St. Louis encephalitis in southern Ontario: laboratory studies for arboviruses

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The first reported outbreak of St. Louis encephalitis in Canada occurred in the summer of 1975 in southern Ontario — in the Windsor–Sarnia–Chatham area, the Niagara region and the city of Toronto. Hemagglutination inhibition and complement fixation testing of serum samples collected during the outbreak confirmed that St. Louis encephalitis virus was the etiologic agent. Furthermore, this virus was isolated from brain tissue of a patient who died. This oubreak was probably an extension of the outbreak that occurred in the United States that summer. It was the first outbreak of arbovirus encephalitis in the province of Ontario.

La première épidémie d'encéphalite de St-Louis à être signalée au Canada est survenue durant l'été de 1975 dans le sud de l'Ontario dans la région de Windsor–Sarnia–Chatham, la région de Niagara et la ville de Toronto. Les épreuves d'inhibition de l'hémagglutination et de fixation du complément des échantillons de sérum prélevés durant l'épidémie ont confirmé que le virus de l'encéphalite de St-Louis était l'agent infectieux. De plus, le virus a pu être isolé du cerveau d'un patient qui est décédé. Cette épidémie est probablement une extension de l'épidémie enregistrée aux États-Unis durant le même été. C'était là la première épidémie d'encéphalite à arbovirus signalée en Ontario.

In the summer of 1975 an outbreak of encephalitis occurred among residents of southern Ontario. Intensive laboratory investigations were carried out during the outbreak. This report is the first of a series dealing with the clinical, epidemiologic and virologic aspects of the outbreak. It describes the arbovirus investigations carried out on specimens submitted from all parts of Ontario during the summer of 1975 to the National Arbovirus Reference Service, a laboratory in Toronto operating in conjunction with the Laboratory Centre for Disease Control of Health and Welfare Canada.

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Epidemiologic and clinical aspects

The outbreak began at the end of July and continued into October. The most severely affected area was the city of Windsor but patients were encountered in other localities in southern Ontario, including the Niagara peninsula and the city of Toronto.

Patients admitted to hospital had symptoms and signs of meningoencephalitis. They complained of fever, severe headaches, chills, malaise, anorexia, pain or stiffness of the neck or both, myalgia, photophobia, nausea, vomiting, drowsiness and confusion. In some patients vomiting, tiredness, sleepiness and depression persisted for several weeks after discharge from hospital. Cerebrospinal fluid (CSF) was abnormal in patients with meningoencephalitis: protein values were normal or moderately elevated and the cellular response was mainly lymphocytic; glucose values were within normal limits. In fatal cases the brain showed the typical histologic features of viral encephalitis — perivascular cuffing and microglial proliferation (N.B. Rewcastle, M. Dietrich, M.J.E. Oxley: personal communications, 1975).

Virologic investigation

Methods

Serum samples from 472 patients suspected of having St. Louis encephalitis (SLE), 420 of whom were from areas thought to be involved in the outbreak, were submitted to the National Arbovirus Reference Service from all parts of the province of Ontario. One CSF and three brain specimens were also received for viral studies. Investigations were confined to tests for arboviruses on the serum samples and attempts to isolate virus from the brain and CSF specimens.

Control reagents and antigens of encephalitis-associated eastern equine encephalomyelitis (EEE), western equine encephalomyelitis (WEE), Powassan (POW) and SLE viruses isolated previously in Canada were prepared in suckling mouse brain by the methods described by Clarke and Casals.[1] Antigen to California encephalitis (CAL)

virus and parallel control material were prepared from vero cell cultures.

Hemagglutination inhibition (HI) tests were performed by the method of Clarke and Casals[1] modified to a microtitre technique described by Sever.[2] Four hemagglutinating (HA) units of each of EEE, WEE, POW and CAL antigens were used routinely in the HI test; 8 HA U of SLE antigen were used in an attempt to eliminate the nonspecific inhibitory reactions obtained sometimes with this antigen.

Complement fixation (CF) tests were carried out by a modification of the microtitre method described by Sever[2] with 2 U of antisheep hemolysin, 2 U of complement and 4 U of antigen; the sheep cell concentration was reduced to 0.4% to provide a more sensitive test.

Attempts to isolate virus were carried out by inoculating 0.02 ml of CSF or brain suspension (20% brain in phosphate-buffered saline containing 0.75% bovalbumin) intracerebrally into suckling mice. The mice were observed for 14 days for evidence of illness.

Results

In the HI tests (Table I) none of the serum samples reacted with EEE and WEE antigens. Serum from 33 patients showed low-titre antibody responses to CAL antigen; however, none of the positive serum reacted by CF with CAL antigen, and diagnostic increases in titre of the antibody were not demonstrated by HI. Twelve serum samples gave positive HI reactions with POW antigen, but all 12 reacted at higher titres with SLE antigen; thus the patients were presumed to be infected with SLE virus, which is antigenically related to Powassan virus — hence, cross-reactions can be expected in serologic testing. Serum samples from 82 patients were positive for SLE by HI testing; 65 of these were also positive for SLE by CF testing.

