*J. Hyg., Camb.* (1977), **79**, 61
*Printed in Great Britain*



# Bunyavirus isolations from mosquitoes in the western Canadian Arctic

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(*Received* 10 *December* 1976)

## SUMMARY

Strains of California encephalitis virus (snowshoe hare subtype) were isolated from 8 of 475 pools comprising 23747 unengorged female mosquitoes of five species collected at three of six locations throughout the Mackenzie Valley of the Northwest Territories, Canada, from latitudes 60 to 69° N between 10 and 24 July 1976. Minimum field infection rates included 1:2734 for *Aedes communis*, 1:256 to 1:3662 for *A. hexodontus* and 1:911 to 1:1611 for *A. punctor*. Northway virus was also isolated from 1 of 3662 *A. hexodontus* mosquitoes collected at Inuvik (69° N, 135° W). Transmission of CE virus by *A. communis* infected by feeding on virus in defibrinated blood and incubation at 0, 13 and 23 °C for 13–20 days clearly demonstrates the importance of this species as a natural vector, and transmission of CE virus by *Culiseta inornata* after incubation at 0 and 13 °C following intrathoracic injection strengthens evidence of its role as a natural vector. Immunofluorescence was less reliable than imunoperoxidase for detection of CE viral antigen in mosquito salivary glands.

## INTRODUCTION

Although infection rates for California encephalitis virus (snowshoe hare subtype) have been determined for mosquitoes in the boreal forest regions of the Yukon Territory (McLean *et al.* 1975), Alberta (Iversen *et al.* 1969) and Saskatchewan (Iversen, Wagner, de Jong, McLintock, 1973) and the tundra near Baker Lake in central Northwest Territories (Wagner *et al.* 1975), the prevalence of arboviruses throughout open woodlands along the Mackenzie Valley of western Northwest Territories adjacent to the Yukon remained unknown. This investigation was undertaken to determine arbovirus infection rates among mosquitoes collected at locations accessible by road or commercial airlines throughout the Mackenzie Valley. Since the summertime atmospheric temperatures in the western Canadian Arctic range from 0 to 23 °C, and since previous experiments involving intrathoracic injection of wild-caught arctic mosquitoes had demonstrated viral replication after incubation at these temperatures (McLean *et al.* 1974, 1975, 1976), attempts were made to transmit virus by wild-caught mosquitoes both after feeding and after injection of virus.

## MATERIALS AND METHODS

### *Mosquito collections in the field*

Adult female mosquitoes were collected by hand aspirators at six locations in the Mackenzie Valley region of the Northwest Territories between 29 May and 24 July 1976. The vegetation was open woodland except near Fort Smith (60° N, 112° W) where it comprised boreal forest. Average daytime temperatures ranged from 15 to 25 °C with overnight temperatures falling to 0–7 °C.

Unengorged mosquitoes were sealed in glass tubes, placed immediately on dry ice at −70 °C, and transported by air once weekly to Vancouver, where they were held frozen at −70 °C until tested for virus content. After speciation (Carpenter & LaCasse, 1955), pools comprising 40–60 mosquitoes of the same species were ground up and extracted with 2 ml diluent (Eagle's minimal essential medium containing 20% newborn calf serum, neomycin 100 μg/ml and amphotericin B 5 μg/ml). After deposition of coarse particles by centrifugation at 2000 rev./min for 3 min, the supernatant was assayed for virus content by intracerebral injection of mice aged 1–3 days.

Live mosquitos were placed in round cardboard cages 11 cm long × 8 cm diameter and air freighted to Vancouver in styrofoam cartons in which the temperature was maintained at 10–13 °C by refrigerant gel packs. Mosquitoes were held in Vancouver at 13 °C to await virus feeding or injection experiments, after which they were incubated at 0, 13 or 23 °C.

### *Mosquito experiments*

Wild-caught *Aedes communis* and *Culiseta inornata* mosquitoes were anaesthetized with carbon dioxide and injected intrathoracically with 0·003 ml California encephalitis (CE) virus (snowshoe hare subtype) strain 74-Y-234 in its first baby mouse passage in doses ranging from 100 to 0·01 mouse LD50. Other mosquitoes were placed in cylindrical cages 2·5 cm long × 2·5 mm diameter, after overnight starvation. They were fed a mixture of virus in defibrinated sheep blood containing 10% sucrose. After 2 h, engorged mosquitoes were removed and held in cylindrical cages 11 × 8 cm, fed on pledgets containing a 10% aqueous solution of sucrose, and placed in sealed plastic bags to ensure high humidity. They were incubated at 0, 13 or 23 °C. Mosquitoes were dissected at weekly intervals after feeding or injection, when half salivary glands and thoraces were assayed individually for virus content by intracerebral injection of weaned mice. Immunofluorescent preparations were made from the other halves of salivary glands.

