

# Imported arbovirus infections in Canada 1974-89

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**H ARTSOB, L SPENCE. Imported arbovirus infections in Canada 1974-89. *Can J Infect Dis* 1991;2(3):95-100.** From 1974 to 1989, sera from symptomatic patients with histories of recent travel outside Canada were tested for antibodies to several arboviruses, principally of the alphavirus and flavivirus families. Diagnostic seroconversions were documented in 84 individuals from six provinces, including one alphavirus (Chikungunya) and 83 flavivirus seroconvertors. Dengue 1 virus was isolated from the blood of one patient. Most flavivirus seroconvertors were likely infected with dengue virus, but infections with tick-borne encephalitis, St Louis encephalitis and Powassan viruses were also recognized. Patients had histories of recent travel to the Caribbean, South America, Asia, Africa, North America (outside Canada), Tahiti, Fiji and Europe. Possible imported infections due to Japanese encephalitis, Ross River, western equine encephalitis and Colorado tick fever viruses were also encountered.

**Key Words:** *Alphavirus, Arbovirus, Dengue, Flavivirus*

## Arboviroses importées aux Canada de 1974 à 1989

**RESUME:** De 1974 à 1989, on a analysé le sérum de patients asymptomatiques revenus sous peu de l'étranger pour rechercher les anticorps dirigés contre plusieurs arbovirus – de la famille des alphavirus et des flavivirus principalement. Des séroconversions diagnostiques ont été établies chez 84 sujets originaires de six provinces, avec une réponse anticorps anti-alphavirus (Chikungunya) et 83 anti-flavivirus. Le virus de la dengue 1 a été isolé chez un patient. La plupart des séroconversions étaient imputables au virus de la dengue, mais le virus de l'encéphalite à tiques, de l'encéphalite américaine de Saint-Louis et le virus Powassan ont également été reconnus. Les patients avaient récemment voyagé aux Caraïbes, en Amérique du Sud, en Asie, en Afrique, en Amérique du Nord (à l'extérieur du Canada), à Tahiti, aux Fidji et en Europe. On a également décelé des infections dues aux virus de l'encéphalite japonaise, de la rivière Ross, de l'encéphalite équine nord-américaine de l'Ouest, et de la fièvre à tiques du Colorado.

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Received for publication August 27, 1990. Accepted December 11, 1990

THE INTERNATIONAL CATALOGUE OF ARBOVIRUSES lists 504 viruses, including 115 proven arboviruses, 357 viruses that are probable or possible arboviruses, and 32 viruses that are probably not or not arboviruses (1). In nature, 110 of these viruses, including 30 flaviviruses, have been shown to be capable of causing human disease. Seven of these arboviruses of 'known human disease'-causing potential have been isolated in Canada, including eastern equine encephalitis, western equine encephalitis, St Louis encephalitis, Powassan, snowshoe hare, Jamestown Canyon and Colorado tick fever viruses (2).

From 1974 to 1989 diagnostic serology was performed at the National Arbovirus Reference Service in Toronto to identify arbovirus infections contracted by individuals while outside of Canada. The results of the study are presented in this report.

## MATERIALS AND METHODS

**Preparation of viral antigens:** Antigens were prepared from Colorado tick fever virus and from the following alpha- and flaviviruses: Chikungunya, eastern equine encephalitis, Semliki forest, Sindbis and western equine encephalitis viruses, all alphaviruses; and dengue type 2, Powassan and St Louis encephalitis viruses, all flaviviruses. All viruses were propagated by intracerebral inoculation of suckling mice with subsequent sucrose-acetone extraction of infected mouse brain (3). An inactivated antigen from tick-borne encephalitis virus was purchased from Behring Diagnostics (Germany).

**Serological tests:** Hemagglutination inhibition tests were performed using the method of Clarke and Casals (3) as modified to a microtitre technique by Sever (4). All sera were acetone-treated and absorbed with packed goose erythrocytes prior to hemagglutination inhibition testing. Four hemagglutinin units were used of each alphavirus antigen, while eight hemagglutinin units were employed for each flavivirus, in an attempt to eliminate the nonspecific hemagglutination inhibition positives more commonly seen with flaviviruses. Initial dilutions of test sera were 1:10.

Complement fixation tests were carried out by a modification of the microtitre method described by Sever (4). Two units of antisheep hemolysin, two units of complement and four units of antigen were used in each test. The sheep cell concentration was reduced to 0.4% in order to provide a more sensitive test. Initial serum dilutions were 1:4.

