JC) virus from Alberta (0607) was also used (Iversen et al. 1969). The hyperimmune sera for these viruses were prepared in domestic rabbits. Neutralization tests were performed in both newborn mice and baby hamster kidney cell culture (BHK_{21} cell line) (Lennette and Schmidt 1969).

Domestic rabbits of the New Zealand White strain (Animal Resources Centre, University of Saskatchewan) were placed as sentinels (McKiel et al. 1966, Iversen et al. 1971) at various locations in Manitoba. As soon as they were acquired, the rabbits were marked, bled for a pre-exposure control serum sample, and then transported in arthropod-proof holding cages to the sentinel site. Upon arrival at their respective sites, the rabbits were placed in wood framed structures (36" x 36" x 24") that had been covered with ¼" mesh metal hardware cloth. These cages were constructed in such a manner as to elevate the bottoms 4" from the forest floor. The rabbits were fed and watered in quantities sufficient to last up to a month in the field without servicing.

At varying intervals from 10 to 28 days, the rabbits were brought back to the laboratory in the arthropod-proof transport cages, held for 3 weeks in anthropod-proof quarters, and then bled for a final serum sample. The pre- and post-exposure sera were tested for SSH virus antibodies by neutralization tests performed in BHK_{21} cell culture. Neutraliza-

tion of 2 dex BHK_{21} LD_{50} was the criterion for seropositive reactions (Theiler and Downs 1973).

RESULTS

A total of 7,491 adult mosquitoes were identified and distributed into 271 pools and were tested for virus (Table 1). One viral isolate was obtained from a pool of 60 female *Ae. communis* group collected on June 20, 1976, near Briggs Spur, Manitoba. Reisolation of the virus was made by a subsequent inoculation of the original mosquito suspension into newborn mice. The isolate was identified as SSH virus by neutralization tests performed in newborn mice (Table 2). The minimum field-infection rate (Sudia et al. 1971) for *Ae. communis* group mosquitoes (viz., *Ae. communis* group, *Ae. communis*, *Ae. pionips*, *Ae. sticticus*) was 0.61 isolates per 1,000 mosquitoes,

Table 1. Female mosquitoes collected for virus isolation attempts, forested areas, Manitoba; summer, 1976.

Species	No. of mosquitoes	Pools	% of all mosquitoes
Aedes stimulans group	3392	70	45
Aedes vexans	823	26	11
Aedes communis group	550	15	7
Aedes pionips	527	19	7
Aedes excrucians	485	24	7
Aedes communis	445	18	6
Aedes intrudens	442	15	6
Aedes sticticus	114	5	2
Aedes fitchii	79	8	1
Aedes cinereus	72	14	1
Aedes canadensis	30	10	<1
Mansonia perturbans	26	4	<1
Aedes implicatus	24	4	<1
Culiseta inornata	17	8	<1
Aedes spencerii	16	2	<1
Aedes punctor	12	4	<1
Aedes dorsalis	11	3	<1
Aedes abserratus	11	3	<1
Aedes barri	9	2	<1
Aedes flavescens	6	4	<1
Aedes riparius	4	3	<1
Indeterminate	396	10	5
Total	7491	271	

Fifty-eight percent of 19 sentinel rabbits became sero-positive (i.e., developed neutralizing antibodies to SSH virus) following deployment in forested areas (Table 3). Three other rabbits that were deployed as sentinels were destroyed by black bear predation.

DISCUSSION

The boreal forest biome characterized by a coniferous forest matrix is still the largest terrestrial bio-region in area in the world (Illies 1974), and it is transcontinental in North America from Newfoundland in the east to Alaska in the west (Shelford 1963). Over 70% of the province of Manitoba falls within this biome. The evidence of a vernal cycle of SSH virus in the forested area of Manitoba further indicates the wide geographical range of SSH virus in the vast mixed-wood forest of Canada (Iversen et al. 1969, McLean 1975). A contiguous geographic distribution of SSH virus as recognized by actual isolation of the virus now stretches from western Alaska to southeastern Ontario (McLean 1975) and Nova Scotia (Embil et al. 1978). The southern limits of recognition are based on various isolations from mosquitoes from Montana, Wisconsin, and New York (Whitney et al. 1969, Sudia et al. 1971). It must be pointed out that occasional isola-

Table 2. Comparative neutralization of the Manitoba isolate (MI) with four California group viruses as revealed by mouse neutralization tests using antisera prepared in rabbits

Antiserum	Virus				
	MI	SSH	LAC	JC	TVT
Manitoba isolate	*4.5**	4.0	1.7	0.5	1.7
Snowshoe hare	3.8	*4.0*	2.0	1.7	1.5
La Crosse	2.5	2.0	*3.8*	1.5	2.0
Jamestown Canyon	0.5	0.7	0.5	*4.5*	1.7
Trivittatus	1.0	1.0	0.5	1.0	*5.0*

* Log_{10} neutralization indices.

Table 3. Development of neutralizing antibodies to SSH virus in sentinel rabbits deployed in Manitoba; summer, 1976.

