ARBOVIRUS STUDIES IN THE TROIS-RIVIÈRES AREA, PROVINCE OF QUEBEC, CANADA

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ABSTRACT. Serological and virus isolation studies were conducted in the Trois-Rivières area, Province of Quebec, from 1974 to 1978. Antibodies to St. Louis encephalitis, Powassan, western equine encephalitis and California serogroup viruses were detected in human and animal sera. Seven strains of snowshoe hare virus were isolated, including four from *Aedes punctor* and one each from *Ae. aurifer, Ae. communis* and *Ae. excrucians*. This is the first reported isolation of snowshoe hare virus from *Ae. aurifer* mosquitoes.

INTRODUCTION

The city of Trois-Rivières (46'20N, 72'32W) is located in the southern part of Québec province. Many mosquito species are present in this zone (Maire and Aubin 1980) which consists mainly of hardwood forests and farms. These include mosquitoes known to be vectors of arboviruses in other parts of Canada (Artsob and Spence 1979). Seven arboviruses of potential human health importance have been isolated in other Canadian provinces (Artsob and Spence 1979) including California (CAL) serogroup viruses, snowshoe hare (SSH), Jamestown Canyon (JC), Powassan (POW), St. Louis encephalitis (SLE), eastern equine encephalitis (EEE), western equine encephalitis (WEE) and Colorado tick fever viruses. However, relatively few arbovirus studies have been reported from the province of Quebec.

Previous findings have included the diagnosis of one human infection with WEE (Pavilanis et al. 1957) and of four with POW viruses (Rossier et al. 1974, Conway et al. 1976, Fauvel, personal communication), the isolation of EEE virus from horses (Bellavance et al. 1973) and the demonstration of human exposure to WEE, CAL and one or more flaviviruses (Artsob et al. 1980). More recently, six humans with central nervous system infections due to CAL serogroup viruses have been reported in Québec province: three in 1978 (Fauvel et al. 1980), one in 1980 (Fauvel et al. 1981) and two in 1981 (Fauvel, personal communication). One human infection of SLE virus (Artsob et al. 1980) and imported flavivirus infections (Artsob et al. 1978a) were also reported. Snowshoe hare (SSH) virus was isolated from rabbits and mosquitoes (Belloncik et al. 1982) and was strongly incriminated in human cases of the California encephalitis.

The growing recognition of arboviruses in the etiology of human disease throughout Canada, and, in particular, Quebec province prompted us to report results of a study conducted from 1974 to 1978 in which numerous human and animal sera were tested for arbovirus antibodies and virus isolation were attempted from mosquitoes collected in the vicinity of the city of Trois-Rivières.

MATERIALS AND METHODS

AREA OF STUDY. The studies were conducted in Trois-Rivières and an area surrounding the city (Fig. 1). Sera were collected from various species of animals chosen at random in the area, as well as sentinel chickens and rabbits. Many locations were investigated. These included wooded areas such as La Mauricie National Park (Fig. 1f) and Les Vieilles Forges (Fig. 1d), residential areas in wooded regions as Trois-Rivières West (Fig.1c) and Cap de la Madeleine, residential areas in the city of Trois-Rivières, and farms including St-Prosper (Fig. 1b), Gentilly (Fig. 1a), St-Maurice (Fig. 1c), Nicolet, Pointe du Lac, Maskinongé, 60 km west of Trois-Rivières (not on Fig. 1) and Champlain.

COLLECTION OF SERA. *Human:* A total of 4,469 sera were obtained from Trois-Rivières area residents. These included 2,554 sera collected from August to December, 1976 and 1,915 sera collected from July to September, 1977. These sera were originally collected for VDRL tests by local hospitals. The negative samples were randomly selected and sent to us by the department of Social Affairs (Service of Dr. S. S. Kasatiya) without any information on the age, sex or medical record of the individuals.

Indicator animals: Sentinel chickens and rabbits were placed in cages (two to five per cage) from 1974 to 1977. These animals were maintained in the field from June to September,

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bled weekly and tested for antibodies to EEE, WEE, POW, SLE and CAL viruses.

