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California encephalitis virus endemicity in the Yukon Territory, 1972

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SUMMARY

Sera from 218 of 1574 (14%) small mammals collected in the Yukon Territory between 14 May and 13 August 1972 neutralized a Yukon strain of California encephalitis virus (snowshoe-hare subtype). These included 133 of 319 (42%) snowshoe hares (*Lepus americanus*), 84 of 1243 (7%) ground squirrels (*Citellus undulatus*) and 1 of 12 (8%) tree squirrels (*Tamiasciurus hudsonicus*). California encephalitis virus (snow-shoe hare subtype) was isolated from four pools of unengorged *Aedes communis* mosquitoes collected near Whitehorse (61° N., 135° W.) and on one occasion each from pools of the same species collected at Hunker Creek (64° N., 138° W.) and at mile 125, Dempster Highway (66° N., 138° W.) during July 1972. Replication of a Yukon strain of California encephalitis virus was observed in wild-caught *Culiseta inornata* and *Aedes canadensis* mosquitoes after intrathoracic injection and holding at temperatures of 80°, 50° and 40° F.

INTRODUCTION

Confirmation of the endemic prevalence of California encephalitis (CE) virus in the Yukon Territory near Whitehorse (61° N., 135° W.) was first obtained during the summer of 1971 by the isolation of strains of CE virus (Montana snowshoe-hare subtype) from 12 of 84 pools of *Aedes canadensis* mosquitoes (McLean *et al.* 1972). This followed the initial demonstration of CE virus activity in mosquitoes and snowshoe hares (*Lepus americanus*) at comparable subarctic latitudes in east-central Alaska after ten human cases of infection of the central nervous system with CE virus were encountered during the spring of 1970 (Sudia, Newhouse, Calisher & Chamberlain, 1971; Feltz *et al.* 1972).

Within British Columbia, CE virus prevalence was suggested by detection of neutralizing antibodies in sera from human residents (Kettyls *et al.* 1972) at Dawson Creek (56° N., 120° W.), and in snowshoe hares or other mammals at Dawson Creek and Williams Lake (52° N., 122° W.) (McLean *et al.* 1971), at Kamloops (51° N., 120° W.) (Newhouse, Burgdorfer, McKiel & Gregson, 1963) and at Penticton (49° 30′ N., 120° W.) (McLean *et al.* 1970, 1971).

Isolation of CE virus from *A. canadensis* and *A. vexans* mosquitoes at Penticton (McLean *et al.* 1970) during the summer of 1969 provided the first definite evidence

of CE virus prevalence in British Columbia at a site some 300 miles north-west of the Bitter Root valley, Montana (46° N., 114° W.), where the snowshoe-hare subtype was isolated initially from the blood of *L. americanus* in 1959 (Burgdorfer, Newhouse & Thomas, 1961), and subsequently from *Aedes fitchii* and *Culiseta impatiens* mosquitoes (Newhouse, Burgdorfer & Corwin, 1971).

Within the boreal forest of northern Alberta near Rochester (54° N., 113° W.), strains of the Jamestown Canyon and snowshoe-hare subtypes of CE virus have been isolated from *Aedes communis* and *A. stimulans* mosquitoes during 1964 and 1965 (Iversen *et al.* 1969). On the prairie portions of southern Alberta near Brooks (50° N., 112° W.), two pools of *Culiseta inornata* mosquitoes yielded the snowshoe-hare subtype of CE virus in 1965 (Morgante & Shemanchuk, 1967).

The prototype strain of CE virus, termed BFS-283, was first isolated from *Aedes melanimon* mosquitoes in a hot irrigated area of the San Joaquin valley, California, during 1943 (Hammon & Sather 1966), more than 1000 miles south of British Columbia and some 2000 miles south of the Yukon. No more CE virus was found in the San Joaquin valley until 1963, when the Jerry Slough strain, BFS-4474 was isolated. Within North America between 1958 and 1964 an additional six serological subtypes of CE virus have been isolated from mosquitoes or man. Consistent but minor antigenic differences have been demonstrated between these strains, principally by immunodiffusion (Sudia *et al.* 1971), but also by complement fixation (Hammon & Sather, 1966). The prevalence of each subtype is confined principally to one geographical area within North America. During the past quarter century, 519 cases of aseptic meningitis or other clinically manifest infections with CE virus, affected human residents of the United States. The incidence increased from 3 cases in 1945 to 90 cases in 1970 (Sudia *et al.* 1971).

