Arbovirus Infections in Several Ontario Mammals, 1975-1980

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ABSTRACT

Serological studies for arboviruses were conducted on 725 animal sera collected in 22 Ontario townships between 1975 and 1980 including 44 coyote (*Canis latrans*), 277 red fox (*Vulpes vulpes*), 192 raccoon (*Procyon lotor*) and 212 striped skunk (*Mephitis mephitis*). Hemagglutination inhibition antibodies to two flaviviruses, namely St. Louis encephalitis and Powassan were found in 50% of coyote, 47% of skunk, 26% of fox and 10% of raccoon sera. Similarly, hemagglutination inhibition antibodies to a California serogroup virus, snowshoe hare, were found in 12% of fox, 7% of skunk, 7% of raccoon and 5% of coyote sera. No antibodies were detected to two alphavirus, namely eastern equine encephalitis and western equine encephalitis, antigens.

This study affirms the endemic presence of Powassan and snowshoe hare virus and further delineates the scope of St. Louis encephalitis activity in Ontario.

Key words: Arboviruses, St. Louis encephalitis, Powassan, snowshoe hare virus, Ontario.

RÉSUMÉ

Cette étude s'étalait sur la période de 1975 à 1980; elle impliquait 22 cantons de l'Ontario et consistait à rechercher des anticorps contre certains arbovirus, dans le sérum des 725 animaux sauvages suivants: 44 coyotes (*Canis latrans*), 277 renards roux (*Vulpes vulpes*), 192 ratons laveurs (*Procyon lotor*) et 212 mouffettes rayées (*Mephitis mephitis*). L'inhibition de l'hémagglutination permit de détecter des anticorps contre deux flavivirus, à savoir : le virus de l'encéphalite de St-Louis et le virus Powassan, chez 50% des coyotes, 47% des mouffettes, 26% des renards et 10% des ratons laveurs. Cette épreuve démontra aussi la présence d'anticorps contre le virus du lièvre, un des virus du sérogroupe de la Californie, chez 12% des renards, 7% des mouffettes, 7% des ratons laveurs et 5% des coyotes. La recherche d'anticorps contre les virus de l'encéphalomyélite équine de l'Est et de l'Ouest, deux alphavirus, donna des résultats négatifs.

Les constatations consécutives à cette étude confirment la présence enzootique du virus Powassan et de celui du lièvre, sur le territoire ontarien, et elle délimite davantage le champ d'activité du virus de l'encéphalite de St-Louis, dans cette province.

Mots clés: arbovirus, encéphalite de St-Louis, virus Powassan, virus du lièvre, Ontario.

INTRODUCTION

Six arboviruses of human disease-causing potential have been isolated in Ontario. These include two alphaviruses — eastern equine encephalitis (EEE) (1,2) and western equine encephalitis (WEE) (3), two flaviviruses, — St. Louis encephalitis (SLE) (4,5) and Powassan (POW) (6-11) and two California (CAL) serogroup viruses — snowshoe hare (SSH) (5,12-15) and Jamestown Canyon (JC) (14). Four of these, namely SLE, POW, SSH and JC, have been shown to cause human disease in Ontario.

In 1975 an outbreak of SLE occurred in southwestern Ontario in which 66 human symptomatic infections were diagnosed (4,16). Four additional cases were recognized in 1976 but no SLE infections have been documented in Ontario since that time. Five human symptomatic infections due to POW virus have been diagnosed in Ontario between 1958 and 1979 with two fatalities (17).

Four human symptomatic infections of California encephalitis have been recognized in Ontario between 1978 and 1981, including three likely due to the SSH and one to the JC serotype (18,19). Human cases were diagnosed also in Ontario in 1982 and 1983 (20).

This study was undertaken on sera from four species in 22 Ontario townships to determine whether these animals can serve as useful monitors of arbovirus activity and to extend our knowledge of the distribution of arboviruses in Ontario.

MATERIALS AND METHODS

COLLECTION OF ANIMAL SERA

Sera were collected from 725 animals in 22 Ontario townships (Fig 1) between 1975 and 1980 including 44 coyote (*Canis latrans*), 277 fox (*Vulpes vulpes*) 192 raccoon (*Procyon lotor*) and 212 skunk (*Mephitis mephitis*). A summary of sera collected by township and year of collection is presented in Table I.