Transmission experiments were performed by inducing individual mosquitoes, placed in 2·5 × 2·5 cm cages following overnight starvation, to imbibe defibrinated sheep blood containing 10% sucrose. Virus excreted in mosquito saliva during the feeding process entered the defibrinated blood which was assayed immediately thereafter for virus content by mouse inoculation. The virus content of mosquito salivary glands and thoraces was assayed similarly.

For immunoflorescent tests, halves of salivary glands were placed in drops of water on microscope slides held on a warming tray at 40 °C. After the preparations

had dried, the glands were fixed with acetone for 10 min, then washed three times in phosphate-buffered saline pH 7·2. Rabbit antiserum to the Yukon CE snowshoe hare prototype strain Marsh Lake 23 (McLean *et al.* 1972), which was conjugated to fluorescein isothiocyanate (Chernesky & McLean, 1969), was added dropwise to each gland, and the slides were held in a humidified chamber for 30 min at 25 °C. After incubation and washing three times with phosphate-buffered saline, the slides were examined at 2000 × magnification under transmitted ultraviolet illumination using a mercury vapour HBO 200 lamp in a Vickers Patholux microscope. Bright areas of yellow-green fluorescence in the cytoplasm of salivary gland acinar cells of test glands, but not in uninfected glands prepared and examined simultaneously, were considered to show positive immunofluorescent reactions. Non-specific background staining of mosquito cells was minimized through absorption of fluorescein-labelled antiserum by activated charcoal followed by powdered arctic mosquito tissues.

## RESULTS

### *Bunyavirus field isolations*, 1976

Among 23747 adult female mosquitoes of five species collected at six locations throughout the Mackenzie Valley of the Northwest Territories between 29 May and 24 July 1976, Bunyavirus strains were recovered from 9 of 475 mosquito pools (Table 1). These comprised 8 of the snowshoe hare subtype of CE virus, antigenically identical with the 1971 Yukon prototype strain (McLean *et al.* 1972), and one of Northway virus, antigenically identical with the 1970 Alaskan prototype strain (Calisher, Lindsey, Ritter & Sommerman, 1974). All isolations were made from mosquitoes collected between 10 and 24 July (27th–29th weeks of 1976). The minimum field infection rates ranged from 1:256 *A. hexodontus* at Fort Simpson (62° N, 122° W) and 1:911 *A. punctor* at Fort Smith (60° N, 112° W) at the southern headwaters of the Mackenzie River to 1:3662 *A. hexodontus* at Inuvik (69° N, 135° W), in the estuarine portion of the Mackenzie River where it flows northward into the Arctic Ocean (Table 2). Thus CE virus has been detected in mosquitoes collected at accessible locations throughout the Mackenzie Valley region of the western Canadian Arctic (Fig. 1). For the first time in Canada, CE virus has been isolated substantially north of the Arctic Circle, at 69° N in an open woodland area, in contrast to virus isolations as far north as 66° N in the boreal forest of the adjacent Yukon Territory during summers 1972–4 (McLean *et al.* 1975).

### *Mammalian serology*

Mouse neutralizing antibodies to CE virus (McLean *et al.* 1975) were found in sera from 2 of 5 snowshoe hares (*Lepus americanus*) collected near Yellowknife and in 1 of 8 red squirrels (*Tamiasciurus hudsonicus*) collected near Fort Simpson throughout July.

Table 1. *Mosquito species assayed for virus in Northwest Territories, summer* 1976

| Locality | Mosquito species | | | | | Total |
|---|---|---|---|---|---|---|
| | *A. com.* | *Cs. in.* | *A. nig.* | *A. hex.* | *A. punc.* | |
| Yellowknife | 0/24 | 0/8 | — | — | — | 0/32 |
| 62° N 115° W | (1121) | (177) | — | — | — | (1298) |
| Fort Providence | 0/28 | 0/6 | — | — | 0/4 | 0/38 |
| 61° N 118° W | (1264) | (149) | — | — | (181) | (1594) |
| Hay River | 0/7 | 0/1 | — | — | — | 0/8 |
| 61° N 116° W | (322) | (26) | — | — | — | (348) |
| Fort Smith | 0/2 | — | — | 1/18 | 1/17 | 2/37 |
| 60° N 112° W | (106) | — | — | (922) | (911) | (1939) |
| Fort Simpson | 2/106 | 0/4 | — | 2/11 | 1/33 | 5/154 |
| 62° N 122° W | (5468) | (114) | — | (512) | (1611) | (7705) |
| Inuvik | 0/81 | 0/1 | 0/55 | 1+1/65* | 0/4 | 1×1/204 |
| 69° N 135° W | (4435) | (11) | (2650) | (3662) | (105) | (10863) |
| Total | 2/248 | 0/20 | 0/55 | 4+1/94 | 2/58 | 8+1/475 |
| | (12716) | (477) | (2650) | (5096) | (2808) | (23747) |

All virus isolations were made between 10 and 24 July 1976.
* 1 CE isolate, 1 NOR isolate.
*A. com.*, *Aedes communis*; *Cs. in.*, *Culiseta inornata*; *A. nig.*, *Aedes nigripes*; *A. hex.*, *Aedes hexodontus*; *A. punc.*, *Aedes punctor*.
Figures in parentheses indicate total number of mosquitoes tested.