This paper reports additional isolations of CE virus strains from wild-caught mosquitoes both in southern and northern portions of the Yukon Territory during summer 1972, together with serological results on small forest rodents collected in the same areas, in an attempt to define more precisely the natural chains of infection of CE virus in a sub-Arctic region.

MATERIALS AND METHODS

Field mosquitoes

Mosquitoes were collected by hand-aspirators at five locations throughout the Yukon Territory between 11 June and 31 July 1972. Unengorged female mosquitoes were pooled in batches of 50–100, sealed in glass tubes in the field, placed immediately in dry ice at −70° C., and air-freighted to Vancouver once weekly, where they were held at −70° C. until tested for virus content. After identification by low-power microscopic examination of every mosquito, pools comprising 50–100 of the same species were ground in mortars suspended with 2·0 ml. diluent,* centrifuged at 2000 rev./min. for 3 min. to deposit coarse particles, and the super-

* The diluent for all virus titrations comprised Earle's saline with lactalbumin hydrolysate 0·5%, yeast extract 0·1%, neomycin 100 μg/ml., and Amphotericin B 5 μg/ml (medium ELY), to which was added 20% newborn-calf serum.

natant assayed for virus content by intracerebral injection of baby mice aged 1–3 days.

Live mosquitoes were placed in round cardboard cages in the field, chilled to about 50° F. and air-freighted to Vancouver, where they were held at 70 ± 2° F. until used for infection experiments.

Infection of mosquitoes in the laboratory

Mosquitoes were anaesthetized with carbon dioxide and injected intrathoracically with 0·003 ml. virus suspension in sterile water, using a finely drawn hard-glass pipette. Mosquitoes were placed in cylindrical cages, pledgets soaked in 15% sucrose solution were added, and the cages were inserted into sealed plastic bags to ensure high humidity. Mosquitoes were held at 80, 50, 40 and 30° F. until examined 2–60 days later for virus content by intracerebral inoculation of weaned mice aged 3–4 weeks.

Mammals

Small wild rodents and lagomorphs were collected by shooting principally within 50 miles of Whitehorse, Y.T. (61° N., 135° W.), but also surrounding Dawson City between Hunker Creek (64° N., 138° W.) and mile 125 on the Dempster Highway (66° N., 138° W.). Blood was collected from shot animals after extravasation into the pleural cavities. After separation of sera from clots at the field station, both samples were held at −20° C. and air-freighted frozen to Vancouver once weekly.

Virus isolation and typing

Brains of baby mice which developed encephalitis after intracerebral injection were removed aseptically, ground in mortars, extracted with 0·5 ml. diluent per brain, centrifuged at 2000 rev./min. for 3 min., and 0·03 ml. volumes of serial tenfold dilutions of supernatant were inoculated intracerebrally into mice aged 3 weeks. The 50% lethal dose for mice (LD 50) was calculated by the method of Reed & Muench (1938). Equal volumes of sodium deoxycholate 1/500 were added to virus suspensions containing at least 10^4 mouse LD 50 per 0·03 ml., held at 25° C. for 30–60 min., diluted 1/100 and injected intracerebrally into mice. Controls contained virus plus deionized water in place of sodium deoxycholate. Antisera prepared in rabbits or guinea-pigs to arboviruses including a Yukon 1971 CE isolate (McLean *et al.* 1972) were mixed in 0·1 ml amounts with equal volumes of virus diluted to contain 10^2 mouse LD 50 per 0·03 ml., held for 30–60 min. at 25° C., and injected into mice.

Serological tests

The neutralizing antibody content of all sera was determined by intracerebral injection of weaned mice with mixtures of undiluted sera plus a dilution of the Marsh Lake 23 strain of CE virus containing 50–100 mouse LD 50 per 0·03 ml. This strain constitutes the prototype of the 12 CE strains isolated from Yukon mosquitoes during 1971 (McLean *et al.* 1972). Sera which inhibited haemagglutination by Powassan (POW) or western equine encephalomyelitis (WEE) viruses were

examined in mouse neutralization tests against 50–100 mouse LD50 of the respective agent.

