STUDIES ON CALIFORNIA SEROGROUP VIRUS ACTIVITY IN NEWFOUNDLAND, CANADA, 1980–83

JOSEPH MOKRY[1], HARVEY ARTSOB[2] AND RALPH BUTLER[3]

ABSTRACT. Arbovirus studies undertaken in Newfoundland between 1980–83 have resulted in the isolation of three California (CAL) serogroup viruses including an isolate of Jamestown Canyon (JC) virus from a mixed pool of *Aedes abserratus* and *Ae. punctor* and two isolates of snowshoe hare (SSH) virus from pools of *Ae. canadensis*. Serological studies on human and animal sera have confirmed the circulation of SSH and JC viruses in Newfoundland with CAL serogroup hemagglutination inhibition antibody rates of 8.6%, 20.9% and 54.5% in 660 human, 43 horse and 11 snowshoe hare (*Lepus americanus*) sera, respectively.

INTRODUCTION

With the large-scale incursion of St. Louis encephalitis (SLE) into Ontario (Mahdy et al. 1979) and repeated outbreaks of western equine encephalitis (WEE) in western Canada (McLintock and Iversen 1975), mosquito-borne viruses have been recognized as a potential health risk associated with the attacks of biting flies. More recently California (CAL) serogroup viruses have been recognized as human pathogens in Canada with clinical cases in Ontario (Artsob et al. 1981, Mahdy et al. 1982), Quebec (Fauvel et al. 1980, Fauvel et al. 1981) and Nova Scotia (Embil et al. 1982).

Antibodies to CAL serogroup viruses have been demonstrated in Eastern Canada including snowshoe hare, horses and moose in Nova Scotia (Embil et al. 1978, McFarlane et al. 1981a), horses, deer and moose in New Brunswick (McFarlane et al. 1982) and horses, snowshoe hare and cattle on Prince Edward Island (McFarlane et al. 1981b).

In an effort to determine the eastern limit of distribution of arboviruses in North America, mosquitoes were collected in Newfoundland on the Avalon Peninsula during the summers of 1980–83 for arbovirus isolation attempts. In addition, human and animal sera were examined for arbovirus antibodies.

MATERIALS AND METHODS

MOSQUITO COLLECTIONS. Collections of mosquitoes were taken at several points within a 20 km radius of St. John's throughout the summers of 1980 to 1983. The specific areas for sampling were determined by the proximity of breeding sites under concurrent study on mosquito ecology by the Research Unit on Vector Pathology. Host seeking mosquitoes were captured in dry ice baited CDC-miniature light traps, and by sweep net of those species attracted to the human collectors. Specimens were transported in cool boxes on ice to the laboratory where they were anesthetized with CO_2 and sorted to species. Individuals of each species were pooled (up to 100/pool) and stored at −70 to −80°C.

VIRUS ISOLATION AND IDENTIFICATION. Virus isolation was attempted by grinding mosquito pools in phosphate buffered saline containing 0.75% bovalbumin and gentamicin (100μg/ml) and clarification of the supernatant at 2000 g for 10 min. Specimens were assayed for virus by inoculation of vero cells with subsequent incubation at 37°C or by intracranial inoculation of 3–5 day old Swiss white mice.

Isolates identified as CAL serogroup viruses were typed by neutralization (NEUT) tests using hyperimmune mouse ascitic fluids prepared in Swiss white mice (Tikasingh et al. 1966) against the following strains—snowshoe hare (SSH) prototype Burgdorfer and Jamestown Canyon (JC) prototype 61 V2235 supplied by Dr C. H. Calisher of Centers for Disease Control, Fort Collins, and trivittatus (TVT) topotype 7941 supplied by Dr. J. R. Polley of the Laboratory Centre for Disease Control, Ottawa.

SERA FOR TESTING

HUMAN SERA. Human sera were provided by the Newfoundland Public Health Laboratories from specimens submitted for various serological screen testing procedures. The 660 sera were proportionately representative of 5 age groups; less than 20 years of age, 20 to 29 years, 30 to 39 years, 40 to 49 years and greater than 50 years of age. No attempt was made to select sera on the basis of sex. Although specimens submitted to the Public Health Laboratories originate from throughout the province, sera selected for arbovirus serology originated from rural areas.

