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Powassan Virus: Vernal Spread During 1965

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ABSTRACT

Powassan virus was isolated from seven pools of *Ixodes cookei* ticks removed from groundhogs *(Marmota monax)* collected near North Bay, Ontario, between May and August 1965, including five pools obtained during spring. Tick pools, each comprising one to nine ticks, contained 2.0 to 5.5 $\log_{10}$ TCD_{50} of virus upon titration in monolayer cultures of primary swine kidney cells. Powassan virus neutralizing antibody prevalence in sera of the current season's groundhogs increased steadily from zero during May to 25% during August but remained relatively unchanged (42% to 58%) in the previous season's groundhogs, thereby confirming that active infection had occurred particularly amongst juvenile groundhogs mainly during spring 1965. Isolation of one strain of Silverwater virus from *Haemaphysalis leporis-palustris* ticks and detection of neutralizing antibody in three of nine snowshoe hares *(Lepus americanus)* confirmed the active spread of this agent during 1965.

SOMMAIRE

On a isolé le virus Powassan de sept complexes ("pools") de tiques *Ixodes cookei* prélevées sur des marmottes *(Marmota monax)* prises près de North Bay, en Ontario, de mai à août 1965. Y figuraient cinq complexes obtenus au cours du printemps. Ces complexes comprenaient chacun de une tique à neuf tiques. La titration sur des cultures monocouches de cellules primaires de rein de porc variait de 2.0 à 5.5 $\log_{10}$ TCD_{50} de virus. Durant la dernière saison, la prédominance des anticorps neutralisants du virus Powassan dans le sérum des marmottes a augmenté régulièrement de zéro durant le mois de mai à 25% durant le mois d'août, mais est restée relativement inchangée (42% à 58%) chez les marmottes de la génération précédente, confirmant ainsi que l'infection avait été sourtout active parmi les jeunes sujets principalement durant le printemps de 1965. L'isolement d'une souche de virus Silverwater sur des tiques *Hæmaphysalis leporis-palustris* et la découverte d'anticorps neutralisants chez trois lièvres "snowshoe" *(Lepus americanus)* a permis de confirmer que la propagation s'était opérée en 1965.

CONTINUING investigations during six previous summers of the mechanisms by which Powassan virus,[1] the sole North American member of the tick-borne encephalitis complex of group B arboviruses,[2] is maintained in nature in a focus of infection near North Bay, Ontario (46°N, 79°30'W), have shown that hard ticks including *Ixodes cookei*[3] and *I. marxi*[4] may serve as vectors, whilst forest rodents especially groundhogs (*Marmota monax*)[3] and red squirrels (*Tamiasciurus hudsonicus*)[4] have been important vertebrate reservoirs of infection. Occasional human infections may occur tangentially to this natural cycle of infection involving ticks and forest fauna, but at present serological evidence of past infection with Powassan virus has been demonstrated in only five of 194 residents of this area,[5] following development of fatal encephalitis in the index case during 1958.[1] Although additional natural foci of Powassan virus infection have been detected in Colorado,[6] South Dakota,[7] Connecticut[8] and upstate New York,[9, 10] no clear evidence of clinically manifest human infections has been obtained in these areas.

Although observations on the serologically related but distinct tick-borne group B arboviruses, louping ill in Scotland[11] and Central European encephalitis in Czechoslovakia[12] and Austria[13] have revealed extensive evidence of virus transmission amongst sheep and forest rodents respectively during the spring months March through June, less detailed information regarding natural transmission of Powassan virus in northern Ontario was available during spring.

The late onset of spring during mid-May 1965 in northern Ontario, in contrast to its usual appearance in mid-April during previous years, provided abundant opportunities for investigations of the spread of Powassan virus in nature during spring.

METHODS AND MATERIALS

During 1965, concerted attempts were directed towards live-trapping a substantial proportion of the total collection of forest fauna, but of 397 animal

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TABLE I.—VIRUS STRAINS ISOLATED FROM TICKS, 1965

Virus type	Strain No.	Ticks		Date collected	Township	Isolation procedure			
						Mice		Swine kidney	
		Species	No.			Date	Result	Date	Titre*
Powassan	3605	*I. cookei*	1	May 24	Bonfield	June 2	+	July 8	4.5
"	3607	"	2	May 24	Bonfield	June 2	+	"	2.0
"	3622	"	9	May 28	Bonfield	June 2	+	"	5.0
"	3626	"	6	May 29	Bonfield	June 2	+	"	5.5
"	3769	"	3	June 2	Laurier	June 10	+	"	3.5
"	4090	"	1	July 1	Chisholm	July 1	+	"	3.5
"	4643	"	1	July 30	Bonfield	Aug. 4	+	Sept. 8	0
Silverwater	4080	HLP	12	June 29	Chisholm	July 10	+	—	—