All sera were tested simultaneously for haemagglutination inhibition (HI) antibodies to 4–8 agglutinating doses of POW (L.B strain) and WEE (McMillan strain). Sera were extracted twice with acetone, absorbed with goose erythrocytes, and serial twofold dilutions were made in borate saline pH 9 containing 0·4% bovalbumin. Disposable Microtiter plates were employed throughout. Haemagglutinins were prepared by extraction of infected baby-mouse brains with sucrose and acetone (Clarke & Casals, 1958). Dilutions containing 4–8 agglutinating doses per 0·025 ml. were added to each cup. After holding serum–virus mixtures for 1 hr. at 25° C., each cup received 0·05 ml. amounts of 0·25% suspensions of goose erythrocytes in appropriate virus-adjusting diluents. The haemagglutination patterns were read after incubation for 1 hr. at 25° C. for POW and 37° C. for WEE, usually at pH 6·4 for both viruses.

Complement-fixing antibodies to CE virus (Marsh Lake 23 strain), using optimal dilutions of borate-saline extracts of infected baby-mouse brains as antigens were sought in all sera after preliminary screening for anticomplementary activity after dilution 1/2·5 in 0·15 M saline. Positively reacting sera were titrated for their antibody contents, using Microtiter plates.

RESULTS

Mosquito isolations

Strains of California encephalitis virus were isolated from 6 of 148 pools of unengorged female mosquitoes, totalling 10,317 insects, which were collected at five Yukon locations between 11 June and 31 July 1972 (Table 1). All six strains were derived from 9048 *Aedes communis* which were tested in 125 pools. This gave an overall minimum field infection ratio (MFIR) (Sudia *et al.* 1971) of 1:1508. Virus isolations were confined to Marsh Lake with MFIR 1:668, Hunker Creek with MFIR 1:1654 and Dempster Highway mile 125 with MFIR 1:2854. Virus was first detected in mosquitoes at Marsh Lake on 3 July and the final isolations were obtained there on 31 July. Isolations from the two northerly areas were obtained only during late July.

Each isolate induced encephalitis 3–4 days after intracerebral inoculation of baby mice, whose brains yielded about 10^6 LD50 per 0·03 ml. upon titration intracerebrally in weaned mice. Infectivity was reduced at least 100-fold by treatment with sodium deoxycholate. All isolates were neutralized completely by antiserum to the Yukon 1971 prototype strain of CE virus (Marsh Lake 23), which was antigenically identical with the Montana snowshoe-hare subtype by immunodiffusion tests (C. E. Calisher, personal communication). Each Yukon 1972 strain was reisolated from mosquito suspensions after storage at − 70° C. for an additional 2–6 weeks after primary isolation.

Table 1. *California encephalitis virus isolations from Yukon mosquitoes*, 1972

Collection site	Species	Week commencing: June 11	June 18	June 25	July 2	July 9	July 16	July 23	July 30	Total: Ratio	Total: MFIR	Total: Mosq. tested
Tagish, 60½° N., 135° W.	*A. communis*	0/6	0/16	—	—	—	—	—	—	0/22	—	1622
	A. canadensis	—	0/2	—	—	—	—	—	—	0/2	—	98
Marsh Lake, 61° N., 135° W.	*A. communis*	0/6	0/7	—	1/9	1/13	0/2	—	2/3	4/40	1:668	2667
	A. canadensis	0/1	—	—	0/7	0/3	0/2	—	0/3	0/16	—	1012
	Cs. inornata	0/1	—	—	—	—	—	—	—	0/1	—	3
Lookout, 61° N., 135½° W.	*A. communis*	—	—	—	0/4	—	—	—	—	0/4	—	251
Hunker Creek, 64° N., 138° W.	*A. communis*	—	—	0/15	—	—	—	1/6	—	1/21	1: 1654	1654
	A. canadensis	—	—	—	—	—	—	0/2	—	0/2	—	141
	Cs. inornata	—	—	0/1	—	—	—	—	—	0/1	—	9
Dempster Hwy., 66° N., 138° W.	*A. communis*	—	—	0/2	—	—	—	1/17	—	1/38	1:2854	2854
	Cs. inornata	—	—	0/1	—	—	—	—	—	0/1	—	6
Total	*A. communis*	0/12	0/23	0/36	1/13	1/13	0/2	2/23	2/3	6/125	1:1508	9048
	A. canadensis	0/1	0/2	—	0/7	0/3	0/2	0/2	0/3	0/20	—	1251
	Cs. inornata	0/1	—	0/2	—	—	—	—	—	0/3	—	18
	All	0/14	0/25	0/38	1/20	1/16	0/4	2/25	2/6	6/148	1:1719	10317

