STUDIES ON A FOCUS OF CALIFORNIA GROUP VIRUS ACTIVITY IN SOUTHERN ONTARIO

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ABSTRACT. California (CAL) group virus activity was studied in the area of Dunnville (42°50'N, 79°40'W), southern Ontario. In 1979, two of five study sites yielded CAL group virus. At one site we isolated Jamestown Canyon (JC) virus from *Aedes euedes, Ae. stimulans-fitchii* and unsorted *Aedes* spp. At the second we isolated JC virus from *Ae. stimulans-fitchii* and snowshoe hare (SSH) virus from *Ae. stimulans-fitchii* and *Ae. canadensis* and demonstrated seroconversions of sentinel rabbits to SSH virus. In 1981 the second site showed continuing virus activity over a 1 km transect of varying terrain including forest, scrub vegetation and open lawn. Seroconversions of sentinel rabbits to SSH and trivittatus viruses were demonstrated and JC and Flanders viruses were recovered from pools of *Ae. stimulans* and *Culex* spp., respectively. Neutralizing antibodies with highest titer to SSH virus were found in 23 (5.7%) and JC virus in 2 (0.5%) of 406 Dunnville residents tested between 1979 and 1982. No clinical infections due to CAL group were documented despite increased diagnositc surveillance during this study.

INTRODUCTION

Evidence for the presence of California (CAL) group viruses has been found in all ten provinces of Canada as well as in the Yukon and Northwest Territories (Artsob 1983). Three CAL group serotypes have been identified in Canada, snowshoe hare (SSH), Jamestown Canyon (JC) and trivittatus (TVT) viruses. In addition, 11 clinical cases of California encephalitis have been documented between 1978 and 1981 including six from Quebec, four from Ontario and one from Nova Scotia (Artsob 1983).

Evidence has been presented for the circulation of three CAL group serotypes in Ontario. Isolations have been made primarily of SSH (Artsob et al. 1978, 1982; McKiel et al. 1966, Thorsen et al. 1980), but also of TVT virus (Thorsen et al. 1980). In addition, a case of California encephalitis likely due to the JC serotype was recognized in Ontario in 1981 (Deibel et al. 1983).

We attempted to identify a site of CAL group virus activity and to study various parameters related to the site, including relative importance of different mosquito vectors, periods of optimal virus activity, extent of virus activity over a given area and over varying terrain and the possible recurrence of virus activity from one year to another. The Dunnville area in southern Ontario was chosen, since high antibody prevalence to SSH virus has been detected in residents of this area (Artsob et al. 1982).

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MATERIALS AND METHODS

STUDY SITES. The town of Dunnville is situated on the Grand River in southern Ontario (42°50'N, 79°40'W). In 1979 caged sentinel rabbits were placed and adult mosquitoes collected at five study sites.

Site 1 was located on the north boundary of town. The topography was nearly flat and the area had poor natural drainage. The soil was dark gray sandy loam over gray mottled sand with clay at about 0.3 to 0.6 m; stone-free. The area immediately adjacent to these residences in this area was heavy bush with many swamp holes or pockets of water. These represented actual areas for the breeding of mosquitoes.

Site 2, north of town, was basically identical to site 1 other than it was less populated with a greater abundance of wild life.

Site 3, east of town, was very similar to the previous two. However, the soil conditions were brown sandy loam over yellow and then mottled sand with clay to a depth of 0.9 to 1.5 m; stone-free. The topography was smooth to undulating and fair to poor surface drainage. This area was the least densely populated of the three sites described so far and was very abundant in wild life such as rabbits, deer and fox.

Site 4 represented basically an agricultural area and heavy bush north of town. The soil was brown loam, fine sandy loam and some clay loam over gray gritty clay with some stones. The topography was basically rolling to hilly with good drainage. However, throughout bush areas, there were low lying swamp hole areas which represented excellent breeding grounds for mosquitoes.

Site 5 was similar to site 4. However, it was located south of town. The soil was gray to light brown clay loam over gray or reddish gritty clay with few stones and some silty knolls. The to-

pography was rolling to smooth upland with fair to poor surface drainage.

Expanded studies were undertaken in 1981 at Dunnville site 3. Sentinel rabbits were placed at four sites within approximately a 1 km transect. The sites were selected over varying terrain including two sites in forested areas about 500 m apart (site 3, the original 1979 study site, and site 3a), one site in scrub vegetation (site 3b) and a fourth site in open lawn (site 3c).

