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Serological Survey of Human Arbovirus Infections in Southeastern British Columbia

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IN British Columbia before 1967, little was known of the occurrence of arboviruses in wild life, and clinical illness in man due to arboviruses in this province has yet to be proved in the laboratory. Encephalitis due to western equine encephalitis (WEE) virus in horses was last reported in 1963 when a single case occurred near Cranbrook, British Columbia, though epidemics had been experienced among the horse population of the Okanagan and Kootenay areas of this province in 1942, 1946 and 1947.[1]

Neutralizing antibodies to the Bitterroot strain of California encephalitis (CE) virus[2] were detected in sera from 13 to 14 snowshoe hares *(Lepus americanus)* collected in 1962 near Kamloops.[3] Nine of 49 human sera collected from the same area in 1962-63 showed neutralizing antibodies for the snowshoe strain of CE virus,[4] though none of the volunteers had central nervous system disease. In 1965 a strain of CE virus was isolated from mosquitoes *(Culiseta inornata)* collected in southern Alberta,[5] providing further evidence of the presence of CE virus in western Canada.

Colorado tick fever (CTF) virus, one of the two tick-borne arboviruses endemic in North America, caused febrile illnesses and occasional cases of encephalitis among residents of 10 states in northwestern U.S.A., including Montana, Idaho and Washington.[6] The distribution of human infections corresponds to that of the ixodid tick *Dermacentor andersoni,*[7] which is the principal natural vector, and the golden-mantled ground squirrel *Citellus lateralis,* the main natural reservoir of this infection.[8] Both *C. lateralis* and *D. andersoni* abound in eastern British Columbia.

Powassan virus, first isolated in Ontario in 1958 from the brain of a child who died from encephalitis,[9] is the only member of the tick-borne complex of group B arboviruses known to occur in North America.[10] This virus is maintained in nature in eastern Canada by a cycle involving principally *Ixodes cookei* ticks as vectors and groundhogs *(Marmota monax)* as reservoirs,[11] though strains of the virus have been recovered from *Ixodes marxi* ticks and from the blood of a red squirrel *(Tamiasciuris hudsonicus).*[12] Foci of Powassan virus infection have since been identified in several areas of the United States.[13, 14] This paper reports that antibodies to WEE virus, CE virus, CTF and Powassan virus were detected in sera from human residents of south eastern British Columbia.

MATERIALS AND METHODS

Blood samples were collected from 1268 adult volunteers resident in three provincial health unit areas in southeastern British Columbia—East Kootenay (Health Unit No. 1), Selkirk (Health Unit No. 2) and West Kootenay (Health Unit No. 3)—between February 1 and May 31, 1967. Headquarters of these health units are located at Cranbrook (116° W.), Nelson (117° W.) and Trail (118° W.) in southeastern British Columbia between the latitudes of 49° and 50° N.

Volunteers were obtained by the medical health officer in each health unit, with the cooperation of local physicians and hospitals. The volunteer group consisted of patients seen in medical clinics or admitted to hospitals for reasons unrelated to arbovirus infections, or men whose daily work kept them outdoors, such as forestry, sawmill and highway employees. Although attempts were made to secure blood samples from persons who had spent their entire lives in southeastern British Columbia, conditions under which health officers worked did not permit at the time of venipuncture the routine elicitation of details about residence that are necessary. Upon conclusion of all laboratory tests on persons who had antibody to one or more arboviruses, histories as to residence were obtained wherever feasible. After the collection of blood samples, alternate procedures were adopted. Either sera were separated from clots

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by centrifugation on the day of collection and shipped frozen to Vancouver, or samples of whole clotted blood were shipped unrefrigerated to Vancouver, where sera were separated. Sera were held at –20° C. until tested.

Serological Procedures

Sera were examined by hemagglutination-inhibition (HI) and complement-fixation (CF) for antibodies to WEE virus, CE virus and Powassan virus, and by CF for antibodies to CTF virus. Sera showing hemagglutinin-inhibiting or complement-fixing antibody for one or more of these antigens were further tested for neutralizing antibody by the inoculation of mice.