MFIR: minimum field infection ratio = (number of isolations)/(mosquitoes tested).

Table 2. *Replication of California encephalitis virus after injection of* Culiseta inornata

(Days of extrinsic incubation at stated temperatures.)

Mouse LD50 injected	Mosq. part	80° F.			50° F.			40° F.				
		5	12	19	7	30	48	5	12	19	43	60
300	SG	2·7*	2·3	3·0	0	1·5	1·9	0	0	2·8	2·0	2·7
	TH	4·0	3·8	4·7	2·2	2·9	1·9	1·5	2·3	3·0	2·3	2·7
30	SG	2·0	—	—	1·8	1·5	—	—	—	0	0	2·0
	TH	3·0	—	—	2·2	3·0	—	—	—	0	2·0	2·3
3	SG	2·0	2·3	—	0	2·3	—	—	0	0	1·8	—
	TH	3·7	1·8	—	1·8	3·0	—	0	0	0	1·5	—
0·3	SG	—	—	—	—	1·5	—	—	—	—	—	—
	TH	—	—	—	0	3·0	—	—	—	—	—	—
0·03	SG	—	—	—	—	1·5	—	—	—	0	—	—
	TH	0	0	—	0	2·0	—	0	0	0	—	—

SG, Salivary glands; TH, thorax.

* Mean log mouse LD50 of CE virus per mosquito part.

Table 3. *Replication of California encephalitis virus after injection of* Aedes canadensis

(Days of extrinsic incubation at stated temperatures.)

Mouse LD50 injected	Mosq. part	80° F.		50° F.					40° F.	
		2	6	5	6	11	17	23	5	17
3·0	SG	3·0*	2·0	0	0	—	2·0	—	0	1·8
	TH	2·3	3·5	0	2·0	—	3·5	—	0	2·0
3	SG	2·7	2·7	0	0	0	—	2·5	0	0
	TH	3·0	3·7	0	1·7	0	—	3·0	0	1·8
0·3	SG	—	1·5	0	0	0	—	—	0	0
	TH	—	3·0	0	0	0	—	—	0	0

SG, Salivary glands; TH, thorax.

* Mean $\log_{10}$ mouse LD 50 of CE virus per mosquito part.

Mosquito infection experiments

During preliminary mosquito collections at Marsh Lake on 15 May 1972, *Culiseta inornata* bit the senior investigator avidly, despite an atmospheric temperature below 32° F. This species therefore provided a model for examination of virus replication in mosquitoes at temperatures below 80° F., which is standard for transmission experiments involving arboviruses from tropical and subtropical areas. Wild-caught *Culiseta inornata* from the Yukon were maintained at 40° F. for 60 days and at 30° F. for 12 days. *Aedes canadensis* from the Yukon were maintained at 40° F. for 17 days. On the contrary, *Aedes aegypti* obtained from a Vancouver mosquito colony could not be maintained at temperatures below 50° F.

