Canad. Med. Ass. J.
Dec. 26, 1964, vol. 91

Powassan Virus: Summer Infection Cycle, 1964

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ABSTRACT

Between May 1, and September 15, 1964, neutralizing antibody to Powassan virus was detected in sera from 163 of 464 forest mammals captured in the Powassan–North Bay area of northern Ontario. These included 159 of 358 groundhogs and four of 43 red squirrels. Acquisition of antibody by juvenile groundhogs occurred principally during July and August. Powassan virus strains were isolated from tick pools containing two to 15 *Ixodes cookei* per pool which were removed from eight of 91 groundhogs in three townships during May, July and August. Virus was also recovered from blood of two groundhogs during May. Powassan virus was re-isolated from five of six tick pools and two blood clots by inoculation of swine kidney tissue cultures. These findings strongly suggest that during 1964 Powassan virus was maintained in nature by a cycle involving groundhogs and *I. cookei* ticks.

SOMMAIRE

Entre le 1er mai et le 15 septembre 1964, on a décelé des anticorps neutralisants de virus de Powassan dans le sérum de 163 mammifères sauvages sur les 464 qui ont été capturés dans le Nord de l'Ontario, dans la région de Powassan–North Bay. On comptait ainsi 159 des 358 marmottes et quatre des 43 écureuils roux capturés. La formation des anticorps chez les jeunes marmottes s'effectue principalement pendant juillet et août. Les souches de virus Powassan ont été isolées d'agglomérats de tiques, contenant de deux à 15 *Ixodes cookei* par agglomérat et qui avaient été récoltés sur des marmottes (de huit à 91) dans trois comtés, en mai, juillet et août. On a également recueilli le virus dans le sang de deux marmottes en mai. On a pu isoler de nouveau le virus Powassan dans cinq ou six agglomérats de tiques et dans deux caillots de sang, par inoculation de cultures sur rein de porc. Ces constatations constituent de fortes présomptions que, durant l'année 1964, le virus Powassan s'est maintenu dans la nature, grâce à un cycle où la marmotte est le réservoir des tiques *I. cookei*, vecteurs du virus.

INTENSIVE field investigations have been undertaken during successive summers to determine the ecology of Powassan virus,[1] a group B tick-borne arbovirus[2] which was first isolated from the brain of a boy living in northern Ontario who contracted fatal encephalitis in September 1958. Surveys of sera from humans and forest rodents revealed foci of infection principally in two areas of northern Ontario, Powassan–North Bay and Manitoulin Island.[3] During late summer 1962,[4] isolation of Powassan virus both from *Ixodes marxi* ticks removed from a red squirrel (*Tamiasciurus hudsonicus*) in Nippissing Township near Powassan, and also from the blood of another red squirrel in the same area, strongly suggested that Powassan virus was maintained in nature by a cycle involving ticks as vectors and red squirrels as reservoirs. Evidence of natural foci of Powassan virus infection has also been obtained in Colorado,[5] South Dakota,[6] upstate New York[7] and Connecticut.[8]

Acquisition of complement-fixing antibodies, in addition to neutralizing antibody, by squirrels, groundhogs (*Marmota monax*) and porcupines (*Erethizon dorsatum*) during the spring of 1963[9] strongly suggested that some animals may have become infected at that time, but conclusive proof was lacking in the absence of virus isolations. Antigenically related, but distinct, members of the group B tick-borne complex of arboviruses such as louping ill[10] and Russian spring-summer encephalitis[11] have been isolated from ticks collected during the spring. The abundance of groundhogs in northern Ontario during the spring and summer of 1964 afforded an unusual opportunity to study their role as natural reservoirs, and their fairly regular infestation by *Ixodes cookei* ticks throughout the spring and summer greatly facilitated the isolation of virus from ticks during the spring, midsummer and late summer.

METHODS AND MATERIALS

Tick pools were held refrigerated at 4° C. until tested within 10 days after collection, by inoculation of extracts of ticks ground up in 10% ox-serum saline into suckling mice aged one to four days as previously described.[4] Blood clots were suspended

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This work was carried out under the sponsorship of the Commission on Viral Infections, Armed Forces Epidemiological Board, and was supported by the U.S. Army Medical Research and Development Command, Department of the Army, under Contract No. DA-49-193-MD-2402.

1:4 in 10% ox-serum saline before injection into suckling mice or tissue cultures. Estimation of Powassan neutralizing antibody was performed by inoculation of serum-virus mixtures into weaned mice,[4] and antihemagglutinin tests on sera were performed in plastic plates using four to eight agglutinating doses of Powassan antigen prepared by the sucrose-acetone method.[12]

Suspensions of ticks and blood clots which had previously yielded Powassan virus following inoculation of suckling mice were inoculated into primary monolayer tissue cultures of pig kidney epithelial cells.[13] These tissue cultures were prepared by trypsinization of kidneys removed aseptically from specific pathogen-free pigs aged eight weeks. The maintenance medium consisted of Medium 597 (CMRL) together with tryptose phosphate broth 15% and fetal calf serum 5%. The cultures were observed daily for evidence of cytopathic effects following incubation in stationary racks at 35° C. for periods up to seven days.

including 36 of 110 (33%) juveniles and 119 of 230 (52%) adult animals (Table II). The low incidence of antibody in juvenile groundhogs during June increased profoundly during the summer to between 45 and 47%, but the prevalence of antibody in adult groundhogs increased only moderately from 43% in May to 60% during August.

