

Powassan Virus: Field Investigations in Northern Ontario, 1959 to 1961

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FOLLOWING the isolation of Powassan virus¹ from the brain of a child living at Powassan, Ontario, who died of encephalitis in September 1958, extensive field studies were undertaken in northern Ontario during the summers of 1959² and 1960.³ The presence of neutralizing antibody to Powassan virus in sera from human residents of Manitoulin Island and Kirkland Lake, in addition to Powassan and the adjacent urban area of North Bay, strongly suggested that these subjects contracted subclinical infections with this virus. Since Powassan virus is a group B arbovirus, antigenically related to, but distinct from, Russian spring-summer encephalitis virus⁴ which is maintained in nature by a cycle involving ticks and small mammals, and since antibody to Powassan virus has been demonstrated repeatedly in squirrels and chipmunks at Powassan and on Manitoulin Island,^{2, 3} it seemed likely that these persons may have acquired their infections following exposure to wild life in forested areas near their homes. The presence of man-biting *Ixodes sp.* ticks on two squirrels captured at Powassan during 1960 provides circumstantial evidence in favour of these ticks as possible vectors of virus from small forest mammals to man. The isolation of an agent antigenically similar to Powassan virus from *Dermacentor andersoni* ticks collected in Colorado during 1952⁵ strengthens the concept that Powassan virus is tick-borne, and demonstrates its presence some 1000 miles south-west of Ontario.

Throughout 1961, studies to determine the presence of antibody in small forest mammals and the relationship between ticks and mammals were undertaken at six test sites in the Powassan area, and at six test areas on the western portion of Manitoulin Island. A serological survey of residents of western and southern Manitoulin Island was undertaken in order to gain further information on the transfer of Powassan virus from nature to man under conditions of frequent exposure to wildlife in a known endemic area.

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METHODS AND MATERIALS

Human blood samples were collected through the kind co-operation of physicians and health laboratory directors in northern Ontario during 1959 and 1960, and by the senior author (D.M.M.) during 1961. Blood samples from domestic animals were collected on several farms near Powassan by a veterinarian (L.W.M.). Wild animals were collected by shooting. Blood samples were collected from each animal immediately, and ectoparasites were removed the next day. All sera were examined for neutralizing antibody to Powassan virus by intracerebral injection of mixtures of unheated serum with virus (final dose 50 mouse LD₅₀) into mice aged three weeks, using methods described previously.^{2, 6} Many sera were also examined for antibodies to Powassan virus and other North American arboviruses in complement-fixation and antihemagglutinin tests.^{2, 3} Blood clots and ectoparasites from wild animals collected near Powassan (by D.M.M. and L.W.M.) and ticks removed from snowshoe hares collected on Manitoulin Island also (by T.H.S. and K.R.) were tested for presence of virus by intracerebral inoculation of newborn mice with suspensions prepared in 10% ox-serum saline.^{2, 3}

RESULTS

Powassan District Animals

Between July 1959 and September 1961, sera from 123 animals in the Powassan district were examined for the presence of neutralizing antibody to Powassan virus (Table I). Sera from six of 29 chipmunks (*Tamias striatus*) and four of 30 red

TABLE I.—NEUTRALIZING ANTIBODY IN ANIMAL SERA, POWASSAN DISTRICT, JULY 1959 TO SEPTEMBER 1961

Species	Number positive	Number tested	Number infested with ticks	Tick species
Chipmunk.....	6	29	0	0
Squirrel.....	4	30	6	<i>Ixodes sp.</i>
Snowshoe hare....	0	20	20	H.L.P.
Groundhog.....	0	3	3	<i>Ixodes cookei</i>
Field mouse.....	0	4	0	0
Sheep.....	0	8	0	0
Cattle.....	0	10	0	0
Horse.....	0	3	0	0
Grouse.....	0	2	1	H.L.P.
Other birds.....	0	14	0	0
Totals.....	10	123	30	3

TABLE II.—NEUTRALIZING ANTIBODY IN SERA OF SQUIRRELS AND CHIPMUNKS COLLECTED NEAR POWASSAN, 1959 - 1961

Species	1959				1960		1961					
	July	August	Sept.	October	April	Sept.	April	May	July	August	Sept.	
Squirrel	0/1	—	0/1	1/7	—	1/5(2)	1/6(1)	0/3(1)	0/1	0/3(1)	1/3(1)	4/30
Chipmunk	2/4	1/4	1/1	0/4	1/2	1/8	—	0/1	0/4	—	0/1	6/29
Total	2/5	1/4	1/2	1/11	1/2	2/13	1/6	0/4	0/5	0/3	1/4	10/59

Numerator: number of sera which neutralized Powassan virus.