SENTINEL RABBIT STUDIES. Four New Zealand white rabbits were placed, four per cage, at each site. Rabbits were bled from the ear weekly and sera were tested by hemagglutination inhibition (HI) for antibodies to CAL group viruses. When seroconversions were observed, reactors were replaced the same week so that four test rabbits were continually maintained per site. Rabbits were kept in the field from May 29 to August 31, 1979 and from May 3 to August 31, 1981.

MOSQUITO COLLECTIONS

ADULT MOSQUITOES. Weekly collections of adult mosquitoes were made using CDC light traps which were suspended ca. 0.5 m from the tops of rabbit hutches. In 1979, collections were made from 5 June to 28 August and in 1981 from 12 May to 25 August. Female mosquitoes were identified by G. S. and J. M. using the keys of Wood et al. (1979).

MOSQUITO LARVAE. *Aedes* spp. larvae were collected from snow melt pools in the vicinity of Dunnville sites 2 and 3 in late April and early May of 1980 and 1981. Larvae were collected by sweeping a standard insect net through the pools. Larvae were reared in the laboratory to fourth instars or adults, identified using the keys of Wood et al. (1979) and tested for virus by inoculation of vero cells or by intracranial inoculation of suckling mice (Artsob et al. 1982).

HUMAN SERA COLLECTIONS. Sera collections, to monitor exposure of Dunnville residents to CAL group viruses, included three sera taken in 1979 from healthy adults residing near site 3 and 380 sera taken from patients without symptoms suggestive of arbovirus infections at Haldimand War Memorial Hospital, Dunnville (280 sera were taken between June 17 and September 9, 1981 and 100 sera were taken in September 1982). In addition, during the summers of 1979–82 paired sera were obtained from 23 patients with illnesses consistent with arbovirus etiology ranging from pyrexia of unknown origin to aseptic meningitis.

IDENTIFICATION OF VIRUS ISOLATES. Ten percent mouse brain suspensions in saline were made from all mice showing signs of illness. These suspensions were clarified by centrifugation (10,000 rpm for 30 min) and tested by complement fixation (CF) against reference grouping ascitic fluids supplied by the National Institutes of Health (NIH), U.S.A.

Isolates identified as CAL serogroup viruses were typed by neutralization (NEUT) tests using mouse ascitic fluid prepared to the following strains: SSH prototype Burgdorfer and JC prototype 61V2235 supplied by Dr. C. H. Calisher of Centers for Disease Control, Fort Collins; LaCrosse (LAC) topotype 3123 supplied by Dr. D. M. McLean, University of British Columbia and TVT topotype 7941 originally isolated at the Rocky Mountain Lab, Montana in 1949 and supplied by Dr. J. R. Polley of the Laboratory Center for Disease Control, Ottawa.

For NEUT typing, ascitic fluids were prepared to JC and TVT by hyperimmunization of mice using four live vaccine doses and sarcoma 180 cells as per Tikasingh et al. (1966). Ascitic fluids were produced to SSH and LAC by one-shot immunization using live vaccine followed by inoculation of sarcoma 180 cells 7 days, and tapping of mice 17 days, after infection. Ascitic fluids produced to SSH and LAC by one-shot immunization were more specific in typing these closely related serotypes than those produced by hyperimmunization.

SEROLOGICAL TESTS. Hemagglutination inhibition tests on acetone treated sera were performed by the method of Clarke and Casals (1958) as modified to a microtiter technique by Sever (1962). Complement fixation tests were carried out by the microtiter method described by Sever (1962).

Neutralization tests were performed by incubating 0.1 ml volume 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

In 1979 six rabbits at site 3 seroconverted to SSH virus by HI tests. In 1981 seroconversions to SSH virus were detected by HI in seven rabbits between July 14 and August 11 including three at site 3, two at site 3a and two at site 3b; no seroconversions occurred in rabbits at site 3c.

Neutralization test results (Table 1) indicated that 8 of 13 CAL group seroconversions were presumed due to SSH virus and 4 most likely

Table 1. California group serology of sentinel rabbits from Dunnville, Ontario, 1979 and 1981.