After intrathoracic injection of *Culiseta inornata* with 300, 30 and 3 mouse LD 50 of Marsh Lake 23 virus, substantial increments of infectivity were demonstrated both in salivary glands and thoraces of mosquitoes tested individually or in

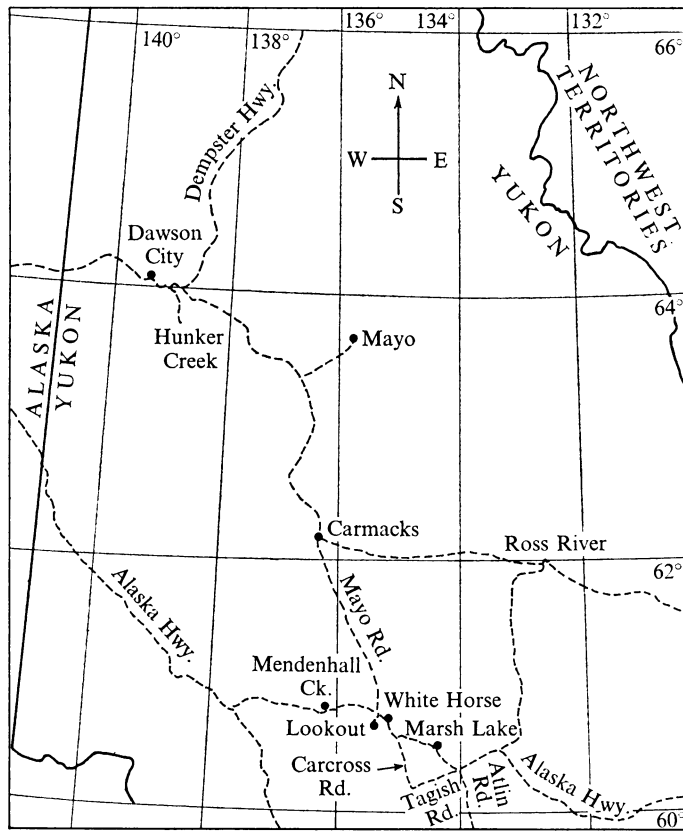

Fig. 1. Field collection sites in the Yukon Territory, summer 1972.

pairs after 5 days of extrinsic incubation at 80° F. Virus titres remained elevated as long as 19 days after injection of 300 mouse LD 50 (Table 2). No infectivity was detected 12 days after injection of 0·03 mouse LD 50. Following incubation at 50° F. substantial increments of virus content were observed in thoraces 30 days after inoculation of as little as 0·3 mouse LD 50, and in each instance the titres increased above those observed at 7 days. However, salivary glands at 30 days contained relatively small quantities of virus. Mosquitoes which received 0·03 mouse LD 50 contained only trace quantities after 30 days of extrinsic incubation. Virus replication was demonstrated after holding for 43 days at 40° F., following injection of 300, 30 and 3 mouse LD 50. However, the time at which substantial increments of infectivity were first detected was prolonged considerably beyond that observed at 80° and 50° F.

After intrathoracic injection of *Aedes canadensis* with 30, 3 and 0·3 mouse LD 50 of Marsh Lake 23 virus, substantial virus titres were detected in salivary glands and thoraces after 6 days of extrinsic incubation at 80° F. (Table 3). Minimal amounts of virus were detected solely in thoraces after 6 days incubation at 50° F. in mosquitoes which received 30 or 3 mouse LD 50. Significant increments of titres both in thoraces and salivary glands were observed at 17 and 23 days respectively.

Table 4. *California encephalitis neutralizing antibody in Yukon mammals*, 1972

Location	May			June			July		August		Total species			Total All
	La	Cu	Th	La	Cu	Th	La	Cu	La	Cu	La	Cu	Th	
Lookout	4/12	7/47	0/1	13/36	6/65	—	15/36	—	1/4	—	33/88	13/112	0/1	46/201
Mayo Road	6/23	0/82	—	2/8	1/16	—	—	2/47	0/1	5/39	8/32	8/184	—	16/216
Carcross Rd	7/13	15/58	—	—	1/48	0/4	—	5/73	—	4/33	7/13	25/212	0/4	32/229
Marsh Lake	30/55	2/45	—	7/29	7/35	0/1	10/18	3/75	—	0/39	47/102	12/194	0/1	59/297
Mendenhall Creek	—	5/32	0/1	0/1	0/24	—	—	0/58	0/1	1/24	0/2	6/138	0/1	6/141
Tagish Road	—	3/61	1/2	1/11	3/82	0/3	—	1/73	2/4	0/23	3/15	7/239	1/5	11/259
Atlin Road	3/12	0/29	—	4/11	2/62	—	—	—	—	7/59	7/23	9/150	—	16/173
Hunker Creek	—	—	—	10/24	—	—	16/18	—	—	—	26/42	—	—	26/42
Dempster Hwy	—	—	—	—	—	—	2/2	4/14	—	—	2/2	4/14	—	6/16
Total no.	50/115	32/354	1/4	37/120	20/332	0/8	43/74	15/340	3/10	17/217	133/319	84/1243	1/12	218/1574
%	43	9	25	31	6	0	58	4	30	8	42	7	8	14

La, *Lepus americanus*; Cu, *Citellus undulatus*; Th, *Tamiasciurus hudsonicus*.