Cases of infection were considered confirmed if a fourfold increase or decrease in antibody titre was demonstrated by testing paired serum samples by CF or HI, or if a single sample had a titre of 1:32 or higher by the CF test. Thus 63 cases of SLE were confirmed serologically: in 20 there was a fourfold increase in titre; in 2, a fourfold decrease in titre; and in 41, a CF titre of 1:32 or greater in a single serum sample or in serial samples. Representative antibody titres in five confirmed cases during the outbreak are presented in Table II.

Antibodies to SLE were demonstrated by HI testing in 19 patients in whom the criteria for diagnosis of SLE infection were not met. Most of these patients had HI titres of 1:10, which could have been either nonspecific reactions or the result of past exposure to a group B arbovirus. Two of the 19 also had antibodies to SLE by CF testing but at titres of less than 1:32. Diagnostic antibody titres of 1:32 or greater were not found even though more than one serum specimen was tested.

Serologically confirmed cases of SLE originated in the following localities: Windsor, Sarnia, Chatham, Hamilton, Toronto, Lincoln, St. Catharines, Welland and Niagara Falls. In addition, one confirmed case was found in northern Ontario (Espanola) but the patient had arrived recently from Ohio, where she was thought to have acquired the disease. Many of the patients were over 40 years of age and deaths occurred only in this group.

Included among the serologically confirmed cases were two fatal cases. In a third fatal case the serum showed antibody responses to SLE antigen by both CF and HI testing, with twofold increases in antibody titre in samples taken 1 day apart. No more samples were available in this case; however, presumptive serologic findings combined with clinical and pathological signs consistent with viral encephalitis confirmed the diagnosis of SLE (L.A. Hatch: personal communication, 1975).

Another case of SLE was diagnosed by the isolation of virus from brain tissue; this was one of the three such specimens tested. The virus was identified in a neutralization test in suckling mice inoculated intraperitoneally. No arboviruses were isolated from CSF.

Discussion

SLE virus, first isolated in the United States in 1933,[3] has been responsible for numerous outbreaks of human disease in that country,[4,5] including an extensive outbreak in at least 25 states during 1975.[6] This virus was first isolated in Canada from *Culex tarsalis* mosquitoes collected in Weyburn, Sask. in 1971,[7] but antibody surveys[8-11] suggested evidence for the presence of SLE virus in Manitoba, Alberta and British Columbia in the past. The outbreak in Ontario is the first recognized outbreak of SLE in Canada and may reflect a northern extension of the large SLE outbreak that occurred in the United States during the same year.[6] This is also the first recorded outbreak of arbovirus encephalitis in humans in Ontario. It is not yet possible to conclude that the virus will persist in this province but further investigations are planned to determine whether it will.

This outbreak should alert physicians to the possibility of arbovirus infection in patients with aseptic meningitis or encephalitis. Any patient may be the first with SLE in an outbreak and early identification of the etiologic agent is desirable in order that appropriate control measures may be instituted.

Facilities for diagnosing arbovirus infections are now available at many of the provincial public health laboratories and at the National Arbovirus Reference Service in Toronto. The most use-

Table I—Results of hemagglutination inhibition (HI) and complement fixation (CF) tests on single or serial serum samples from 472 patients with suspected St. Louis encephalitis in Ontario, 1975

Antigen to encephalitis-associated virus	No. positive/total no. tested*	
	HI	CF
Eastern equine	0/472	NT
Western equine	0/472	NT
California	33/472	0/33
Powassan	12/472	NT
St. Louis	82/472	65/82

*NT = not tested

Table II—Representative titres of antibody to antigen of St. Louis encephalitis virus from five serologically confirmed cases of St. Louis encephalitis

Patient no.	Date of onset of symptoms	Date serum collected	Titre	
			HI	CF
1	Aug. 28	Sept. 8	1:20	1:64
		Sept. 19	1:160	1:256
2	Aug. 1	Sept. 14	1:20	1:32
		Sept. 18	1:20	1:64
3	Sept. 12	Sept. 15	1:10	< 1:4
		Sept. 29	1:20	1:32
		Oct. 23	1:40	1:64
4	Aug. 20	Aug. 27	1:160	1:256
		Sept. 8	1:160	1:256
5	Sept. 16	Sept. 19	< 1:10	< 1:4
		Sept. 29	1:320	1:128
		Oct. 17	1:80	1:128

ful specimens for study are clotted blood specimens collected in a sterile container during both the acute and convalescent stages of illness. Since enteroviruses may produce aseptic meningitis, stool and CSF specimens should also be submitted.

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