Table 2. *Bunyavirus isolations from Northwest Territories mosquitoes,* 1976

| Location | Week no. | Species | Species type | Ratio* | MFIR† |
|---|---|---|---|---|---|
| Fort Smith | 29 | *A. hexodontus* | CE | 1/18 | 1:922 |
| 60° N 112° W | 29 | *A. punctor* | CE | 1/17 | 1:911 |
| Fort Simpson | 29 | *A. hexodontus* | CE | 2/11 | 1:256 |
| 62° N 112° W | 28 | *A. punctor* | CE | 1/33 | 1:1611 |
| | 28 | *A. communis* | CE | 2/106 | 1:2734 |
| Inuvik | 27 | *A. hexodontus* | NOR | 1/65 | 1:3662 |
| 69° N 135° W | 27 | *A. hexodontus* | CE | 1/65 | 1:3662 |

A total of 23747 unengorged female mosquitoes were tested in 475 pools, of which 8 yielded CE virus (snowshoe hare subtype) and 1 yielded Northway virus.
* Ratio: number of mosquito pools which yielded virus/number of pools tested.
† MFIR: minimum field infection rate.

### *Transmission of CE virus by Arctic mosquitoes*

#### (i) *Culiseta inornata*

Wild-caught *Culiseta inornata* mosquitoes were injected intrathoracically with CE virus doses ranging from 100 to 0·1 mouse LD50. Infectivity titrations of salivary glands and thoraces at weekly intervals after injection revealed minimum virus titres ($10^{1\cdot8}$–$10^{2}$ mouse LD50 per anatomical unit) at 6 days, which increased approximately tenfold after an additional 1–2 weeks of incubation at 13 °C

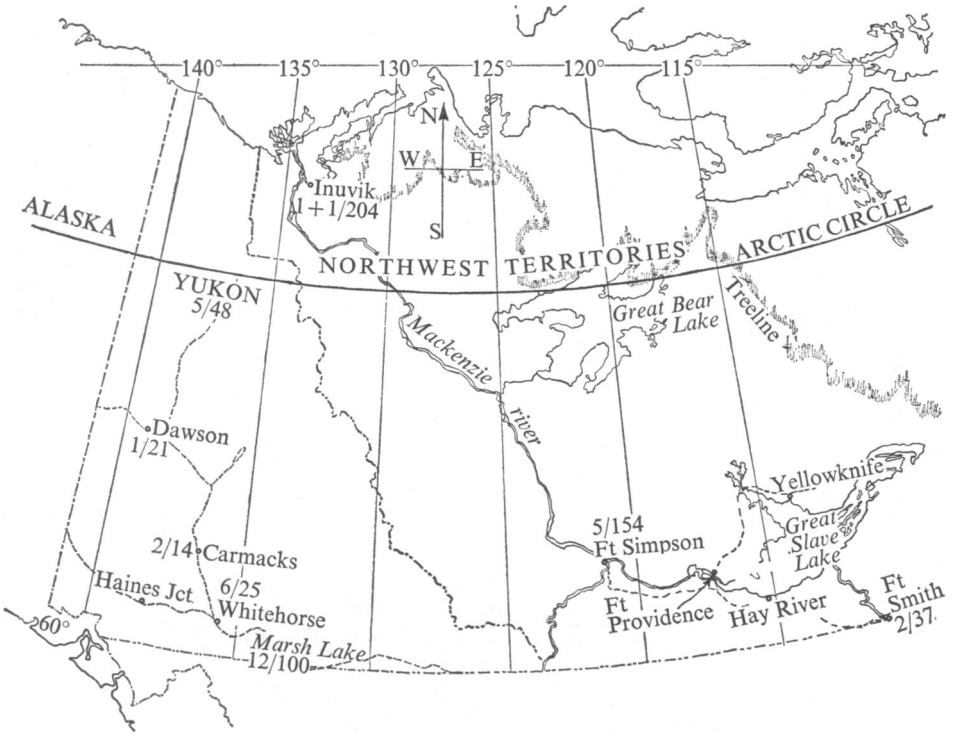


Fig. 1. Ratios: virus-positive mosquito pools/pools tested.

(Table 3). In mosquitoes incubated at 0 °C, virus replication was detected after 27 days incubation, but not after 6 and 13 days, following injection of 1 or 0·1 mouse LD 50. Virus persisted in salivary glands and thoraces at least 96 days.

Virus transmission was achieved as early as 20 days after injection of 10 or 100 mouse LD 50 following incubation at 13 °C, and after 27 days incubation at 0 °C following injection of 1 mouse LD 50. Transmission was also demonstrated 96 days after injection of 10 or 100 mouse LD 50 following incubation at 0 °C.

