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Arbovirus Prevalence in the East Kootenay Region, 1968

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ARBOVIRUSES comprise at least 204 catalogued agents,[1] 40 of which have been recovered directly from humans. Illnesses in patients have been attributed to 38 arboviruses, which induce syndromes varying from mild to moderate febrile upsets with or without rashes to severe or fatal conditions such as hemorrhagic fever, liver damage or encephalitis.[2] Within the Nearctic zoogeographic region which includes most of North America, activity of 31 arboviruses has currently been demonstrated and two agents, Powassan[3] and Silverwater,[4] were first isolated in Canada. Encephalitis in Canadian patients has been associated with two agents: Powassan virus in eastern Canada,[3] which is maintained in nature by a cycle involving ixodid ticks and wild rodents;[5] and western equine encephalomyelitis virus on the prairies of western Canada[6] whose natural cycle involves virus transfer between culicine mosquitoes and birds.[7]

In a survey of 1268 sera from human residents of southeastern British Columbia during the winter of 1967, hemagglutination inhibition (HI) antibodies to Powassan virus were detected in 25 subjects, including 15 from the East Kootenay Health Unit, but none of these sera neutralized this agent.[8] HI tests also detected antibodies against western equine encephalomyelitis virus in 21 subjects, and 16 of these sera also neutralized the viral agent; a further 21 sera inhibited hemagglutination by California encephalitis (BFS-283 strain), which was neutralized by three of the sera. Only one serum each from the Selkirk and West Kootenay Health Units fixed complement in the presence of Colorado tick fever virus, and neither serum neutralized this virus. However, during 1965 and 1966 the isolation of Colorado tick-fever virus from 8 of 22 pools of *Dermacentor andersoni* adult ticks collected by dragging in the Selkirk District suggests that this virus is endemic in eastern British Columbia.[9] Detection of Powassan neutralizing antibodies in sera from 37 of 699 small forest rodents in the East Kootenay, West Kootenay and South Okanagan Districts of eastern British Columbia during the summer of 1967, and complement fixing antibodies to California encephalitis virus in 8 of 117 animals, further strengthens the hypothesis that these agents are endemic in nature in these regions.[10]

This paper reports further evidence of the endemic prevalence of two group B arboviruses, tick-borne Powassan virus and mosquito-borne St. Louis encephalitis virus, in wildlife in the East Kootenay Region of southeastern British Columbia during 1968, together with some evidence of human infection with St. Louis encephalitis virus.

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METHODS AND MATERIALS

Field Specimens

Between March 27 and August 27, 1968, serum samples were collected from 1243 small forest rodents within a 30-mile radius of Cranbrook, British Columbia (116°W, 49° 30'N). Most collection sites were alongside logging roads through forests in mountainous areas covered with sparse to dense stands of conifers, but occasional sites such as F and S were undulating grassy areas beside main roads. Altitudes ranged from 2700 to 4400 feet above sea level.

Whenever possible, animals were collected in live traps, bled by cardiac puncture under ether anesthesia, marked with ear tags and released for attempts at recapture subsequently. However, the majority of animals were collected by shooting, and blood was collected after extravasation into the pleural cavities. After separation of sera from clots by centrifugation, both components were held at –20° C. until shipped under refrigeration to Vancouver, where they were again stored at –20° C. until tested.

All captured animals were examined for the presence of ticks, which were removed and held at 4° C. until tested. Ticks were also collected between March and June by dragging the forest floor and grassy roadside areas with flannelette flags.

No attempts were made to collect birds, since it was not apparent until the virtual conclusion of the summer field program that mosquito-borne St. Louis encephalitis may also be prevalent in this district. Unusually low populations of mosquitoes during late summer provided no opportunity for the execution of a systematic program of collection of mosquitoes.

Serological Tests

All sera were extracted twice with acetone, absorbed with goose erythrocytes and examined simultaneously for hemagglutination inhibition (HI) antibodies against four to eight agglutinating doses of three group B arbovirus antigens, Powassan, Murray Valley encephalitis (MVE) and Modoc, together with one group A arbovirus antigen, western equine encephalomyelitis (WEE).[10]

Previous experience has shown that sufficient antigenic overlap between MVE and St. Louis encephalitis (SLE) viruses occurs in HI tests to permit the use of MVE virus in serological surveys.[5] Modoc virus is endemic in Californian wild rodents.

