Canad. Med. Ass. J.
Jan. 26, 1963, vol. 88

Powassan and Silverwater Viruses: Ecology of Two Ontario Arboviruses

DONALD M. McLEAN, M.D. and R. P. BRYCE LARKE, M.D.,
Toronto

ABSTRACT

Powassan virus was isolated from a pool of Ixodes marxi ticks collected during late August 1962, from a red squirrel, Tamiasciurus hudsonicus, and from blood obtained from a red squirrel during early October 1962 near Powassan, Ontario, where a child contracted fatal encephalitis due to this virus in September 1958. The frequent detection of Powassan virus neutralizing antibody in sera of squirrels captured during autumn, but rarely at other seasons, and the frequent I. marxi infestation of squirrels, some of which contain antibody, but the lack of occurrence of I. marxi on other forest rodents, suggest that I. marxi ticks are vectors and squirrels are reservoirs of Powassan virus infection. Isolation of Silverwater virus from Haemaphysalis leporis-palustris ticks which infested a snowshoe hare Lepus americanus near Powassan demonstrates the presence of this agent in the Powassan area also.

THE isolation of Powassan virus, a newly recognized Group B arbovirus, from the brain of a child, a resident of Powassan, Ontario (46° N, 79° 30′ W), who contracted fatal encephalitis in September 1958,[1] has stimulated extensive investigations in the natural history of this agent in northern Ontario during successive summers.[2-4] Demonstration of a fairly close antigenic relationship between Powassan virus and Russian spring-summer encephalitis,[5] which is tick-borne, suggested strongly that Powassan virus may also be transmitted by ticks. This hypothesis was further strengthened by the isolation of Powassan virus from *Dermacentor andersoni* ticks collected in Colorado during 1952[6] and in South Dakota during 1960.[7] In the latter area, Powassan neutralizing antibodies have been found in sera from chipmunks and other wild rodents. This communication reports the isolation of Powassan virus from *Ixodes marxi* ticks removed from a red squirrel and viremia in another red squirrel collected near Powassan during 1962, thus providing good evidence that the virus may be maintained in nature by a cycle involving ticks and squirrels.

Silverwater virus, a new arbovirus which has not as yet shown any antigenic relationship with other arboviruses of North America and elsewhere, was first isolated from a pool of *Haemaphysalis leporis-palustris* (H.L.P.) ticks removed from two snowshoe hares collected on western Manitoulin Island near Silverwater, Ontario (46° N, 82° 30′ W) in July 1960.[8] Another strain of this agent was isolated from a pool of H.L.P. ticks removed from two additional snowshoe hares captured in the same vicinity shortly afterwards.[3] Although complement-fixing antibody to Silverwater virus has been detected in seven of 85 snowshoe-hare sera during the summer of 1960, and in 10 of 107 hare sera during the summer of 1961, it has not been detected in 105 sera from human residents of Manitoulin Island.[4] This suggests that Silverwater virus is maintained in nature by a cycle involving snowshoe hares and H.L.P. ticks, but apparently man is not involved in this cycle. A survey of ectoparasites of Manitoulin Island fauna has been reported recently.[9] Investigations of H.L.P. ticks on snowshoe hares in the Powassan area between 1959 and 1960 failed to demonstrate the existence of Silverwater virus in this area. The isolation of Silverwater virus from H.L.P. ticks from a snowshoe hare which was obtained near Powassan during July 1962, as described in this report, indicates that this virus is also present in the Powassan area.

METHODS AND MATERIALS

Animals were collected in the Powassan area by shooting. Test sites were located six to eight miles east, 10 to 12 miles west, 12 to 14 miles north, and 15 to 20 miles south of Powassan in thickly forested country containing mixed spruce and deciduous vegetation. The areas had not been logged for many years. Since areas from which most animals were collected between 1959 and 1961 carried smaller populations during 1962, the principal collection sites during 1962 were located one to two miles from former test areas.