Virus isolation attempts were undertaken on selected acute sera of patients for whom a seroconversion was demonstrated and whose acute

**TABLE 1**  
**Arbovirus infections contracted outside of Canada**

Year	Probable infecting virus	Area of travel (number of cases)
1974	Chikungunya or O'Nyong Nyong	Uganda (1)
1975	St Louis encephalitis	United States (1)
1976	Flavivirus	Nigeria, Ghana and Dahomey (1)
1977	Dengue	Jamaica (12); Haiti (1); French Guinea, Surinam and Guadeloupe (1); Unknown (5)
	Flavivirus	India (1); India, Thailand and Indonesia (1); Sri Lanka (1)
1978	Dengue	Trinidad (3); Jamaica (1); Tahiti (1)
	Flavivirus	Nigeria (1); Southeast Asia (1); Unknown (3)
	Powassan	United States (1)
1979	Dengue	Barbados (1); Haiti (1); Tahiti (1); Thailand (1)
	Flavivirus	Sri Lanka (2); Unknown (2)
1980	Dengue	Fiji (2); Jamaica (1)
	Flavivirus	Guyana (1); India (1)
1981	Dengue	Haiti (1)
	Flavivirus	Unknown (1)
1982	Dengue	India (2); Jamaica (2)
	Flavivirus	India (1); Martinique (1); Unknown (3)
	Tick-borne encephalitis	Austria (1)
1983	Dengue	Haiti (1); Tahiti (1)
1984	Dengue	Haiti (3); Trinidad (1)
1985	Flavivirus	India, Nepal and Malaysia (1); Korea and Philippines (1)
1986	Dengue	Mexico (1)
	Flavivirus	Chad (1)
1987	Dengue	Haiti (1); Mexico (1)
	Flavivirus	Thailand (2); Guyana (1)
1988	Dengue	Dominican Republic (2); Haiti (1)
	Flavivirus	Unknown (1)
1989	Dengue	St Martens (1)
	Flavivirus	Philippines (1); Southeast Asia (1); Unknown (1)

serum was found to be hemagglutination inhibition negative. Sera were generally stored at 4°C until virus isolation was attempted. Sera were inoculated into *Aedes albopictus* C6/36 and Vero cells with subsequent incubation of cultures at 30°C and 37°C, respectively.

Seven sera were submitted to Dr Jordi Casals of the Yale Arbovirus Research Unit (YARU), who undertook hemagglutination inhibition serology for Ross River virus. Three sera from a patient with suspected Japanese encephalitis were also submitted to YARU, where plaque reduction neutralization tests using the Nakayama strain of Japanese encephalitis virus and Japanese encephalitis IgM capture enzyme-linked immunosorbent assay (ELISA) were performed.

An isolate, presumptively identified as dengue virus, was submitted to Gladys Sather, Centers for Disease Control, Puerto Rico, for typing. This isolate was inoculated into *Aedes pseudoscutellaris* cells and identified by plaque reduction neutralization tests.

## RESULTS

**Confirmed cases:** From 1974 to 1989, diagnostic seroconversions were obtained in sera from 84 patients, including 59 for whom fourfold or greater rises in antibody titres were demonstrated, and 25 for whom diagnostic declines in titres were documented (Table 1). Thirty-seven individuals had histories of travel to the Caribbean and/or South America, 17 to Asia, four to Africa, four to North America (outside Canada), three to Tahiti, two to Fiji and one to Europe. The travel histories of 15 seroconvertors were unknown.

Confirmed cases were diagnosed during every year of testing, with peak numbers of cases recognized in 1977 (22 cases), 1978 (11 cases) and 1982 (10 cases). The numbers of diagnosed cases by province of submission were as follows: Ontario 43, Quebec 17, British Columbia 10, Alberta eight, Manitoba four and Nova Scotia two.

One patient seroconverted to Chikungunya antigen by hemagglutination inhibition and complement fixation serology (Table 2). The patient was a 45-year-old female who had onset of fever, chills, generalized rash and headache eight days after leaving Uganda. In the absence of neutralization tests, it was not possible to conclude whether the infection was due to Chikungunya or O'Nyong-Nyong virus.

The remaining 83 seroconvertors were to flavivirus antigens. These included 49 patients diagnosed as having dengue fever and thus reported as probable dengue cases, 31 flavivirus seroconvertors for whom the probable infecting flavivirus could not be surmised, and one patient each with tick-borne encephalitis, Powassan and St Louis encephalitis virus infections.