All tests were performed in Microtiter*[11] plates using 0.025-ml. amounts of sera and antigen diluted in borate-saline buffer containing 0.4% bovalbumin at pH 9.0, plus 0.05-ml. portions of 0.25% suspensions of goose erythrocytes in appropriate virus adjusting diluents. Antigens were prepared by acetone-sucrose extraction of brains of suckling mice infected with the prototype strain of each arbovirus.[12]

Attempts were made to examine all sera with group B arbovirus HI activity by neutralization tests performed on the same day against the prototype LB strain of Powassan virus[3] and the Parton strain of SLE virus.† Neutralization tests were conducted by intracerebral inoculation of groups of three weaned mice aged 3 to 4 weeks, with 0.03-ml. amounts of mixtures of undiluted serum with 100 mouse 50% lethal doses (LD_{50}) of virus.[5] Whenever possible, sera which inhibited hemagglutination by WEE virus were examined in mouse neutralization tests against the McMillan strain of WEE virus. Sera from 59 of 61 *Citellus lateralis* were examined by intracerebral inoculation of families of suckling mice aged 1 to 4 days, using 100 LD_{50} of the Florio strain of Colorado tick fever virus.

Complement fixation tests were performed in Microtiter plates using two hemolytic units of complement and optimal dilutions of antigens for SLE, California encephalitis (BFS-283 strain) and Colorado tick fever virus. All sera were screened for anticomplementary activity before use in these tests.

Virus Isolation Attempts

All tick pools were ground in mortars and suspended in 2 ml. of Earle's saline containing 0.5% lactalbumin hydrolysate, 0.1% yeast extract and 20% newborn-calf serum. These undiluted suspensions were injected intracerebrally into families of suckling mice aged 1 to 4 days, but suspensions from pools of blood-engorged ticks, diluted 1:10 and 1:100, were also injected.

All blood clots were extracted with 2 ml. of Earle's saline containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract, centrifuged at 2500 r.p.m. for 30 minutes to deposit bacteria introduced inadvertently in the field, and 1.0-ml. amounts were inoculated into each of two primary monolayer tube cultures of swine kidney cells which were incubated at 37° C. for one week.[13]

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TABLE I.—ARBOVIRUS ANTIBODIES BY SPECIES AND MONTH, CRANBROOK, 1968

	C. columbianus				*C. lateralis*				*T. hudsonicus*				*E. amoenus*				*Others*				
Test	*May*	*June*	*July*	*Aug.*	*May*	*June*	*July*	*Aug.*	*May*	*June*	*July*	*Aug.*	*May*	*June*	*July*	*Aug.*	*May*	*June*	*July*	*Aug.*	*Total*
NT (P)	2	9	12	2	0	0	0	0	0	0	..	0	0	1	0	0	0	0	0	0	26
	100	90	99	21	8	7	4	5	10	7		5	5	7	11	6	8	5	1	10	409
NT (S)	21	9	18	4	0	0	1	0	3	3	..	1	0	1	0	0	0	0	0	0	61
	99	90	99	21	6	8	4	5	9	7		5	3	7	11	6	3	5	1	10	399
HI (P)	39	21	21	8	6	6	0	0	7	3	0	0	1	4	1	1	3	2	0	2	125
HI (M)	17	29	28	3	0	0	1	0	0	1	0	1	2	1	6	2	1	0	0	4	96
HI (Mod)	9	4	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	17
HI (Het.)	35	36	50	9	2	2	3	5	4	3	0	4	1	1	4	3	4	2	1	4	173
HI (all)	100	90	99	21	8	8	4	5	11	7	0	5	5	7	11	6	8	5	1	10	411
	230	301	217	74	12	30	11	8	47	24	7	31	34	25	42	31	34	29	9	47	1243

NT = Neutralization of virus. S = St. Louis encephalitis virus. HI = Hemagglutination inhibition. P = Powassan virus. M = Murray Valley encephalitis virus. Mod = Modoc virus. Het. = More than one agent.