Site[1]	Date of conversion	Titer of neutralizing antibody to: SSH[2]	LAC[2]	JC[2]	TVT[2]	Probable infecting serotype
3	25.VI.1979	80	—[3]	—	—	SSH
3	25.VI.1979	≥640	≥640	—	—	SSH or LAC
3	30.VII.1979	≥640	≥640	—	40	SSH or LAC
3	30.VII.1979	320	80	—	—	SSH
3	30.VII.1979	≥640	160	—	—	SSH
3	7.VIII.1979	320	320	—	—	SSH or LAC
3	14.VII.1981	320	80	—	—	SSH
3	21.VII.1981	160	40	—	—	SSH
3	21.VII.1981	≥640	320	—	—	SSH
3a	4.VIII.1981	≥640	40	—	—	SSH
3a	11.VIII.1981	80	80	—	40	SSH or LAC
3b	21.VII.1981	—	—	—	160	TVT
3b	21.VII.1981	320	40	—	—	SSH

[1] See Materials and Methods for site descriptions.
[2] SSH = Snowshoe hare virus, LAC = LaCrosse virus, JC = Jamestown Canyon virus, TVT = Trivittatus virus.
[3] — = < 1:40.

due to infection with this virus. One rabbit in the scrub vegetation showed highest NEUT titers to TVT virus.

VIRUS ISOLATIONS FROM MOSQUITOES—ISOLATES OBTAINED. A total of 44,713 adult mosquitoes was collected, 17,081 in 1979 (Table 2) and 27,632 in 1981 (Table 3). From 895 pools, 12 virus isolates were obtained including 11 CAL group isolates and one isolate of Flanders (FLA) virus (Table 4). Ten of 11 CAL group isolates were from mosquitoes collected in 1979, including three from site 2 and seven from site 3.

All CAL group isolates were obtained from *Aedes* spp. mosquitoes, including six from *Ae. stimulans-fitchii,* three from *Ae. canadensis*

Table 2. Mosquitoes collected in CDC light trap from Dunnville, Ontario, 1979.

Mosquito species	Site number 1	2	3	4	5	Total
Aedes aurifer	2(1)[1]	4(2)	—	1(1)	—	7(4)
Ae. canadensis	65(4)	841(14)	1938(25)[5]	41(8)	81(5)	2966(56)
Ae. cinereus	5(2)	37(7)	43(6)	10(4)	2(2)	97(21)
Ae. euedes	43(4)	775(13)[2]	118(6)	2(1)	—	938(24)
Ae. flavescens	—	3(1)	—	—	—	3(1)
Ae. provocans	—	1(1)	—	—	—	1(1)
Ae. stimulans-fitchii	866(15)	4807(55)[3]	2680(33)[6]	63(7)	40(7)	8456(117)
Ae. stimulans-fitchii-euedes	62(1)	344(4)	—	—	—	406(5)
Ae. trivittatus	1(1)	—	9(2)	—	—	10(3)
Ae. triseriatus	4(3)	21(3)	7(3)	8(4)	1(1)	41(14)
Ae. vexans	100(9)	328(13)	934(16)	10(4)	43(9)	1415(51)
Aedes spp.	—	1275(13)[4]	—	—	—	1275(13)
Anopheles spp.	—	9(3)	113(10)	—	5(1)	127(14)
Culex spp.	231(10)	156(9)	254(10)	9(3)	15(6)	665(38)
Culiseta spp.	1(1)	6(6)	6(2)	—	—	13(5)
Coquillettidia perturbans	21(6)	112(6)	204(8)	304(10)	20(5)	661(35)
Total	1401(57)	8719(146)	6306(121)	448(42)	207(36)	17081(402)

[1] ( ) = Number of pools screened.
[2] = A California group virus was isolated from a pool of 100 *Aedes euedes* collected on June 12.
[3] = A California group virus was isolated from a pool of 100 *Aedes stimulans-fitchii* collected on June 12.
[4] = A California group virus was isolated from a pool of 100 *Aedes* spp. collected on June 5.
[5] = California group isolates were obtained from pools of 104 and 100 *Aedes canadensis* collected on July 4 and a pool of 100 *Ae. canadensis* collected on July 17.
[6] = A California group isolate was obtained from a pool of 101 *Ae. stimulans-fitchii* collected on July 4, from pools of 100 and 98 *Ae. stimulans-fitchii* collected on July 17 and from a pool of 100 *Ae. stimulans-fitchii* collected on July 31.

Table 3. Mosquitoes in CDC light trap from Dunnville, Ontario, 1981.

