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Enzyme-Linked Immunosorbent Assay Typing of California Serogroup Viruses Isolated in Canada

HARVEY ARTSOB,* LESLIE P. SPENCE, AND CECELIA TH'NG

National Arbovirus Reference Service, Department of Microbiology, University of Toronto, Toronto, Ontario M5G 1L5, Canada

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A procedure was developed to type California serogroup viruses by an antibody-capture, enzyme-linked immunosorbent assay. Seven California serogroup members from North America were distinguished, including snowshoe hare, La Crosse, California encephalitis, San Angelo, Jamestown Canyon, Keystone, and trivittatus. Extensive cross-reactions were observed between Jamestown Canyon and the closely related South River strain. The enzyme-linked immunosorbent assay method was successfully applied to the typing of 77 California serogroup viruses isolated in Canada, including 61 snowshoe hare, 13 Jamestown Canyon, and 3 trivittatus topotypes.

The enzyme-linked immunosorbent assay (ELISA) for quantitative measurement of antibody-antigen reactions was developed in 1971 by Engvall and Perlmann (5) and by Van Weemen and Schurrs (14). The ELISA system utilizes enzyme-linked immunoreactants that are bound to a solid phase by a series of antigen-antibody reactions and measured by an enzyme-substrate reaction. ELISA has been used in the study of a variety of biological compounds, including hormones, haptens, normal and disease-specific antigens, and antibodies of various classes.

Our laboratory attempted to adapt the ELISA method to the typing of California (CAL) serogroup viruses. The CAL serogroup comprises 13 mosquito-transmitted viruses within the genus *Bunyavirus* (4). Three members of the CAL serogroup have been isolated in Canada, including snowshoe hare (SSH), Jamestown Canyon (JC), and trivittatus (TVT) (1).

Various methods have been employed to type CAL serogroup viruses, including immunodiffusion (9, 15) and complement fixation (10, 11), but the test most commonly used is a serum-dilution, plaque-neutralization test with single-immunization hamster sera (8).

An attempt was made to develop an ELISA typing scheme that would distinguish the CAL serogroup members known to occur in North America (4) and to apply the method to type 77 CAL serogroup topotypes isolated in Canada.

MATERIALS AND METHODS

Virus strains used in study. The CAL serogroup members used to immunize mice to produce reference antibody for development of our ELISA typing system were as follows: SSH, Burgdorfer, passage 21; La Crosse (LAC), original, passage 8; California encephalitis (CE), BFS 283, passage 6; San Angelo (SA), C13995, passage 19; JC, 61V-2235, passage 5; South River (SR), NJO-94F; Keystone (KEY), B64-5587.05, passage 7; and TVT, 7941, passage 5.

Typing was undertaken against CAL serogroup topotypes kindly provided by the following individuals: S. Belloncik, Institut Armand Frappier, Laval-des-Rapides, Quebec; C. H. Calisher, Centers for Disease Control, Fort Collins, Colo.; M. Grayson, New York State Public Health Laboratory, Albany; J. Iversen, Western College of Veterinary Medicine, Saskatoon, Saskatchewan; J. A. McKiel, Laboratory Center for Disease Control, Ottawa, Ontario; D. M. McLean, University of British Columbia, Vancouver; J. Mokry, Memorial University, St. John's, Newfoundland; L. Sekla, Cadham Public Health Laboratory, Winnipeg, Manitoba; and J. Thorsen, Ontario Veterinary College, Guelph. In addition, some topotypes studied were isolated by the National Arbovirus Reference Service, University of Toronto.

Production of hyperimmune mouse ascitic fluids. Immune ascitic fluids were produced by hyperimmunization of Swiss white mice by using four live vaccine doses and sarcoma 180 cells (13). Vaccines consisted of 10% clarified suspensions of infected suckling mouse brains. The first two vaccines consisted of equal volumes of virus mixed with Freund complete adjuvant, and the final two vaccines consisted only of virus.

Mice were inoculated intraperitoneally with 0.5 ml of vaccine on days 1, 7, 21, and 28 and with sarcoma 180 cells on day 25 postinitiation of immunization. Mice were tapped when they became distended, generally between days 35 and 40 postinitiation of immunization, and the ascitic fluids collected were clarified by centrifugation at 3,500 × *g* for 10 min.