1. HI tests were performed in Microtiter plates using 0.025-ml. volumes of reagents, against four to eight agglutinating doses of WEE virus (McMillan strain), CE virus (BFS-283 strain) and Powassan virus (L.B. strain). Antigens were prepared by the sucrose-acetone method from infected suckling mouse brains.[15] Equal volumes of 0.25% suspensions of erythrocytes from the white domestic goose *(Anser cinereus)* were added in appropriate virus adjusting diluents to serum-virus mixtures. 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, CE virus, CTF virus and Powassan virus 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.

3. Neutralization tests (NT) were performed on selected sera against 50-100 mouse LD_{50} of WEE, CE and Powassan virus stocks, by intracerebral injection of weaned mice aged 28 days with mixtures of virus and undiluted serum;[16] NT employing CTF virus were conducted by intracerebral injection of suckling mice aged 1 to 3 days.

RESULTS

Of the total of 1268 human sera tested, HI or CF antibodies for WEE virus were detected in 21, for CE virus in 23, for CTF virus in 2, for Powassan virus in 26. Neutralizing antibodies were detected in 16 of 21 of the WEE-positive sera and in 3 of 23 of the CE-positive sera, but not in 2 of the CTF-positive sera, and in none of 26 of the Powassan-positive sera (Table I).

TABLE I.—PREVALENCE OF HEMAGGLUTININ-INHIBITING, COMPLEMENT-FIXING AND NEUTRALIZING ANTIBODIES TO WESTERN EQUINE ENCEPHALITIS, CALIFORNIA ENCEPHALITIS, COLORADO TICK FEVER AND POWASSAN VIRUSES IN MAN IN SOUTHEASTERN BRITISH COLUMBIA, 1967

		ANTIGEN											
		WEE			*CE*			*CTF*		*POW*			
Health unit	*No.*	*HI*	*CF*	*NT*	*HI*	*CF*	*NT*	*CF*	*NT*	*HI*	*CF*	*NT*	*Total tested*
East Kootenay	1	5	0	2/5*	8	0	1/8	0	—	15	1	0/16	312
Selkirk	2	7	0	7/7	1	2	0/3	1	0/1	3	0	0/3	395
West Kootenay	3	9	0	7/9	12	0	2/12	1	0/1	7	0	0/7	561
Number positive		21	0	16	21	2	3	2	0	25	1	0	
Number tested		1268	1268	21	1268	1268	23	1268	2	1268	1268	26	1268

HI: Sera containing hemagglutinin-inhibiting antibodies.
CF: Sera containing complement-fixing antibodies.
NT: Incidence of neutralizing antibodies.
WEE: Western equine encephalomyelitis virus.
CE: California encephalitis virus.
CTF: Colorado tick fever virus.
POW: Powassan virus.
* numerator: number positive; denominator: number of sera tested.

A history of residence was obtained from 13 of 16 volunteers with neutralizing antibodies to WEE virus and in all of the volunteers with neutralizing antibodies for CE virus. Eleven of 13 volunteers with neutralizing antibodies to WEE had lived for significant periods of their lives in one or more of the prairie provinces, where WEE virus is endemic.[1] Of the remaining two of 13 volunteers, one had lived for a brief period of time in the Crows Nest Pass, on the border between British Columbia and Alberta, before moving permanently to British Columbia, while the second has lived all his life in this province. While two of three volunteers with neutralizing antibodies for CE virus had spent significant periods of time on the prairies where CE virus has been demonstrated in nature,[5] the remaining volunteer had come to this province

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in 1914 in the first year of his life and has remained in British Columbia ever since.

DISCUSSION

Detection of neutralizing antibody to WEE virus in two indigenous residents of southeastern British Columbia who had not lived outside the area, and to CE virus in one life-long resident, strongly suggests that occasional sub-clinical human infections by these agents occur in this province.