*Log_{10} TCD_{50}/ml. tick suspension.

sera tested, 141 were obtained from trapped animals and 256 from shot specimens. Trapped animals were anesthetized in the field, their ages were estimated, they were bled by cardiac puncture, labelled ear tags were placed on both ears, ticks were removed, and the animals were then released. Live traps were set daily for approximately one week at a particular test site, and these sites were revisited for subsequent trapping at about monthly intervals. Shot animals were bled from the heart, and ticks were collected from them in snap-cap vials. Sera were separated from clots and both were stored frozen at –20° C. to await the once-weekly shipment to Toronto in refrigerated containers. Ticks were held at 4° C. while awaiting shipment. A tick pool comprised all ticks removed from one animal.

In Toronto, as described previously,[4] virus isolations from unfrozen tick pools were attempted by intracerebral inoculations of groups of eight or more suckling mice aged 1 to 4 days with 0.02-ml. amounts of suspensions of ground-up ticks in 10% ox serum saline diluent. Suspensions were prepared usually within one day following receipt of the shipment from the field station. Tick suspensions were held frozen at –70° C. in an electric refrigerator until tested one to six weeks later by inoculation of primary monolayer tube cultures of swine kidney epithelial cells.[3] Blood clots were held frozen at –70° C. for as long as two months before being tested by intracerebral inoculation of suckling mice. All fresh isolates were typed by neutralization tests in mice using antisera prepared in guinea-pigs against known arboviruses.[4]

All sera were examined for the presence of neutralizing antibodies to 100 mouse LD_{50} of Powassan virus (prototype strain L.B.) by intracerebral inoculation of groups of five weaned mice aged 3 weeks with 0.03-ml. quantities of mixtures of virus and undiluted unheated serum.[4] If sufficient sera were available, antihemagglutinin tests using kaolin-treated sera and rooster erythrocytes were performed against four to eight agglutinating doses of antigens prepared by the sucrose-acetone method[14] from Powassan and eastern equine encephalomyelitis (EEE) viruses. Selected sera were also examined against Murray Valley encephalitis (MVE) antigen. Complement fixation tests were performed whenever serum supplies permitted, using optimal dilutions of the following viral antigens: Powassan, EEE and California.

RESULTS

Virus Isolations from Ticks

Strains of Powassan virus were isolated by inoculation of suckling mice with suspensions prepared from seven of 122 pools of *I. cookei* ticks removed from groundhogs (Table I). Positive tick pools contained between one and nine adult or nymphal ticks, virtually all of which were engorged with blood. Virus was reisolated from frozen suspensions of six of seven tick pools upon inoculation of primary monolayer tissue cultures of swine kidney

TABLE II.—VIRUS ISOLATIONS FROM TICKS, AND POWASSAN NEUTRALIZING ANTIBODIES IN 397 ANIMAL SERA, 1965

Species	May	June	July	August	Totals
Groundhog	45/87 (4/48)	33/92 (2/31)	48/112 (1/31)	22/59 (0/12)	148/350 (7/122)*
Squirrel	0/10	0/6	0	2/5	2/21
Snowshoe hare	0/2 (0/2)	1/6 (1/6)	1/5 (0/5)	0	2/13 (1/13)†
Others‡	0/3	2/9	0/1	0	2/13
Totals	45/102	36/113	49/118	24/64	154/397

Numerator: number of sera with antibody or tick pools which yield virus.
Denominator: number of sera or tick pools tested.
*Powassan virus in *I. cookei* ticks.
†Silverwater virus in HLP ticks.
‡Chipmunks 0/7. Porcupines 2/6.

