Arbovirus infections in man in British Columbia

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Summary: During the summer of 1971, the first laboratory-proved cases of acute encephalitis in man due to any of the known arboviruses occurred in the south-central region of British Columbia. Five human cases of encephalitis with two deaths were diagnosed; three of these patients, including one of the fatalities, were proven in the laboratory to have contracted western equine encephalitis.

During 1968 and 1969, a human serum survey undertaken in approximately 2000 life-long residents of the province discovered low levels of hemagglutinin-inhibiting and/or complement-fixing as well as neutralizing antibodies for western equine encephalitis, St. Louis encephalitis, Powassan encephalitis, California encephalitis and Colorado tick fever. Evidence of recent subclinical infection was detected in some cases.

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The proximity of the province to known sites of endemic prevalence of arboviruses in Alberta, Montana and Washington suggested that arboviruses might also be prevalent in British Columbia. The possibility was supported by the reports of encephalitis among human residents and horses in the south central regions of the province during several summers following the initial outbreak among horses in 1942.[1] The endemic prevalence of group B agents in the East Kootenay district of British Columbia was suggested by detection of hemagglutinin-inhibiting (HI) antibodies of Powassan (POW) and/or St. Louis encephalitis (SLE) in sera from 411 of 1243 wild rodents collected during 1968;[2] 26 of these sera neutralized POW virus and 61 neutralized SLE virus. Furthermore, sera from two of 21 residential school students showed HI and neutralizing (NT) antibodies to SLE virus. Neutralizing antibodies to the Montana snowshoe hare strain of California encephalitis (CE) virus were detected in sera from 13 of 14 snowshoe hares (*Lepus americanus*) collected near Kamloops, B.C. in 1962.[3] In a survey of 1268 human sera from residents of southeastern British Columbia during the winter of 1967, HI antibodies to CE virus were detected in 21 subjects; three of these sera also neutralized this agent.[4] Strains of this virus were isolated from mosquitoes collected near Penticton, B.C. in 1969[5] and Whitehorse, Yukon Territory in 1971.[6] Colorado tick fever (CTF) virus was isolated from *Dermacentor andersoni* ticks collected in the Selkirk region of B.C. during 1965,[7] suggesting the endemicity of this virus in British Columbia.

This paper reports the first laboratory-proven clinical cases of western equine encephalitis (WEE) in human residents of British Columbia, and the isolation of a strain of WEE virus from the brain of a horse. Also recorded are results of the first extensive human serum survey designed to determine the prevalence of certain arbovirus infections in man in British Columbia.

Materials and methods

Clinical material

Specimens consisting of throat and rectal swabs, cerebrospinal fluid (CSF) and serum were collected from three human cases of encephalitis. All were residents of the Okanagan valley of British Columbia. At least two other patients from this area contracted encephalitis, but

adequate specimens for laboratory study were not available. Portions of brain from an affected horse were obtained immediately after death and quick-frozen. All specimens were refrigerated with wet ice and shipped to Vancouver.

Virus isolation

The specimen of horse brain was triturated in medium CMRL HB597*. After centrifugation the supernatant was inoculated in 0.02 ml. aliquots intracerebrally and intraperitoneally into newborn mice aged 1 to 3 days. Mice subsequently observed moribund were sacrificed and quick-frozen. The frozen newborn mice were allowed to thaw and the brain was withdrawn into a 6 ml. disposable syringe using a 19-gauge needle. A 50% brain-HB597 suspension was prepared and inoculated into a second group of newborn mice. Brains were harvested at the appropriate time and supernatant obtained as described for horse brain was stored at −70°C. as stock isolate.

The horse brain supernatant, in volumes of 0.2 ml., was also inoculated into tubes of primary cynomolgus monkey kidney tissue culture. Tubes subsequently showing a cytopathic effect (CPE) were harvested and passed into fresh tissue cultures.

Specimens consisting of throat swabs, rectal swabs and CSF from the three human cases of encephalitis were prepared as previously described[8] and inoculated into tissue culture, embryonated eggs and newborn mice.