ELISA. (i) Preparation of antigens. Antigens employed in the ELISA test consisted of supernatant fluid from virus-infected Vero cells harvested at times of maximum cytopathic effect. Antigens were clarified by centrifugation at 3,500 × *g* for 10 min and inactivated by treatment overnight at 4°C with 0.3% Betaprone (Chromalloy Pharmaceuticals Inc).

Vero control antigen was prepared by freezing and thawing uninfected Vero cells three times, followed by clarification of antigen by centrifugation at 3,500 × *g* for 10 min and overnight treatment at 4°C with 0.3% Betaprone.

(ii) Preparation of antibodies. Gamma globulins were precipitated from mouse ascitic fluids by the dropwise addition of an equal volume of saturated ammonium sulfate. The precipitates were collected by centrifugation at 3,500 × *g* for 5 min, washed two times with 50% ammonium sulfate, reconstituted to the original volume in phosphate-buffered saline (PBS), and then dialyzed against normal saline for 48 to 72 h at 4°C. Complement fixation titers of the immune gamma globulins ranged from 1:80 to 1:5,120.

(iii) Conjugation of antibodies. Conjugation of mouse gamma globulins to horseradish peroxidase (type VI, RZ [Reinheitszahl; measure of hemin content as provided by Sigma

* Corresponding author.

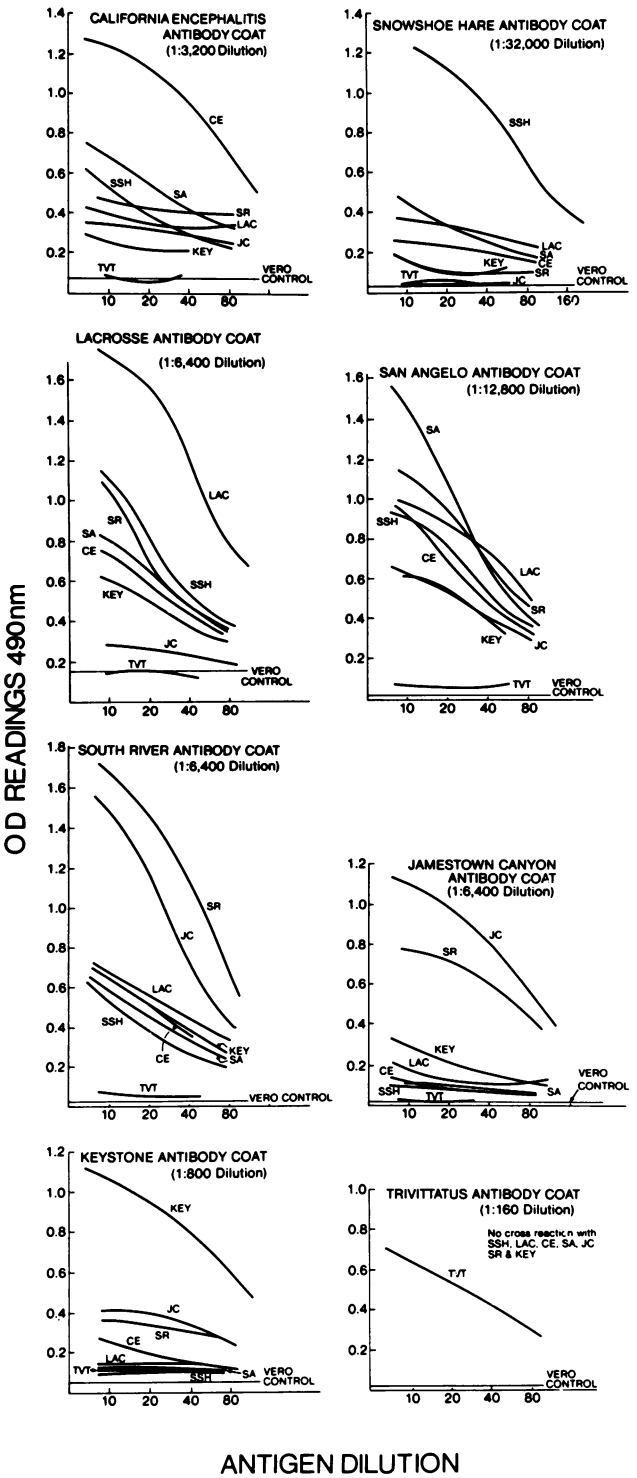

FIG. 1. Homologous and heterologous reactions of CAL serogroup viruses by the ELISA system. In the first ELISA system, plates were coated with immunoglobulin G of anti-CAL serogroup antibodies prepared in mice. The enzyme conjugate was horseradish peroxidase-conjugated anti-CAL serogroup antibodies prepared in mice.