In mosquitoes incubated at 40° F., minimal quantities of virus were detected 17 days after injection of 30 and 3 mouse LD 50.

Mammalian serology

Between 14 May and 13 August 1972, sera were collected from 1574 wild mammals at seven general locations near Whitehorse and two areas near Dawson City (Fig. 1). Neutralizing antibodies to the Marsh Lake 23 strain of CE virus were found in 218 (14 %) animals (Table 4), including 133 of 319 (42 %) snowshoe hares (*Lepus americanus*), 84 of 1243 (7 %) ground squirrels (*Citellus undulatus*) and 1 of 12 (8 %) red squirrels (*Tamiasciurus hudsonicus*). The incidence of antibody in snowshoe hares was highest during July (58 %), but a substantial proportion (43 %) of hares collected during May neutralized CE virus. Antibody prevalence exceeded the overall mean of 42 % at Carcross Road (52 %), Marsh Lake (47 %), Hunker Creek (62 %) and along the Dempster Highway to mile 125 (100 %).

Complement-fixing antibodies to CE virus were found in 78 of 133 snowshoe hare sera which neutralized this agent, and in an additional 33 sera which were devoid of neutralizing antibody. Complement-fixing antibodies were also found in 7 of 84 sera from ground squirrels which neutralized CE virus, and in a further 15 without netralizing antibody.

Haemagglutination-inhibition antibodies to POW virus were detected in sera from 44 mammals, including 4 snowshoe hares and 40 ground squirrels, whilst WEE antibodies were found in 34 mammals, including 3 hares and 31 squirrels. Sera from 4 animals inhibited haemagglutination by both viruses. However, none of these sera neutralized their respective viruses.

Blood clots between 11 June and 29 July from 38 mammals devoid of CE neutralizing antibodies were examined for virus content by intracerebral injection of suckling mice. No virus was isolated.

DISCUSSION

Isolation of California encephalitis virus from *Aedes communis* mosquitoes collected along the Dempster Highway in the Yukon Territory at 66° N., 138° W., together with the demonstration of CE neutralizing antibodies in showshoe hares and ground squirrels from the same location, demonstrates clearly the existence of a natural endemic focus of CE virus near the Arctic Circle. These results extend northwards by two degrees of latitude the previously established northern limit of CE virus activity in North America near Tok, Alaska (64° N., 144° W.), about 150 miles west of Dawson City, Y.T. (Feltz *et al.* 1972; Sudia *et al.* 1971). Additional isolations from mosquitoes during 1972 confirm our 1971 demonstration of the endemic prevalence of CE virus at Marsh Lake (61° N., 135° W.) and other locations near Whitehorse (McLean *et al.* 1972). They substantiate our proposition that CE-neutralizing antibody in 22 % of mammals collected near Dawson City (64° N., 138° W.) arose from natural infection by this agent.

Although all six CE virus strains from mosquitoes during 1972 were isolated from

A. communis, in contrast to *A. canadensis*, from which all 12 strains were recovered during 1971, both these mosquito species have yielded strains of the snowshoe-hare subtype of CE virus in more southerly portions of western Canada (Iversen *et al.* 1969, McLean *et al.* 1970). The minimum field infection ratio of 1:2854 for *A. communis*, at the northern fringe of the boreal forest (66° N.) during 1972, approached that of 1:2168 for this species near Rochester, Alberta (54° N.), at the southern extremity of the boreal forest, where wooded areas merge into the Canadian prairie. Whilst the maximum prevalence of CE virus in the Yukon during 1972, in common with 1971, was found at Marsh Lake, where the MFIR was 1:668, the overall rate of 1:1508 for *A. communis* collected at all Yukon test sites compares favourably with the MFIR of 1:1518 for the principal vector species *A. fitchii* in the Bitter Root valley of Montana (Newhouse *et al.* 1971) where the snowshoe-hare subtype of CE virus was first isolated.