Hemagglutinin-inhibiting antibody was detected in 159 animals whose sera also neutralized Powassan virus, but a further 70 sera which showed antihemagglutinin contained no neutralizing antibody. This latter finding is currently under investigation. Neutralizing antibody alone was detected in 10 sera. No antibody was demonstrated by either test in 174 sera.

Ticks

Ixodes cookei ticks from 97 groundhogs and three porcupines, and *I. marxi* ticks from three squirrels were examined for virus content by inoculation of suckling mice. A tick pool comprised all the ticks removed from one animal, and the number of ticks per pool ranged between one and 15. Powassan virus strains were isolated from eight tick pools removed from groundhogs during the months of May, July and August (Tables II and III). Virtually all ticks were engorged with blood, and although most ticks were in the nymphal stage, some adults were also found. In East Ferris Township, three of 26 tick pools containing an average of 2.5 ticks per pool yielded virus, or approximately 4.5% of ticks were virus-infected. In Chisholm Township, where Powassan virus was isolated from five of 39 tick pools each containing an average of 2.5 ticks, about 5.2% of ticks carried virus.

Powassan virus strains were also isolated from the blood of two groundhogs collected during May in South Himsworth and East Ferris Townships, respectively. No additional virus strains have been isolated from a further 232 blood samples tested to date (September 28, 1964).

Powassan virus strains were reisolated from five of six tick suspensions and two suspensions of groundhog blood clot following inoculation of pig

TABLE I.—POWASSAN VIRUS ISOLATIONS AND NEUTRALIZING ANTIBODIES BY TOWNSHIPS, SUMMER 1964

Township	*Squirrel antibody*	*Groundhog*		*Porcupine antibody*	*Other* animals antibody*	*Totals*	
		Virus	*Antibody*			*Virus*	*Antibody*
Nipissing	1/5	—	0/1	0/1	0/4	—	1/11
N. Himsworth	0	—	0/5	0	0/7	—	0/12
S. Himsworth	1/6	1 blood	2/4	0/2	0/4	1 blood	3/16
Bonfield	0/2	—	58/123	0/2	0/4	—	58/131
E. Ferris	0/5	3 ticks 1 blood	24/50	0/1	0/4	3 ticks 1 blood	24/60
Chisholm	2/14	5 ticks	73/167	0	0/17	5 ticks	75/198
7 others	0/11	—	2/8	0/5	0/2	—	2/26
Totals	4/43	2 bloods 8 ticks	159/358	0/11	0/42	2 bloods 8 ticks	163/454

*Includes: 18 snowshoe hares, 18 chipmunks, two muskrats, two mice (*Peromyscus leucopus*), one grey squirrel (*Sciurus carolinensis*) and one redback vole.

RESULTS

Serological Studies

Sera from 454 forest rodents collected in the Powassan—North Bay area of northern Ontario between May 1 and September 15, 1964, were examined for evidence of neutralizing antibody to Powassan virus by inoculation of mice. Antibody was detected in 163 animals, including four of 43 red squirrels and 159 of 358 groundhogs, but none was found in 11 porcupines or 42 other animals (Table I). Animals were collected principally from the Townships of Bonfield, East Ferris and Chisholm on account of (i) the demonstration of a high incidence of antibody in these areas during the spring, first by hemagglutination inhibition tests and subsequently by neutralization tests; and (ii) the relative abundance of rodents, especially groundhogs, which was observed in this region and elsewhere in Ontario during 1964; the squirrel population, however, was reduced below that of previous years.

Within the Townships of Bonfield, East Ferris and Chisholm, neutralizing antibody was detected in 155 of 340 groundhogs in these townships. The groundhog population was increased throughout,

TABLE II.—MONTHLY INCIDENCE OF POWASSAN ANTIBODY IN 340 GROUNDHOGS AND POWASSAN VIRUS IN 91 TICK POOLS IN THREE TOWNSHIPS, SUMMER 1964