Chemical Co.] = 3.2; Sigma Chemical Co.) was done by the method of Wilson and Nakane (16).

Antibody-capture ELISA. Wells of microtiter plates (Immulon II; Dynatech) were filled with 100 μl of mouse gamma globulin containing an average of 1 μg of protein diluted in 0.05 M sodium carbonate-bicarbonate buffer (pH 9.6). Plates were incubated overnight at 4°C and washed three times with PBS containing 0.5% Tween 20 (PBS-T), and the wells were filled with 1% bovine serum albumin diluted in PBS. After 1 h at room temperature, the bovine serum albumin was removed, and the plates were washed three times with PBS-T.

One hundred microliters of antigen, diluted in PBS-T containing 1% bovine serum albumin, was added to each well, and the plates were incubated overnight at 4°C. Plates were washed five times with PBS-T, and 100 μl of conjugate diluted 1:100 to 1:200 was added to each well. The optimum concentration of each conjugate was predetermined by homologous checkerboard titrations. Plates were incubated at room temperature for 1 h and washed five times with PBS-T, and 100 μl of freshly prepared substrate (40 mg of *ortho*-phenylenediamine in 100 ml of citrate buffer–0.1 M citric acid–0.2 M dibasic sodium phosphate containing 20 μl of 30% hydrogen peroxide) was added to each well.

Plates were incubated in the dark for 30 min, and the reactions were terminated by the addition of 50 μl of 2 M sulfuric acid. Readings were taken at an optical density (OD) at 490 nm with a Dynatech MR590 single-beam reader. Each sample was tested in duplicate, and an average of both readings was recorded. OD differences between duplicates were in the range of 0.03 to 0.06 and rarely exceeded 0.10.

Negative controls included the following: (i) conjugate control in which wells were not coated with antibody and antigen was not added, (ii) Vero control in which virus antigen was replaced by uninfected Vero cell antigen, and (iii) antigen control in which four serologically unrelated arboviruses, western equine encephalitis, St. Louis encephalitis, Powassan, and Cache Valley, were tested.

Neutralization. Neutralization tests were conducted with hyperimmune mouse ascitic fluids prepared to the three CAL serogroup members isolated previously in Canada, SSH, JC, and TVT. Neutralization tests were performed by incubating 0.1-ml volumes of heat-inactivated (56°C for 30 min) ascitic fluids of various dilutions with 0.1 ml containing 200 50% tissue culture infective doses of virus at 4°C overnight and inoculating Vero cells with 0.1 ml of the mixture (challenge dose of 100 50% tissue culture infective doses). Ascitic fluids were considered to contain neutralizing antibodies at the dilutions tested if complete inhibition of cytopathic effect was obtained.

RESULTS

Specific reactions were obtained with all eight CAL serogroup viruses, SSH, LAC, CE, SA, JC, SR, KEY, and TVT, although various degrees of cross-reactions were observed between the related CAL serogroup members. No reactions were obtained with unrelated arboviruses.

Homologous box titrations by ELISA for each system showed high antibody titers in the immune ascitic fluids. Antibody titers measured by ELISA were generally several hundred- or thousand-fold higher than those obtained by hemagglutination inhibition or neutralization.

To study cross-reactions between related CAL serogroup strains, dilutions of antibody coat (capture antibody) were selected that gave OD readings of ca. 1.0 when tested against

antigen dilutions of 1:10 to 1:20. The results are summarized in Fig. 1.

The SSH, LAC, CE, KEY, and TVT serotypes gave clearly identifiable distinctions between homologous and heterologous antigens. JC and SR, although clearly distinct from other strains, showed similar reactions and could not be confidently separated from each other by ELISA typing. The SA serotype showed specific reactions only at high antigen concentration. However, as the antigen concentration decreased, extensive cross-reactions were observed.

