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POWASSAN VIRUS IN *Ixodes cookei* AND MUSTELIDAE IN NEW ENGLAND [1]

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Abstract: Powassan virus was recovered from a pool of 3 nymphal and 1 adult female *Ixodes cookei* removed from a striped skunk *(Mephitis mephitis)* trapped in Massachusetts during 1967 and from a pool of 9 nymphal *I. cookei* from a long-tailed weasel *(Mustela frenata)* captured in Connecticut during 1978. Virus was detected in the blood of both mammals. Hemagglutination-inhibiting (HI) antibody to Powassan virus was demonstrated in 16.0% of the skunks sampled in Connecticut, and neutralizing antibody was detected in 83.3% of the skunks tested from Massachusetts. HI antibody was found in 1 of 6 long-tailed weasels from Connecticut and 1 of 6 short-tailed weasels *(Mustela erminea)* from Maine.

INTRODUCTION

Powassan virus was first reported from the brain of a 5-year-old boy who died of encephalitis in Ontario in 1958.[14] Since that time, 14 additional confirmed or presumptive cases have been recognized in the northeastern United States and eastern Canada.[11]

Like other members of the tick-borne encephalitis complex, Powassan virus is suspected of being transmitted among small- to medium-sized mammals by one or more species of scutate ticks with man as an accidental host.[11] This virus has been isolated from *Ixodes cookei* on several occasions in Ontario and New York State.[2] The only published report of this virus from a mustelid was from the kidney of a spotted skunk *(Spilogale gracilis)* captured in California.[2] Neutralizing (N) and hemagglutination-inhibiting (HI) antibody was detected in a striped skunk *(Mephitis mephitis)* and 2 long-tailed weasels *(Mustela frenata)* from New York State;[24] one of 3 short-tailed weasels *(Mustela erminea)* had N but not HI antibody.

This paper reports isolations of Powassan virus from *I. cookei* a striped skunk, and a long-tailed weasel in Massachusetts and Connecticut and serological evidence of infections in mustelids from New England.

MATERIALS AND METHODS

Mustelids were live-trapped as part of a tag-and-release study to collect serial blood and ectoparasite samples from mammals in southern New England. Animals were anesthetized with ether, blood sampled by cardiac puncture, examined for ectoparasites, and tagged with a small numbered metal band clipped to the ear. Additional serum samples were collected on filter paper discs from road kills or mammals trapped for their pelts. The serum was extracted from the filter paper with normal saline.

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Virus isolations were made by intracerebral inoculation of 1- to 4-day old Swiss mice by standard techniques.[20,21] Ticks were triturated either in 5% fetal calf serum with antibiotics (penicillin, streptomycin, neomycin, amphotericin B) in tris buffered Hank's balanced salt solution (pH 7.6) (Massachusetts isolates) or in 10% fetal calf serum with penicillin and streptomycin in phosphate buffered saline (pH 7.2) (Connecticut isolates). Pools were lightly centrifuged and the supernatant injected into suckling mice. Serum samples were diluted approximately 1:10 with the fetal calf serum diluents described above prior to suckling mouse inoculations. The Massachusetts isolates were identified by neutralization tests in VERO cells while the Connecticut strains were identified by complement-fixation (CF) and HI tests on microtiter plates.

HI tests were done with acetone extracted sera and 8 hemagglutinating units of sucrose-acetone extracted mouse brain antigens.[5] The Byers strain of Powassan was used until 1978 when it was replaced with the local Connecticut isolate (Ar-218-78). N tests were performed by incubating equal volumes of serum and 400 to 500 LD_{50}/ml of virus (Byers strain) for 1 h at 37 C; mixtures were then injected into suckling mice by the intraperitoneal route.[22] CF tests were done on microtiter plates with heat inactivated (56 C for 20 minutes) sera, 2 units of guinea pig complement, and sucrose-acetone extracted mouse brain antigens. Grid titrations with the initial serum and antigen dilutions of 1:4 were incubated 18 h at 4 C before the hemolytic system was added.[4]

RESULTS

A total of 583 *I. cookei* was collected from mustelids trapped in southern New England between 1964 and 1979 (Table 1). Two pools of these ticks yielded Powassan virus. The first pool consisted of 3 nymphs and 1 adult female tick taken from a striped skunk trapped in Berkley (Bristol Co.), Massachusetts on 23 October 1967. This skunk was viremic making it impossible to determine if the virus was present in the tick tissues or merely in the ingested blood. No virus or CF, HI, or N antibody to Powassan virus was detected in serum from this same skunk on 3 occasions prior to 23 October or in 1 sample taken 10 days later.