Immunofluorescence was demonstrated regularly in the cytoplasm of acinar cells of salivary glands of mosquitoes treated with fluorescein labelled CE antiserum which were incubated at 13 °C for 6–96 days after injection of 100 mouse LD 50, but irregularly after injection of smaller virus doses and incubation at 0 or 13 °C. Usually immunofluorescence affected cells throughout the salivary glands of virus-positive mosquitoes, but it was not observed in salivary glands of uninfected mosquitoes tested simultaneously. In some instances, however, immunofluorescence was observed in mosquitoes in which virus remained undetected, despite the absence of immunofluorescence in controls.

After *Cs. inornata* mosquitoes imbibed 100 mouse LD 50 virus in a blood meal, virus was detected in minimal quantities only in salivary glands and thoraces of mosquitoes incubated for 6 and 13 days at 0 and 13 °C, and no virus was demonstrated after 20 days incubation at either temperature.

Table 3. *Transmission of mosquito CE isolate* 74-Y-234 *by* Culiseta inornata *mosquitoes*

| Dose Mouse LD 50 | | 0 °C | | | | | 13 °C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 6 | 13 | 20 | 27 | 96 | 6 | 13 | 20 | 27 | 96 |
| 100 | F | $\frac{2}{2}$ | $\frac{1}{2}$ | $\frac{2}{4}$ | — | $\frac{1}{2}$ tr $\frac{2}{2}$ | $\frac{4}{4}$ | $\frac{3}{4}$ | $\frac{6}{6}$ tr $\frac{1}{1}$ | — | $\frac{1}{1}$ |
| (Inj.) | SG | $\frac{1}{2}$ (2·0) | $\frac{0}{2}$ | $\frac{3}{4}$ (2·0) | — | $\frac{2}{2}$ (2·0) | $\frac{4}{4}$ (1·8) | $\frac{4}{4}$ (2·5) | $\frac{6}{6}$ (3·0) | — | $\frac{1}{1}$ (2·5) |
| | Th. | $\frac{2}{2}$ (2·0) | $\frac{0}{2}$ | $\frac{4}{4}$ (3·0) | — | $\frac{2}{2}$ (2·5) | $\frac{4}{4}$ (2·0) | $\frac{4}{4}$ (2·5) | $\frac{6}{6}$ (3·0) | — | $\frac{1}{1}$ (3·5) |
| 10 | F | $\frac{1}{2}$ | $\frac{2}{4}$ tr $\frac{0}{1}$ | $\frac{2}{6}$ | $\frac{0}{2}$ | $\frac{0}{2}$ tr $\frac{1}{1}$ | $\frac{0}{2}$ | $\frac{2}{4}$ | $\frac{0}{6}$ tr $\frac{3}{3}$ | $\frac{0}{2}$ | |
| (Inj.) | SG | $\frac{2}{2}$ (1·8) | $\frac{1}{4}$ (1·8) | $\frac{4}{6}$ (2·5) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (3·0) | $\frac{2}{2}$ (2·0) | $\frac{4}{4}$ (2·0) | $\frac{6}{6}$ (3·5) | $\frac{2}{2}$ (3·5) | |
| | Th. | $\frac{2}{2}$ (1·8) | $\frac{4}{4}$ (1·5) | $\frac{6}{6}$ (3·2) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (3·2) | $\frac{2}{2}$ (2·0) | $\frac{4}{4}$ (2·0) | $\frac{6}{6}$ (3·2) | $\frac{2}{2}$ (2·5) | |
| 1 | F | $\frac{0}{2}$ | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{0}{1}$ tr $\frac{1}{1}$ | — | $\frac{0}{2}$ | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{0}{1}$ | |
| (Inj.) | SG | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | $\frac{0}{2}$ | $\frac{1}{1}$ (2·0) | — | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (1·8) | $\frac{1}{2}$ (2·8) | $\frac{1}{1}$ (2·0) | |
| | Th. | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | $\frac{0}{2}$ | $\frac{1}{1}$ (2·8) | — | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·0) | $\frac{1}{1}$ (2·8) | |
| 0·1 | F | $\frac{0}{2}$ (1·8) | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{2}{2}$ | $\frac{1}{1}$ | $\frac{0}{2}$ | $\frac{1}{2}$ | $\frac{2}{2}$ | $\frac{0}{2}$ tr $\frac{1}{2}$ | |
| (Inj.) | SG | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{0}{2}$ | $\frac{2}{2}$ (2·5) | $\frac{1}{1}$ (4·0) | $\frac{2}{2}$ (2·0) | $\frac{1}{2}$ (1·8) | $\frac{1}{2}$ (2·0) | $\frac{2}{2}$ (4·0) | |
| | Th | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | $\frac{0}{2}$ | $\frac{2}{2}$ (2·8) | $\frac{1}{1}$ (4·0) | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (3·0) | $\frac{2}{2}$ (3·5) | $\frac{2}{2}$ (3·5) | |
| 100 | F | $\frac{0}{1}$ | $\frac{1}{2}$ | $\frac{0}{2}$ | — | — | $\frac{1}{1}$ | $\frac{1}{2}$ | $\frac{1}{2}$ tr $\frac{0}{2}$ | | |
| (Fed.) | SG | $\frac{1}{1}$ (2·0) | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | — | — | $\frac{1}{1}$ (2·0) | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | | |
| | Th | $\frac{1}{1}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | — | — | $\frac{1}{1}$ (1·5) | $\frac{2}{2}$ (2·0) | $\frac{0}{2}$ | | |