[1] Research Unit on Vector Pathology, Memorial University, St. John's, Newfoundland, Canada A17 5S7. Present address: Vector Research & Control Consulting, P.O. Box 966, St. John's, Newfoundland, Canada A1C 5M3.

[2] National Arbovirus Reference Service, Department of Microbiology, University of Toronto, Ontario, Canada, M5G 1L5.

[3] Newfoundland Public Health Laboratories P.O. Box 8800, St. John's, Newfoundland Canada A1B 3T2.

ANIMAL SERA. Serum samples collected from 11 snowshoe hares (*Lepus americanus*) caught in Terra Nova National Park were provided to this study by Parks Canada Wildlife Officers. Equine samples exclusively represented the Newfoundland pony, which is recognized as a distinct breed of horse. All ponies had been bred on the Avalon Peninsula and none had ever been off the island of Newfoundland. Whole-blood samples were drawn by the Provincial Veterinary Department for this study. Samples were returned to the laboratory, centrifuged and the serum removed. Sera of both horses and hares were stored at −20°C until tested.

SEROLOGICAL TESTS. Sera were tested by hemagglutination inhibition (HI) against the following antigens: SSH topotype R2929, JC topotype Mn 256–260, WEE isolate from *Culex tarsalis* (Coq.) in Manitoba, SLE Parton strain, eastern equine encephalitis (EEE) isolate from horse brain in Quebec and Powassan (POW) isolate from *Ixodes* ticks in Ontario. Tests were performed on acetone treated sera by the method of Clarke and Casals (1958) as modified to a microtiter technique by Sever (1962).

Neutralization tests were undertaken against SSH prototype Burgdorfer and JC prototype 61 V 2235 by incubating 0.1 ml volumes of heat inactivated (56°C for 30 min) sera or ascitic fluids with 0.1 ml containing 200 $TCID_{50}$ of virus at 4°C overnight and inoculating of vero cells with 0.1 ml of the mixture (100 $TCID_{50}$ challenge dose). Sera or ascitic fluids were considered to contain neutralizing antibody if complete inhibition of cytopathic effect was obtained.

RESULTS

A total of 18,658 mosquitoes were collected and processed for virus from 1980 to 1983 (Table 1). Three CAL serogroup isolates were obtained including an isolate of JC from a mixed pool of 45 *Aedes abserratus* (Felt and Young) and *Aedes punctor* (Kirby) taken about 8 km from St John's on July 17, 1980 and two isolates of SSH, one from a pool of 55 *Ae. canadensis* (Theobald) collected at Clarenville on Aug. 18, 1982 and the second from a pool of 96 *Ae. canadensis* collected on Aug. 20, 1983.

The isolates were identified as JC and SSH viruses by NEUT tests against CAL serogroup viruses previously isolated in Canada—SSH, JC and TVT (Table 2). Identification was confirmed by testing the isolates against SSH, JC, TVT, California encephalitis, LaCrosse, Keystone and San Angelo hyperimmune fluids by an Enzyme Linked Immunosorbant Assay tying procedure developed in the laboratory of one of the co-authors (H. Artsob, L. Spence and C. Thing, unpublished data).

SEROLOGY. Fifty-seven of 660 human, 9 of 43 horse and 6 of 11 snowshoe hare sera had antibodies to SSH or JC by HI for positive rates of 8.6%, 20.9% and 54.5% respectively (Table 3). No HI antibodies were detected to EEE, WEE, SLE or POW in any of the sera tested.

Neutralization tests on HI positive sera against the two CAL serotypes isolated during our study, JC and SSH, indicated that most human and horse reactors had been infected with the JC serotype. In contrast, all snowshoe hare reactors appear to have been infected with the SSH serotype. The 34 horse and 5 snowshoe hare sera negative by HI to SSH and JC

Table 1. Newfoundland mosquitoes collected for virus screening, 1980–83.