A second pool contained 9 nymphs taken from a long-tailed weasel captured on 7 October 1978 in Old Lyme (New London Co.), Connecticut. Again, virus was recovered from the blood of the

TABLE 1. *Ixodes cookei* from mammals trapped in Massachusetts (1964-1970) and Connecticut (1975-1978).

hosts	number of hosts	larvae	nymphs	adults	
				males	females
Mephitis mephitis	91	74	274	3	103
Mustela frenata	6	107	20	0	2
Total Mustelidae (2 species)	97	181	294	3	105
Other Mammals* (8 species)	64	151	94	1	35
TOTAL MAMMALS (10 species)	161	332	388	4	140

*Raccoon *(Procyon lotor)*, Gray Fox *(Urocyon cinereoargenteus)*, Opossum *(Didelphis virginiana)*, Woodchuck *(Marmota monax)*, Porcupine *(Erethizon dorsatum)*, Eastern Cottontail *(Sylvilagus floridanus)*, Dog *(Canis familiaris)*, and Cat *(Felis domestica)*.

weasel. However, the titer of virus was higher in the tick than in the blood suggesting that the tick was infected. No virus was recovered from a pool of 23 larval *I. cookei* or 4 larval *I. dammini* removed from the weasel at the same time as the nymphs.

Virus was not isolated from an additional 69 *I. cookei* (3 larvae, 39 nymphs, 1 adult male, 26 adult females) collected in Massachusetts between 1966 and 1970 or in 573 *I. cookei* (240 larvae, 271 nymphs, 1 adult male, 61 adult females) from Connecticut between 1974 and 1979.

HI antibody was detected in 12 of 75 (16.0%) skunks sampled in Connecticut since 1962 and N antibody was demonstrated in 10 of 12 (83.3%) skunks from Massachusetts in 1965 (Table 2). In addition to the one viremic weasel, HI antibody was found in 1 of 6 (16.7%) long-tailed weasels from Connecticut and 1 of 6 (16.7%) short-tailed weasels from Maine (Table 2).

In a study in southcentral Connecticut in 1978, 33 skunks were sampled at least once (range 1-9, samples, mean 2) over a 20-week observation period (14 June 1978-29 October 1978). Nine (27.3%) of these skunks had Powassan HI antibody (Table 3). Eight of the 9 seropositive skunks were captured only once. The ninth was recaptured once, 13 weeks after the initial capture; HI antibody (1:20) was detected in both serum samples. In contrast, more than half (54.2%) of the seronegative skunks were recaptured at least once (mean number of times captured was 2.3) (Table 4). Antibody was demonstrated in immature (14.3%) and adult (36.8%) skunks. Antibody rates were higher in the males (35.3%) than in the females (18.8%) (Table 3).

DISCUSSION

Powassan virus is widely distributed across the northern Nearctic Region in a broad band from Nova Scotia and Pennsylvania west to British Columbia and California.[2,11] Virus isolations from the Soviet Union suggest a holoarctic distribution.[9,10] The present study is the first demonstration of Powassan virus in New England.

In eastern North America, where all of the human cases of Powassan encephalitis have been reported, epidemiologic evidence strongly implicates *I. cookei* was an important vector. Nearly all of the virus isolations from naturally infected ticks in this area have been from this species,[2,11] although at least one isolation was reported from *I. marxi*, the squirrel tick.[15] *I. cookei* occurs throughout most of eastern North America and it does occasionally feed on man.[1,6,8]

TABLE 2. Hemagglutination-inhibiting (HI) and neutralizing (N) antibody in Mustelidae from New England, 1962 to 1978.

	Connecticut		Maine	Massachusetts
	HI	HI	HI	N
	1962-1966	1975-1978	1962-1963	1965
Mephitis mephitis	3/41*	9/34 (10/67)**	-	10/12
Mustela frenata	0/3	1/4 (1/5)	0/1	-
Mustela erminea	-	0/1	1/6	-
Mustela vison	0/1	-	-	-
TOTAL	3/45	10/39 (11/73)	1/7	10/12
% positive	6.7	25.6	14.3	83.3

*number of seropositive animals/number of animals tested.
**number of positive sera/number of sera tested in parenthesis.