Occurrence of CE antihemagglutinins in 21 of 1268 (1.6%) British Columbia residents is comparable to the presence of HI antibodies in 25 of 949 (2.6%) of inhabitants of the Tampa Bay area of Florida.[17] Both in British Columbia and in Florida sera were treated with kaolin to remove non-specific inhibitors of hemagglutination, because it was shown that inhibitors were frequently present after acetone extraction but not after adsorption with kaolin.[17] Results of serological surveys of residents of British Columbia and Florida contrast sharply with those obtained in Kern County, California, during 1963 where CE antihemagglutinins were detected in 60 of 118 (51%) subjects, and 38 of 60 HI-positive sera also neutralized CE virus.[18] However, CE virus was first isolated in this area in 1943,[19] and the recovery of the Jerry Slough strain BFS-4474 during 1963[20] showed the continued endemic prevalence of members of the CE complex in southern California.

The presence of WEE neutralizing antibody in sera of 16 subjects, 14 of whom had resided in or travelled through the prairie provinces where WEE virus is endemic,[21] suggests that many of these subjects received subclinical infections outside British Columbia. In portions of Washington State immediately south of the test areas, WEE antihemagglutinins were detected in sera from 86 of 293 (29%) school children between 1961 and 1964.[22] It seems likely that the northerly extension of WEE virus activity may occur occasionally, thereby inducing subclinical infections in the two British Columbia residents with neutralizing antibody who had spent their entire lives in the province. Lack of WEE complement-fixing antibodies suggests that all positive reactors had acquired their subclinical infections at least four to six years previously.[23]

In a serological survey of 699 small wild rodents carried out in this same test area in 1967 by McLean, Ladyman and Purvin-Good,[24] Powassan-neutralizing antibodies were found in 37 animals. Hemagglutinin-inhibiting antibodies were found in 129 of 447 of these mammals, while 88 of 99 sera inhibited hemagglutination by Murray Valley and St. Louis encephalitis viruses in addition to Powassan virus. CF antibodies for CE virus were detected in eight of 117 animals and neutralizing antibodies in eight of 66 additional mammals.

These findings suggest that CE and Powassan viruses are endemic in wild life in southeastern British Columbia. These animals serve as reservoirs from which residents may contract clinical or subclinical infections as revealed by detection of antibodies in these human sera.

Summary In a human serum survey of 1268 volunteers carried out in southeastern British Columbia during the spring of 1967, hemagglutinin-inhibiting antibodies for western equine encephalomyelitis virus were found in 21 of the test sera, for California encephalitis virus in 21, and for Powassan virus in 25. Complement-fixing antibodies for western equine encephalomyelitis virus were discovered in no sera, for California encephalitis virus in two, for Colorado tick fever virus in two, and for Powassan virus in one. Neutralizing antibodies for western equine encephalomyelitis virus were detected in 16 of 21 sera positive by hemagglutination-inhibition or complement fixation, for California encephalitis virus in 3 of 23 sera, for Colorado tick fever virus in neither of 2 sera, and for Powassan virus in none of 26 sera. These findings suggest that human infection due to these arboviruses may occur in British Columbia.

Résumé Les auteurs analysent les résultats d'une étude des sérums humains entreprise en Colombie Britannique du sud-est, durant le printemps de 1967 et portant sur 1268 volontaires. Des anticorps inhibiteurs des hémagglutinines ont été découverts dans 21 des sérums essayés pour l'encéphalomyélite équine occidentale (EMEW), dans 21 sérums pour l'encéphalite californienne (EC) et dans 25 sérums pour le virus Powassan. Concernant les anticorps de fixation du complément, ils n'ont été découverts dans aucun des sérums pour l'EMEW, mais trouvés dans deux sérums pour l'EC, dans deux sérums pour le virus de la fièvre à tique du Colorado et dans un sérum pour le virus Powassan. Dans 16 des 21 sérums positifs par le test d'inhibition des hémagglutinines ou par celui de fixation du complément, existaient des anticorps neutralisants pour l'EMEW, dans trois des 23 sérums pour le virus de l'EC, dans aucun des deux sérums pour le virus de la fièvre à tique du Colorado et dans aucun des 26 sérums pour le virus Powassan. Les auteurs croient pouvoir conclure de ces constatations que l'infection causée par ces arbovirus chez l'homme peut survenir en Colombie Britannique.

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