Populations of *A. communis* and *A. canadensis* became extremely abundant from mid-June to late July, when daytime temperatures usually exceeded 80° F. *Culiseta inornata*, however, was prevalent during May, when daytime temperatures were cooler, and it was observed to feed on humans even at temperatures below 32° F. The propagation of CE virus in *A. canadensis* and *Cs. inornata* following intrathoracic injection of 3–30 mouse LD50, at temperatures as low as 40° F., following extrinsic incubation periods 2–5 weeks longer than those encountered after incubation at 80° F., suggests that these mosquito species may provide both an effective overwintering mechanism in addition to serving as summertime natural vectors.

The high incidence of CE-neutralizing antibodies in sera from snowshoe hares collected in the Yukon during 1971 (25 %) and also 1972 (42 %) strongly suggests that *Lepus americanus* provides the principal vertebrate reservoir of infection, although a small proportion of ground squirrels, comprising 8 % in 1971 and 7 % in 1972, acquired antibody presumably following infection in nature. Isolation of the snowshoe-hare subtype of CE virus from the blood of *L. americanus* collected at Tok, Alaska (Feltz *et al.* 1972), Rochester, Alberta (Hoff, Yuill, Iversen & Hanson, 1969) and Hamilton, Montana (Burgdorfer *et al.* 1961), confirms the role of this species as a natural reservoir. The ability of chipmunks and squirrels collected in Wisconsin to develop viraemia, after peripheral inoculation with the serologically related La Crosse subtype of CE virus, at a titre sufficient to infect *Aedes triseriatus* mosquitoes which subsequently transmitted this agent by biting mice (Pantuwatana, Thompson, Watts & Hanson, 1972), suggests that the zoologically related Arctic ground squirrel, *Citellus undulatus*, may also serve as a vertebrate reservoir of CE virus in the Yukon.

Although serological surveys of small wild mammals revealed extensive evidence of infection both at southern and northern Yukon locations, the incidence of infection among the sparse human population of these areas remains undetermined through lack of serological investigation. However, in adjacent portions of Alaska near Tok, detection of CE neutralizing antibody in 72 % of 325 human residents during 1968 and the acquisition of HI antibodies by all seronegative subjects during the subsequent year, despite the onset of headache and fever in only 10 of 95

persons shortly before sero-conversion (Feltz *et al.* 1972), strongly suggests the occurrence of mild or subclinical infections with CE virus tangentially to the natural cycle of infection between mosquitoes and rodents in sub-Arctic regions of Alaska and the Yukon Territory.

California encephalitis virus appears to be the sole arbovirus which is at present prevalent in the Yukon Territory, where *Aedes* and *Culiseta* mosquitoes assume high summertime population densities, but *Culex tarsalis* has not been collected. Although WEE neutralizing antibodies suggesting past virus infections have been detected in 8 of 160 reindeer collected as far north as Atkinson Point, Northwest Territories (70° N., 132° W.), during 1968 (Burton & McLintock, 1970), no evidence of WEE infection has been detected in Yukon mammals during 1971 and 1972. The northern limit of *Culex tarsalis*, the principal natural vector of WEE on the Canadian prairies and in other portions of the western United States, is about 53° N. (Burton & McLintock, 1970). Usually *Aedes* mosquitoes alone are insufficient to maintain WEE virus in nature. In a transitional zone between prairie and boreal forest at Rochester, Alberta (54° N.), some serological evidence of WEE infection was detected in snowshoe hares during 1963 and 1965 when human cases occurred frequently throughout Alberta. However, only *Aedes* mosquitoes were collected at Rochester (Yuill, Iversen & Hanson, 1969). Possibly, snowshoe hares became infected tangentially to a natural cycle involving *C. tarsalis* mosquitoes in adjacent prairie portions of the terrain. The absence of WEE infection from Yukon mammals correlates well with the lack of collection of *C. tarsalis* in that region.