RESULTS

Group B Arbovirus Antibody Reactions

Hemagglutination inhibition reactions against one or more group B arbovirus antigens were detected in 411 of 1243 (33%) sera collected from 1210 small forest rodents near Cranbrook during the spring and summer of 1968 (Table I). Although daily collections of animals were commenced regularly on May 3 and discontinued on August 27, results of tests on 15 sera from animals obtained between March 27 and April 3 have been included in the total of 357 sera recorded for May. Positive reactors included 310 of 822 (38%) columbian ground squirrels (*Citellus columbianus*), 25 of 61 (41%) golden mantled ground squirrels (*Citellus lateralis*), 23 of 109 (21%) red squirrels (*Tamiasciurus hudsonicus*), 29 of 132 (22%) yellow pine chipmunks (*Eutamias amoenus*) and 24 of 119 (20%) other mammals. These other species included 11 of 93 deer mice (*Peromyscus maniculatus*), 11 of 21 bushytail woodrats (*Neotoma cinerea*), 1 of 2 snowshoe hares (*Lepus americanus*), 1 of 1 domestic rat (*Rattus rattus*), 0 of 1 Townsend chipmunk (*Eutamias townsendi*) and 0 of 1 northern bog lemming (*Synaptomis borealis*) (Table II).

Of the 411 sera which inhibited hemagglutination by one or more group B arboviruses, 125 inhibited Powassan virus exclusively, 96 reacted solely with MVE virus, 17 reacted exclusively with Modoc virus and 173 inhibited hemagglutination by more than one agent (Table I). Of these 411 sera with group B antihemagglutinins, 26 of 409 neutralized Powassan virus and 61 of 399 neutralized SLE virus, including 18 which neutralized both viruses. Of the latter 18 sera, 2 inhibited hemagglutination by Powassan virus exclusively, 4 inhibited MVE hemagglutinin and 12 showed heterologous reactions to more than one antigen. None of 14 sera tested from the 17 which inhibited hemagglutination by Modoc virus exclusively, neutralized this agent, none of these 17 neutralized Powassan virus, and 2 of 17 neutralized SLE virus.

TABLE II.—GROUP B ANTIHEMAGGLUTININ PREVALENCE IN 119 OTHER ANIMALS

	Month				
Species	*May*	*June*	*July*	*August*	*Total*
Peromyscus maniculatus	4/27	3/21	0/8	4/37	11/93
Neotoma cinerea	2/4	2/6	1/1	6/10	11/21
Lepus americanus	1/1	0/1	..	..	1/2
Rattus rattus	1/1	..	..	..	1/1
Eutamias townsendi	0/1	..	..	..	0/1
Synaptomis borealis	..	0/1	..	..	0/1
Total	8/34	5/29	1/9	10/47	24/119

Although the incidence of neutralizing antibodies to both Powassan and SLE viruses in HI-positive sera was low, it seemed unlikely that a substantial number of HI-negative sera would neutralize Powassan virus. During May, only 3 of 225 sera devoid of group B antihemagglutinins neutralized this agent.

Sera were collected at 12 general sampling areas within 30 miles of Cranbrook. Some areas such as F (a roadside stone quarry flanked by sparsely forested rocky bluffs) and T (a rock slide secluded from the highway by pine trees) were localized to about two acres each. Other areas such as D and N consisted of undulating forested country with some grassy slopes which were traversed by logging roads for two to five miles. The altitudes of all these areas ranged from 2700 to 3000 feet above sea level. Area P, on the other hand, was a relatively circumscribed area of sloping grassland at 4000 feet altitude adjacent to a dense stand of tall conifers.

The incidence of group B arbovirus antihemagglutinins ranged from 24% at area E to 51% at area J, but in most test areas the proportion of HI-positive sera was within five percentage points of the overall incidence of 33% (Table III). The proportion of sera which neu-