Mosquito species	Site number 3	3a	3b	3c	Total
Aedes canadensis	3172(34)[1]	761(12)	68(6)	87(6)	4088(58)
Ae. cinereus	219(6)	149(7)	49(4)	102(5)	519(22)
Ae. dorsalis	—	—	2(1)	8(2)	10(3)
Ae. provocans	6(3)	—	4(1)	1(1)	11(5)
Ae. stimulans	1238(15)	1228(16)	392(11)	1439(25)[3]	4297(67)
Ae. triseriatus	2(1)	3(1)	—	—	5(2)
Ae. trivittatus	13(2)	13(2)	5(2)	24(4)	55(10)
Ae. vexans	1203(17)	2375(30)	3444(40)	3192(38)	10214(125)
Aedes spp.	2238(28)	110(6)	20(3)	156(7)	2524(44)
Aedes + *Coquillettidia perturbans*	227(3)	1525(16)	—	100(1)	1852(20)
Anopheles spp.	126(12)	292(18)	415(19)	393(21)	1226(70)
Culex spp.	24(5)	54(5)[2]	19(2)	11(4)	108(16)
Culiseta spp.	15(4)	—	42(5)	9(2)	66(11)
Cq. perturbans	436(7)	340(10)	1273(11)	608(12)	2657(40)
Total	8919(137)	6850(123)	5733(105)	6130(128)	27632(493)

[1] ( ) = Number of pools screened.
[2] = Flanders virus was isolated from a pool of 41 *Culex* spp. collected on June 23.
[3] = A California group virus was isolated from a pool of 100 *Ae. stimulans* collected on June 16.

(Theobald), one from *Ae. euedes* (Howard, Dyar and Knab) and one from a pool of unsorted *Aedes* spp. mosquitoes. The CAL group isolates from site 2 had a Minimum Field Infection Ratio (MFIR) of 1:775, 1:4807 and 1:1275 for *Ae. euedes, Ae. stimulans-fitchii* and unsorted *Aedes* spp., respectively. Similarly, at site 3 the MFIR for *Ae. stimulans-fitchii* and *Ae. canadensis* was 1:670 and 1:646, respectively. The MFIR for the CAL group isolate from *Ae. stimulans* (Walker) at site 3c in 1981 was 1:1439 and for FLA virus from *Culex* spp. at site 3a was 1:54.

No virus isolates were obtained from 991 *Ae. canadensis* and 1276 *Ae. stimulans* larvae collected and reared in the laboratory to fourth instar or adult.

TYPING OF CALIFORNIA GROUP ISOLATES. The CAL group isolates were initially identified by CF testing against CAL grouping ascitic fluid supplied by N.I.H. Two of 11 isolates were sub-

Table 4. Neutralization typing of California group isolates from Dunnville, Ontario, 1979 and 1981.

Isolate typed	Isolated from	Antibody titer[1] SSH[2]	LAC[2]	JC[2]	TVT[2]	Serotype
9–54	*Aedes* spp.	—[3]	—	160	—	JC
9–68	*Ae. euedes*	—	—	320	—	JC
9–71[4]	*Ae. stimulans-fitchii*	—	—	320	—	JC
9–178[4]	*Ae. stimulans-fitchii*	—	—	80	—	JC
9–180	*Ae. canadensis*	320	40	40	—	SSH
9–192	*Ae. canadensis*	640	40	40	—	SSH
9–237	*Ae. stimulans-fitchii*	—	—	320	—	JC
9–252	*Ae. stimulans-fitchii*	N.T.[5]	N.T.	N.T.	N.T.	CAL
9–254	*Ae. canadensis*	N.T.	N.T.	N.T.	N.T.	CAL
9–312	*Ae. stimulans-fitchii*	160	20	40	—	SSH
1–237-81	*Ae. stimulans*	—	—	80	—	JC
SSH Burgdorfer		320	40	20	—	
LAC 3123		40	640	40	—	
JC 61V2235		—	—	160	—	
TVT 7941		—	—	—	160	

[1] Reciprocal of highest antibody dilution that neutralized 100 $TCID_{50}$ of virus.
[2] CAL = California group untyped, JC = Jamestown Canyon, LAC = LaCrosse, SSH = Snowshoe hare, TVT = trivittatus.
[3] – = < 1:20.
[4] Isolates 9–71 and 9–178 were independently typed as JC virus by Dr. C. H. Calisher of the Centers for Disease Control, Fort Collins.
[5] N.T. = Not typed.

sequently lost on storage and could not be typed. Neutralization typing of the remaining nine isolates showed that six were JC and three were SSH viruses (Table 4). Jamestown Canyon isolates were obtained from *Ae. euedes, Ae. stimulans-fitchii* and unsorted *Aedes* spp. mosquitoes. The SSH isolates were obtained from *Ae. canadensis* and *Ae. stimulans-fitchii.*

The 1979 isolates included three JC from site 2 as well as two JC, three SSH and two CAL group untyped strains from site 3. The 1981 isolate from site 3c was JC virus.