Representative OD readings of the various CAL serogroup strains at 1:10 antigen dilutions and dilutions of antibody that maximized serotype differentiation are presented in Table 1.

ELISA typing of Canadian CAL group topotypes. (i) SSH. A total of 61 SSH topotypes isolated from five Canadian provinces (Manitoba, Newfoundland, Ontario, Quebec, and Saskatchewan) and the Yukon were typed by ELISA (Table 2). All topotypes reacted to the highest titer with SSH ascitic fluid and could be confidently identified as SSH. Greater cross-reactions were observed with ascitic fluids produced to members of the CE subcomplex (CE, LAC, SA) than to members of the Melao (JC, SR, KEY) and TVT subcomplexes.

OD readings of all SSH topotype reactions with Melao and TVT subcomplex antibodies never exceeded 50% of the SSH OD readings, with strongest cross-reactions to the SR ascitic fluid. In contrast, 19 of the 61 SSH topotypes reacted to antibodies of one or more members of the CE subcomplex, with OD readings greater than 50% of the homologous SSH readings. OD readings exceeded 80% of the SSH homologous reactions only twice, with strains 8843 and 134A12 (Table 2). However, both topotypes were still clearly identifiable as SSH.

Neutralization tests confirmed the identity of all 61 topotypes as SSH.

(ii) JC. Thirteen topotypes of JC isolated from five Canadian provinces (Manitoba, Newfoundland, Ontario, Quebec, and Saskatchewan) showed the highest ELISA titers to JC, SR, or both (Table 3). Eleven topotypes showed highest OD readings to both JC and SR. However, topotypes 137A17 and 140A18 from Quebec, although showing the highest OD to SR, displayed higher OD reactions to CE and SA than to JC.

OD readings of the 13 topotypes to related CAL serogroup ascitic fluids (excluding JC) never exceeded 50% of the SR reactions, with the exception of SA in which OD readings ranged up to as high as 80% of the SR readings.

Neutralization tests with hyperimmune mouse ascitic fluids showed all topotypes to be JC virus.

(iii) TVT. Only three topotypes of TVT, all isolates from Ontario, were available for ELISA typing. All three strains showed clearly higher titers to TVT (Table 3) and were confirmed as TVT virus by neutralization tests.

TABLE 1. ELISA typing of reference CAL serogroup viruses[a]

Antigen[b]	OD[c] with hyperimmune mouse ascitic fluid[d] to:							
	SSH	LAC	CE	SA	JC	SR	KEY	TVT
SSH	1.63	0.22	0.14	0.23	0.05	0.10	0.10	0.05
LAC	1.05	1.61	0.61	0.94	0.16	0.68	0.47	0.04
CE	0.50	0.36	1.27	0.66	0.31	0.43	0.23	0.03
SA	0.89	0.96	0.90	1.49	0.62	1.11	0.60	0.05
JC	0.09	0.15	0.10	0.11	1.11	0.79	0.30	0.03
SR	0.48	0.62	0.60	0.59	1.51	1.62	0.56	0.07
KEY	0.05	0.09	0.21	0.07	0.36	0.31	1.01	0.08
TVT	0	0	0	0	0	0	0	0.86

[a] The following virus strains were used: SSH, Burgdorfer; LAC, original; CE, BFS 283; SA, C13995; JC, 61V-2235; SR, NJO-94F; KEY, B64-5587.05; TVT, 7941.

[b] All antigens used were 1:10 dilutions of tissue culture supernatant fluid (see text).

[c] OD readings represent an average of duplicate samples from which Vero control background was subtracted.

[d] The following dilutions of antibody coat were employed: SSH, 1:32,000; LAC, 1:6,400; CE, 1:3,200; SA, 1:12,800; JC, 1:6,400; SR, 1:6,400; KEY, 1:800; TVT, 1:160.

DISCUSSION

The ELISA typing system was able to distinguish seven CAL serogroup members, including SSH, LAC, CE, SA, JC, KEY, and TVT. Cross-reactions, observed between SA and other serogroup members, may possibly be minimized by production of single-immunization mouse ascitic fluid. However, under the conditions employed, the SA ascitic fluid was effective in typing CAL serogroup topotypes isolated in Canada.