Blood samples were collected from each animal immediately and held at 4° C. in vacuum flasks containing ice, as described previously.[3] All sera were examined for neutralizing antibody to Powassan virus prototype strain L.B. by intracerebral injection of mice aged three weeks with mixtures of unheated serum and virus, to give a final dose of 30 to 100 mouse LD_{50}.[2, 4] Blood clots

From the Virology Department, The Research Institute of The Hospital for Sick Children, Toronto.
Assisted with funds allocated by the Province of Ontario under the National Health Grants Program, Department of National Health and Welfare, Ottawa.

were examined for presence of virus by inoculation of 1:3 suspensions of clots in 10% ox-serum saline into suckling mice.[3, 4]

Ectoparasites were removed from animals four to eight hours after collection and held at 4° C. until tested. Ticks from each animal were pooled, ground in mortars, extracted with 1.5 ml. of 10% ox-serum saline per pool, and centrifuged at 1800 r.p.m. for three minutes; 0.02 ml. aliquots of supernatant were inoculated intracerebrally into groups of eight or nine suckling mice each aged one to four days.[3] The remaining supernatant was stored in sealed ampoules at –70° C. Other ectoparasites were examined similarly. It now appears likely[9] that *Ixodes sp.* ticks removed from squirrels during 1960 and 1961 should all be classified as *I. marxi.*

RESULTS

Isolation of Powassan Virus from Ticks and Squirrel Blood

On August 29, 1962, a pool of 35 *I. marxi* ticks, both larval and nymphal stages, was collected from a red squirrel, *Tamiasciurus hudsonicus,* which was captured about 12 miles west of Powassan. This site was about five miles from the farm on which the index case was living in 1958. Inoculation of eight suckling mice, each aged four days, with a suspension of these ticks in ox-serum saline induced encephalitis in all mice six days later. Suspensions of these mouse brains in the proportion of one brain per 0.25 ml. ox-serum saline showed intracerebral LD_{50} titres of $10^{-7.5}$ per 0.03 ml. in both weaned and suckling mice. This new virus isolate was designated strain No. 1427 (Table I).

TABLE I.—ISOLATION OF POWASSAN VIRUS FROM *I. marxi* TICKS, AND SEASONAL INCIDENCE OF POWASSAN NEUTRALIZING ANTIBODY IN SQUIRREL SERA, POWASSAN AREA, 1959 - 1962

	1959		1960		1961		1962		Total	
	Sera*	Ticks†	Sera	Ticks	Sera	Ticks	Sera	Ticks	Sera	Ticks
April	—	—	—	—	1/6	0/1	0/1	0	1/7	0/1
May	—	—	—	—	0/3	0/1	—	—	0/3	0/1
June	—	—	—	—	—	—	—	—	—	—
July	0/1	0	—	—	0/1	0	0/1	0/1	0/3	0/1
August	—	—	—	—	0/3	0/1	0/14	1/5	0/17	1/6
September	0/1	0	1/5	0/2	1/3	0/1‡	—	—	2/9	0/3
October	1/7	0	—	—	—	—	3/12	0/2‡	4/19	0/2

*Numerator: number of sera which neutralized Powassan virus. Denominator: number of sera tested.

†Numerator: number of pools of ixodid ticks which yielded Powassan virus. Denominator: number of tick pools tested.

‡*Ixodes marxi* ticks were removed from animals which had Powassan antibody.