		May		*June*		*July*		*August*		*September*		*Total*	
Township		*Juv.*	*Adult*	*Juv.*	*Adult*	*Juv.*	*Adult*	*Juv.*	*Adult*	*Juv.*	*Adult*	*Juv.*	*Adult*
Bonfield	Antibody.....	0/1*	5/10	0/18	16/34	5/14	15/25	2/5	15/16	—	—	7/38	51/85
	Virus in tick.....	0/1*	—	0/6	0/3	0/8	0/6	—	0/2	—	—	0/15	0/11
	Ticks in pool†.....	1	—	2	1	6	2	—	1	—	—	—	—
E. Ferris	Antibody.....	1/1	10/23	0/1	5/9	1/1	4/8	2/4	1/3	—	—	4/7	20/43
	Virus in tick.....	—	2/17	0/1	0/2	0/1	0/1	1/2	0/2	—	—	1/4	2/22
	Ticks in pool.....	—	3	1	1	2	1	3	2	—	—	—	—
Chisholm	Antibody.....	0/1	3/9	1/12	17/33	11/23	8/18	10/21	17/36	3/8	3/6	25/65	48/102
	Virus in tick.....	—	0/2	0/2	0/7	2/12	0/4	0/5	3/6	0/1	—	2/20	3/19
	Ticks in pool.....	—	2	3	1	3	2	2	4	1	—	—	—
Total	Antibody.....	1/3	18/42	1/31	38/76	17/38	27/51	14/30	33/55	3/8	3/6	36/110	119/230
percent	Antibody.....	33	43	3.2	50	45	53	47	60	37	50	33	52
Total	Virus in ticks.....	2/20		0/21		2/32		4/17		0/1		8/91	

*Numerator: number of animals with antibody or tick pools yielding virus.
Denominator: number of animals or tick pools tested.
†Average number of *I. cookei* ticks per pool.

kidney cultures one to eight weeks after their initial isolation by inoculation of suckling mice. Cytopathic effects were observed on the fifth or sixth day. The amounts of virus per tick pool ranged from 0.5 to 3.7 log TCD_{50} per 0.1 ml., and the blood clots yielded 0.7 to 1.2 log TCD_{50} per 0.1 ml. (Table III).

TABLE III.—STRAINS OF POWASSAN VIRUS ISOLATED DURING SUMMER 1964

Strain number	*Source*	*Date collected*	*Township*	*Technique* *Mice**	*Technique* *Log_{10} TCD_{50}†*
1963.....	4 ticks§	19 May	E. Ferris	+	2.2
1973.....	blood	21 May	S. Himsworth	+	0.7
1981.....	9 ticks	22 May	E. Ferris	+	0
1982.....	blood	22 May	E. Ferris	+	1.2
2881.....	9 ticks	17 July	Chisholm	+	+
3015.....	4 ticks	20 July	Chisholm	+	+
3219.....	6 ticks	6 Aug.	Chisholm	+	0.5
3336.....	2 ticks	18 Aug.	E. Ferris	+	3.7
3446.....	2 ticks	18 Aug.	Chisholm	+	n.t.
3668.....	15 ticks	25 Aug.	Chisholm	+	n.t.

*Intracerebral inoculation of suckling mice aged one to three days.
†Log_{10} TCD_{50} per 0.1 ml. tick suspension using primary monolayer tissue cultures of pig kidney.
§Number of *I. cookei* nymphs and/or adults in pool.
n.t.: Not tested.

DISCUSSION

Isolation of Powassan virus from *I. cookei* ticks removed from eight groundhogs which were captured on various occasions throughout the spring and summer 1964 in the Powassan—North Bay area indicates clearly that this tick species is an important vector of Powassan virus, in addition to *I. marxi* which was found to be naturally infected near Powassan during the sumer of 1962.[4] In a focus of infection in the Black Hills of South Dakota, isolation of Powassan virus from wild caught *I. spinipalpus* ticks removed from *Peromyscus sp.* mice points to the likely role of this species as a vector also.[6] The serologically related tick-borne encephalitis viruses of Europe and Asia have been isolated repeatedly from *I. ricinus*[14-16] or *I. persulcatus*[11, 17] ticks collected in natural foci.

The sharply increased incidence of Powassan neutralizing antibody in juvenile groundhogs during the summer provides good evidence that they acquired active infections during that season. The isolation of Powassan virus from the blood of two groundhogs during the spring of 1964 indicates that active infection has occurred at that time. These findings suggest that the relatively frequent occurrence of complement-fixing antibody in groundhogs and squirrels captured during the spring of 1963[9] resulted from infection during the same season, and not during the previous summer. These observations also show that groundhogs, in addition to squirrels,[4] are important forest reservoirs of Powassan virus infection in northern Ontario. However, in contradistinction to recent reports of isolation of the serologically related louping ill virus from *Apodemus sylvaticus* mice in Scotland[18] and tick-borne encephalitis virus from this species in Austria,[14] no evidence has been obtained so far to indicate that field mice (*Peromyscus sp.*) serve as natural reservoirs of Powassan virus in northern Ontario.

Although hemagglutination-inhibition provided a rapid and convenient technique for location of foci of infection in the test area, thereby enabling the field workers to concentrate their collection of animals in antibody-positive localities, confirmation that this antibody resulted from infection with Powassan virus must await results of neutralization tests about 10 days subsequently.

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