TABLE 3. Serological* results on serial blood samples from striped skunks *(Mephitis mephitis)* trapped in southcentral Connecticut during 1978.

	Immatures		Adults		Total	
	males	females	males	females	males	females
Individual Skunks						
Site I	0/2**	2/7 (28.6)	1/4 (25.0)	0/4	1/6 (16.7)	2/11 (18.2)
Site II	0/1	-	0/2	0/2	0/3	0/2
Site III	0/2	0/1	1/1 (100)	0/1	1/3 (33.3)	0/2
Other Sites	0/1	-	4/4 (100)	1/1 (100)	4/5 (80.0)	1/1 (100.0)
All Sites	0/6	2/8 (25.0)	6/11 (54.5)	1/8 (12.5)	6/17 (35.3)	3/16 (18.8)
Serum Samples						
Site I	0/2	2/15 (13.3)	1/6 (16.7)	0/17	1/8 (12.5)	2/32 (6.2)
Site II	0/3	-	0/6	0/2	0/9	0/2
Site III	0/3	0/2	2/2 (100)	0/2	2/5 (40.0)	0/4
Other Sites	0/1	-	4/4 (100)	1/1 (100)	4/5 (80.0)	1/1 (100.0)
All Sites	0/9	2/17 (11.8)	7/18 (38.9)	1/22 (4.5)	7/27 (25.9)	3/39 (7.7)

*Hemagglutination-inhibition test.
**number positive/number tested (% positive in parenthesis).

TABLE 4. Recapture data on striped skunks *(Mephitis mephitis)* trapped in south-central Connecticut during 1978.

	Immatures		Adults		Total	
	males	females	males	females	males	females
seronegative skunks						
range*	1-3	1-4	1-5	1-9	1-5	1-9
mean**	1.5	2.5	2.2	3.0	1.8	2.8
number***	6	6	5	7	11	13
seropositive skunks						
range	-	1	1-2	1	1-2	1
mean	-	1.0	1.2	1.0	1.2	1.0
number	0	2	6	1	6	3

*range = The minimum to maximum number of times individual skunks were = captured.
**mean = average number of times individual skunks were captured.
***number = number of individual skunks

I. cookei has been reported from a wide variety of small - (eg: *Peromyscus, Microtus)* and medium - (e.g. *Marmota, Erethizon, Mephitis, Mustela, Martes, Procyon, Vulpes, Urocyon)* sized mammals;[1,6,8] many of these mammals have been implicated by virus isolations and/or serosurveys as possible vertebrate hosts for Powassan virus.[12,14 19,24] Mustelids are an important component of the host-list for *I. cookei* although published reports of Powassan virus infections in skunks and weasels are scarce. Johnson[2] reported an isolation of this virus from the kidneys of a spotted skunk in California (outside the range of *I. cookei)* and neutralizing antibody was detected in a striped skunk, a long-tailed weasel, and 1 of 3 short-tailed weasels tested in New York State.[24]

The population dynamics of the skunks and weasels ensure that a large number of new "susceptibles" are available each year to maintain the virus in nature. This involves a short life span (usually less than 2 or 3 years) coupled with a high reproductive rate (2 to 10 progeny each year).[3,23] Verts[23] estimates a mortality rate of more than 65% among skunks in their first year and that few individuals live beyond 3 years in nature.

Denning and movement habits are important behavioral patterns which influence the ability of an animal to serve as a host for the ticks. Dens occupied by skunks are often utilized by several different mammal species, including woodchucks, badgers, and foxes, in succession,[23] thus providing the opportunity for interspecific exchange of ticks and possibly tick-borne viruses. The use of a single den by several individual skunks, such as a female with her offspring or a male with several females, provides the opportunity for intraspecific exchange.

The effects of Powassan virus on the mustelids have not been observed. The difference in recapture rates among seronegative and seropositive skunks in Connecticut during 1978 might suggest that infection is associated with morbidity or mortality. However, a more likely explanation may relate to the wandering behavior of adult males when compared with females or juveniles. The distance traveled by skunks varies with age, sex, season, and such life history events as winter denning, breeding, pregnancy, lactation, and postbreeding recovery.[23] The antibody rates in adult males (54.5%) was greater than those of adult females (12.5%) and immatures (14.3%).

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