TABLE III.—ARBOVIRUS ANTIBODY PREVALENCE AT TEST AREAS AROUND CRANBROOK, 1968

Area	*C. columbianus*				*C. lateralis*				*T. hudsonicus*				*E. amoenus*				*Others*				*Total No.*		%
	May	*June*	*July*	*August*	*May*	*June*	*July*	*August*	*May*	*June*	*July*	*August*	*May*	*June*	*July*	*August*	*May*	*June*	*July*	*August*	*HI*	*NT*	*HI*
A	..	..	*3 (1)	2 (1)†	..	..	..	..	1 (0)	..	..	2 (0)	1 (0)	..	..	0	1 (0)	..	..	..	10	(2)	28
			4 (0)	10 (1)					4 (0)			7 (0)	5 (0)			5	1 (0)				36	(1)	
D	9 (0)	41 (5)	39 (0)	3 (0)	..	..	..	..	..	..	..	..	1 (0)	..	..	5 (0)	..	..	..	..	98	(5)	36
	17 (1)	148 (5)	87 (2)	8 (0)									1 (–)			8 (0)					269	(8)	
E	12 (0)	1 (0)	4 (2)	0	1 (0)	..	..	..	5 (0)	0 (0)	0	..	0	0	0	0	..	..	..	..	23	(2)	24
	25 (4)	19 (0)	27 (2)	1	1 (0)				6 (3)	3 (0)	3		5	1	1	3					95	(9)	
F	4 (1)	5 (0)	..	0	1 (0)	0	..	0 (0)	1 (0)	..	..	..	1 (0)	0	..	0	3 (0)	0	..	..	15	(1)	29
	9 (3)	6 (0)		3	4 (0)	3		1 (0)	6 (0)				4 (–)	1		2	11 (0)	2			52	(3)	
H	2 (0)	6 (1)	21 (1)	..	..	..	..	..	1 (0)	1 (0)	0	..	0	1 (0)	5 (0)	0	..	..	..	..	37	(2)	29
	15 (2)	23 (0)	40 (5)						11 (–)	1 (0)	1		3	4 (0)	25 (0)	2					125	(7)	
J	13 (0)	8 (2)	8 (4)	..	..	..	0	..	1 (0)	1 (0)	..	..	1 (0)	..	..	..	..	..	..	..	32	(6)	51
	26 (5)	15 (1)	12 (2)				3		2 (0)	1 (0)			4 (0)								63	(8)	
N	16 (1)	..	7 (1)	..	..	..	..	..	0	..	0	1 (0)	1 (0)	..	0	1 (0)	..	..	..	..	26	(2)	30
	55 (3)		10 (1)						8		2	1 (0)	2 (0)		6	2 (0)					86	(4)	
P	..	9 (1)	1 (0)	1 (0)	..	0	..	..	..	2 (0)	0	..	..	1 (1)	0	0	..	..	..	..	14	(2)	27
		23 (2)	7 (0)	2 (0)		5				8 (2)	1			4 (1)	1	1					52	(5)	
Q	..	3 (0)	9 (3)	..	..	0	..	..	..	0	..	0	..	..	..	1 (0)	..	..	..	..	13	(3)	38
		3 (1)	16 (6)			1				1		9				4 (0)					34	(7)	
S	25 (0)	7 (0)	2 (0)	..	1 (0)	6 (0)	..	..	0	..	..	1 (0)	0	0	..	1 (0)	0	2 (0)	0	1 (0)	46	(0)	29
	42 (2)	17 (0)	6 (0)		2 (0)	15 (0)			1			9 (0)	4	5		7 (0)	7	15 (0)	1	26 (0)	157	(2)	
T	16 (0)	3 (0)	5 (0)	1 (0)	5 (0)	2 (0)	4 (0)	5 (0)	1 (0)	0 (0)	..	0	0	2 (0)	1 (0)	1 (0)	4 (0)	3 (0)	1 (0	)9 (0)	63	(0)	38
	31 (1)	22 (0)	8 (0)	6 (0)	5 (0)	6 (0)	8 (1)	7 (0)	7 (0)	2 (0)		2	1	2 (0)	1 (0)	2 (0)	15 (0)	12 (0)	8 (0)	21 (0)	166	(2)	
V	3 (0)	7 (0)	..	14 (1)	..	..	..	..	1 (0)	3 (0)	..	1 (0)	0	3 (0)	..	2 (0)	..	..	..	..	34	(1)	32
	10 (0)	25 (0)		44 (3)					2 (0)	8 (0)		3 (1)	5	8 (0)		3 (0)					108	(5)	
All	100 (2)	90 (9)	99 (12)	21 (2)	8 (0)	8 (0)	4 (0)	5 (0)	11 (0)	7 (0)	0	5 (0)	5 (0)	7 (1)	11 (0)	6 (0)	8 (0)	5 (0)	1 (0)	10 (0)	411	(26)	33
	230 (21)	301 (9)	217 (18)	74 (4)	12 (0)	30 (0)	11 (1)	8 (0)	47 (3)	24 (3)	7	31 (1)	34 (0)	25 (1)	42 (0)	31 (0)	34 (0)	29 (0)	9 (0)	47 (0)	1243	(61)	

*Numerator: number of sera with group B antihemagglutinins. Denominator: number of animal sera tested. †Parentheses: upper and lower numbers signify group B inhibitory sera which neutralized Powassan and St. Louis encephalitis virus respectively.

tralized Powassan virus approached 10% at area J (51% antihemagglutinins) and area Q (38% inhibitory sera), but at area T with an equally elevated incidence of HI reactors (38%) no serum neutralized Powassan virus. Area F, which yielded the highest proportion of Powassan neutralizing antisera during 1967, showed a relatively minute incidence of Powassan neutralizing antibody during 1968. In all areas except A, more sera neutralized SLE virus than Powassan virus.