Non-indicator animals: Sera from the following animals were collected from the Trois-Rivières region: horses (with no known previous history of vaccination with EEE or WEE), wild birds, snowshoe hares, chipmunks, cattle, goats, sheep, pigs and dogs and chickens.

Mosquito collections: Adult mosquitoes were collected during 1977 and 1978 at five different sites (Fig. 1) using CDC light traps supplemented with carbon dioxide. The locations sampled were chosen close to where seroconversions occurred. The mosquitoes were identified and pooled in groups of 50 according to species and collection site.

SEROLOGICAL TESTS. All sera were tested by hemagglutination-inhibition (HI) against EEE, WEE, SLE, POW and SSH virus antigens. Sera were kaolin treated and HI tests performed by the method of Clarke and Casals (1958) modified by Sever (1962). Four units of antigen

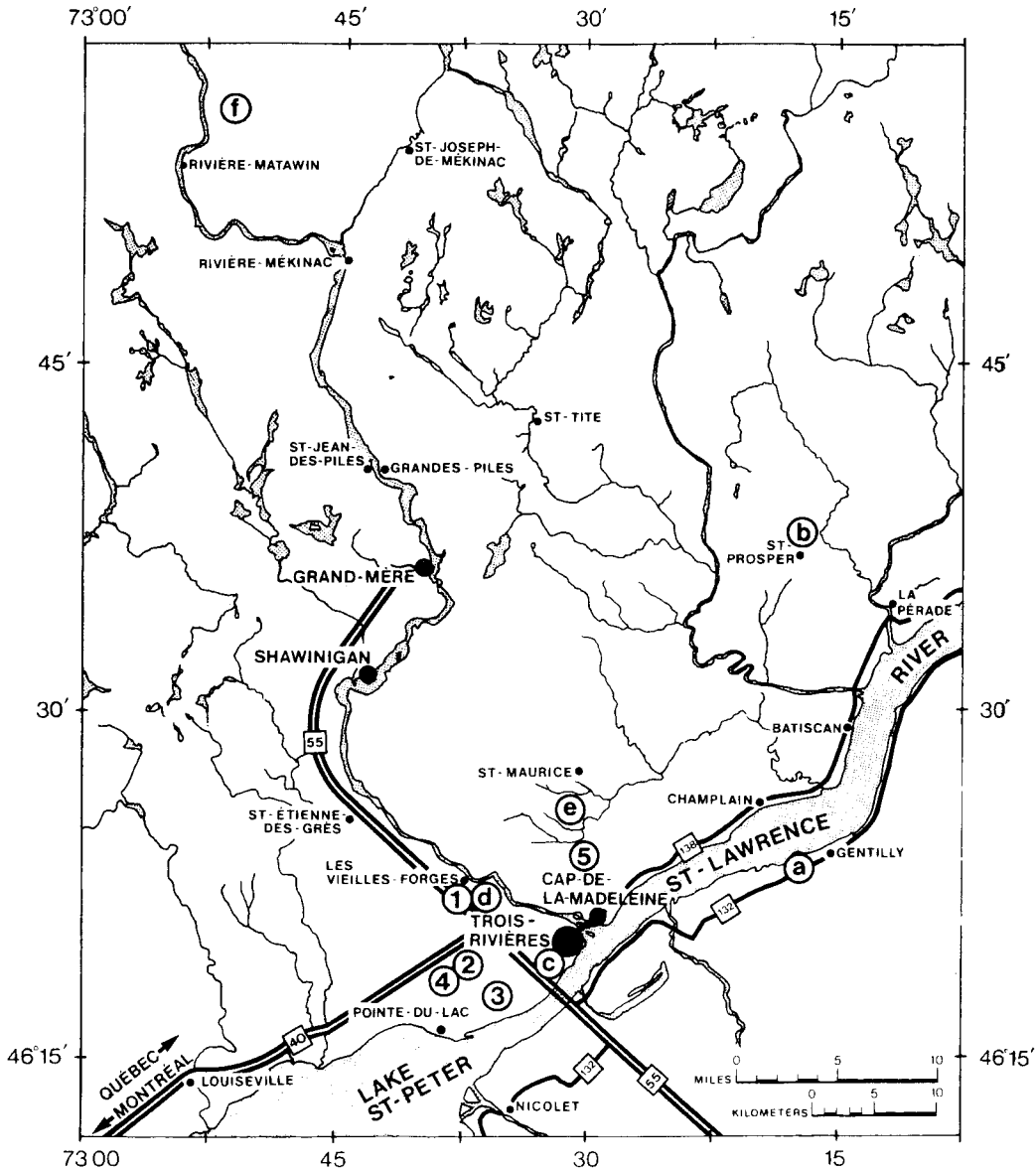

Fig. 1. Investigation area in the Province of Quebec. Sites of arbovirus detections by serology (a to f) and viral isolation (1 to 5).

were used for all viruses except for SLE, for which eight units were employed. All sera inhibiting antigen in preliminary tests were acetone treated and retested prior to being considered as genuine HI positives. Bird sera were treated with protamine sulphate to remove additional non-specific inhibitors.

Neutralization (N) tests were conducted on all sera positive by HI to SSH antigen. These tests were undertaken by the cell culture infective dose technique described elsewhere (Artsob et al. 1978b) against snowshoe hare (SSH), La Crosse (LAC), trivittatus (TVT) and Jamestown Canyon (JC) viruses.

VIRUS ISOLATION AND IDENTIFICATION. Attempted virus isolation from mosquitoes and identification of the isolates obtained was carried out as described elsewhere (Belloncik et al. 1982).

RESULTS

Arbovirus activity was demonstrated between 1974 and 1978 in the area of Trois-Rivières. Analysis of human sera revealed arbovirus antibodies (Table 1). Six of 2,554 of these sera (0.23%) reacted to SSH antigen by HI; neutralizing antibodies to SSH virus were confirmed in three of these six sera. Human exposure to flaviviruses was demonstrated in sera collected in 1976. 