F, proportion of salivary glands with cytoplasmic immunofluorescent foci in acinar cells.
SG, proportion of salivary glands containing virus (log mouse LD 50 titres per gland in parentheses).
Th., proportion of thoraces containing virus.
tr., proportion of mosquitoes which transmitted virus whilst feeding on defibrinated blood in pledgets.
Inj. intrathoracic injection.
Fed, feeding on virus in defibrinated blood.

Table 4. *Transmission of mosquito CE isolate* 74-Y-234 *by* Aedes communis *mosquitoes after intrathoracic injection*

| Mouse LD 50 injected | | 0 °C | | | | | | 13 °C | | | | | | 23 °C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 13 | 20 | 43 | 73 | 3 | 6 | 13 | 20 | 27 | 74 | 3 | 6 | 13 | 20 |
| 100 | F | $\frac{1}{4}$ | $\frac{3}{6}$ | $\frac{3}{6}$ | $\frac{1}{4}$ tr $\frac{4}{2}$ | $\frac{1}{2}$ | $\frac{2}{2}$ | $\frac{4}{4}$ | $\frac{5}{8}$ | $\frac{5}{8}$ | $\frac{4}{8}$ | $\frac{2}{4}$ | — | $\frac{4}{4}$ | $\frac{3}{6}$ | $\frac{0}{6}$ | $\frac{0}{1}$ |
| | SG | $\frac{2}{4}$ (1·8) | $\frac{5}{6}$ (2·0) | $\frac{5}{6}$ (2·0) | $\frac{4}{4}$ (2·5) | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·8) | $\frac{3}{4}$ (1·8) | $\frac{8}{8}$ (2·5) | $\frac{8}{8}$ (2·0) | $\frac{8}{8}$ (3·0) | $\frac{4}{4}$ (2·0) | — | $\frac{4}{4}$ (2·5) | $\frac{6}{6}$ (3·0) | $\frac{5}{6}$ (2·5) | $\frac{1}{1}$ (2·0) |
| | Th. | $\frac{3}{4}$ (2·3) | $\frac{6}{6}$ (2·0) | $\frac{6}{6}$ (2·0) | $\frac{4}{4}$ (2·5) | $\frac{2}{2}$ (2·5) | $\frac{2}{2}$ (3·5) | $\frac{4}{4}$ (2·3) | $\frac{8}{8}$ (3·0) | $\frac{8}{8}$ (2·5) | $\frac{8}{8}$ (3·0) | $\frac{4}{4}$ (2·0) | — | $\frac{4}{4}$ (2·8) | $\frac{6}{6}$ (3·0) | $\frac{5}{6}$ (2·3) | $\frac{1}{1}$ (2·0) |
| 10 | F | $\frac{3}{6}$ | $\frac{4}{6}$ | $\frac{4}{6}$ | $\frac{3}{5}$ tr $\frac{0}{1}$ | $\frac{1}{1}$ tr $\frac{1}{1}$ | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{1}{8}$ | $\frac{7}{8}$ | $\frac{5}{8}$ tr $\frac{1}{3}$ | $\frac{2}{3}$ tr $\frac{0}{1}$ | — | $\frac{2}{6}$ | $\frac{1}{6}$ | $\frac{0}{6}$ tr $\frac{1}{1}$ | $\frac{1}{3}$ tr $\frac{0}{1}$ |
| | SG | $\frac{3}{6}$ (1·8) | $\frac{4}{6}$ (2·0) | $\frac{4}{6}$ (2·0) | $\frac{3}{5}$ (2·5) | $\frac{1}{1}$ (2·5) | $\frac{2}{2}$ (1·8) | $\frac{5}{6}$ (1·8) | $\frac{7}{8}$ (2·3) | $\frac{5}{8}$ (2·5) | $\frac{8}{8}$ (2·8) | $\frac{3}{3}$ (3·0) | — | $\frac{6}{6}$ (3·0) | $\frac{6}{6}$ (2·3) | $\frac{5}{6}$ (2·5) | $\frac{2}{3}$ (2·5) |
| | Th. | $\frac{2}{6}$ (1·8) | $\frac{5}{6}$ (2·0) | $\frac{6}{6}$ (3·0) | $\frac{4}{5}$ (3·0) | $\frac{1}{1}$ (2·5) | $\frac{2}{2}$ (3·0) | $\frac{6}{6}$ (2·5) | $\frac{8}{8}$ (2·5) | $\frac{8}{8}$ (3·3) | $\frac{8}{8}$ (2·8) | $\frac{3}{3}$ (3·0) | — | $\frac{6}{6}$ (3·5) | $\frac{6}{6}$ (2·3) | $\frac{5}{6}$ (2·5) | $\frac{2}{3}$ (2·8) |
