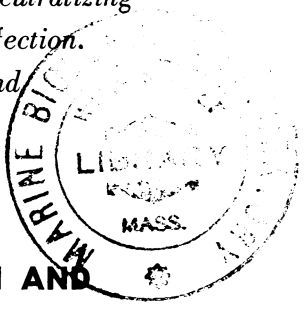
In 1958, a newly described agent of encephalitis, Powassan virus, was isolated in Canada. Serological surveys of residents of northern Ontario showed that 3 per cent of those tested had neutralizing antibody to this virus, indicating past subclinical infection. Presence of neutralizing antibody in chipmunks and squirrels in this area suggests that these rodents may be natural reservoirs of infection.



POWASSAN VIRUS: SURVEYS OF HUMAN AND ANIMAL SERA

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ALTHOUGH THE ARTHROPOD-BORNE viral encephalitides[1] have presented public health problems in many areas of western United States[2] and Canada[3] over the past three decades, their occurrence in eastern North America has been less frequent and their distribution was confined mainly to the eastern seaboard, from Texas to Massachusetts.[2] In Montreal during 1957, a child who developed encephalitis showed serological evidence of infection with western equine encephalomyelitis virus.[4] In 1958, a newly described agent, Powassan virus,[5] was isolated from the brain of a child dying of encephalitis who lived at Powassan, Ontario.

Powassan virus behaves as a member of Casals' group B of the arthropod-borne viruses.[6] In complement-fixation and hemagglutination inhibition tests, it showed fairly close antigenic relationship to Russian spring-summer encephalitis (RSSE) virus.[7] Although this relationship was also evident on neutralization tests, the viruses were distinct from each other. In addition, Powassan virus gave comparatively weak cross-reactions with St. Louis encephalitis virus, which is the only other group B arthropod-borne virus known to infect man in North America.

On account of this antigenic relationship between Powassan virus, and RSSE virus which is known to be transmitted by ixodid ticks,[8,9] it was considered likely that ticks may also be vectors of Powassan virus. Since ground squirrels and to a lesser extent chipmunks[10] are important natural reservoirs of Colorado Tick fever, the only arthropod-borne virus disease in North America which is known to be transmitted by ticks,[11] it was considered that forest rodents may also be reservoirs of Powassan virus. It should be emphasized that the vector of Powassan virus is at present unknown and, consequently, possible vectors including ticks and mosquitoes were investigated.

Methods and Materials

Serological surveys of healthy residents of several northern Ontario communities were carried out through the kind cooperation of Dr. J. E. Dillane, Powassan, and Dr. P. N. Karnauchow, North Bay.

Neutralization and complement-fixation tests were performed on all human sera received, according to methods described previously.[5] In neutralization tests, unheated sera (0.15 ml aliquots) were mixed with equal quantities of Powassan virus diluted to give 50 mouse LD_{50} per 0.03 ml of serum-virus mixture. After standing for one hour at room temperature, 0.03 ml aliquots were inoculated intracerebrally into groups of five mice each aged three weeks. Sera which neutralized 50 LD_{50} or more of virus were considered positive. Complement-fixation tests were performed in plastic hemagglutination plates using as antigens borate-saline extracts of infected suckling mouse brain. Antigens prepared from Powassan, St. Louis encephalitis, eastern equine and western equine encephalomyelitis viruses were used at their optimal dilutions which were determined in preliminary "box" titrations with their homologous antisera in the presence of two complete hemolytic units of complement.

In northern Ontario, mammals, excepting farm animals, and birds, excepting those from Algonquin Park, were obtained by shooting. Upon retrieving the specimen, the chest was opened and blood was pipetted out. After holding the blood overnight at 4° C, the serum was separated and transported to the Toronto laboratory at 4° C in a vacuum flask. In the laboratory, blood was obtained from hamsters and chickens by cardiac puncture. Blood from farm animals was obtained by venipuncture.

Ticks were sought along roadways and on the forest floor by dragging with flannelette flags two feet square. In addition, all animals shot in the field were placed in canvas bags. An examination for ticks and other ectoparasites was conducted the following morning.

Mosquitoes were captured while feeding on human beings by means of a sucking tube. They were held at room temperature and fed on an aqueous solution of sucrose until tested. For injection experiments, mosquitoes were anesthetized with CO_2 and injected using a finely drawn hard glass pipette as described previously.[12]

Table 1—Incidence of Neutralizing Antibody in Human Residents of Northern Ontario

Locality	Number Positive	Number Tested
Powassan	1	6
North Bay	2	65
Manitoulin Island	3	52
Sault Ste. Marie	0	57

Results

Sera obtained from 180 healthy residents of northern Ontario between April and August, 1959, were examined for antibody to Powassan virus (Table 1). Of six healthy family contacts of the Powassan index case described previously,[5] one had neutralizing and complement-fixing antibody to Powassan virus. This person and three other contacts who did not have antibody used to participate frequently with the index case in skinning chipmunks and squirrels which they had shot in forests adjacent to the homestead. The other two family contacts were not exposed to forest fauna.