Neutralization tests

Undiluted WEE antiserum* in a volume of 0.15 ml. was mixed with 0.15 ml. of stock isolate, calculated to contain 50 to 100 mouse LD_{50} per 0.03 ml. The serum-virus mixture was incubated at room temperature for 30 minutes and 0.03 ml. aliquots were inoculated intracerebrally into four-week-old mice. This procedure was duplicated using the same WEE antiserum and the McMillan strain of WEE virus. Control animals received matching dilutions of virus, with diluent replacing WEE antiserum.

Serial ten-fold dilutions of the isolate in tissue culture fluid were prepared using CMRL HB597; an aliquot of WEE antiserum at a dilution of 1:10 was added to each dilution. After incubation at room temperature for 30 minutes, 0.2 ml. volumes of each mixture were inoculated into four tubes of primary cynomolgus monkey kidney tissue culture. Matching controls were prepared, using diluent in place of the WEE antiserum.

Acute and convalescent phase sera collected from the three human cases of encephalitis were tested for WEE antibody by hemagglutination-inhibition (HI) and complement-fixation (CF) tests as described in the following section, and by mouse neutralization (NT) tests. Both the McMillan strain of WEE and the horse brain isolate were diluted with 50% ox serum-HB597 so that 0.1 ml. contained 50 to 100 mouse LD_{50}. Acute and convalescent sera, undiluted and at dilutions of 1:10 were combined with equal volumes of virus suspension. These were left at room temperature for 30 minutes and inoculated into groups of three mice, aged four weeks.

Human serum survey, 1968-1969

Blood samples were obtained from 2202 volunteers during the first year of the study (1968) and from 1947 subjects during the second year (1969); with the exception of 266 adult outdoor workers included in the first year's sample, all volunteers were secondary school students 10 to 14 years of age who had lived all their lives in British Columbia. Subjects were selected to provide a fair representation of the major geographic regions and climatic conditions of this province (Fig. 1).

More than half the specimens collected in 1969 were from children who had participated in the 1968 survey. All blood samples were shipped to Vancouver where sera were separated from clots by centrifugation and held at −20°C. until tested.

All sera were examined by HI and CF tests for antibodies to WEE virus, POW virus, SLE virus and CE virus, and by CF test for antibodies to CTF virus. Sera showing HI or CF antibody for one or more of these antigens were further tested for NT antibody by mouse inoculation.

1. HI tests were performed after the method of Clarke and Casals[9] adapted to microtiter plates.[10] Reagents were used in 0.025 ml. volumes against four to eight agglutinating doses of WEE virus (McMillan strain), POW virus (L.B. strain), SLE virus (Parton strain) and CE virus (LaCrosse strain). All antigens with the exception of CE virus were prepared by the sucrose-acetone method from infected suckling mouse brains;[9] CE virus antigen was prepared in tissue culture and supplied by the Center for Disease Control, Atlanta, Georgia. Equal volumes of 0.25% suspensions of goose erythrocytes (*Anser cinereus*) were added to serum-virus mixtures.[9] Sera were treated with a 25% suspension of kaolin and absorbed with goose erythrocytes before titrations were begun. Initial dilutions of sera were 1:10.

2. CF tests were performed against optimal dilutions of antigens for WEE virus, POW virus, SLE virus, CE virus and CTF virus (Florio strain) using two hemolytic units of complement. Tests were performed in Microtiter† plates using 0.025 ml. volumes of reagents. All sera were heated at 56°C. for 30 minutes before testing. Initial dilutions were 1:4.

†Cooke Engineering Co., Alexandra, Virginia, U.S.A.