The proportion of HI-positive sera among *C. columbianus* of all ages fluctuated from 43% during May through 30% in June, 46% in July and 28% in August (Table IV). Except during May when the incidence of HI reactors in the current season's animals was somewhat higher than in older specimens, presumably owing to transplacental transfer of maternal antibody, relatively minor variations of antibody incidence were noted during each month for the three age groups. Powassan virus was neutralized by a somewhat higher proportion of sera from adult and juvenile animals collected during July and August than from animals of comparable age which were collected during May and June. A comparable trend was also noted for the incidence of SLE neutralizing antibody.

At areas F, S and T, 27 animals which were trapped initially during May or June were retrapped 1 to 12 weeks subsequently. These in-

TABLE IV.—AGE INCIDENCE OF ARBOVIRUS ANTIBODIES IN *C. columbianus* CRANBROOK, 1968

	May		*June*		*July*		*Aug.*		*Total*	
Age	*HI*	*NT*	*HI*	*NT*	*HI*	*NT*	*HI*	*NT*	*HI*	*NT*
Adults	67	2	57	4	59	7	15	2	198	15
	166	13	195	5	134	8	45	4	540	30
Juvenile	15	0	11	1	23	5	6	0	55	6
	34	3	36	1	44	7	29	0	143	11
Current	18	0	22	4	17	0	..	..	57	4
	30	5	70	3	39	3			139	11
Total	100	2	90	9	99	12	21	2	310	25
	230	21	301	9	217	18	74	4	822	52

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TABLE V.—GROUP B ANTIHEMAGGLUTININ RESPONSE IN 27 RETRAPPED ANIMALS

Species	Total tested	Weeks between samples	Initial and final antibody status			
			Rise	High	Fall	Absent
C. columbianus	7	3	1	1	1	..
		6	1	..	2	1
C. lateralis	12	1	..	..	..	1
		3	1	1	5	1
		6	..	..	1	1
		8	1	..	..	..
E. amoenus	1	2	1	..	..	..
P. maniculatus	4	3	..	..	1	1
		6	..	..	..	1
		12	..	..	..	1
N. cinerea	3	3	3	..	..	..
Total	27	1–12	8	2	10	7

cluded 22 which were retrapped once, 4 on two subsequent occasions and 1 on three occasions, but only the results of antibody determinations on the initial and final sera from each animal are recorded. Although 8 animals showed fourfold or greater increments of group B arbovirus antihemagglutinin and another 2 had unchanged titres, 10 animals showed declining antibody levels and 7 remained HI-negative (Table V). None of the HI-positive sera neutralized Powassan or SLE viruses.

Sera were collected by C. R. Schornhurst, D.V.M., from 65 domestic animals including 59 dogs and 6 cats, whose owners resided within 60 miles of Cranbrook. Hemagglutination by Powassan virus exclusively was inhibited by three dog sera and HI reactions to all three group B arboviruses were observed with one dog serum.

Through the collaboration of several practising veterinarians in the Vancouver metropolitan area, sera were collected from 55 dogs. Inhibition of hemagglutination by Powassan virus only was noted in four sera, and two sera showed HI cross-reactions with all three group B arboviruses. Sera from the two animals with heterologous HI responses did not neutralize Powassan or SLE viruses.

Other Arbovirus Antibodies

Complement fixation tests using antigens for SLE, California encephalitis and Colorado tick fever viruses were conducted on 1079 wild animal sera from Cranbrook. Anti-complementary activity occurred in 256 sera (24%), so that 823 were available for assay for the occurrence of arbovirus antibodies at 1:5 or higher dilutions. Although reactions occurred exclusively against SLE antigen in nine sera, California encephalitis in another nine and Colorado tick fever in four, none of seven SLE reactors for which sera were available neutralized this virus, and none of five reactors with California encephalitis antigen neutralized this agent.