Denominator: number of sera tested.

Number in parenthesis indicates numbers of squirrels carrying Ixodid ticks.

squirrels (*Tamiasciurus hudsonicus*) contained antibody, but none of 27 sera from wild animals, including 20 snowshoe hares (*Lepus americanus*), three groundhogs (*Marmota monax*) and four field mice (*Peromyscus maniculatus*), neutralized Powassan virus, nor was antibody detected in sera from eight sheep, 10 cattle, three horses or 16 birds including two ruffed grouse (*Bonasa umbellus*), four sparrows, eight finches and two domestic fowls. Antibody was detected in mammals taken during the summer and fall of 1959, and in the spring and fall of both 1960 and 1961 (Table II).

grouse was infested heavily with H.L.P. ticks. No virus was isolated from any of the ectoparasites.

Manitoulin Island Animals

During the summer of 1961, sera samples were obtained from 187 animals collected on the western portion of Manitoulin Island including 19 squirrels, 13 chipmunks, 128 hares and 27 other mammals (Table III). Neutralizing antibody to Powassan virus was detected in sera from two squirrels. Powassan complement-fixing antibody has not been detected in sera from 14 squirrels, 8 chipmunks,

TABLE III.—ANTIBODIES IN SERA OF ANIMALS COLLECTED ON MANITOULIN ISLAND, 1960 AND 1961

Species	Summer 1960			February 1961			May 1961			Summer 1961		
	Powassan NT	Powassan CF	Silv. ¹ CF	Powassan NT	Powassan CF	Silv. CF	Powassan NT	Powassan CF	Silv. CF	Powassan NT	Powassan CF	Silv. CF
Squirrel†	3/56*	0/3	0/3	0/23	0/5	0/5	1/1	—	—	2/19	0/14	—
Chipmunk	2/18	0/2	0/2	—	—	—	0/1	0/1	0/1	0/13	0/8	—
Snowshoe hare‡	5/178	0/84§	7/85	0/16	0/13	2/13	0/4	0/4	0/4	0/128	0/121	10/107
Other mammals	0/91	0/14	0/14	—	—	—	—	—	—	0/27**	0/27	0/27
Total	10/343	0/103	7/104	0/39	0/18	2/18	1/6	0/5	0/5	2/187	0/170	10/134

¹Silverwater virus

CF: Complement fixation test.

§Sera from two animals neutralized Powassan virus.

†14 squirrels taken during summer 1960 carried *Ixodes* sp. ticks, and one squirrel carried H.L.P. ticks.

‡All hares collected during summer 1960, May 1961 and summer 1961 were infested with H.L.P. ticks.

**Includes 22 deer, two *Microtus*, one beaver, one skunk, one groundhog.

Blood clots obtained from 22 squirrels, 14 chipmunks, 16 snowshoe hares, three groundhogs and one grouse were examined for virus content by intracerebral injection of suckling mice aged one to four days. No virus was isolated from any of these 56 specimens.

Ixodes sp. ticks, both larval and nymphal forms, were recovered in small numbers from six squirrels, two during September 1960, and one each during April, May, August and September 1961. In only one instance did a tick-infested squirrel have antibody to Powassan virus. Fleas were found on 11 squirrels and two chipmunks during 1961, and on two squirrels and two chipmunks during 1960, and antibody was found in one flea-infested squirrel. All 20 snowshoe hares were heavily infested with *Haemaphysalis leporis-palustris* (H.L.P.) ticks, and all three groundhogs carried *Ixodes cookei* or *I. kingi* ticks in all stages. One

121 snowshoe hares or 27 other mammals. However, complement-fixing antibody to Silverwater virus has been detected in sera from 10 of 107 snowshoe hares, including two of 16 which were collected in the same area as the ticks which yielded Silverwater virus during 1960.

Ixodes sp. ticks were found on five squirrels, but none of these animals had Powassan neutralizing antibody. *Hoplopleura erraticus* lice were found on six squirrels, five chipmunks, one groundhog and one *Microtus*.