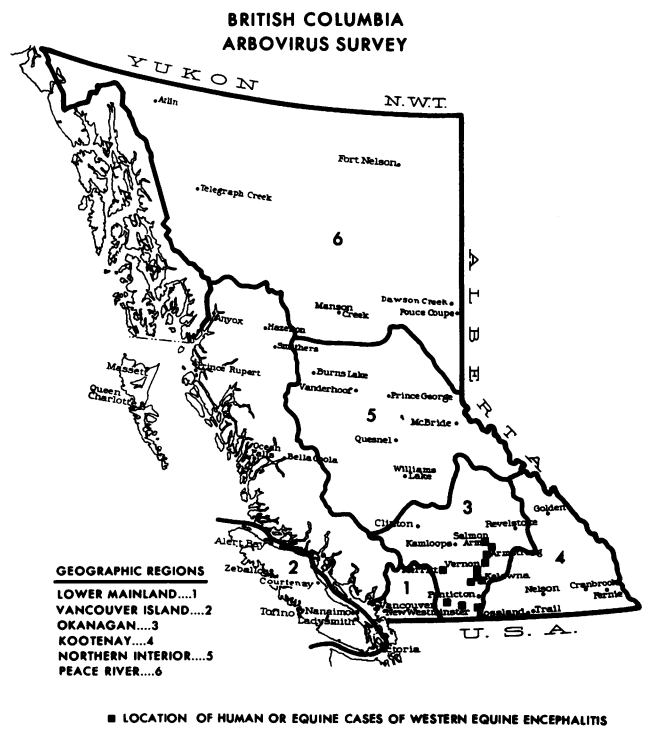

FIG. 1—Location of human or equine cases of western equine encephalitis

*Connaught Medical Research Laboratories, Toronto.

3. Neutralization tests were performed on selected sera against 50 to 100 mouse LD_{50} of WEE, POW, SLE and CE virus stocks by intracerebral injection of weaned mice aged 28 days with mixtures of virus and undiluted serum;[11] NT tests employing CTF virus were conducted by intracerebral injection of newborn mice.

Clinical and epidemiological data

The WEE outbreak, 1971

During August and September of 1971, five human cases of acute encephalitis occurred among residents of the Okanagan valley in south central British Columbia (49° to 51°N, 119° to 121°W). Of the three patients investigated serologically, one died; death also occurred in one of the two additional patients who were not investigated in the laboratory. Clinical features of the three laboratory-confirmed patients were typical of arbovirus encephalitis (Table I).

Encephalitis was reported in 60 horses in the Okanagan valley between July 27 and September 7, and was fatal in 15 cases. Laboratory confirmation of infection was obtained in two horses, and brains of seven other fatal cases showed histological features typical of arbovirus encephalitis.[12]

Heavy rainfall occurred throughout the Okanagan valley during June and early July, followed by five weeks of hot weather with daily temperatures of 95° to 100°F. (Fig. 1).

Results

Virus isolation and serology, 1971

Within four days following inoculation of the horse brain suspension into mice, all animals had developed signs of acute encephalitis. The mice inoculated with the horse brain isolate-WEE antiserum mixture and those which received the McMillan strain of WEE virus-WEE antiserum mixture were alive and well at 11 days. All control animals had expired by seven days.

Cytopathic effect was observed in tissue cultures within five days of inoculation and progressed to complete cellular destruction. This effect was neutralized by WEE antiserum.

Paired sera collected from one equine encephalitis case showed rising levels of HI antibody to WEE virus. Elevated HI antibody titres of 5 to 160 were demonstrated in single sera collected from 39 equine cases after onset of encephalitis.[13]

Rising titres of WEE antibody in the sera of the three human patients were demonstrated by CF, HI and mouse NT tests (Table II). Serologic studies to detect rises in antibody to herpes simplex virus, enteroviruses, and the viruses of SLE, POW, CE and CTF were all negative.

Human serum survey, 1968-1969

Results of HI, CF and NT tests conducted on sera collected from 1936 students during 1968, and from 1947 students in 1969, are set forth in Table III.

WEE virus antibodies

Six students were positive by HI test in 1968, while in 1969, three had detectable HI antibody and three had CF antibody. The positive CF tests suggest recent infection. Ten of the 12 students positive by HI or CF tests were also positive by NT tests in mice. All students with NT antibody were residents of the Okanagan or Kootenay regions.

SLE and POW antibodies

All sera possessing HI or CF antibody for one of these group B agents were tested in mice for NT antibody to both. No NT antibody to either agent was detected in these sera, suggesting the possibility of other group B virus activity in British Columbia.

CE virus antibodies

In 1968, 35 sera were positive by HI test, 14 by CF test

Table I

Summary of clinical and laboratory studies of three human cases of western equine encephalitis in British Columbia, 1971

	Patient 1	Patient 2	Patient 3
Date of onset	August 18	August 24	September 19
Age and sex	85 (female)	66 (female)	60 (male)
Signs and symptoms	Fever, stiff neck progressing rapidly to coma.	Malaise, nausea and vomiting, progressing to confusion and disorientation.	Malaise, nausea and vomiting, progressing to unconsciousness within 3 days.
Leukocyte count	19,400; lymphocytes 48%	12,500; lymphocytes 20%	12,000; lymphocytes 30%
Spinal fluid cell count	28 WBC; lymphocytes 72%	Normal limits	420 WBC; lymphocytes 98%
Clinical course	Death	Recovery; apparently normal.	Recovery slow but progressive.