Two patterns of flavivirus seroconversion were seen, exemplified by three dengue cases reported in Table 3. One pattern indicating primary exposure to flaviviruses (patient 899, Table 3) was characterized by relatively low hemagglutination inhibition titres, delayed antibody responses and limited cross reactions between flavivirus antigens, particularly in the complement fixation test. By contrast, in secondary flavivirus infections, antibody titres rose quickly and were extremely high, and extensive cross reactions occurred between flavivirus antigens.

Symptoms most often accompanying confirmed dengue cases included fever, headache (usually

TABLE 2

Alphavirus seroconversion in patient with recent travel to Uganda

Date serum taken*	Chikungunya serology	
	HI†	CF
01/04/1974	1:10	<1:4
06/05/1974	1:80	1:16

\*Onset of symptoms on 30/03/1974. †Hemagglutination inhibition serology negative for eastern equine encephalitis, Sindbis and western equine encephalitis antigens. CF Complement fixation; HI Hemagglutination inhibition

TABLE 3

Representative serology of confirmed flavivirus seroconvertors likely infected with dengue virus

Patient no.	Date taken	HI			CF		
		DEN	POW	SLE	DEN	POW	SLE
899	26/09/77*	—	—	—	—	—	—
	15/10/77	20	—	40	—	—	—
	14/11/77	20	—	40	16	—	—
1181	13/03/79	2,560	2,560	1,280	1,024	256	512
	26/03/79	10,240	10,240	5,120	16,384	1,024	4,096
1269	18/10/79	320	40	320	128	—	8
	29/10/79	2,560	160	5,120	1,024	—	256

\*Dengue 1 virus was isolated from this blood sample. — Less than 1:10 by hemagglutination inhibition and less than 1:4 by complement fixation. CF Complement fixation; DEN Dengue virus; HI Hemagglutination inhibition; POW Powassan virus; SLE St Louis encephalitis virus

TABLE 4

Flavivirus serology of confirmed tick-borne encephalitis case

Date taken*	HI			CF			
	DEN	POW	SLE	DEN	POW	SLE	TBE
20/08/82	—	—	—	—	—	—	—
31/08/82	20	40	40	—	4	4	16
19/10/82	20	40	40	—	4	4	32

\*History of tick bite on 12/08/82. — Less than 1:10 by hemagglutination inhibition and less than 1:4 by complement fixation. CF Complement fixation; DEN Dengue virus; HI Hemagglutination inhibition; POW Powassan virus; SLE St Louis encephalitis virus; TBE Tick-borne encephalitis virus

described as frontal), eye pain, muscle and joint pains, and rash. Less commonly reported symptoms included nausea, vomiting and diarrhea. One of the patients who seroconverted with a secondary pattern of infection (patient 1269, Table 3) was a 22-month-old Laotian male from a Thai refugee camp, who was diagnosed as having dengue hemorrhagic fever (5). This patient was hospitalized because of melena, hematemesis and persistent fever. Petechiae were noted on the lower extremities, followed the next day by a generalized maculopapular rash and palpebral edema. The patient had disseminated intravascular coagulation with bleeding into the skin, gastrointestinal tract, genitourinary tract and possibly central nervous system. The patient recovered following appropriate treatment.

The patient who seroconverted to tick-borne encephalitis virus was a 55-year-old male who had onset of symptoms seven days after history of a tick bite while in Austria (Table 4). Symptoms included ongoing severe fatigue, anorexia and malaise. Serology and history of travel and tick bite clearly indicated that the patient had been infected with tick-borne encephalitis virus.

The Powassan and St Louis encephalitis cases were likely contracted in the states of New York and Ohio, respectively, and have been reported previously (6,7).

**Virus isolation:** A virus, isolate 899a, was obtained from the blood of a patient with a history of recent travel to Jamaica (patient 899, Table 3). Cytopathic effects from isolate 899a were first noted in *A albopictus* C6/36 cells at 11 days post inoculation. Isolate 899a underwent further passage in *A albopictus* C6/36 cells but was not pathogenic to three-day-old suckling mice challenged by the intracerebral route. Isolate 899a was identified by plaque reduction neutralization tests as dengue 1 virus.

**Possible arbovirus infections:** In addition to the 84 seroconvertors, 186 of 723 patients with histories of recent travel outside Canada had flavivirus antibodies, while a further 18 reacted to alphavirus antigens. The flavivirus reactors included 124 patients from whom a second serum could not be obtained, and 62 patients with static titres in two or more sera. These patients could not be categorized as confirmed cases, but several interesting possible arbovirus infections were documented.