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REFERENCES

Burgdorfer, W., Newhouse, V. F. & Thomas, L. A. (1961). Isolation of California encephalitis virus from the blood of a snowshoe hare (*Lepus americanus*) in western Montana. *American Journal of Hygiene* **73**, 344.

Burton, A. N. & McLintock, J. (1970). Further evidence of western encephalitis infection in Saskatchewan mammals and birds and in reindeer in northern Canada. *Canadian Veterinary Journal* **11**, 232.

Clarke, D. H. & Casals, J. (1958). Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene* **7**, 561.

Feltz, E. T., List-Young, B., Ritter, D. G., Holden, P., Noble, G. R. & Clarke, P. S. (1972). California encephalitis virus: serological evidence of human infections in Alaska. *Canadian Journal of Microbiology* **18**, 757.

Hammon, W. McD. & Sather, G. E. (1966). History and recent reappearance of viruses of the California encephalitis group. *American Journal of Tropical Medicine and Hygiene* **15** 199.

Hoff, G. L., Yuill, T. M., Iversen, J. O. & Hanson, R. P. (1969). Snowshoe hares and the California encephalitis virus group in Alberta 1961–1968. *Bulletin of the Wildlife Disease Association* **5**, 254.

IVERSEN, J., HANSON, R. P., PAPADOPOULOS, O., MORRIS, C. V. & DE FOLIART, G. R. (1969). Isolation of viruses of the California encephalitis virus group from boreal *Aedes* mosquitoes. *American Journal of Tropical Medicine and Hygiene* **18**, 735.

KETTYLS, G. D., VERRALL, V. M., WILTON, L. D., CLAPP, J. B., CLARKE, D. A. & RUBLEE, J. D. (1972). Arbovirus infections in man in British Columbia. *Canadian Medical Association Journal* **106**, 1175.

McLEAN, D. M., BERGMAN, S. K. A., GODDARD, E. J., GRAHAM, E. A. and PURVIN-GOOD, K. W. (1971). North–South distribution of arbovirus reservoirs in British Columbia, 1970. *Canadian Journal of Public Health* **62**, 120.

McLEAN, D. M., CRAWFORD, M. A., LADYMAN, S. R., PEERS, R. R. & PURVIN-GOOD, K. W. (1970). California encephalitis and Powassan virus activity in British Columbia, 1969. *American Journal of Epidemiology* **92**, 266.

McLEAN, D. M., GODDARD, E. J., GRAHAM, E. A., HARDY, G. J. & PURVIN-GOOD, K. W. (1972). California encephalitis virus isolations from Yukon mosquitoes, 1971. *American Journal of Epidemiology* **95**, 347.

MORGANTE, O. & SHEMANCHUK, J. (1967). Virus of California encephalitis complex: isolation from *Culiseta inornata*. *Science, New York* **157**, 292.

NEWHOUSE, V. F., BURGDORFER, W. & CORWIN, D. (1971). Field and laboratory studies on the hosts and vectors of the snowshoe hare strain of California virus. *Mosquito News* **31**, 401.

NEWHOUSE, V. F., BURGDORFER, W., McKIEL, J. A. & GREGSON, J. D. (1963). California encephalitis virus: serologic survey of small wild mammals in northern United States and southern Canada and isolation of additional strains. *American Journal of Hygiene* **78**, 123.

PANTUWATANA, S., THOMPSON, W. H., WATTS, D. M. & HANSON, R. P. (1972). Experimental infection of chipmunks and squirrels with La Crosse and Trivittatus viruses and biological transmission of La Crosse virus by *Aedes triseriatus*. *American Journal of Tropical Medicine and Hygiene* **21**, 476.

REED, L. J. & MUENCH, H. (1938). Simple method of estimating 50 per cent endpoints. *American Journal of Hygiene* **27**, 493.

SUDIA, W. D., NEWHOUSE, V. F., CALISHER, C. H. & CHAMBERLAIN, R. W. (1971). California group arboviruses: isolations from mosquitoes in North America. *Mosquito News* **31**, 576.

YUILL, T. M., IVERSEN, J. O. & HANSON, R. P. (1969). Evidence for arbovirus infections in a population of snowshoe hares: a possible mortality factor. *Bulletin of the Wildlife Disease Association* **5**, 248.