Treatment of strain No. 1427 diluted in 10% ox-serum saline with equal parts of 1:500 sodium deoxycholate in saline reduced its infectivity for mice by at least 4 $\log_{10}$ LD_{50}. Inactivation by deoxycholate is a characteristic of all arboviruses.[10] Guinea-pig antiserum obtained after three intraperitoneal injections of prototype Powassan virus (strain L.B.) neutralized 100 mouse LD_{50} of both strain No. 1427 and strain L.B., whereas the fresh isolate was not neutralized by guinea-pig antisera to the following arboviruses: St. Louis encephalitis (SLE), eastern equine encephalomyelitis (EEE) and western equine encephalomyelitis (WEE), Bunyamwera and Silverwater. Antigen prepared from suckling mouse brains infected with strain No. 1427 by extraction with borate-saline followed by protamine treatment[11] fixed complement readily in the presence of antisera to Powassan virus strain L.B., but not in the presence of antisera to SLE, EEE, Bunyamwera and Silverwater viruses. These tests demonstrate clearly that strain No. 1427 is antigenically identical with Powassan virus strain L.B. Powassan virus strain No. 1427 was re-isolated from the tick suspension by intracerebral inoculation of suckling mice on September 6, 1962, following storage of the suspension in sealed ampoules at –70° C.

No virus was isolated either from a pool of four *Monopsyllus sp.* fleas or blood collected from the same squirrel whose ticks yielded Powassan virus. Similarly, no virus was isolated from *I. marxi* ticks removed from seven other squirrels during 1962, fleas from 20 other squirrels, or H.L.P. ticks from three or four snowshoe hares.

A further strain of Powassan virus (No. 1828) was isolated from blood clot which was obtained from a squirrel on October 3, 1962, about 12 miles west of Powassan. No virus was isolated from blood clots obtained from 23 other squirrels, two chipmunks and four snowshoe hares.

Powassan Neutralizing Antibody in Animal Sera

Neutralizing antibody to Powassan virus was detected in sera from three of 12 squirrels collected during early October 1962 (Table I). *I. marxi* ticks were removed from two of these squirrels which had antibody. Of 58 squirrels tested between July 1959 and October 1962, seven had antibody, and six of these were collected during September or October.

TABLE II.—VIRUS ISOLATIONS FROM TICKS AND NEUTRALIZING ANTIBODY TO POWASSAN VIRUS IN SERA FROM ANIMALS, POWASSAN DISTRICT, JULY 1950 TO OCTOBER 1962

					Virus in ticks	
Species	Number positive	Number tested	Number infested with ticks	Tick species	Number positive	Virus type
Squirrel	7	58	14	*I. marxi*	1	Powassan
Chipmunk	6	35	0	0	0	0
Snowshoe hare	0	24	24	H.L.P.	1	Silverwater
Groundhog	0	3	3	*I. cookei*	0	0
Field mouse	0	4	0	0	0	0
Sheep	0	8	0	0	0	0
Cattle	0	10	0	0	0	0
Horse	0	3	0	0	0	0
Grouse	0	3	1	H.L.P.	0	0
Other birds	0	14	0	0	0	0
Totals	13	162	42	3	2	2

Between 1959 and 1962, Powassan neutralizing antibody has been detected in sera from a total of seven of 58 squirrels and six of 35 chipmunks, but it has not been found in 24 snowshoe hares, three groundhogs, four field mice, eight sheep, 10 cattle, three horses, three grouse or 14 other birds (Table II).

Isolation of Silverwater Virus from H.L.P. Ticks

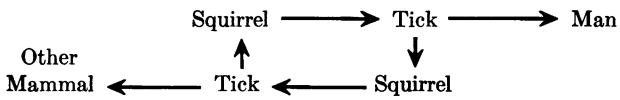
A pool comprising about 50 *Haemaphysalis leporis-palustris* ticks in all stages was removed from a snowshoe hare collected some eight miles east of Powassan on July 10, 1962. Inoculation of suckling mice, aged two days, with an extract of the tick pool in ox-serum saline was followed by encephalitis in one mouse after 10 days. The brain of the affected mouse was extracted with 1 ml. ox-serum saline. Intracerebral injection of a 10^{-1} dilution of supernatant induced encephalitis in suckling mice five to six days later, but no illness was observed in weaned mice. The intracerebral suckling mouse LD_{50}-titre of second-mouse-brain-passage virus was $10^{-5.5}$. This new isolate was termed strain No. 1081.