Extensive cross-reactions were observed between JC and SR, and these strains could not be separated by ELISA with hyperimmune mouse ascitic fluid. This was not unexpected, as SR is serologically indistinguishable from JC by complement fixation and very closely related by neutralization (11). The SR strain, in fact, is not currently recognized in the arbovirus catalog (3, 7) as a separate CAL serogroup member but is considered as a variety of JC (4).

Incorporation of SR in the ELISA typing was important in that SR ascitic fluid was able to recognize JC strains (previously identified by neutralization) that would have been missed if only JC ascitic was employed. These results suggest serological heterogeneity among CAL serogroup topotypes found in Canada and currently identified as JC. Studies are in progress to characterize more clearly serological heterogeneity among these strains.

The pattern of cross-reactions observed between CAL serogroup members corresponded to what might be anticipated based on current classification (4), with more cross-reactions between viruses within the CE (SSH, LAC, CE, SA), Melao (JC, SR, KEY), and TVT subcomplexes than between subcomplexes. Similarly, Hildreth et al. (6) developed enzyme immunoassay systems for LAC antigen and showed stronger cross-reactivity to SSH than to JC, KEY, or TVT antigens. The absence of reactions with serologically unrelated arboviruses provides further evidence of the specificity of the ELISA reactions.

This ELISA typing method has several advantages over the currently favored serum-dilution neutralization test (8) in that it is more rapid, does not require careful antigen standardization, and can employ inactivated antigens. Previously, LAC antigen has been demonstrated by enzyme immunoassay in infected *Aedes triseriatus* pools (6), and preliminary studies in our laboratory have shown that ELISA can be used to type directly CAL serogroup viruses in ground mosquito pools.

Strain 109 (Table 3), which was shown initially by ELISA and subsequently by neutralization to be TVT virus, is of interest. This strain was isolated from the blood of a sentinel rabbit maintained on the Caradoc Indian Reserve in Ontario in 1970 (M. Garvie, personal communication) and now represents the earliest documented recognition of the TVT serotype in Canada. Subsequent isolates of TVT were obtained from *Aedes trivittatus* collected in Essex County in