TABLE III.—NEUTRALIZING AND HEMAGGLUTININ INHIBITION ANTIBODIES TO POWASSAN VIRUS IN 350 GROUNDHOG SERA, 1965

		May		June		July		Aug.		
Township	*Test*	*Adult*	*Juvenile*	*Adult*	*Juvenile*	*Adult*	*Juvenile*	*Adult*	*Juvenile*	*Totals*
Bonfield	NT†	17/24	0/4	2/5	1/5	13/21	4/20	2/10	1/4	40/93
	HI	15/21	0/4	4/5	1/5	16/21	8/20	4/10	3/4	52/90
Chisholm	NT	17/29	0	17/35	0/11	19/35	4/19	11/23	2/8	70/160
	HI	12/29	0	17/34	0/11	24/35	5/19	11/22	4/8	73/158
E. Ferris	NT	7/13	0	6/13	2/7	3/5	0/4	0	0/1	18/42
	HI	9/13	0	7/13	5/7	4/4	1/4	0	0/1	26/42
Eight others*	NT	4/17	0	4/14	1/2	4/7	1/2	5/10	1/3	20/55
	HI	6/17	0	3/12	2/2	4/5	1/2	5/8	1/3	22/49
Totals—No.	NT	45/83	0/4	29/67	4/25	39/67	9/45	18/43	4/16	148/350
%		54	0	42	16	58	20	42	25	42
No.	HI	42/80	0/4	31/64	8/25	48/65	15/45	20/40	8/16	173/339
%		52	0	48	32	74	33	50	50	51

*Other townships: Armour, Calvin, Gurd, North Himsworth, South Himsworth, Laurier, Nipissing, Novar.
†NT: incidence of neutralizing antibody.
HI: incidence of hemagglutinin inhibiting antibody.

cells, and infective titres of pools ranged from 2.0 to 5.5 $\log_{10}$ TCD_{50}/ml.

Most Powassan virus strains were isolated between May 24 and June 2. Virus strains were recovered more frequently from Bonfield Township than elsewhere. The proportion of tick-infested groundhogs decreased steadily throughout the summer (Table II), and virus was not isolated after July 30.

Silverwater virus was isolated from a pool of 12 *Haemaphysalis leporis-palustris* (HLP) ticks in all stages which were removed from a snowshoe hare (*Lepus americanus*) in Chisholm Township on June 29. No virus was recovered from pools of HLP ticks removed from 12 other hares (Tables I and II).

No strains of virus were isolated from blood clots of 397 animals whose sera were examined for Powassan neutralizing antibody.

Antibodies to Powassan Virus

Neutralizing antibody to Powassan virus was detected in 154 of 397 animal sera collected between May 8 and September 9, 1965 (Table II). These included sera from eight groundhogs which were retrapped once and from one groundhog which was retrapped on two subsequent occasions. Since only seven sera were collected during September, these were added to the total for August. Of 350 groundhog sera, 148 (42%) neutralized Powassan virus. However, the incidence of antibody in juvenile groundhogs which were born during 1965 increased steadily from zero during May to 25% during August, in contrast to the relatively steady prevalence of antibody (42 to 58%) throughout summer in sera from adult groundhogs born before 1965.

Hemagglutination inhibition antibodies were found in 173 of 339 (51%) groundhog sera. In common with the prevalence of neutralizing antibodies, the incidence of antihemagglutinins in juvenile groundhogs increased steadily to a maximum of 50% during August, but the proportion of adult groundhogs remained elevated to between 48 and 74% throughout the season.

On account of the high populations of groundhogs in the Townships of Bonfield, Chisholm and East Ferris, and the high incidence of Powassan virus in ticks and antibodies in vertebrates which were observed during previous years,[3] efforts to collect animals were concentrated within these townships.

Of nine groundhogs which were retrapped one day to two months after being trapped and tagged, five had both neutralizing antibody and antihemagglutinin and four were devoid of neutralizing antibody, two of which also showed no antihemagglutinin. Serum from one adult animal inhibited hemagglutination upon capture initially, but no inhibition was found in serum collected the next day. A juvenile groundhog which was devoid of antihemagglutination on July 17 inhibited hemagglutination on August 11, but no neutralizing antibody was demonstrated.

Sera from 95 groundhogs were also examined by complement-fixation tests in addition to neutralization and hemagglutination inhibition. A steady increase in the proportion of juvenile groundhogs

TABLE IV.—POWASSAN ANTIBODIES IN 95 GROUNDHOGS BY THREE TESTS

Month	*Total*	*Age*	*NT*	*CF*	*HI*
May	8	Adult	3	1	4
	2	Juvenile	0	0	0
June	9	Adult	0	0	1
	17	Juvenile	0	1	6
July	8	Adult	4	2	7
	29	Juvenile	5	4	9
August	12	Adult	4	2	5
	10	Juvenile	4	4	4
Total	95	—	20	14	36

which developed complement-fixing antibody as summer progressed was observed in parallel to the increased proportion of positive reactors by the other two tests (Table IV). However, the application of the complement-fixation test was severely restricted by the extraordinarily frequent occurrence of anticomplementary sera (Table V), especially in sera from adult animals.