Sodium deoxycholate at 1:1000 final concentration reduced the infectivity of dilutions of strain No. 1081 by at least 3 $\log_{10}$ mouse LD_{50}. Guinea-pig anti-serum to prototype Silverwater virus strain No. 131 neutralized 30 LD_{50} of strains No. 1081 and No. 131, but strain No. 1081 was not neutralized by Powassan or Bunyamwera antisera. Antigen prepared from suckling-mouse brains infected with strain No. 1081 by the borate-protamine method[11] fixed complement readily in the presence of guinea-pig antisera to strain No. 1081 and Silverwater virus No. 131, but not in the presence of antisera to Powassan, Bunyamwera or EEE viruses. This demonstrates that strain No. 1081 is antigenically identical with the prototype Silverwater virus strain No. 131.

Neutralizing antibody to Silverwater virus strain No. 1081 was detected in sera of two snowshoe hares captured during July and August, but it was not found in sera of two hares collected during April 1962. Complement-fixing antibody was detected in four of 13 hares captured during 1960.

DISCUSSION

Isolation of Powassan virus from *I. marxi* ticks collected from a red squirrel about five miles from the former residence of a child who contracted fatal encephalitis due to Powassan virus four years previously, together with detection of virus and neutralizing antibody in sera of squirrels taken in the vicinity, strongly suggests that Powassan virus is maintained in nature by a cycle of infection involving ixodid ticks as vectors and squirrels as reservoirs. Man, and small mammals including chipmunks and possibly snowshoe hares, may become infected tangentially, and apparently play no part in the maintenance of the virus in nature, as shown in the diagram:



The occurrence of encephalitis due to Powassan virus in a child during September, the isolation of this virus from ticks during late August at a time of maximum tick infestation of squirrels, and the detection of neutralizing antibody in squirrels during September and early October, but not during early summer, strongly suggests that the maximum spread of this virus occurs during late summer. It seems likely that squirrels undergo symptomless viremia for several days following bites by infected ticks, after which antibody is produced.[2] The demonstration of viremia in a single squirrel in this study, together with the repeated demonstration of neutralizing antibody in these animals during four successive autumns, leaves little doubt that squirrels acquire infection during late summer, thereby serving as natural reservoirs of infection. Antibody which was detected in one squirrel during April was probably acquired during the previous summer, since all squirrels taken during April were born during the previous summer, and no antibody was detected in the current year's squirrels which were captured in May, July or August.

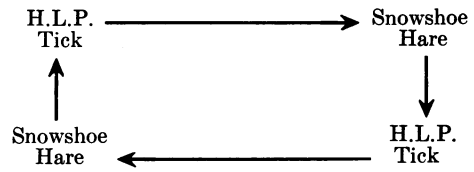
Human infection may be acquired by the bite of *I. marxi* ticks which are transferred during the skinning of squirrels, which is a common pastime of children in northern Ontario. Inadvertent inoculation of viremic blood into knife wounds sustained while skinning squirrels could also result in human infection. Although infected ticks resting on underbrush may attach themselves to man while he is walking through the forest, this seems unlikely, since the senior author (D.M.M.) has not yet encountered ticks on clothing or by dragging the forest floor with flannelette flags. The low incidence of Powassan antibody in local residents[4] suggests that relatively few persons contract subclinical infections.

Although some chipmunks have circulated Powassan antibody, ticks have not yet been found on these animals, even at a time of heavy infestation of squirrels. It appears unlikely that chipmunks serve as significant reservoirs for the maintenance of Powassan virus in nature; they are probably merely tangential hosts. Powassan antibody has been detected in a small proportion of sera from snowshoe hares on Manitoulin Island,[3] but none has been found in Powassan animals. Since hares become infested regularly with H.L.P. ticks, but only rarely by *I. marxi*, and since H.L.P. ticks infest hares almost exclusively,[9] snowshoe hares appear to be tangential hosts only in the Powassan virus cycle.