These included a 35-year-old female who in August 1982 developed meningoencephalitis soon after her return to Canada from Manchuria. Paired sera taken four and 31 days post onset of symptoms showed neutralization titres of 1:160 and 1:80, respectively, to Japanese encephalitis virus. Both sera were positive by IgM capture ELISA. Serology was considered consistent with, but not diagnostic of, Japanese encephalitis virus (personal communication).

A possible case of Ross River virus infection was encountered in 1979 in a woman who had travelled to Fiji. Symptoms included headache, fever and muscle and joint pains. Sera taken 32 and 42 days post onset of symptoms showed hemagglutination inhibition titres of 1:40 and 1:20, respectively, to Ross River virus, and were negative for related alphaviruses (eastern and western equine encephalitis viruses).

Possible infections with two additional arboviruses were noted in patients recently returned from the United States. These included a possible case of western equine encephalitis in a 48-year-

old woman who had been in Oregon in late June 1986 and who developed fever and headache 10 days after her return. A single serum taken 20 days post onset of symptoms revealed a hemagglutination inhibition titre of 1:640 to western equine encephalitis virus.

Finally, a 38-year-old woman developed febrile illness and headaches after visiting Colorado in July 1989. Paired sera taken two to three months post onset of symptoms showed static titres of 1:16 to Colorado tick fever virus by the complement fixation test.

## DISCUSSION

Reports of imported arbovirus infections in Canada date back to at least the late 18th century, when yellow fever cases were recorded on ships coming into Halifax from the West Indies (8). A report in January 1862 stated that several cases of yellow fever had actually originated in Halifax harbour aboard infected ships arriving from the West Indies, and in the hospital hulk *Pyramus*, which was anchored off the dockyard.

Despite these early reports, the degree to which arbovirus infections are contracted by Canadians abroad and/or are being incubated by visitors to Canada has not been extensively documented. Mahdy et al reported serological cases consistent with dengue fever in 11 patients returning to Ontario between 1976 and 1978 (9) and another probable dengue case in Ontario in 1983 (10). In addition, two probable dengue cases in patients returning to Quebec from Haiti were reported (11,12), and four of the 49 dengue cases reported in this paper have been published previously as case reports (5,13-15).

This large number of imported dengue cases is not a surprise, since dengue and dengue hemorrhagic fever are major public health problems in most tropical countries (16). It is, in fact, likely that many of the 31 flavivirus seroconvertors, for whom no specific viral agents were ascribed, were dengue cases.

The increased number of dengue cases in 1977 and 1978 reflects the occurrence of a dengue pandemic in the Caribbean due to the introduction of dengue 1 virus imported from either Asia or Africa (17). This pandemic started in Jamaica in February 1977, and subsequently spread throughout the Caribbean. Cases were recognized in Canadians returning from the Caribbean approximately three to four months after the first cases occurred in the respective countries. In addition to these confirmed dengue cases, many patients were encountered who had high flavivirus titres in single or static titres in paired sera during this time period.

The isolation of dengue 1 virus from the blood of a patient recently returned from Jamaica, and dengue 4 virus from the blood of another patient from Haiti, highlight the fact that viremic patients may be encountered (11). Under normal circumstances this would not be of great concern, since dengue virus likely cannot multiply in Canadian mosquitoes. However, the relatively recent introduction of *A albopictus* to the United States (18,19) raises the possibility of local amplification cycles of dengue virus in Canada, should *A albopictus* ever become established in this country.

There was only one probable case of Japanese encephalitis despite the prominence of this virus as a cause of encephalitis in Asia. The authors are not aware of any confirmed Japanese encephalitis cases having been identified in Canada, although there is a report of a patient with encephalitis in Manitoba in 1938 who had antibodies that showed questionable protection against St Louis encephalitis and definite protection against Japanese encephalitis virus (20). It has been speculated that appropriate mosquito vectors and vertebrate hosts exist, particularly on the west coast of Canada, to allow for the establishment of local amplification cycles of Japanese encephalitis virus (21,22).

**ACKNOWLEDGEMENTS:** The authors acknowledge the following institutes which provided seropositive specimens: Victoria General Hospital, Halifax; Laboratoire de Santé Publique du Québec; Institut Armand Frappier; McGill Centre for Tropical Diseases; Tropical Diseases Clinic, Toronto General Hospital; Ontario Public Health Laboratory; Cadham Public Health Laboratory; Provincial Laboratory of Public Health for Northern Alberta; and British Columbia Centre for Disease Control.

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