Mosquito species	Year of collection				Total
	1980	1981	1982	1983	
Aedes punctor	744[1]	1,010	2,591	4,634	8,979
Ae. abserratus	1,990[1]	633	844	515	3,982
Ae. canadensis	295	117	299[2]	406[3]	1,117
Ae. communis	—	—	1,850	1,472	3,331
Ae. decticus	19	51	70	6	146
Ae. diantaeus	18	—	—	6	24
Ae. cantator	29	4	14	276	323
Ae. excrucians	52	13		5	111
Ae. cinereus	39	20	37	308	404
Ae. intrudens	13	—	11	—	24
Culiseta spp.	17	32	130	38	217
Total	3,216	1,880	5,896	7,666	18,658

[1] Jamestown Canyon virus was isolated from a mixed pool of 18 *Ae. punctor* and *Ae. abserratus* collected on July 17.

[2] Snowshoe hare virus was isolated from a pool of 55 *Ae. canadensis* collected on August 18.

[3] Snowshoe hare virus was isolated from a pool of 96 *Ae. canadensis* collected on August 20.

Table 2. Neutralization typing of California group isolates from Newfoundland mosquitoes, 1980–83.

Isolate typed	Isolated from	Antibody titer[1]			Serotype
		SSH[2]	JC[2]	TVT[2]	
78–80	*Aedes abserratus*/ *Aedes punctor*	40	160	—[3]	JC
185–82	*Ae. canadensis*	20,480	40	—	SSH
154–83	*Ae. canadensis*	20,480	—	—	SSH
SSH Burgdorfer		40,960	40	—	
JC 61V2235		40	320	40	
TVT 7941		—	—	320	

[1] Reciprocal of highest antibody dilution that neutralized 100 $TCID_{50}$ of virus.
[2] SSH= snowshoe hare, JC= Jamestown Canyon, TVT= Trivittatus.
[3] – = < 1:20.

Table 3. California group serology of Newfoundland human, horse and showshoe hare sera.

Sera tested	Hemagglutination inhibition		Neutralization titer highest to:		
	Sera tested	Sera positive to SSH +/or JC	SSH[2]	JC[2]	SSH/JC[3]
Human	660	57[4](8.6%)	6	43	7
Horse	43	9[5](20.9%)	1	8	0
Snowshoe hare	11	6[6](54.5%)	6	0	0

[1] Refers only to neutralization tests undertaken on hemagglutination inhibition positive sera.
[2] SSH= snowshoe hare, JC= Jamestown Canyon.
[3] SSH/JC= equal neutralizing titers to snowshoe hare and Jamestown Canyon virus.
[4] One hemagglutination inhibition positive serum had no California group neutralizing antibodies.
[5] 12 of 34 hemagglutination inhibition negative horse sera had California group neutralizing antibodies including 2 with highest titer to SSH and 10 with highest titer to JC.
[6] 1 of 5 hemagglutination inhibition negative snowshoe hare sera had neutralizing antibodies with highest titer to SSH.

were tested by NEUT with 12 additional horse and 1 additional snowshoe hare reactors detected.

There appeared to be no significant difference in the positive rates amongst the age groups of human sera tested (Table 4). Although no attempt was made to select sera for testing by sex, 72% of bloods tested were from females, due primarily to the preponderance of female sera submitted to the Newfoundland Public Health Laboratories for various screen testing procedures. Despite this, nearly equal numbers of male and female sera were positive for CAL group antibodies giving positive reactor rates of 15.1% (28/185) and 6.1% (29/475) for males and females, respectively.

DISCUSSION

This study reports the first isolations of mosquito transmitted arboviruses from Newfoundland and documents the easternmost distribution of CAL serogroup viruses in North America. The isolation of SSH virus in Newfoundland is not surprising since it has been demonstrated in all other Canadian provinces

Table 4. Human sera tested by hemagglutination inhibition for CAL group virus antibodies, 1983.