Specimens were obtained from trappers during the normal fall trapping season, roughly October 10 to November 15. Blood samples were

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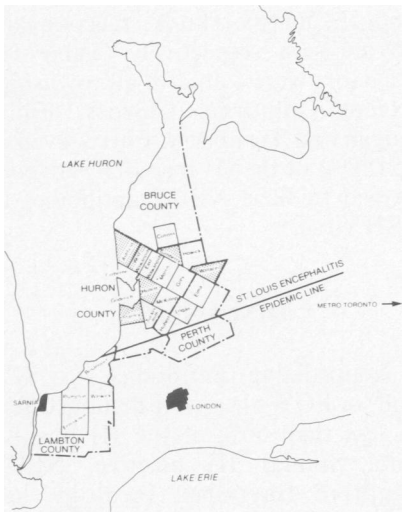

Fig. 1. Townships from which mammalian sera were collected for arbovirus studies. The St. Louis encephalitis epidemic line was designated in 1975 by the Committee on Programs for the Prevention of Mosquito-borne Encephalitis in the province of Ontario to divide the province into a nonepidemic area (north of the line) and a southwestern belt of proven or high risk St. Louis encephalitis activity (16). The dotted areas represent townships in which animals with neutralizing antibodies to St. Louis encephalitis were found.

TABLE I. Breakdown of Township and Year of Mammalian Sera Collected for Arbovirus Testing

Ontario Township of Collection	Year Sera Collected 1975	1976	1977	1979	1980	Total Sera Collected
Ashfield	6	0	1	0	5	12
Bosanquet	0	0	0	0	17	17
Colborne	11	1	1	0	1	14
Culross	2	1	0	0	0	3
East Wawanosh	7	0	0	0	2	9
Elma	6	0	0	0	0	6
Enniskillen	0	0	0	0	9	9
Goderich	22	6	2	49	66	145
Grey	48	1	6	30	9	94
Hibbert	0	0	1	0	0	1
Howick	5	0	4	1	0	10
Hullett	21	2	5	24	40	92
Logan	10	0	0	0	0	10
McKillop	1	0	5	12	25	43
Morris	19	1	4	13	5	42
Plympton	0	0	0	0	14	14
Stanley	17	3	8	33	55	116
Tuckersmith	4	0	2	5	18	29
Turnberry	3	0	0	0	0	3
Unknown	1	0	0	0	1	2
Wallace	8	0	2	0	0	10
Warwick	0	0	0	0	9	9
West Wawanosh	8	0	1	0	26	35
Total	119	15	42	167	302	725

taken before animals were killed. Most samples were obtained by cardiac puncture, using Vacutainer® (Becton Dickinson Canada, 2464 South Sheridan Way, Mississauga, Ontario, L5J 2M8) equipment. Samples were centrifuged within 12-24 hours, and the serum stored frozen until assayed.

SEROLOGICAL TESTS

Virus Strains — The alphavirus strains used in this study: EEE, isolated from horse brain in the province of Quebec, and WEE, strain 440-66, isolated from a pool of *Culex tarsalis* collected in Manitoba, were supplied by Dr. J.R. Polley (Laboratory Center for Disease Control, Ottawa). St. Louis encephalitis, strain M6868, was obtained from Dr. C.H. Calisher (Centers for Disease Control, Fort Collins, Colorado), POW, LB prototype strain M794, was received from Dr. D.M. McLean (University of British Columbia, Vancouver, British Columbia) and snowshoe hare, strain R2929, was supplied by Dr. J.A. McKiel (Laboratory Center for Disease Control, Ottawa).

Hemagglutination Inhibition Test — Hemagglutination inhibition (HI) tests were performed on acetone treated sera by the method of Clarke and Casals (21) as modified to a microtiter technique by Sever (22). Four to eight hemagglutinating units were used of the following antigens: EEE, WEE, SLE, POW and SSH. Initial sera dilutions for the HI test were 1 in 10.

Neutralization Test — Neutralization (NEUT) tests were undertaken against SLE, POW and SSH viruses by incubating 0.1 mL volumes of heat inactivated (56°C for 30 min) sera with 0.1 mL containing 200 $TCID_{50}$ of virus at 4° C overnight and inoculating of tissue culture cells with 0.1 mL of the mixture (100 $TCID_{50}$ challenge dose). Neutralization tests for SLE, POW and SSH were conducted in BHK-21, WI-38 and vero cells respectively. Sera were considered as neutralization positive if complete inhibition of cytopathic effect was obtained.