Date	Birch River (Balsam poplar)	Renwer (Aspen poplar)	Brigg's Spur (Jack pine)	Grandview (Spruce spp.)	Ochre River (Mixed grazed)	
May 20–May31	0	–	+	+	–	2/4
May 31–June 10	–	–	p	+	+	2/4
June 10–June 20	–	+	+	+	+	4/5
June 20–June 30	–	–	o	p	o	0/2
June 30–July 20	+	+	o	o	o	2/2
July 20–Aug 17	–	+	o	p	o	1/2
	1/5	3/6	2/2	3/3	2/3	11/19

o=none deployed.
–=seronegative.
+=seropositive.
p=destroyed by black bear predation.

tions of SSH virus have come from communities outside the boreal forest biome, viz., isolations from *Culiseta inornata* from the prairie grassland in Alberta (Morgante and Shemanchuk 1967) and isolations from tundra mosquitoes of the *Ae. hexodontus-punctor* group collected in the Keewatin District, Northwest Territories (Wagner et al. 1975).

Serologic evidence obtained through sentinel rabbit deployment indicated that SSH virus cycled within a variety of dominant climax stands within the boreal forest biome in Manitoba (viz., balsam or aspen poplar, jack pine, black or white spruce, and grazed stands of mixed-wood forest). The seropositive conversion rate for Manitoba sentinel rabbits (58% of 19) was slightly below that observed in sentinel rabbits stationed in southeastern Ontario (69% of 29), but it was somewhat in excess of the seropositive rate in sentinel rabbits stationed in Saskatchewan (33% of 79) (McKiel et al. 1966, Wagner 1975). In the boreal forest of North America, the snowshoe hare (*Lepus americanus*) is an important host of SSH virus (Hoff et al. 1969). In general, small wild mammals presumably function as amplifying hosts of the CE group viruses, since the small mammals are frequently infected and are characterized by relatively rapid population turnover (Henderson and Coleman 1971). The recognized range of the virus is roughly similar to that of the snowshoe hare, the vertebrate from which this virus was first isolated (Burgdorfer et al. 1961).

A variety of *Aedes* species have appeared to be the primary vector for most of the subtypes of California encephalitis virus. In the forested regions of the prairie provinces, SSH virus isolations have been obtained mainly from the predominant snowpool univoltine *Aedes* species (Iversen et al. 1969, Iversen et al. 1973). Of the 14 species of mosquitoes from which SSH virus has been obtained elsewhere (Sudia et al. 1971), 10 were numbered in the collections obtained in the forested areas of Manitoba. Many of the northern isolates of the California en-

cephalitis group of arboviruses have been isolated from the *Ae. communis* group (Sudia et al. 1971, Brummer-Korvenkontio et al. 1973). It is noteworthy that the minimum vector-infection rate of 0.46 isolates per 1,000 *Ae. communis* group reported in Alberta (Iversen et al. 1969) is comparable to the minimum vector-infection rate (viz., 0.61/1,000 *Ae. communis* group mosquitoes) demonstrated in Manitoba.

The apparent early seasonal transmission (viz., seroconversion in sentinel rabbits deployed during 16 – 22 May) provides circumstantial evidence of transovarial or "delayed biological transmission" (McLintock et al. 1976). Similar phenological relationships have been recognized in forested Alberta (Iversen et al. 1969) and Saskatchewan (Iversen et al. 1973). The initial emergence of the univoltine black-legged aedines coincided with initial transmission to the sentinels. There is a considerable recent literature on transovarial transmission of California group arboviruses (Watts and Eldridge 1975), and field evidence of such transmission of SSH virus has been reported (McLean 1975, McLintock et al. 1976). "Delayed biological transmission" of SSH virus would provide a mechanism of enzootic infection in the boreal forest, and it would compensate for the following: 1) the well-known reluctance of boreal *Aedes* to take more than one blood meal during their lifetime (McLean 1975) and/or 2) periodic host scarcity due to fluctuating rodent or lagomorph populations in boreal environments (McLintock et al. 1976).

In the classical terms of "landscape epidemiology" (Pavlovsky 1966), SSH virus is a "natural-nidal" agent, and the "pathobiocenose" includes the virus, boreal *Aedes*, the snowshoe hare, and their *Populus* spp.-*Picea* spp. associates. Poplar and spruce would represent "indicator" species of the "nidii" of the virus. In Canada, the exercise in SSH virus biogeography lacks data only from the island of Newfoundland. Of further biogeographical interest are the isolations of Inkoo virus of the CE group, an agent initially isolated from *Ae. communis* and/or *punctor* collected in the forested areas of Fennoscandia (Brummer-Korvenkontio et al. 1973). The occurrence of antibodies to Inkoo virus up to the extreme north of Finland (70°N) led to the hypothesis that similar viruses may be present in other parts of boreal Eurasia (Brummer-Korvenkontio 1973). The hypothesis is supported by the holarctic distribution of the boreal forest (Shelford 1963, Illies 1974), the evolution and dispersal of boreal *Aedes* (Ross 1964), and the ecologic similarities between the Paleoarctic and the Neoarctic (Happold 1965, Brummer-Korvenkontio et al. 1971).

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