Age (yrs)	Sera tested			Reactive sera		
	F[1]	M[1]	Total	F[1]	M[1]	Total
<20	95	40	135	7	1	8
20–29	90	10	100	4	4	8
30–39	82	18	100	4	5	9
40–49	60	40	100	2	6	8
>50	49	51	100	5	5	10
Not available	99	26	125	7	7	14
	475	185	660	29(6.1%)	28(15.1%)	57(8.6%)

F[1] and M[1] refer to female and male sera, respectively.

as well as the Yukon and Northwest Territories (Artsob 1983). Although antibodies to JC virus have been detected in moose in Nova Scotia and New Brunswick (McFarlane et al. 1981a, McFarlane et al. 1982), this is the first clear documentation of JC virus in Atlantic Canada. Previous Canadian isolations of JC virus have been in Alberta, Saskatchewan and Ontario (Artsob 1983).

Serology of snowshoe hare suggests that they have been infected with SSH virus. This is consistent with previous data implicating snowshoe hares as the primary reservoir of SSH virus in Canada (Artsob 1983). Similarly the high CAL antibody levels in horses is consistent with previous observations that horses are good monitors of CAL serogroup, specifically SSH, activity in Canada (Artsob et al. 1979, McFarlane et al. 1980). However, it is pertinent to note that the greatest number of CAL serogroup reactors in the Newfoundland horse population was to JC rather than SSH virus.

The exposure of Newfoundland residents (8.6% HI reactors) to CAL serogroup viruses is of interest. Previous studies have documented infection of Canadians in six provinces to CAL serogroup viruses with exposure rates varying from 0.5 to 31.9% (Artsob 1983, Belloncik et al. 1983). In fact, the 8.6% positive rate while high, is likely an underestimation of the number of infected individuals since subsequent studies on horse and snowshoe hare sera indicated that NEUT was a more sensitive screening test than HI. Males in particular showed high infection rates with nearly 2.5 times the rate in females. This is consistent with previous studies in Ontario (Artsob et al. 1982) and Minnesota (Monath et al. 1970).

The high incidence of reactors to JC virus differs from previous Canadian studies in which SSH has usually been surmised as the infecting serotype. However, both SSH and JC have been documented to cause human disease in Canada (Artsob 1983). Therefore, although CAL serogroup viruses are generally considered to be of low virulence, this study does indicate the desirability of surveillance of possible clinical cases of California encephalitis that may be occurring during periods of mosquito activity.

These periods of mosquito activity generally began in mid-June for *Ae. abserratus* and *Ae. punctor*, whereas *Ae. canadensis* is not usually common until July. Epidemiologically, it is interesting that the three virus isolations reported here were from mosquitoes caught after mid-July or later. The mean physiological age of both *Ae. abserratus* and *Ae. punctor* was 1-par by mid-July by which time 75–90% of the females of these two species had already been caught (Mokry 1984). This suggests the possibility that the isolation of JC from a mixed batch of *Ae. abserratus* and *Ae. punctor* may represent part of the virus amplification cycle in vertebrates and not necessarily an overwintered transovarial infection (Berry et al.1977). The August 18 and 20 isolations of SSH from *Ae. canadensis* coincided with the time when females of this species averaged 2-par and only about 5% of the female population was still alive (Mokry 1984). Transovarial transmission has been demonstrated for SSH as well (McLean et al.1975) and it it surprising that we repeatedly have no isolations from any mosquito species earlier in the summer. Belloncik et al. (1982, 1983) reported 21 isolations of CAL serogroup viruses in Quebec, at least 18 of which were SSH, and all of which were isolated from mosquitoes caught before mid-July. Their data show a temporal distribution of species very similar to our Newfoundland mosquito data and it is thus likely that most of their isolations came from nulliparous females, an indication of transovarial transmission. In contrast, our results to date suggest that the greatest activity in the transmission of SSH and JC may take place later in the summer. While our isolations may still represent overwintered virus in older females, there is an increasing likelihood of their being acquired infections.

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