Trombiculid mites were removed from eight squirrels, three chipmunks, one groundhog and one *Microtus*, and *Megabothris acerbus* fleas were found on seven squirrels, two chipmunks, one groundhog and one *Microtus*.

In February 1961, sera from 23 squirrels and 16 snowshoe hares collected in the Gore Bay and Silverwater areas of western Manitoulin Island

were examined in neutralization tests, but no antibody to Powassan virus was detected. No complement-fixing antibody to Powassan virus or Silverwater virus^{3, 7} was detected in sera of five squirrels, and two of 13 snowshoe hares had antibodies to Silverwater virus and not Powassan virus. No ectoparasites were removed from any animals.

In May 1961, four snowshoe hares and one chipmunk were collected on the southern end of Manitoulin Island near South Bay Mouth. Serum from one squirrel which was taken on Birch Island, northeast of Manitoulin Island, neutralized Powassan virus. All four snowshoe hares were infested with H.L.P. ticks, but no ectoparasites were found on other animals.

Human Sera

During 1961, sera were obtained from 105 adult human residents of western and southern Manitoulin Island, 87 men and 18 women. Neutralizing antibody to Powassan virus was detected in sera of two men who lived on western Manitoulin Island, and, in common with most Manitoulin inhabitants, they frequently entered forested areas during all seasons. Complement-fixing antibody to Powassan virus or Silverwater virus was not detected in any of the 105 sera. In 1959, sera from three of 52 Manitoulin Island residents neutralized Powassan virus.²

Between 1959 and 1961, neutralizing antibody to Powassan virus was detected in sera from 11 of 1008 residents of northern Ontario (Table IV). Complement-fixing antibody to Powassan virus has been detected in one family contact at Powassan whose serum also neutralized Powassan virus,² which suggests that he contracted a subclinical infection at the time the index case became ill. The absence of complement-fixing antibody in the remaining 10 sera with neutralizing antibody suggests subclinical infection more than one year previously. At present, infection in man with Powassan virus has been demonstrated almost exclusively in the Powassan-North Bay area and on Manitoulin Island.

DISCUSSION

The presence of neutralizing antibody to Powassan virus in squirrels or chipmunks captured in the Powassan district during each of three years following the occurrence of a case of encephalitis due to infection with this virus in September 1958, together with the demonstration of neutralizing antibody in 5 of 194 humans living within 25 miles of Powassan, strongly suggests that a focus of virus infection exists in this area. The most likely natural reservoirs⁸ are squirrels, since antibodies have been detected in this species repeatedly, and *Ixodes sp.* larval and nymphal ticks, some of which were recently engorged with blood, were found on six squirrels, one of which had antibody to Powassan virus. The presence of antibody in one squirrel

TABLE IV.—NEUTRALIZING ANTIBODY IN HUMAN SERA, NORTHERN ONTARIO 1959 - 1961

Locality	Number positive	Number tested
Powassan (family).....	1	6
Powassan (others).....	0	20
North Bay.....	4	168
Kirkland Lake.....	1	182
Timmins.....	0	126
Sudbury.....	0	227
Manitoulin Island.....	5	157
Sault Ste. Marie.....	0	122
Total.....	11	1008

during April 1961 and in another taken during September, and the absence of antibody from animals captured during intervening months, suggests that many animals acquire infection during the summer. Squirrels taken during April appeared to be born during the previous summer, while animals collected in May and July appeared immature. No ticks have yet been found on chipmunks, which suggests that although they may acquire infection following bites by infected ticks, the ticks may not remain attached for long periods.

Although all three groundhogs harboured all stages of *I. cookei* or *I. kingi* ticks, the lack of antibody in their sera offers no evidence in favour of their role as natural reservoirs of infection. *I. angustus*, *I. cookei* and *I. kingi* parasitize a wide range of small forest mammals and they will attack man. Therefore on circumstantial evidence they may be postulated as natural vectors of Powassan virus both amongst forest fauna and from animals to man.

On Manitoulin Island during the summer of 1960, *Ixodes sp.* ticks were collected from 14 of 56 squirrels, although no tick-infested squirrel circulated antibody to Powassan virus. No ticks were detected on chipmunks. All 178 snowshoe hares were infected with H.L.P. ticks, all stages of which feed almost exclusively on hares. The presence of Powassan neutralizing antibody in five hares suggests that they may have become infected by the bite of *Ixodes sp.* ticks which became infected by feeding on squirrels. Should H.P.L. ticks become infected while feeding on snowshoe hares during the viremic phase, these ticks would transmit virus to other hares only. The lack of antibody to Powassan virus in both squirrels and snowshoe hares during February 1961 suggests lack of virus transmission during the winter months.