| 1 | F | $\frac{2}{6}$ | $\frac{2}{6}$ | $\frac{2}{6}$ | $\frac{2}{6}$ tr $\frac{2}{2}$ | — | $\frac{1}{2}$ | $\frac{3}{6}$ | $\frac{4}{8}$ | $\frac{4}{8}$ tr $\frac{1}{1}$ | $\frac{2}{7}$ tr $\frac{1}{1}$ | $\frac{2}{2}$ tr $\frac{1}{1}$ | $\frac{1}{1}$ | $\frac{2}{4}$ | $\frac{0}{4}$ | $\frac{2}{4}$ tr $\frac{1}{1}$ | |
| | SG | $\frac{2}{6}$ (1·5) | $\frac{5}{6}$ (1·8) | $\frac{3}{6}$ (1·8) | $\frac{5}{6}$ (2·3) | — | $\frac{2}{2}$ (3·5) | $\frac{2}{6}$ (1·5) | $\frac{6}{8}$ (1·8) | $\frac{8}{8}$ (2·0) | $\frac{7}{7}$ (2·8) | $\frac{2}{2}$ (3·0) | $\frac{1}{1}$ (3·0) | $\frac{4}{4}$ (1·8) | $\frac{4}{4}$ (2·5) | $\frac{4}{4}$ (2·5) | |
| | Th. | $\frac{2}{2}$ (1·8) | $\frac{5}{6}$ (1·8) | $\frac{3}{6}$ (1·8) | $\frac{5}{6}$ (2·3) | — | $\frac{2}{2}$ (4·0) | $\frac{2}{6}$ (1·8) | $\frac{8}{8}$ (2·0) | $\frac{7}{8}$ (2·0) | $\frac{7}{7}$ (2·5) | $\frac{2}{2}$ (3·0) | $\frac{1}{1}$ (1·8) | $\frac{4}{4}$ (2·5) | $\frac{4}{4}$ (2·5) | $\frac{4}{4}$ (2·5) | |
| 0·1 | F | $\frac{1}{4}$ | $\frac{0}{4}$ | $\frac{0}{2}$ | $\frac{2}{2}$ tr $\frac{1}{1}$ | — | — | $\frac{2}{4}$ | $\frac{2}{7}$ | $\frac{3}{6}$ | $\frac{3}{8}$ tr $\frac{1}{1}$ | $\frac{0}{4}$ | $\frac{1}{1}$ | $\frac{0}{2}$ | $\frac{0}{4}$ | — | $\frac{0}{1}$ |
| | SG | $\frac{0}{4}$ | $\frac{1}{4}$ (2·0) | $\frac{0}{2}$ | $\frac{1}{2}$ (1·8) | — | — | $\frac{0}{4}$ | $\frac{4}{8}$ (1·8) | $\frac{5}{6}$ (1·8) | $\frac{7}{8}$ (2·0) | $\frac{2}{4}$ (1·8) | $\frac{1}{1}$ (3·0) | $\frac{1}{2}$ (1·8) | $\frac{4}{4}$ (3·0) | — | $\frac{1}{1}$ (2·0) |
| | Th. | $\frac{0}{4}$ | $\frac{1}{4}$ (1·8) | $\frac{0}{2}$ | $\frac{1}{2}$ (2·0) | — | — | $\frac{0}{4}$ | $\frac{5}{8}$ (1·8) | $\frac{5}{6}$ (1·8) | $\frac{8}{8}$ (2·0) | $\frac{2}{4}$ (2·0) | $\frac{1}{1}$ (2·8) | $\frac{1}{2}$ (2·0) | $\frac{4}{4}$ (3·0) | — | $\frac{1}{1}$ (3·0) |
| 0·01 | F | $\frac{2}{6}$ | $\frac{2}{3}$ | $\frac{2}{2}$ tr $\frac{2}{2}$ | $\frac{2}{2}$ | — | $\frac{2}{2}$ | $\frac{2}{2}$ | $\frac{2}{6}$ | $\frac{2}{2}$ tr $\frac{1}{1}$ | $\frac{3}{6}$ tr $\frac{1}{1}$ | $\frac{0}{4}$ tr $\frac{1}{1}$ | — | $\frac{0}{2}$ | $\frac{1}{6}$ | $\frac{2}{2}$ | $\frac{1}{1}$ |
| | SG | $\frac{0}{6}$ | $\frac{3}{3}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (1·8) | — | $\frac{2}{2}$ (1·8) | $\frac{0}{2}$ | $\frac{5}{6}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{6}{6}$ (1·8) | $\frac{4}{4}$ (1·8) | — | $\frac{1}{2}$ (2·0) | $\frac{3}{6}$ (1·8) | $\frac{2}{2}$ (1·5) | $\frac{1}{1}$ (2·0) |
| | Th. | $\frac{0}{6}$ | $\frac{3}{3}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (1·8) | — | $\frac{2}{2}$ (2·0) | $\frac{0}{2}$ | $\frac{5}{6}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{6}{6}$ (1·8) | $\frac{4}{4}$ (1·8) | — | $\frac{1}{2}$ (2·0) | $\frac{3}{6}$ (1·8) | $\frac{2}{2}$ (2·0) | $\frac{1}{1}$ (1·8) |