125 out of 2,554 sera (4.9%) reacted by HI to SLE antigen including 66 (2.6%) with a titer higher than 1/10. Nine sera reacted to POW (titer 1/20) including three which reacted to both POW and SLE. No evidence was found for infection with EEE virus. Three sera showed HI antibody to WEE virus (titers 1/10–1/20). Seventeen of 105 horses (16.2%) had HI antibodies to SSH antigen with reactors detected in sera taken from 1974 to 1976 (Table 1). Neutralizing antibodies to CAL group viruses were confirmed in 13 of these 17 HI reactors with titers generally highest to SSH and LAC viruses. However, one serum showed highest antibody titer to JC virus. One horse showed HI antibodies to EEE virus.

Indicator rabbits in three different wooded areas as well as two other animals taken randomly from farms were found to have antibodies to CAL viruses during 1974 and 1975 (Table 1). Neutralization tests showed the probable infecting agent to be SSH or LAC virus. Seroconversions occurred from the beginning to the end of July in three locations: the city of Trois-Rivières West, the suburb of Trois-Rivières and the village of St-Maurice (Fig 1 C, D, E).

Hares from the wooded area of La Mauricie National Park (Fig. 1) were found to have antibodies to SSH virus. Sera of five of nine ani-

Table 1. Hemagglutination inhibition reactions of sera collected in the Trois-Rivières area (Province of Quebec) to arbovirus antigens.

Host	Collection dates	Number of sera tested	Number of HI positive sera to antigen: SSH	EEE	WEE	SLE	POW	Location[1]
Human	Aug.–Dec. 1976	2554	6	0	3	125(66)[2]	9[3]	*
"	July–Sept. 1977	1915	0	0	0	0	0	*
Horses	Aug. 1974	4	1	0	0	0	0	a
"	July–Aug. 1975	25	4	0	0	0	0	a,b
"	March–May 1976	41	12	1	0	0	0	b
"	Sept. 1977	35	0	0	0	0	0	a
Hares	July–Oct. 1974	9	5	0	0	0	0	f
Rabbits	July and Aug. 1974	4(4)[4]	(1)[5]	0	0	0	0	d
"	June–Aug. 1975	56(21)[4]	2(2)[5]	0	0	0	0	c,e
"	June–Aug. 1976 and 1977	96(82)[4]	0	0	0	0	0	
Wild	Oct.–Nov. 1976	51	0	0	0	3	1[6]	**
Birds	July–Aug. 1977	150[7]	0	0	0	0	0	
Chicken	June–Sept. 1975, 1976 and 1977	441(224)[4]	0	0	0	0	0	**
Other	June–Aug. 1975 and 1976	158	0	0	0	0	0	*

[1] Localization on map (Fig. 1) of sera positive animals *: Several locations **: Maskinongé, (not on the map).