TABLE 2. ELISA typing of SSH topotypes isolated in Canada

Province of isolation	Strain designation[a]	Isolated from	OD[b] with hyperimmune ascitic fluid[c] to:								Source[d]
			SSH	LAC	CE	SA	JC	SR	KEY	TVT	
Manitoba	26-N-83	Mosquito	1.26	0.41	0.81	0.40	0.14	0.26	0.16	0.02	CPHL
	32-N-83	Mosquito	1.26	0.38	0.66	0.40	0.14	0.25	0.16	0.05	CPHL
Newfoundland	185-82	Mosquito	1.56	1.02	0.56	0.67	0.18	0.67	0.06	0	MU
Ontario	9-180-79	Mosquito	1.64	0.87	0.92	1.21	0.12	0.64	0	0	NARS
	9-192-79	Mosquito	1.57	0.67	0.70	0.68	0.08	0.41	0	0	NARS
	9-312-79	Mosquito	1.62	0.49	0.70	1.06	0.14	0.53	0	0	NARS
	R2929	Rabbit	1.61	0.61	0.60	0.93	0.07	0.47	0	0	LCDC
	436-454-76	Mosquito	1.60	0.42	0.98	0.73	0.02	0.28	0	0	OVC
	852-845-76	Mosquito	1.58	0.70	0.67	0.68	0.06	0.27	0.03	0.03	OVC
	84-75	Mosquito	1.68	0.53	0.73	0.38	0.09	0.35	0.01	0	NARS
	27	Rabbit	0.45	0.10	0.09	0.03	0	0.09	0.08	0	LCDC
	30	Rabbit	1.35	0.26	0.17	0.30	0.01	0.19	0.06	0	LCDC
	36	Rabbit	1.63	0.48	0.54	0.58	0	0.35	0.02	0	LCDC
	37	Rabbit	1.61	0.39	0.43	0.51	0	0.30	0.07	0	LCDC
	38	Rabbit	1.51	0.48	0.73	0.19	0.11	0.44	0.01	0.03	LCDC
	101	Rabbit	1.50	0.50	0.44	0.50	0	0.31	0.14	0	LCDC
	105	Rabbit	1.58	0.61	0.72	0.27	0.12	0.46	0.04	0.02	LCDC
	108	Rabbit	1.54	0.28	0.51	0.36	0	0.13	0.03	0.09	LCDC
	110	Rabbit	1.59	0.46	0.38	0.52	0	0.32	0.03	0	LCDC
	111	Rabbit	1.29	0.23	0.29	0.36	0	0.21	0.06	0	LCDC
	113	Rabbit	1.58	0.47	0.53	0.47	0.01	0.33	0.12	0	LCDC
	115	Rabbit	0.83	0.11	0.16	0.02	0	0.19	0.08	0.14	LCDC
	391-78	Mosquito	0.35	0.04	0.06	0.05	0	0.04	0.14	0	OVC
	74-75	Mosquito	1.64	0.60	0.65	0.67	0	0.45	0.03	0	NARS
	70-214-213-78	Mosquito	1.62	0.47	0.65	0.85	0.03	0.35	0.18	0	OVC
	973-974-78	Mosquito	1.60	0.54	0.51	0.63	0	0.40	0.04	0	OVC
	942-938-78	Mosquito	1.63	0.44	0.52	0.60	0	0.39	0.05	0.06	OVC
Quebec	27B1B2	Mosquito	1.31	0.56	0.67	0.72	0.14	0.44	0.01	0	IAF
	25A3	Mosquito	1.20	0.65	0.77	0.78	0.14	0.49	0.01	0	IAF
	36C1	Mosquito	1.39	0.21	0.41	0.51	0	0.33	0.05	0.01	IAF
	36C3	Mosquito	1.61	0.48	0.57	0.72	0.02	0.39	0.09	0	IAF
	37A1	Mosquito	1.52	0.41	0.48	0.49	0.15	0.37	0.06	0	IAF
	532	Rabbit	1.70	0.56	0.81	0.43	0.07	0.38	0	0	IAF
	534	Rabbit	1.53	0.46	0.50	0.37	0.06	0.39	0.01	0.05	IAF
	8843	Rabbit	1.37	0.81	1.09	0.93	0.15	0.60	0.03	0.01	IAF
	28C137E150C	Mosquito	1.54	0.55	0.59	0.80	0.30	0.71	0.20	0	IAF
	48C1158B	Mosquito	1.50	0.32	0.21	0.28	0	0.21	0.07	0	IAF
	134A12	Mosquito	1.39	0.35	0.95	1.18	0.08	0.50	0.03	0	IAF
	136B2	Mosquito	1.55	0.42	0.55	0.57	0.07	0.19	0	0	IAF
	140B1	Mosquito	1.68	0.69	0.97	0.79	0.12	0.52	0.04	0.05	IAF
	141B2	Mosquito	1.70	0.43	0.39	0.52	0.05	0.33	0	0	IAF
	74-31162/3	Mosquito	1.57	0.55	0.75	0.57	0.07	0.38	0.03	0.03	NYSL
Saskatchewan	WM 55-59-75	Mosquito	1.45	0.31	0.52	0.96	0.06	0.30	0.12	0	WCVM
Yukon	71-Y-23	Mosquito	1.49	0.37	0.52	0.51	0.07	0.29	0.03	0	UBC
	72-Y-121	Mosquito	1.55	0.47	0.78	0.65	0.08	0.27	0.04	0.01	UBC
	73-Y-347	Mosquito	1.63	0.40	0.71	0.65	0.08	0.21	0.04	0.07	UBC
	74-Y-48	Mosquito	1.62	0.40	0.67	0.49	0.02	0.25	0.18	0	UBC
	74-Y-234	Mosquito	1.58	0.45	0.32	0.46	0	0.29	0.11	0	UBC
	74-Y-235	Mosquito	1.41	0.34	0.47	0.60	0.02	0.25	0.07	0	UBC
	74-Y-237	Mosquito	1.64	0.49	0.50	0.52	0.12	0.33	0.17	0.04	UBC
	74-L-82	Mosquito	1.49	0.42	0.72	0.79	0.02	0.52	0.10	0	UBC
	76-Y-286	Mosquito	1.69	0.61	0.96	0.82	0.07	0.38	0.01	0	UBC
	76-Y-316	Mosquito	0.92	0.15	0.24	0.18	0.03	0.15	0.09	0	UBC
	78-Y-120	Mosquito	1.59	0.39	0.71	0.53	0.05	0.21	0.03	0	UBC
	80-Y-1	Mosquito	1.59	0.49	0.60	0.75	0.03	0.27	0.09	0	UBC
	80-Y-20	Mosquito	1.69	0.59	1.01	0.72	0.05	0.31	0.01	0	UBC
	82-Y-21	Mosquito	1.62	0.42	0.63	0.44	0	0.26	0.12	0	UBC
	82-Y-22	Mosquito	1.63	0.62	0.71	0.51	0.06	0.37	0.10	0	UBC
	82-Y-46	Mosquito	1.63	0.54	0.93	0.45	0.19	0.42	0.14	0.10	UBC
	82-Y-69	Mosquito	1.63	0.53	0.69	0.55	0	0.31	0.12	0.09	UBC
	82-Y-188	Mosquito	1.64	0.46	0.71	0.54	0.07	0.28	0.12	0	UBC