DYNAMICS OF CALIFORNIA GROUP ACTIVITY AT SITE 3. The temporal sequence of CAL group virus activity at site 3 in 1979 is presented in Fig. 1. Evidence for SSH activity was obtained as early as June 26 with two sentinel rabbit seroconversions. However no CAL group strains were recovered from mosquitoes taken in June.

The earliest isolates were obtained from mosquitoes taken on July 4 with two isolates of SSH and one isolate of JC virus. Mosquitoes taken on July 17 yielded one JC isolate as well as two CAL group strains that were not typed. One isolate of SSH virus was obtained from mosquitoes taken on July 31.

Sentinel rabbit conversions on July 31 and August 8 appeared to reflect SSH activity in mosquitoes in July. No antibodies to JC virus were demonstrated in any of the sentinel rabbits. Disappearance of CAL group activity at site 3 paralleled the disappearance of *Ae. canadensis* and *Ae. stimulans-fitchii.*

HUMAN SEROLOGY. A total of 406 Dunnville residents were tested with 25 NEUT confirmed CAL group reactors including 23 (5.7%) with highest NEUT titers to SSH and 2 (0.5%) having highest NEUT titers to JC virus.

One of three healthy adults (two female, one male) residing near site 3 and bled in August, 1979 showed antibodies. This was a 39 year old male who had highest NEUT titers to SSH virus.

Of 280 sera collected in 1981, 25 had HI antibody to SSH virus. Neutralization tests confirmed 18 of these, with 16 having highest NEUT titers to SSH and two having highest NEUT titers to JC virus. The 100 sera collected in 1982 were tested for NEUT antibodies to CAL group viruses. Five (5%) of the individuals had NEUT antibodies to SSH. No antibody to JC virus was detected.

Twenty-three patients with possible illness of arbovirus etiology were screened during summers of 1979–82. One patient had HI and