[2] ( ): Number of positive sera with titer > 1/10.

[3] Three sera of nine reacted also to SLE antigen.

[4] ( ): Number of indicator animals.

[5] ( ): Number of seroconversions.

[6] Serum positive to SLE also.

Other: Sera of 41 sheep, 20 dogs, 78 cows, 10 pigs, 8 squirrels and 1 goat.

SSH: Snowshoe hare, SLE: St. Louis encephalitis, EEE: Eastern equine encephalitis, POW: Powassan, WE: Western equine encephalitis.

mals bled in 1974, reacted to SSH virus by HI and N tests (Table 1).

Three birds showed HI antibodies to SLE virus (Table 1). The birds, (two rusty blackbirds *(Euphagus carolinus)* and one house sparrow *(Passer domesticus)* were caught at Maskinongé between October and November 1976. One of the two rusty blackbirds reacted also to POW virus.

No arbovirus antibodies could be demonstrated in chipmunk, bovine, goat, sheep, pig, dog or chicken sera (Table 1).

Seven isolates of SSH virus were obtained from 17,615 adult mosquitoes collected in 1978 (Table 2 and 3). Four mosquitoes species from five collection sites yielded SSH virus including one isolate from each of *Aedes aurifer* (Coquillett), *Ae. communis* (De Geer), *Ae. excrucians* (Walker) and *Ae. punctor* (Kirby). No viral isolates were obtained from 2,889 mosquitoes collected during the dry 1977 summer.

DISCUSSION

The main objective of this study was to expand our knowledge of the presence of arboviruses in the province of Quebec. This study in the Trois-Rivières area revealed the exposure of humans to SSH, to SLE viruses or a closely related flavivirus, and possibly to WEE virus. In addition, indicator rabbit and hare exposure to CAL serogroup viruses, horse exposure to CAL serogroup viruses and possibly EEE, and flavivirus antibodies in wild birds was demonstrated. Finally, SSH activity was conclu-

Table 2. Isolation of California serogroup viruses from mosquitoes collected in the Trois-Rivieres area, Quebec during 1978.

	Mosquitoes		Isolates			
Species	No. of mosquitoes caught	% of mosquitoes caught	No. of isolates	Collection dates[1]	Location[2]	MFIR[3]
Ae. aurifer	993	5.64	1	*	1	1.01
Ae. communis	1280	7.27	1	**	2	0.78
Ae. excrucians	2017	11.45	1	June 9	3	0.50
Ae. punctor	2384	13.53	1	May 30	4	1.68
			1	June 9	2	
			2	June 9	5	
Other[4]	10941	62.11	0			
Total	17615	100	7			

* Pool of mosquitoes collected June 20 and July 7.

** Pool of mosquitoes collected June 1, 9, 22 and July 12.

[1] Collection date of mosquito pools from which virus was isolated.

[2] Locations on map (fig. 1).

[3] Minimum Field Infection Rate.

[4] Other: *Aedes abserratus* (Felt and Young), *Ae. canadensis* (Theobald), *Ae. cinereus* Meigen, *Ae. decticus* Howard, Dyar and Knab, *Ae. diantaeus* H.D. and K., *Ae. fitchii* (Felt and Young), *Ae. intrudens* Dyar, *Ae. provocans* (Walker), *Ae. stimulans* (Walker), *"Culex-Culiseta", Cx. pipiens-restuans, Culiseta melanura* (Coquillett), *Cs. morsitans* (Theobald), *Coquillettidia perturbans* (Walker), *Anopheles* sp.

Table 3. Identification of virus isolates from the Trois-Rivières area, Quebec by neutralization tests.