Table II

Serologic tests for WEE antibody in three human residents of British Columbia, 1971

Test	Patient 1 1st serum	2nd serum	Patient 2 1st serum	2nd serum	Patient 3 1st serum	2nd serum
Hemagglutination-inhibition	<10*	20	<10	160	<10	160
Complement-fixation	< 2	2	64	256	< 2	128
Mouse neutralization	Negative	Positive	Negative	Positive	Negative	Positive

*Figures are reciprocals of antibody titres.

and 18 by NT test; in 1969, there were nine HI-positive sera, 11 were CF-positive while 19 sera had significant levels of NT antibody. With one exception the students with CE antibodies were all residents of the Okanagan, Kootenay, Northern Interior or Peace River areas. Three students positive by NT test in 1969 had been negative in 1968, indicating a sub-clinical infection following the 1968 bleeding.

CTF virus antibodies

Of the six sera positive by CF test over these two years, one was shown in 1969 to possess NT antibody. However, this student's serum had been negative in 1968, indicating infection subsequent to the first test.

During 1968, a group of 266 adult outdoor workers was included in the survey (Table IV). Although antibody was detected in a number of these patients, only one adult with NT antibody for SLE virus and one with NT antibody for CE virus were life-long residents of British Columbia.

Discussion

Serological confirmation of infection due to WEE virus in three human residents of the Okanagan valley of British Columbia who contracted encephalitis during the summer of 1971, provided the first clear evidence of overt human infections in this province with any known arbovirus. All three patients were aged 60 years or more, which parallels observations in Saskatchewan[14] on the severity of illness due to WEE virus among elderly patients.

The serological survey which was conducted during 1968 and 1969 documents for the first time the prevalence of human arbovirus infections in British Columbia, a province occupying almost 370,000 square miles of North America. Evidence of sub-clinical infection by WEE virus in human residents of the south central area of this province is not surprising; in portions of Washington State immediately south of the Okanagan-Kootenay regions of British Columbia, WEE antihemagglutinins were detected in sera from 29% of 293 school children between 1961 and 1964.[15] The presence of WEE virus activity in the Okanagan region in 1969 is indicated by the demonstration of CF antibody in the sera of two subjects residing in the area where eight clinical equine cases were reported; WEE was proven by serological tests in two of these animals.[16] The occurrence of sub-clinical infections in life-long residents of this region as demon-

Table III

Prevalence of HI, CF and NT antibodies to five arboviruses in human residents of British Columbia—1968 and 1969

Positive reactors by region

		ANTIGEN															
		WEE			SLE			POW			CE			CTF		TOTAL	
REGION		HI	CF	NT	HI	CF	NT	HI	CF	NT	HI	CF	NT	CF	NT	TESTED	
Lower mainland	1968	0	0	0/0*	7	0	0/7	3	0	0/7	7	2	1/9	0	0/0	298	
	1969	0	1	0/1	3	0	0/3	1	0	0/3	0	0	1/1	2	0/2	297	
Vancouver Island	1968	0	0	0/0	0	0	0/0	0	0	0/0	0	0	0/0	0	0/0	91	
	1969	0	0	0/0	0	0	0/0	0	0	0/0	0	1	0/1	0	0/0	70	
Okanagan Valley	1968	2	0	2/2	3	0	0/3	1	0	0/3	12	5	8/16	0	0/0	670	
	1969	3	2	3/4	4	0	0/4	0	0	0/4	7	6	10/16	1	0/1	747	
Kootenay	1968	4	0	4/4	7	0	0/7	0	0	0/7	10	4	5/14	2	0/2	555	
	1969	0	0	1/1	3	0	0/3	0	0	0/3	0	2	3/5	1	1/1	504	
Northern interior	1968	0	0	0/0	4	0	0/4	0	0	0/4	2	3	0/5	0	0/0	187	
	1969	0	0	0/0	2	0	0/2	0	0	0/2	2	1	1/3	0	0/0	192	
Peace river	1968	0	0	0/0	0	0	0/0	0	0	0/0	4	0	4/4	0	0/0	135	
	1969	0	0	0/0	0	0	0/0	0	0	0/0	0	1	4/5	0	0/0	137	
Number positive	1968	6	0	6	21	0	0	4	0	0	35	14	18	2	0		
	1969	3	3	4	12	0	0	1	0	0	9	11	19	4	1		
Number tested	1968	1936	1936	6	1936	1936	21	1936	1936	21	1936	1936	48	1936	2	1936	
	1969	1947	1947	6	1947	1947	12	1947	1947	12	1947	1947	31	1947	4	1947	