TABLE V.—ANTICOMPLEMENTARY ACTIVITY AND AGE

Reaction	*Adult*	*Juvenile*
Complementary	37	58
Anticomplementary	83	4

Antibodies to Other Arboviruses

Of 39 groundhog sera whose Powassan antihemagglutinin titres were 40 or greater, four inhibited Murray Valley encephalitis (MVE) antigen to titres higher than those against Powassan antigen, eight showed equal titres to both antigens, 17 showed MVE antibody titres lower than those against Powassan antigen and 10 did not react with MVE antigen when diluted 1:40. Of the latter 10 sera, seven did not neutralize Powassan virus (Table VI). These findings provide no clear evidence of activity of a mosquito-borne group B arbovirus in the test area during 1965.

TABLE VI.—HEMAGGLUTINATION INHIBITION REACTIONS OF 39 GROUNDHOG SERA AGAINST TWO GROUP B ANTIGENS

	HI reactions (initial serum dilution 1:40)			
Powassan NT	*MVE neg.*	*MVE <Pow*	*MVE =Pow*	*MVE >Pow*
Positive	3	15	8	3
Negative	7	2	0	1
Total	10	17	8	4

Of 290 groundhog sera which were examined simultaneously in hemagglutination inhibition tests against Powassan and EEE antigens, 24 inhibited EEE antigen at titres exceeding 10. No EEE neutralizing antibody was detected in 17 of these 24 inhibitory sera which were tested. Powassan antihemagglutinin was found in 23 sera, 17 of which also neutralized Powassan virus. Complement-fixation tests were performed on 95 sera, but none reacted with EEE antigen. These results do not suggest the presence of EEE virus in the test area.

Neutralizing antibodies to California encephalitis virus were found in seven of 10 snowshoe hare sera. Complement-fixing antibodies were detected in one of four snowshoe sera tested, and this sample also neutralized California virus, but two other sera which had neutralizing antibodies did not fix complement in the presence of California antigen. None of the 95 groundhog sera showed complement-fixing antibody to California virus.

Neutralizing antibodies to Silverwater virus[15] were demonstrated in three of nine snowshoe hare sera, including the animal from which ticks which yielded Silverwater virus were removed. This, together with the isolation of Silverwater virus from HLP ticks, confirms the continued presence of a natural cycle of infection between ticks and hares in the test area during 1965, in common with previous years.[4]

DISCUSSION

The isolation of Powassan virus from five pools of *I. cookei* ticks removed from groundhogs during spring, followed by a steadily increasing incidence of antibody in groundhogs from early to late summer, provides clear evidence of active transfer of virus between ticks and groundhogs in northern Ontario during the warmer months of 1965. In Bonfield Township, where substantial proportions of groundhogs collected during 1963, 1964 and 1965 had antibody, no virus was recovered from ticks until 1965, during which five of a total of seven ticks isolates were achieved from this township. This contrasts with observations during 1964 when of a total of nine tick isolates, five were derived from Chisholm Township and three were from East Ferris Township.[3] These findings exemplify the focal distribution of Powassan virus within the test area in northern Ontario, in parallel with observations on the serologically related European arboviruses transmitted principally by *I. ricinus* ticks which included louping ill in Scotland[11] and Central European encephalitis in Czechoslovakia,[12] Austria,[13] Poland,[16] Latvia[17] and Denmark.[18]

In northern Ontario during 1965, the high proportion of virus isolations from ticks during May, and lack of virus recovery beyond July, contrast sharply with 1964 observations when the peak prevalence of virus in ticks occurred during August.[3] The delayed onset of spring during 1965, as shown by the loss of the ice coating on Lake Nipissing on May 11,[19] about three to four weeks later than usual, may have permitted more detailed investigation of virus transfer at this season than was possible in former years. The rapid decline of collections of groundhogs and ticks during August, possibly associated with a dry spring and a cool early summer,[19] may have contributed to the lack of virus isolations from ticks during late summer. The vernal spread of Powassan virus parallels the natural transfer of louping ill virus in Ayrshire, Scotland, during spring, as revealed by virus isolations from small wild rodents during March and April[20] and acquisition of antibody by at least 30% of sheep between March and June.[11] Similarly in Neunkirchen, Austria, the peak of human infections of the central nervous system with Central European encephalitis virus occurs about one month after the peak populations of *I. ricinus* ticks have been encountered during May.[13]