RESULTS

ALPHAVIRUS

No HI reactors were found to EEE or WEE virus.

FLAVIVIRUS

Flavivirus reactors were found in all animal species with HI antibodies to SLE and/or POW antigen in 50% of coyote, 47% of skunk, 26% of fox and 10% of raccoon sera. Reactors were detected in sera collected from 1975 to 1980 (Table II). Many reactors were found to both SLE and POW but with titers generally higher to POW antigen.

ST. LOUIS ENCEPHALITIS

All 21 coyote SLE HI reactors were tested by NEUT with no specific antibodies detected for this virus (Table II). Forty-six of 47 fox SLE HI reactors were tested and NEUT antibodies were demonstrated in three animals. Nine of ten raccoon SLE HI reactors were tested by NEUT and SLE virus antibodies were confirmed in one raccoon. Finally, 56 of 60 skunk SLE HI reactors were tested by NEUT and antibodies to SLE virus demonstrated in ten of these animals.

Neutralizing antibodies to SLE virus were demonstrated in 14 animals from 12 Ontario townships. However, eight of these animal sera also neutralized POW, a related flavivirus, making interpretation of these reactions difficult. A summary detailing

TABLE II. Arbovirus Serology of Ontario Mammals, 1975-1980

Animal	Year of Collection	Number of Sera Tested	Hemagglutination Inhibition SLE	Hemagglutination Inhibition POW	Hemagglutination Inhibition SSH	Neutralization[a] SLE	Neutralization[a] POW	Neutralization[a] SSH
Coyote	1975	11	7[c]	5	1	0[d]/7[e]	3/5	—
	1976	13	7	6	0	0/7	0/6	—
	1977	10	4	4	1	0/4	0/4	0/1
	1979	6	3	3	0	0/3	3/3	—
	1980	4	0	1	0	—	0/1	—
	Total	44	21(86)[f]	19(95)[g]	2	0/21	6/19	0/1
Fox	1975	78	20	21	9	1/19	9/18	7/9
	1976	2	0	0	0	—	—	—
	1979	52	6	12	3	0/6	7/10	3/3
	1980	145	21	31	21	2/21	11/19	17/21
	Total	277	47(85)	64(63)	33	3/46	27/47	27/33
Raccoon	1975	56	6	8	12	1/6	2/5	9/9
	1979	72	3	6	0	0/2	4/5	—
	1980	64	1	2	1	0/1	1/2	1/1
	Total	192	10(70)	16(44)	13	1/9	7/12	10/10
Skunk	1975	54	27	33	9	5/23	9/24	5/7
	1977	32	10	17	2	2/10	4/10	1/1
	1979	37	11	19	2	1/11	12/14	2/2
	1980	89	12	30	2	2/12	11/15	2/2
	Total	212	60(98)	99(60)	15	10/56	36/63	10/12

[a]Neutralizing tests were conducted only on sera positive by hemagglutination inhibition to the corresponding virus
[b]SLE = St. Louis encephalitis; POW = Powassan; SSH = Snowshoe hare
[c]Number of hemagglutination inhibition reactors
[d]Number of sera showing neutralizing antibodies
[e]Number of sera tested
[f]Percent SLE positives also reacting by HI with POW antigen
[g]Percent POW positives also reacting by HI with SLE antigen

the six animals showing NEUT antibodies only to SLE virus is presented in Table III.

POWASSAN

Six of 19 coyote POW HI reactors showed NEUT antibodies to the virus. Similarly NEUT antibodies to POW virus were demonstrated in 27 of 47 fox, 7 of 12 raccoon and 36 of 63 skunk POW HI reactors (Table II). Neutralization confirmed POW virus reactors were shown in all townships except Ashfield, Culross, Enniskillen, Howick, Turnberry and Wallace.

CALIFORNIA SEROGROUP SNOWSHOE HARE

Hemagglutination inhibition reaction rates to SSH antigen were 12, 7, 7 and 5% in fox, skunk, raccoon and coyote sera respectively (Table II). Reactors were found in all townships except Colborne, Culross, Elma, Logan and Turnberry. Forty-seven of 55 (86%) of the HI reactors were confirmed to have NEUT antibodies to SSH virus.