Although 59 of the 61 *C. lateralis* collected were examined for neutralizing antibody to Colorado tick fever virus by inoculation of suckling mice, no antibody was detected.

Antihemagglutinin to WEE virus was detected in 30 of 1243 sera, 15 of which also inhibited hemagglutinin by one or more group B arboviruses. None of 21 sera with positive HI reactions to WEE antigen, which were available for testing, neutralized this agent.

Ticks

Dermacentor andersoni ticks, principally engorged nymphs, were removed from 29 animals including three *C. columbianus* during May, 16 *C. lateralis* (four in May, nine in June, three in July), six *T. hudsonicus* (two each in May, June and August), three *E. amoenus* (two in May, one in June) and one *N. cinerea* during May. The mean number of ticks which infested each animal was 2.5, but actual collections ranged from one to nine per rodent. Intracerebral inoculation of suckling mice with suspensions of 29 ground-up tick pools, each of which was made from all the ticks from one rodent, failed to yield any virus.

Inoculation of suckling mice with 28 pools of unengorged *D. andersoni* nymphs and adults, collected by dragging the underbrush with flannelette flags, failed to yield virus. Pool sizes for dragged ticks averaged 7.0 and ranged from 1 to 36 specimens. Seven pools were collected between March 26 and April 3, 15 pools were obtained between May 3 and May 19 and six pools were secured between June 1 and June 24.

Animal Blood

Blood clots which were separated from 800 sera examined by HI and other serological tests were assayed for virus content by inoculation of primary monolayer tissue cultures of swine kidney cells in roller tubes. No virus was isolated.

Human Sera

During April 1968, sera were obtained from 20 students aged 10 to 14 years and also from one teacher, at a residential school located on farmland near area A. During the summer holidays three students returned to their homes which were located in rural areas of the East Kootenay District more than 50 miles from Cranbrook. Serum from one student inhibited hemagglutination by SLE virus at a titre of 20, and the teacher's serum showed a HI titre of 10 against this antigen. Both of these sera neutral-

ized SLE virus following inoculation of mice. No serum inhibited hemagglutination by other group B antigens including Powassan, MVE and Modoc viruses, or by WEE antigen from group A.

DISCUSSION

Detection of group B arbovirus antihemagglutinins in 411 of 1243 wild rodents in the East Kootenay District of British Columbia, including 26 sera which neutralized Powassan virus and 61 which neutralized St. Louis encephalitis virus, points strongly towards the endemic prevalence of these two arboviruses in this region. This evidence is strengthened by the demonstration of neutralizing and hemagglutinin inhibiting antibodies to SLE virus in sera from 2 of 21 members of a residential school near Cranbrook, which suggests that human infections may occur tangentially to a natural cycle involving wild vertebrates and arthropods. Furthermore, detection of SLE neutralizing antibodies in 5 of 81 animal sera with group B arbovirus antihemagglutinins which were collected during 1967[10] provides additional evidence of activity of SLE virus, together with Powassan virus, at Cranbrook.

Natural reservoirs of group B tick-borne arboviruses consist mainly of rodents such as groundhogs (*Marmota monax*) and to a lesser extent red squirrels (*Tamiasciurus hudsonicus*) for Powassan virus in eastern Canada[5] and Upstate New York,[14] but in Scotland[15] and Austria[16] smaller rodents such as mice and voles are important vertebrate reservoirs of the related but antigenically distinct louping ill and Central European encephalitis virus respectively. In western Canada however, such as the East Kootenay Region of British Columbia where *M. monax* is not found regularly, it seems likely that ground squirrels, *Citellus columbianus*, in addition to *Tamiasciurus hudsonicus*, may be important natural reservoirs of Powassan virus. The acquisition of Powassan neutralizing antibody by increasing proportions of adult and juvenile *C. columbianus* with the progress of summer suggests that they become infected during May and June at the time of maximum tick activity, but the low rate of antibody acquisition by the current season's animals suggests relatively little virus activity in 1968. However, their low rate of infestation by the prevalent species of ixodid tick, *D. andersoni*, in contrast to the higher rate of infestation of *M. monax* by *Ixodes cookei* in eastern Canada, may explain the low total incidence of Powassan neutralizing antibody in *C. columbianus*, both in 1967 and 1968.