Neutralizing antibody to Powassan virus was detected in sera of two persons out of 65 who lived in the North

Bay district. Sera from three residents of Manitoulin Island out of 52 tested contained antibody. However, no antibody was detected in sera donated by 57 residents of Sault Ste. Marie. None of these sera contained complement-fixing antibodies against Powassan, St. Louis, eastern equine or western equine encephalitis antigens. This suggests that foci of infection with Powassan virus exist in Powassan extending northwards to North Bay, and on Manitoulin Island.

During August and September, 1959, we examined sera from 28 wild and six domestic mammals, 12 wild birds and two domestic fowl, in the Powassan area, and from five wild birds including one ruffed grouse (Bonasa umbellus) and one squirrel on Manitoulin Island for evidence of neutralizing antibody to Powassan virus. Through the courtesy of Dr. A. M. Fallis, Ontario Research Foundation, sera from 11 wild birds which were kept in Algonquin Park during the summer of 1958 or 1959 were tested for Powassan antibody.

Neutralizing antibody was detected in sera of four chipmunks (Tamias striatus) out of 13 examined from the Powassan area (Table 2). Antibody was detected in the serum of one squirrel (Tamasciurus hudsonicus) from the Powassan district out of nine examined, but antibody was not detected in four mice (Peromyscus maniculatus); two snow-shoe rabbits (Lepus americanus); two horses, two cows, two dogs, and two fowl on the farm at which the index case resided; or in 17 birds mainly finches and sparrows near Powassan and on Manitoulin Island. All 11 Algonquin Park birds comprising five ruffed grouse, one spruce grouse (Canachites canadensis), three grackles (Quiscalus versicolor), and two Canada jays (Perisoreus canadensis) gave negative results in a neutralization test.

Table 2—Incidence of Neutralizing Antibody in Mammalian and Avian Sera

Locality	Species	Number Positive	Number Tested
Powassan	Chipmunk	4	13
	Squirrel	1	9
	Field mouse	0	4
	Rabbit	0	2
	Farm mammal	0	6
	Bird	0	14
Manitoulin Island	Squirrel	0	1
	Bird	0	5
Algonquin Park	Bird	0	11

These results show that chipmunks and squirrels are likely reservoirs of Powassan virus in nature. The absence of antibody in sera of 30 birds tested so far renders unlikely the possible role of birds as reservoirs of infection. Final assessment of this role of birds as reservoirs will await the result of tests on further bird sera.

All rodents and birds shot in the field were examined closely for ectoparasites. No ticks were found on ground game or birds, but Haemaphysalis leporis-palustris larvae, nymphs, and adult ticks were found on two rabbits taken near Powassan. Apart from occasional fleas which were found on some chipmunks and mites on one mouse, no blood-sucking ectoparasites were found either on animals or on the ground.

Pools of approximately 50 ticks were ground in chilled mortars and extracted with 10 per cent ox serum saline containing antibiotics. The clear supernatant following centrifugation at 1,500 RPM for five minutes was injected intracerebrally into groups of nine newborn mice. Virus was not isolated from any of four pools examined.

Aedes spp. mosquitoes were collected

Table 3—Response of Chickens to Subcutaneous Injection with Powassan Virus

Virus Dose Mouse LD_{50}	Viremia Days After Injection						Antibody 23 Days
	1	3	4	5	7	9	
1	0/3*	8/10	3/3	6/6	6/7	0/4	4/6
10	0/3	9/9	3/3	6/6	4/6	2/3	4/5
100	1/2	4/4	3/3	4/4	1/1	n.t.*	2/3

* Numerator: Number of chickens with viremia or antibody.
Denominator: Number of chickens tested.
n.t.: Not tested.

while attempting to feed on the investigators in a forest five miles east of Powassan during early September, 1959. No virus was isolated by inoculation of newborn mice with an extract of approximately 50 mosquitoes in ox serum saline.

Groups of two-day old chickens were inoculated subcutaneously with 1, 10, and 100 mouse LD_{50} of Powassan virus. Blood was obtained by cardiac puncture at intervals after inoculation. Blood was diluted 1:3 in sterile Alsevers' solution. Its virus content was titrated by intracerebral injection of three-week old mice in groups of five. Each mouse received 0.03 ml of serial tenfold dilutions of citrated blood in ox serum saline.