TABLE III. Ontario Mammals with Neutralizing Antibodies to St. Louis Encephalitis Virus

Species	Year Trapped	Age (years)	Sex	Township of Collection
Fox	1975	>1	male	Turnberry
Skunk	1975	1.5	male	Wallace
	1977	3.5	male	Hullett
	1979	1.5	female	Stanley
	1980	3.5	female	West Wawanosh
Raccoon[a]	1975	2.5	male	Ashfield

[a]Neutralizing antibodies to SLE virus in this raccoon were confirmed by Dr. M.S. Mahdy of the Ontario Ministry of Health using plaque reduction technique in primary duck embryo cells

DISCUSSION

Neutralizing antibodies to SLE and/or POW virus were demonstrated in less than 50% of the HI positive sera. Similar HI positive, NEUT negative flavivirus serology has resulted from surveys in Newfoundland (23), Quebec (24), British Columbia (25,26) and the Yukon (27). This likely indicates that use of flavivirus antigens results in a large number of nonspecific HI positives. However other possible explanations are that cross-reactions occurred with a flavivirus not included in the survey or that the tissue culture NEUT tests employed in this study were of limited sensitivity.

This study did demonstrate extensive infection of Ontario mammals with POW and SSH, two arboviruses known to occur endemically in Ontario, and antibodies to SLE virus. St. Louis encephalitis activity was last clearly demonstrated in Ontario in 1976 when four clinical cases were detected in southwestern Ontario (16) and two isolates were obtained from *Culex* sp. mosquitoes (5).

The presence of several SLE positive animals is especially interesting. Animals with confirmed NEUT antibodies to SLE alone were all taken north of the area of known SLE activity during the 1975 and 1976 outbreaks (Table III, Fig. 1). Radio telemetry studies of movements of these species (D. Voigt, unpublished data) have shown that it is possible, but unlikely, that all the positive individuals had moved north from the outbreak area. Radio-collared foxes were observed to disperse up to 122 km (straight line from point of origin). However from a sample of 45 radio-tracked juveniles, males dispersed an average of 27 km and females only 8 km. Adult foxes rarely dispersed. Of 30 skunks tracked, none dispersed more than 10 km. The average home range of

foxes was less than 10 km^2, but of skunks was only 1.6 km^2. Therefore, the most plausible explanation is that the SLE positive individuals were infected within 20 km of the capture site.

The demonstration of NEUT antibodies strongly suggests that Ontario mammals such as skunk, fox and raccoon had been infected with SLE virus. Although large numbers of specimens were obtained in 1979 and 1980, the prevalence of SLE infection was very low. Three of six animals showing NEUT antibodies only to SLE virus were captured after 1976 (Table III). However, all the post-1976 skunk specimens were adults which had, therefore, been exposed through more than one mosquito season. All these facts suggest that the probability of infection with SLE was much less in 1977-1980 than it had been in 1975.

This report extends the known range of POW virus in Ontario. Previously, POW virus has been isolated from Guelph (6) and the Powassan-North Bay-Manitoulin region of northern Ontario (7-11), and clinical cases have been diagnosed, likely contracted near Powassan (8), Kingston (28,29), Lower Buckhorn Lake (30) and possibly near Ottawa (31). In addition, serological studies have suggested POW activity in several other communities including Warkworth, Orangeville, Barrie, Halton Hills, Hamilton, Milton and Troy (6).

The high POW reaction rates, particularly in coyote, fox and skunk, show that these mammals may serve as useful monitors for POW activity. It would be of interest to determine whether any of these mammals can serve as actual amplifying hosts of the virus.

Demonstration of antibodies to SSH virus in all species and most townships tested is not surprising. Snowshoe hare virus has been documented in all ten Canadian provinces as well as the Yukon and Northwest Territories and antibodies reported in four domestic and sixteen wild animal species in Canada including coyote and raccoon but not fox or skunk (18). While it is still unclear at present which mammalian species in Ontario serve as amplifying hosts of SSH virus, it is helpful to note that all four animal species examined in this study can be used to monitor for SSH virus activity in various localities.

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