The spread of Powassan virus during the late summer closely resembles the peak incidence of human infection with the antigenically related Central European encephalitis (CEE) virus in Czechoslovakia, during July and August, when large numbers of urban dwellers walk through tick-infested forest and scrubland. However, in Czechoslovakia, bites by the vector tick *I. ricinus* account for only 60.2% of human infections, the remainder being contracted by ingestion of raw milk obtained

from animals which become infested while grazing in tick-infested scrubland adjacent to pastures.[12] The absence of Powassan antibody from domestic sheep and cattle[3] and the paucity of ticks in northern Ontario scrubland render the possibility of infection of humans[2, 4] and domestic animals considerably less common in Ontario than in Czechoslovakia. The peak incidence of Powassan virus infections of man and forest rodents is considerably later than the June peak for *I. persulcatus*—transmitted Russian spring-summer encephalitis in the Tartar Republic,[13] or the May and June peak for *D. andersoni*—transmitted Colorado tick fever infections in the western U.S.A.[14]

Although Silverwater virus has now been isolated from H.L.P. ticks collected both on Manitoulin Island and also in the Powassan area, its spread appears to be confined entirely between snowshoe hares as reservoirs and H.L.P. ticks as vectors, as shown in the diagram:



In view of the absence of Silverwater antibody from sera of 105 human residents of Manitoulin Island, and the absence of human infestation by H.L.P. ticks, it seems likely that infection with Silverwater virus is not a significant hazard for man.

SUMMARY

Powassan virus was isolated from *Ixodes marxi* ticks collected from a red squirrel, *Tamiasciurus hudsonicus*, and from the blood of another squirrel, near Powassan, Ontario, during late August 1962.

Neutralizing antibody to Powassan virus was found in seven of 58 squirrels. The antibody-positive squirrels were captured principally during September and October, and *I. marxi* ticks were found on three antibody-positive squirrels.

Silverwater virus was isolated from *Haemaphysalis leporis-palustris* ticks infesting a snowshoe hare, *Lepus americanus*, near Powassan during July 1960.

REFERENCES

1. McLEAN, D. M. AND DONOHUE, W. L.: *Canad. Med. Ass. J.*, **80**: 708, 1959.
2. McLEAN, D. M. *et al.*: *Amer. J. Public Health*, **50**: 1539, 1960.
3. McLEAN, D. M. *et al.*: *J. Infect. Dis.*, **109**: 19, 1961.
4. McLEAN, D. M. *et al.*: *Canad. Med. Ass. J.*, **86**: 971, 1962.
5. CASALS, J.: *Ibid.*, **82**: 355, 1960.
6. THOMAS, L. A., KENNEDY, R. C. AND EKLUND, C. M.: *Proc. Soc. Exp. Biol. Med.*, **104**: 355, 1960.
7. EKLUND, C. M.: Personal communication.
8. McLEAN, D. M.: *Fed. Proc.*, **20**: 443, 1961 (abstract).
9. SCHOLTEN, T. H., RONALD, K. AND McLEAN, D. M.: *Canadian Journal of Zoology*, **40**: 605, 1962.
10. THEILER, M.: *Proc. Soc. Exp. Biol. Med.*, **96**: 380, 1957.
11. CLARKE, D. H. AND CASALS, J.: *Amer. J. Trop. Med.*, **7**: 561, 1958.
12. BLASKOVIC, D.: *Trans. N.Y. Acad. Sci.*, s. 2, **23**: 215, 1961.
13. CHUEVA, S. V.: *Med. Parazit. (Moskva.)*, **27**: 308, 1958.
14. EKLUND, C. M., KENNEDY, R. C. AND CASEY, M.: *Rocky Mountain Med. J.*, **58**: 21, 1961.