*Numerator: Number positive; Denominator: Number of sera tested

WEE: Western equine encephalitis virus
SLE: St. Louis encephalitis virus
POW: Powassan virus
CE: California encephalitis virus
CTF: Colorado tick fever virus
HI: sera containing hemagglutinin-inhibiting antibodies
CF: sera containing complement-fixing antibodies
NT: sera containing neutralizing antibodies

Table IV

Prevalence of HI, CF and NT antibodies for five arboviruses in a group of adult outdoor workers in southeastern British Columbia

	Antigen															
	WEE†			SLE			POW			CE			CTF		Total
Region	HI	CF	NT	HI	CF	NT	HI	CF	NT	HI	CF	NT	CF	NT	tested
Kootenay 1968	3	0	1/3*	31	0	7/33	33	0	2/33	17	8	7/24	0	—	266
Number positive	3	0	1	31	0	7	33	0	2	17	8	7	0	—	
Number tested	266	266	3	266	266	33	266	266	33	266	266	24	266	—	266

*Numerator: Number positive; Denominator: Number of sera tested
†Key as for Table III

strated by serum survey, together with the outbreaks of WEE in 1969 and 1971, indicate that this virus is presently well established in wild-life in south central British Columbia.

In spite of the obvious activity of WEE virus in the south central portion of the province, there was more evidence of infection in man due to CE virus than due to any other arbovirus investigated. That this should be true in the Okanagan and Kootenay areas might be anticipated. In 1961, strains of CE virus were isolated in northwestern Montana from ticks removed from several species of small wild mammals,[3] and in 1962 and 1963 from mosquitoes;[17] this agent was isolated from mosquitoes collected in 1965 in southern Alberta[18] and in 1969 near Penticton, B.C.[5] Activity of CE virus in man and wild-life has been demonstrated in the northern interior and Peace River regions to 56°N. latitude and in mosquitoes at 61°N. near Whitehorse, Y.T.[6]

The single student with NT antibody for CTF virus resides in that area of British Columbia from which several strains of this agent were isolated from ticks collected in 1965.[7]

The lack of NT antibody for either SLE or POW virus, in the presence of HI antibody to both agents in the sera of a number of students, indicates the need to screen for additional group B agents in any future survey. However, ample evidence that these two agents are endemic in wild-life in central British Columbia is on record.[2] The presence of NT antibody to SLE virus in one of the adult outdoor workers (Table IV) who is a life-long resident of British Columbia provides evidence that clinical or subclinical infection in man due to SLE virus also occurs in this province.

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Résumé

Infections à arbovirus chez l'homme en Colombie-Britannique

Au cours de l'été 1971, les premiers cas confirmés par le laboratoire d'encéphalite aiguë chez l'homme causés par l'un des arbovirus classés, sont survenus dans la région centrale et méridionale de la Colombie-Britannique. On a de la sorte diagnostiqué cinq cas humains d'encéphalite, suivis de deux décès. Chez trois de ces malades, y compris un des deux malades décédés, il a été prouvé par le laboratoire qu'ils avaient contracté l'encéphalite équine de l'Ouest.

Pendant les années 1968 et 1969, on a découvert dans le sérum de près de 2000 très anciens résidents de la province, de faibles niveaux d'anticorps inhibiteurs de l'hémagglutinine et de fixateurs de complément (ou l'un des deux) ainsi que des anticorps neutralisants pour l-encéphalite équine occidentale, l'encéphalite de Saint-Louis, l'encéphalite Powassan, l'encéphalite de Californie et l'encéphalite à tiques du Colorado. Des signes d'infection sub-clinique ont été décelés en certains cas.

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