F, proportion of salivary glands with cytoplasmic immunofluorescent foci in acinar cells.
SG, proportion of salivary glands containing virus (log mouse LD 50 titres per gland in parentheses).
Th., proportion of thoraces containing virus.
tr., proportion of mosquitoes which transmitted virus whilst feeding on defibrinated blood in pledgets.
Inj., intrathoracic injection.
Fed., feeding on virus in defibrinated blood.

Table 5. *Transmission of mosquito CE isolate* 74-Y-234 *by* Aedes communis *mosquitoes after feeding*

| Mouse LD 50 fed | | 0 °C | | | | 13 °C | | | | | 23 °C | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 6 | 13 | 20 | 3 | 6 | 13 | 20 | 27 | 13 | 20 |
| 1000 | F | — | — | — | — | — | — | $\frac{1}{4}$ tr $\frac{1}{1}$ | $\frac{0}{4}$ tr $\frac{2}{2}$ | — | $\frac{0}{2}$ tr $\frac{2}{2}$ | |
| | SG | — | — | — | — | — | — | $\frac{4}{4}$ (2·0) | $\frac{4}{4}$ (2·0) | — | $\frac{2}{2}$ (2·0) | |
| | Th. | — | — | — | — | — | — | $\frac{4}{4}$ (2·5) | $\frac{4}{4}$ (2·0) | — | $\frac{2}{2}$ (1·8) | |
| 100 | F | $\frac{0}{2}$ | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{0}{2}$ tr $\frac{1}{1}$ | $\frac{0}{2}$ | $\frac{1}{2}$ | $\frac{2}{5}$ tr $\frac{1}{1}$ | $\frac{1}{4}$ tr $\frac{2}{3}$ | $\frac{0}{2}$ | — | |
| | SG | $\frac{0}{2}$ | $\frac{2}{2}$ (1·5) | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·0) | $\frac{0}{2}$ | $\frac{2}{4}$ (1·8) | $\frac{4}{5}$ (3·0) | $\frac{4}{4}$ (3·3) | $\frac{2}{3}$ (2·0) | — | |
| | Th. | $\frac{0}{2}$ | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·5) | $\frac{0}{2}$ | $\frac{2}{4}$ (1·8) | $\frac{4}{5}$ (3·5) | $\frac{4}{4}$ (3·3) | $\frac{2}{3}$ (2·0) | — | |
| 1 | F | — | — | — | — | — | — | $\frac{0}{2}$ tr $\frac{1}{1}$ | $\frac{0}{1}$ | — | — | $\frac{0}{1}$ |
| | SG | — | — | — | — | — | — | $\frac{2}{2}$ (2·0) | $\frac{0}{1}$ | — | — | $\frac{1}{1}$ (2·0) |
| | Th. | — | — | — | — | — | — | $\frac{2}{2}$ (2·0) | $\frac{1}{1}$ (1·8) | — | — | $\frac{1}{1}$ (1·5) |
| 0·1 | F | | — | — | — | — | — | $\frac{2}{2}$ | $\frac{0}{2}$ | $\frac{0}{2}$ | — | $\frac{0}{1}$ tr $\frac{1}{1}$ |
| | SG | — | — | — | — | — | — | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (1·8) | — | $\frac{1}{1}$ (1·8) |
| | Th. | — | — | — | — | — | — | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (1·8) | — | $\frac{1}{1}$ (1·5) |
| 0·01 | F | — | — | — | — | — | — | $\frac{0}{2}$ tr $\frac{1}{1}$ | $\frac{0}{2}$ | $\frac{0}{2}$ | — | $\frac{0}{2}$ tr $\frac{2}{2}$ |
| | SG | — | — | — | — | — | — | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (1·8) | $\frac{2}{2}$ (2·0) | — | $\frac{2}{2}$ (1·8) |
| | Th. | — | — | — | — | — | — | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·0) | $\frac{2}{2}$ (2·0) | — | $\frac{2}{2}$ (1·8) |

For notes, see Table 4.