[a] All antigens used were 1:10 dilutions of tissue culture supernatant fluid (see text).
[b] OD readings represent an average of duplicate samples from which Vero control background was subtracted.
[c] Antibody coat dilutions as in Table 1, footnote *d*.
[d] CPHL, Cadham Public Health Laboratory; MU, Memorial University; NARS, National Arbovirus Reference Service; LCDC, Laboratory Center for Disease Control; OVC, Ontario Veterinary College; IAF, Institut Armand Frappier; NYSL, New York State Public Health Laboratory; WCVM, Western College of Veterinary Medicine; UBC, University of British Columbia.

TABLE 3. ELISA typing of JC and TVT topotypes isolated in Canada

Province of isolation	Strain designation[a]	OD[b] with hyperimmune ascitic fluid[c] to:								Source[d]
		SSH	LAC	CE	SA	JC	SR	KEY	TVT	
JC topotypes										
Manitoba	MN256-260	0.01	0.31	0.46	0.86	1.15	1.56	0.24	0.02	CPHL
Newfoundland	78-80	0.29	0.14	0.56	0.32	0.73	1.32	0.35	0.17	MU
Ontario	9-54-79	0.14	0.39	0.37	0.82	1.18	1.54	0.38	0	NARS
	9-68-79	0.11	0.38	0.35	0.65	1.10	1.57	0.18	0	NARS
	9-71-79	0.11	0.43	0.48	0.70	1.17	1.58	0.15	0.03	NARS
	9-178-79	0.17	0.41	0.38	0.97	1.17	1.64	0.28	0	NARS
	9-237-79	0.16	0.43	0.40	0.76	1.18	1.59	0.19	0	NARS
	1-237-81	0.21	0.17	0.24	0.20	0.67	1.29	0.05	0	NARS
Quebec	136A3	0.22	0.25	0.67	0.87	1.23	1.41	0.35	0	IAF
	137A17	0.42	0.26	0.64	0.84	0.60	1.37	0.27	0	IAF
	140A8	0.28	0.18	0.51	1.15	0.50	1.44	0.25	0	IAF
Saskatchewan	YGMC216-82	0.19	0.32	0.46	0.79	1.17	1.57	0.34	0	WCVM
	YGMC300-82	0.26	0.84	0.65	0.87	1.28	1.66	0.28	0.01	WCVM
TVT topotypes										
Ontario	109	0.09	0.02	0	0.02	0.08	0.13	0.01	0.92	LCDC
	658-674-76	0.03	0	0.02	0.01	0.02	0.01	0	0.25	OVC
	663-671-76	0.06	0	0.04	0.03	0.01	0.05	0	0.61	OVC

[a] All antigens used were 1:10 dilutions of tissue culture supernatant fluid (see text).
[b] OD readings represent an average of duplicate samples from which Vero control background was subtracted.
[c] Antibody coat dilutions as in Table 1, footnote *d*.
[d] Sources as in Table 2, footnote *d*.

1976 (12), and serological conversion to TVT virus was demonstrated in a sentinel rabbit maintained in the Dunnville area of southern Ontario in 1981 (2).

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