In Scotland, Austria and Poland,[16] field mice and related small rodents appear to be important sylvan reservoirs of infection for group B tick-borne

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arboviruses, and sheep or goats which graze in tick-infested pastures or scrublands provide additional domestic reservoirs of infection, from which humans may receive infection by tick-bite, or in central Europe by consumption of raw milk.[12] However, in northern Ontario, larger forest rodents such as groundhogs and squirrels appear to be the major reservoirs of Powassan virus, and neither small rodents such as field mice nor domestic animals on farms have been incriminated so far as reservoirs.[5, 21] The lack of domestic animals as reservoirs, together with the low (35%) rate of infestation of groundhogs, often by five ticks or less per animal, may account for the relatively rare occurrence of subclinical infections in only five of 194 human residents of the Powassan–North Bay area[5] in contrast to 37% of persons living in Ostrava, Czechoslovakia,[22] and 16% of residents of Bialowieza, Poland,[16] who had inapparent infections with Central European encephalitis virus.

SUMMARY

Powassan virus was isolated from seven of 122 pools of *Ixodes cookei* ticks removed from groundhogs (*Marmota monax*) which were collected in northern Ontario between May 8 and September 9, 1965. Virus isolations from ticks were achieved principally during spring. Neutralizing antibody to Powassan virus was detected in 148 of 350 groundhogs. The incidence of antibody in groundhogs born during 1965, which increased steadily from zero during May to 25% during August, suggested active infection during spring and summer.

Silverwater virus was isolated from one of 13 pools of *Haemaphysalis leporis-palustris* ticks removed from snowshoe hares (*Lepus americanus*), and sera from three of nine neutralized this virus.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

INCIDENTS IN A LIFE

In my boyhood, at fifteen years, I began noting the results of my father's wide practice in Glengarry as a physician. He frequently rode miles in that district, and occasionally was absent days at a time. On one occasion an old patient of his called stating his wife was very ill, and wished to know if, in my father's absence, I could do anything for her. I invited him to take a seat, and said I would give him a few powders until my father returned. I at once secured a tablespoon of flour, which the cook browned in good form, and I made into twelve powders, to be taken night and morning in sugar and water. In about a week the farmer returned to secure an additional supply of the medicine, stating his wife never got anything that did her so much good. "What did you give her?" asked my father. "Simple brown flour." "What a remedy," said he, "and how remarkable the result." This was my first experience in the art of prescribing, and in my lifetime I never experienced more marked success. The influence of mind over matter is truly remarkable, and the most successful physicians are those who have the power of imparting confidence, which, as a factor of relief, actually knows no bounds.

In 1874 I was suddenly called to Basketong Station, Gilmour and Company, Gatineau, fifty miles distant, mid snow, frost and heavy roads. On arrival found the case was not fracture of the thigh, as informed, but paralysis, right side of body, complete, with retention of urine, very acute. The case was imperfectly reported, and I had every appliance but no catheter. What to do under the circumstances, and at such a distance from Ottawa, was the problem. Quietly resting in my chair to decide on my course of action, an idea struck me that many years past, when reading the life of Dr. Clutterbuck, of New York, as a student, I had adopted a suggestion of a practical character to cut a hole in the lining of an old warm winter coat, and insert an elastic catheter, held safely in the coat's tail. I at once examined closely my tried friend and there to my delight discovered the catheter, which at once afforded relief and saved the life of my patient *pro tem.* Any port in a storm, and it is a safe expedient under such circumstances to have a catheter near at hand.

My first case of stone in the bladder. In 1860 took charge of a ward in the General Protestant Hospital, Ottawa, where my attention was called to a patient labouring under a severe attack of whooping cough. A young lad aged sixteen, thin, pale, and distressed, owing to inability to retain his urine when coughing. On examination the urine was discovered to be quite ammonical, voided in considerable quantity, and this irregularity noted particularly since cough developed. I at once concluded a foreign body was the source of difficulty, sounded the bladder and defined the presence of stone, which I removed successfully in a few days by the lateral operation. The chief source of difficulty was non-union of the incision, owing to the spasmodic action of the patient's coughing on the wound, which, after several weeks, closed completely, and patient was discharged entirely free from both cough and stone.—J. Grant, *Canad. Med. Ass. J.*, 6: 300, 1916.