(ii) *Aedes communis*

After injection of *A. communis* mosquitoes with virus doses ranging from 100 to 0·1 mouse LD50, evidence of viral replication was demonstrated in some or all mosquitoes of each batch incubated for 6 days at 0, 13 and 23 °C (Table 4). Virus infectivity was not detected after 3 days incubation at 0 and 13 °C, following inoculation with 0·1 or 0·01 mouse LD50, and in 50% of those incubated at 23 °C. Virus transmission was achieved as early as 13 days after incubation at 0, 13 or 23 °C, following inoculation with as small amounts of virus as 0·01 mouse LD50. Virus was detected in salivary glands and thoraces of mosquitoes for as long as 73–74 days incubation at 0 or 13 °C, and transmission occurred after 43 days incubation at 0 °C. Localization of CE antigen by direct immunoflorescence was demonstrated fairly regularly following incubation at 13 °C after mosquitoes were injected with 100 or 10 mouse LD50 but irregularly after mosquitoes were incubated at the other two temperatures.

After *A. communis* mosquitoes imbibed a blood meal containing 1000, 100, 1 or 0·01 mouse LD50, virus transmission was demonstrated following 13 days incubation at 13 °C (Table 5). Virus transmission was attained 20 days after mosquitoes imbibed 100 mouse LD50, following incubation at 0 °C. Immunofluorescent foci were observed irregularly in mosquito salivary glands which supported virus replication.

## DISCUSSION

Recovery of CE and NOR viruses from *A. hexodontus* mosquitoes collected at Inuvik (69° N, 135° W) represents the northernmost documented isolations of arboviruses in the Canadian Arctic. Inuvik is situated in an open woodland area on the estuary of the Mackenzie River about 300 miles east and somewhat south of the northernmost isolation of CE virus in Alaska at a tundra location along the Arctic Ocean at (70° N, 143° W) immediately west of the Alaska–Yukon border (Ritter & Feltz, 1974). For the first time also in the open woodland terrain of the western Canadian Arctic, *A. hexondontus* and *A. punctor* have yielded Bunyavirus isolations, both CE and NOR in the case of *A. hexodontus*, thus bridging the gap between isolations in the boreal forest of east-central Alaska (Ritter & Feltz, 1974) and on the tundra of the central Canadian Arctic at Baker Lake (62° N, 98° W) (Wagner *et al.* 1975). Consistent with previous experience in the Yukon Territory (McLean *et al.* 1975), the peak incidence of Bunyavirus isolations from mosquitoes throughout the Mackenzie Valley, N.W.T., during 1976 was during mid and late July.

Serological results from the small number of wild mammals which were able to be collected, owing to unduly low wildlife population in 1976, strongly suggested that *Lepus americanus* was a significant natural reservoir of CE virus, and that *Tamiasciurus hudsonicus* may become infected in nature, consistent with findings in the Yukon Territory (McLean *et al.* 1975) and elsewhere.

Transmission of CE virus by *A. communis* mosquitoes was demonstrated for the first time during 1976, employing modifications of a technique used by Rosen & Gubler (1974) for dengue virus experiments, whereby mosquitoes restrained in

2·5 cm cylindrical cages were induced to imbibe defibrinated blood. During this process, mosquito saliva containing virus was ejected into the blood, which was assayed promptly for virus content. Transmission of CE virus by *A. communis* which was achieved after incubation at temperatures which extended over its entire viable range of 0–23 °C, following infection of mosquitoes which fed initially on virus-laden blood, clearly demonstrated the high significance of this species as a natural virus vector, thus extending earlier observations of the high frequency of virus isolations from wild-caught specimens in the Yukon (McLean *et al.* 1975), Alaska (Ritter & Feltz, 1974) and Alberta (Iversen *et al.* 1969). Similarly the present findings that *Cs. inornata* which was infected by intrathoracic injection, transmitted CE virus after incubation at 0° and 13 °C in addition to 27 °C reported previously (McLean *et al.* 1974), strengthens the probability that this species also serves an important role as a natural vector. Since both these arctic mosquito species were infected by virus doses as small as 0·1 to 0·01 mouse LD50 after incubation at temperatures as low as 0 °C, it seems probable that the vector potential for a particular mosquito is governed primarily by its ability to survive at frigid temperatures.

Localization of antigen of the snowshoe hare subtype of CE virus in the salivary glands of *A. communis* and *Cs. inornata* mosquitoes did not provide a sufficiently reliable indicator of infection to permit abandonment of infectivity assays for detection of virus, in contradistinction to the high degree of correlation of virus-specific immunofluorescence which was observed in thoracic contents of *A. triseriatus* mosquitoes infected with the La Crosse subtype of CE virus (Beaty & Thompson, 1975). Titres of the snowshoe hare subtype of CE virus in salivary glands and thoraces of *A. communis* infected by intrathoracic injection and incubated at 0, 13 and 23 °C were comparable during experiments conducted in summer 1975 (McLean *et al.* 1976) and 1976, but the direct immunoperoxidase technique applied during 1975 revealed viral antigen somewhat more regularly than the direct immunofluorescent technique during 1976.

This work was supported by the National Research Council, Canada, Contract 031-604 and the Medical Research Council, Canada, Grant MT-2811.

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