The isolation of Silverwater virus from two pools of H.L.P. ticks removed from snowshoe hares captured on Manitoulin Island during 1960, together with the presence of complement-fixing antibody to this agent in seven of 85 hares during summer 1960 and two of 13 hares during February 1961, suggests that this virus is maintained exclusively by a cycle of infection involving snowshoe hares as reservoirs and H.L.P. ticks as vectors. However, H.L.P. ticks do not normally bite man. The absence of antibody to Silverwater virus from

sera of 105 humans resident in the endemic area suggests either that this virus is not conveyed to man or that it is non-infective to man.

SUMMARY

Near Powassan, Ontario, between 1959 and 1961, neutralizing antibody to Powassan virus has been detected in sera from four of 30 squirrels, six of which were infested with *Ixodes sp.* ticks. Sera from six of 29 chipmunks neutralized Powassan virus, but ticks were not found on any animal. No antibody was detected in sera from 20 snowshoe hares, three ground-hogs, four field mice, 21 domestic mammals or 16 birds.

On Manitoulin Island, neutralizing antibody to Powassan virus has been detected on three of 56 squirrels, two of 18 chipmunks and five of 178 snowshoe hares captured during 1960, and in two of 19 squirrels but not in 13 chipmunks captured in 1961. Complement-fixing antibody to Silverwater virus has been detected in nine of 98 snowshoe hare sera, and this virus has been isolated from two pools of H.L.P. ticks.

Detection of neutralizing antibody in sera from five of 194 human residents of the Powassan-North Bay area and five of 157 humans living on Manitoulin Island, but in only one of 657 residents of other districts of northern Ontario, suggests that human infection with Powassan virus occurs principally in the first two regions. Evidence has been presented in favour of the existence of natural cycles of infection involving ticks and small mammals in both regions.

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Treatment of Intraepithelial Carcinoma of the Cervix

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UNTIL recently the accepted treatment for intraepithelial carcinoma of the cervix has been total hysterectomy including a wide cuff of vagina, with or without removal of the uterine appendages, for all patients with this disease.¹⁹ The rationale for such a major operation is the belief that intraepithelial carcinoma frequently develops into invasive carcinoma if left untreated¹⁵ or if treated by less radical procedures.

However, many of the data quoted in support of this contention appear to be based on what must now be considered unscientific evidence, in that the original diagnosis was made from single or multiple biopsies *without* conization of the cervix. Some of these cases may therefore have been invasive at the time, the invasive portion of the cancer having been missed by the biopsy. Most authorities now agree^{2, 7, 20} that the diagnosis of intraepithelial carcinoma of the cervix should be made only after careful study of a wide cone biopsy taken with a cold knife.

This leads to two important conclusions: (1) The data purporting to show the progress of intraepithelial carcinoma of the cervix left untreated are invalid, or at least require reappraisal. (2) The necessity for such drastic treatment in *all cases* must be questioned, though few would deny that it is the treatment of choice for most.

Perusal of the recent literature reveals the fact that others are evidently thinking along the same lines,^{11, 13, 16, 17, 20} and that more conservative or non-definitive therapy has a place in the management of this disease, though patients who are subjected to such treatment require most careful selection and follow-up.^{12, 21}

In order to decide rationally how to treat each patient as an individual, it is essential to know how effective non-definitive procedures are in eradicating the disease. Since the average gynecologist sees only a few cases of this condition each year, such information can best be obtained by pooling the results from many sources.

The figures in Table I refer to all of the 36 cases of intraepithelial carcinoma of the cervix diagnosed by cone biopsy in the two general hospitals of Victoria, British Columbia, during the period 1953-1960. The presenting symptom (if any), the cytology of the cervical smear, and the method of treatment are indicated. It is interesting to note that 50% of these patients were symptomless. An attempt has also been made to classify the cone specimens into those which may be considered adequate and those which may not. A cone has been quite arbitrarily classed as adequate if it consisted of one or two pieces only and measured at least 2.0 cm. in length. Fragmentary specimens and those measuring less than 2.0 cm. are classed as inadequate. The writer does not wish to imply that he considers this to be a satisfactory way of

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