Viremia was detected in virtually all chickens between the third and seventh days after inoculation (Table 3). However, few chickens were circulating virus when bled on the first or ninth days after inoculation. Maximum titers of viremia ($10^{5.0}$ mouse LD_{50} per 0.03 ml) were attained on the third and fourth days but they fell to titers of $10^{1.5}$ on the seventh day. The titers of viremia was independent of the virus dose administered. Thus the recently hatched chicken was as sensitive an indicator of virus in a seed lot as the weaned mouse inoculated intracerebrally. Antibody which neutralized to 50 LD_{50} or more of Powassan virus was detected in sera of most birds when bled 23 days after inoculation. At no time did any chicken develop signs of encephalitis.

Viremia was produced in hamsters regularly on the second, fourth, and fifth days after subcutaneous injection of 10^4 mouse LD_{50} of Powassan virus, but viremia was not detected six or eight days after inoculation (Table 4). However, of six hamsters which received merely 10 LD_{50} of virus, only three had viremia when tested four, five, or six days after inoculation.

Two laboratory rabbits were injected subcutaneously with 10^4 mouse LD_{50} of Powassan virus. One rabbit produced a low titer viremia four days after inoculation. Neutralizing antibody was detected in sera of both animals when tested 14 days after inoculation.

Culex fatigans mosquitoes captured near Toronto were injected with 10^3 mouse LD_{50} of Powassan virus. Although most mosquitoes contained virus immediately after injection, none was detected in two mosquitoes which were tested after being held at 80° F for four days, nor in a further two mosquitoes which were tested after seven days of extrinsic incubation.

Discussion

Isolation of Powassan virus, a group B member of the arthropod-borne

viruses, from the brain of a child who succumbed to encephalitis during September, 1958,[5] stimulated investigations of possible natural reservoirs and vectors. Following subcutaneous injection of small doses of Powassan virus in the laboratory, young chickens and hamsters produced a transient, asymptomatic viremia. The titer and duration of viremia of Powassan virus in two-day old chickens approximated closely the results of St. Louis encephalitis virus in 0.5-day-old chickens[13] or Murray Valley encephalitis virus in two-day-old chickens.[14] Production of neutralizing antibody followed the disappearance of viremia in the case of each virus. It is possible that forest rodents and birds react similarly to injection of small doses of Powassan virus by syringe or by the bite of an infected arthropod. Since neutralizing antibody was detected in sera obtained from four chipmunks and one squirrel near Powassan, it seems likely that these rodents may be natural reservoirs of infection.

The mechanism of transfer of infection from natural reservoirs to man remains an enigma. Ixodid ticks which bite man such as Dermacentor variablis are seen by local residents most infrequently. During the summer of 1959, the only ixodid ticks found by us were Haemaphysalis leporis-palustris, and these infested two rabbits which were shot. These rabbits did not circulate Powassan antibody. Only rarely do these ticks bite man, and they have not so far been found on chipmunks or squirrels. The low rate of inapparent infection amongst the local human population is in favor of a vector which bites man occasionally. It is possible that Haemaphysalis ticks may perpetuate a rodent-tick-rodent cycle in the same manner as Haemaphysalis spinigera ticks are vectors of Kyasanur Forest disease virus among forest primates and rodents in India.[15] Since our index case at Powassan was exposed to ground game repeatedly, it is conceivable that he may have become infected by the bite of a vector which alighted from a recently captured rodent.

It seems unlikely that culicine mosquitoes may act as vectors of Powassan virus. Virus was not isolated from mosquitoes captured near Powassan. Mosquitoes were not abundant during late summer at which time the case of encephalitis occurred. Virus did not multiply following injection into the body cavity of mosquitoes, unlike Murray Valley encephalitis[12] or other mosquito-borne viruses. Furthermore the close serological relationship which exists between Powassan virus and RSSE virus which is transmitted by ticks and not by mosquitoes favors ticks rather than mosquitoes as vectors of Powassan virus.

Table 4—Response of Hamsters to Subcutaneous Injection with Powassan Virus

Virus Dose	Viremia Days After Injection					Antibody 23 Days		
Mouse LD_{50}	2	4	5	6	8	14	17	36
10	0/3*	1/3	1/3	2/3	0/6	1/1	2/2	2/2
10^4	3/3	3/3	3/3	0/2	0/6	1/1	2/2	2/2

* Numerator: Number of hamsters with viremia or antibody.
Denominator: Number of hamsters tested.

Summary

In serological surveys of residents of northern Ontario, 3 per cent of persons tested had neutralizing antibody to Powassan virus, which indicates past subclinical infection with this virus. The presence of neutralizing antibody in four out of 13 chipmunks and one out of ten squirrels captured in these districts suggests that these rodents may be natural reservoirs of infection.

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