Hemagglutination inhibition tests by the Microtiter technique appeared to afford the optimal serological screening procedure for evidence of group B arbovirus infections. Upon testing 225 sera without group B arbovirus antihemagglutinins, only three neutralized Powassan virus. However, the high proportion of Cranbrook sera which inhibited hemagglutination by group B arboviruses in the absence of detectable neutralizing antibody to Powassan and SLE viruses parallels findings obtained for antibody prevalence of these agents in wildlife of upstate New York.[14]

Dermacentor andersoni ticks, which are known vectors of Colorado tick fever in the western United States,[17] were also removed from 29 animals near Cranbrook during 1968. Although ticks infested *C. lateralis* more frequently than other wild animals, Powassan neutralizing antibody was not detected in this species, in contradistinction to its finding in 15 of 822 *C. columbianus* whose serum neutralized this agent, despite a substantially lower rate of tick infestation. Recently it has been shown that Powassan virus has been transferred transstadially to nymphs from larvae which were infected by feeding on a rabbit rendered viremic by intravenous injection of Powassan virus 48 hours after the ticks became attached.[18] Moreover, virus was transmitted to a guinea-pig following the bite of nymphs which emerged from these larvae, and an enhancement of virus content was noted in the adult ticks which emerged from these infected nymphs. This suggests that *D. andersoni* may be a natural vector of Powassan virus.

Summary Between May and August 1968 hemagglutinin inhibiting (HI) antibodies to one or more group B arboviruses were detected in 411 of 1243 sera from wild rodents collected in forested mountainous terrain near Cranbrook, B.C. These 411 sera included 125 with HI reactions against Powassan virus exclusively, 96 to Murray Valley encephalitis, 17 to Modoc and 173 to more than one agent. Sera from 26 of 409 animals with group B arbovirus antihemagglutinins neutralized tick-borne Powassan virus and 61 of 399 group B inhibitors neutralized mosquito-borne St. Louis encephalitis virus. The HI prevalence rates among commonly encountered animal species included 310 of 822 (38%) *Citellus columbianus*, 25 of 61 (41%) *Citellus lateralis*, 23 of 109 (21%) *Tamiasciurus hudsonicus*, 29 of 132 (22%) *Eutamias amoenus* and 24 of 119 (20%) other mammals. Neutralizing antibody to Colorado tick fever virus was not found in sera of 59 *C. lateralis*. No virus was isolated from 57 pools of *Dermacentor andersoni* ticks.

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Sera from two of 21 persons at a residential school contained HI and neutralizing antibodies to St. Louis encephalitis virus. These results suggest the endemic prevalence of Powassan and St. Louis encephalitis viruses in the East Kootenay Region.

Résumé Du mois de mai au mois d'août 1968, on a recueilli 1243 spécimens de sérum chez des rongeurs sauvages habitant dans les montagnes boisées près de Cranbrook, C. B. Sur 411 de ces échantillons, on a découvert des anticorps inhibant l'hémagglutinine (IH) d'un ou de plusieurs virus Arbor (arbovirus) du groupe B. Parmi ces 411 sérums, on trouvait 125 spécimens ayant une réaction IH contre le virus Powassan exclusivement, 96 contre l'encéphalite de Murray Valley, 17 contre le Modoc et 173 contre plus d'un pathogène. Le sérum de 26 des 409 animaux ayant des antihémagglutinines du groupe des arbovirus du groupe B ont neutralisé le virus Powassan (dont le vecteur est une tique) et 61 des 399 inhibiteurs du groupe B ont neutralisé le virus de l'encéphalite de St. Louis (vecteur: moustique). Les proportions de prédominance de IH chez les animaux fréquemment piégés étaient les suivantes: 310 *Citellus colombianus* sur 822 (38%), 25 *Citellus lateralis* sur 61 (41%), 23 *Tamiasciurus hudsonicus* sur 109 (21%), 29 *Eutamias amoenus* sur 132 (22%) et 24 autres mammifères sur 119 (20%). Dans les spécimens de sérum de 59 *C. lateralis*, on n'a trouvé aucun anticorps neutralisant de la fièvre à tique du Colorado. On n'a pas non plus décelé de virus de 57 "pools" de tique de la fièvre pourprée des Montagnes Rocheuses *(Dermacentor andersoni)*.

Le sérum de deux des 21 élèves fréquentant une école privée contenait des anticorps IH et neutralisants de l'encéphalite de St. Louis. Ces résultats laissent croire à une endémie des virus Powassan et de l'encéphalite de St. Louis dans la région de Kootenay est.

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