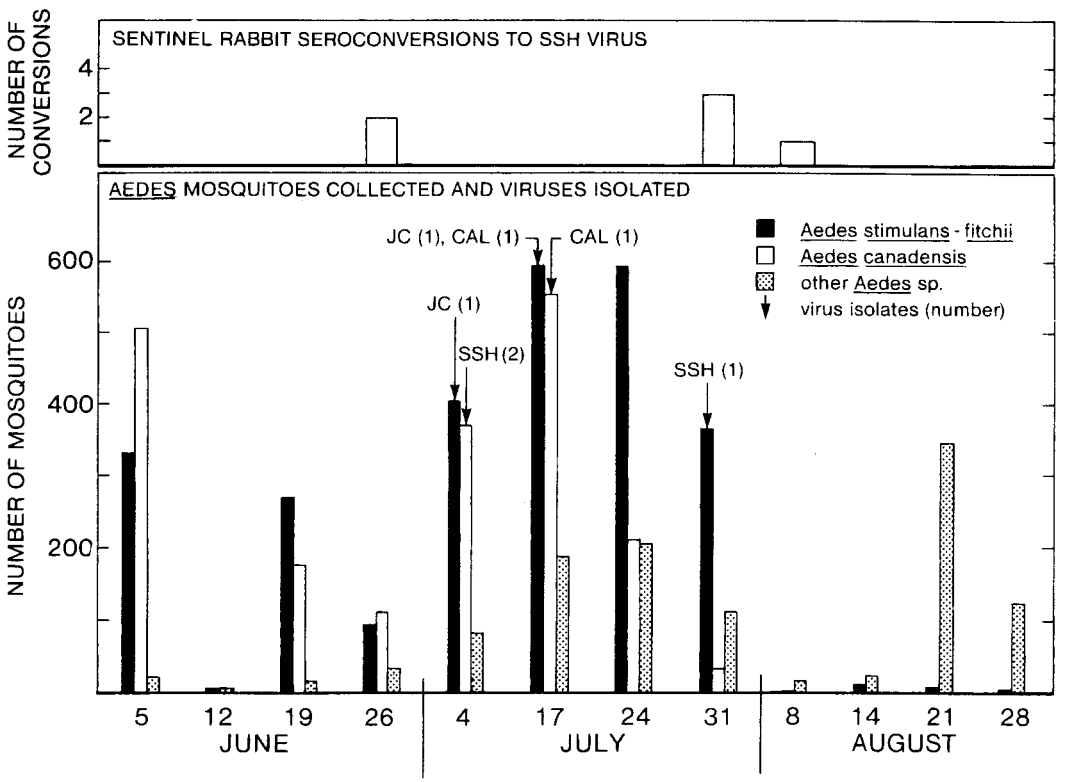

Figure 1. Temporal sequence of California group virus activity at Dunnville site 3, 1979.

NEUT titers of 1:10 and 1:40, respectively to SSH virus but no CF antibodies. However antibody titers remained static in three sera drawn over a three mo period.

DISCUSSION

These studies confirm a previous serological survey (Artsob et al. 1982) which indicated that Dunnville is a focus of CAL group virus activity. Two of five sites chosen for study in 1979 revealed CAL group virus activity with JC virus isolations from mosquitoes at one site (site 2) and sentinel rabbit conversions as well as JC and SSH virus isolations at a second site (site 3).

The pattern of CAL group virus activity in Dunnville suggests that humans may be infected with CAL group viruses from early June to early August; JC activity was demonstrated from June 5 to July 17, SSH from early June to July 31 and TVT in July.

The fact that both JC and SSH were actively circulating at site 3 but seroconversions in rabbits occurred only to SSH virus indicates that these sentinels are poor monitors of JC virus activity. This agrees with Seawright et al. (1974) who inoculated ten New Zealand white rabbits intravenously with JC virus and found antibody response in only one. Only one common vector, *Ae. stimulans-fitchii,* was identifed for both JC and SSH at site 3, and a possible role for vector preference, such as *Ae. canadensis* vs *Ae. stimulans-fitchii* feeding on sentinel rabbits, cannot be excluded.

This study provides the first clear documentation of JC virus in Ontario and complements the diagnosis of a clinical case of California encephalitis in Ontario in 1981 due likely to the JC serotype (Deibel et al. 1983). This is of particular interest in view of the recently recognized role of JC virus as a causative agent of human disease in Michigan and New York as well as Ontario (Deibel et al. 1983, Grimstad et al. 1982).

Studies to more fully document the presence and distribution of JC virus in Ontario could include testing of deer, a known amplifying host of JC virus (Issel et al. 1972, Issel 1973) and possible screening of various additional Diptera from which JC has been isolated (DeFoliart et al. 1969).

Although no isolates were obtained from field collected larvae reared to fourth instars or adults, the demonstration of CAL group virus activity at site 3 in 1979 and 1981 suggests that both SSH and JC strains over-wintered at this site. The demonstration of multiple CAL group activity (SSH, JC and possibly TVT) at one site, apparently persisting over a prolonged period of time, as well as the isolation of two serotypes (SSH and JC) from a common vector, *Ae. stimulans-fitchii,* provide a natural setting for the potential generation of genetic recombinants, as has been demonstrated under laboratory conditions (Gentsch et al. 1978).

The discovery of extensive CAL group activity over a 1 km range and over differing terrain parallels observations of Keystone virus in the Pocomoke Cypress Swamp (LeDuc 1978) and of SSH virus at several study sites in Entrelacs, Quebec (Belloncik et al. 1982). LaCrosse virus has been shown to recur yearly at specific sites (Clark et al. 1983, Pantuwatana et al. 1974, Watts et al. 1974). However the primary vector of LAC virus, *Ae. triseriatus* (Say) does not travel far from its breeding sites, primarily tree holes, and LAC virus activity has been shown to be very localized (Balfour et al. 1976). The Dunnville focus of activity extends over a wide area and numerous *Aedes* species were involved indicating that control measures to eliminate virus activity may not be practical.

The isolation of FLA virus from a pool of *Culex* spp. expands our knowledge of the distribution of this virus in Ontario. Previously, a total of 34 FLA isolates were reported from Essex, Lambton, Niagara and Wellington counties (Thorsen et al. 1980). Flanders virus is not considered to be a pathogen for man.

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