		Antisera			
Isolate	Origin	SSH	LAC	JC	Identification
1	*Ae. aurifer*	640[1]	80	20	SSH
2	*Ae. communis*	640	20	40	SSH
3	*Ae. excrucians*	320	80	20	SSH
4	*Ae. punctor*	640	160	20	SSH
5	*Ae. punctor*	640	20	20	SSH
6	*Ae. punctor*	320	80	20	SSH
7	*Ae. punctor*	640	40	20	SSH
WML55-59-75	SSH topotype	640	40	20	
M3123	LAC topotype	40	160	20	
61V-2235	JC Prototype	40	40	320	

[1] Reciprocal of serum antibody titer.

SSH: Snowshoe hare; LAC: La Crosse; JC: Jamestown Canyon.

sively documented with the isolation of seven strains from *Aedes* spp. mosquitoes.

Our results suggest the widespread distribution of CAL serogroup viruses in the Trois-Rivières area as already shown in other provinces of Canada (Artsob 1983). From serological and viral isolation studies we demonstrated in the Trois-Rivières area CAL group activity from the end of May to the end of July. Comparative neutralization titration with SSH, LAC, TVT and JC viruses generally suggested SSH or LAC as the infecting serotype. However, one horse reactor with the highest titer to JC virus was noted. Jamestown Canyon virus has been already isolated in Alberta, Ontario and Saskatchewan (Artsob 1983) and human exposure to this agent has been shown in Entrelacs, Quebec (Fauvel, personal communication). It will be of interest to further investigate the presence of JC virus in Quebec province because it is known to cause human disease (Grimstad et al. 1982).

The low CAL infection rate of humans is similar to that found by Artsob et al. (1980) studying the same area during 1971–74. However, the percentage of horse sera positive to CAL viruses was found to be high. This fact is in agreement with the statement of Artsob et al. (1978b) that horses are very good monitors of SSH activity.

As demonstrated by McLean et al. (1975, 1977, 1979, 1981), SSH virus infections occur in natural foci. In our region we identified some foci in wooded areas but not in the city of Trois-Rivières due to the absence of mosquitoes. However, SSH virus activity was found in Tros-Rivières West which has residential wooded areas and a high density of *Aedes* species. Therefore, risk of human infection by CAL viruses is present.

CAL virus activity was not detected in 1977 either by serological or viral isolation studies. The dry summer and subsequent low density of mosquitoes could explain the relative absence of arboviruses. An absence of CAL viruses was also noted in Ontario in 1977 (Thorsen et al. 1980).

It was shown that *Ae. punctor* had a high SSH minimal field infection rate followed by two other species: *Ae. communis* and *Ae. excrucians*. This is the first reported isolation of SSH virus from *Ae. aurifer*, a very abundant species along the shore of the St. Lawrence river (Maire et al. 1978). It was also clearly demonstrated that infected mosquitoes were in the proximity of the areas where seroconversions occurred (Fig. 1: locations c, d., e and 1 to 5).

Several studies in western Canada reported exposure of animals or human to flaviviruses (McLean et al. 1969, Kettyls et al. 1972, McLintock 1976). In the Province of Québec, Artsob et al. (1980) found 1% of the human sera, collected in Trois-Rivières from 1971 to 1974, positive to SLE antigen by HI but no HI antibodies to SLE in a 1978 sampling. In our study, 2.6% and 0% of the population had HI antibodies to SLE in 1976 and 1977, respectively. Despite the fact that an appreciable number of indicator chickens were bled in 1975 and 1976, no seroconversion to SLE virus was noted, showing no evidence for recent SLE virus activity in the Trois-Rivières area.

Three HI reactors to WEE virus were detected. Artsob et al. (1980) observed one resident of Quebec city who had antibodies to WEE virus, likely Highlands J (Calisher et al. 1980). Neutralization tests would be useful to confirm our reactors and to determine whether they were infected with the Highlands J strain.

Intensive research done, in another part of the province, from 1978 to 1982 (Belloncik et al. 1982, Belloncik et al. unpublished results) demonstrated the high frequency and persistence of SSH virus in an infected area. For a more complete understanding of the ecology of arboviruses in the province of Québec, further investigations in areas of demonstrated virus activity would be productive.

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