INCRIMINATION OF *AEDES PROVOCANS* AS A VECTOR OF JAMESTOWN CANYON VIRUS IN AN ENZOOTIC FOCUS OF NORTHEASTERN NEW YORK

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ABSTRACT. A 2-year field study was conducted in southern Saratoga County, New York, to determine which species of the *Aedes communis* group mosquitoes were potential vectors of Jamestown Canyon (JC) virus. A total of 23,890 mosquitoes (890 pools) were processed for virus isolation in 1988–89, yielding 17 JC virus isolates from *Ae. provocans* and one isolate each from *Ae. communis*, *Ae. intrudens* and *Ae. punctor*. Minimum field infection rates (MFIR) and daily MFIRs as high as 1:219 and 1:38, respectively, were found in adult female *Ae. provocans*. Virus isolation attempts from an additional 394 individual *Ae. provocans* produced a seasonal field infection rate (FIR) of 1:131 and daily FIRs of 1:71 and 1:22. Evidence of transovarial transmission of JC virus was demonstrated by the isolation of virus from 2 pools each of 50 male *Ae. provocans* reared in the insectary from pupae collected at the study site in 1989. We conclude that *Ae. provocans* is a potentially important vector of JC virus in northeastern New York.

INTRODUCTION

Jamestown Canyon (JC) virus, a member of the California (CAL) serogroup (Bunyaviridae: *Bunyavirus*), is recognized as a human pathogen in New York State. Sixty-seven percent (62/93) of patients with central nervous system infection and evidence of CAL serogroup antibody from 1971 to 1982 in New York were infected with JC virus (Srihongse et al. 1984). The first 10 documented human cases of JC virus encephalitis were described from New York residents where an antibody prevalence rate of 3.5–12.5% was reported (Deibel et al. 1983).

The majority of JC virus isolates in New York have been obtained from mosquitoes of the *Aedes communis* group. The similarity in morphology within this group makes specific identification problematic. In the literature these mosquitoes are seldom identified beyond *Ae. communis* group, especially in studies of vector potential for viral encephalitides. Grayson et al. (1983) reported 36 JC virus isolates from 45,876 *Ae. communis* group mosquitoes, with a minimum field infection rate (MFIR) of 1:1,274. This group accounted for 42% of all JC virus isolates obtained in New York State from 1972 to 1980. Identification of species in the *Ae. communis* group is essential for understanding the part this mosquito group plays in the natural transmission cycle of JC virus.

This investigation examined the role of *Ae. communis* group mosquitoes in the vector ecology of JC virus in a previously identified enzootic focus of infection (J. McKeon, personal communication). The goals of this study were to: 1) determine the species composition of the *Ae. communis* group, 2) determine the MFIR for JC virus, 3) demonstrate evidence for the transovarial transmission of JC virus, and 4) evaluate the vector potential of this mosquito group for JC virus in northeastern New York.

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MATERIALS AND METHODS

Study site: The study site was situated in a northern hardwood and coniferous forest with numerous woodland pools located on Gurnsprings Road in the town of Wilton, Saratoga County, approximately 45 miles north of Albany, New York. This area of New York was ecologically classified as the Hudson-Mohawk lowland region (Thompson 1977).

Field and insectary techniques: Human-baited landing/biting collections of host-seeking female mosquitoes were made from May 12 to June 21, 1988, using a hand-held aspirator. Adult mosquitoes were collected from May 23 to June 1, 1989, by sweeping vegetation with a battery-powered field aspirator (Nasci 1981). These mosquitoes were transported to the insectary on dry ice and stored at −70°C in a mechanical freezer.

Mosquito larvae and pupae were collected with a white plastic dipper from vernal woodland pools, stored in 1-liter glass jars, held on wet ice and transported to the insectary from May 1 to 9, 1989. The 4th instar larvae and detritus collected at the study site were transferred to white enamel pans (38 × 23 × 7 cm) containing 2 liters of tap water. The pans were held at room temperature (22°C) until pupation occurred. Approximately 200 pupae were put into 500-ml drinking cups and placed in aluminum mosquito cages (46 × 46 × 46 cm) containing 10% sucrose-soaked cotton balls. The cages were stored at room temperature in large plastic bags to pre-

vent dehydration; newly emerged adult mosquitoes were collected with a hand-held aspirator every morning until all had emerged.

Mosquitoes were identified using keys of Means (1979) and Darsie and Ward (1981) and pooled in the insectary according to species, sex, date and method of collection. Voucher specimens of adult mosquitoes were deposited in the reference collection at the New York State Museum, Albany, New York.

Laboratory methods: Tests for virus isolation from mosquitoes were performed in duplicate Vero cell cultures as described in Srihongse et al. (1979), with the following modifications: after centrifugation of the mosquito suspensions, 0.45 ml of each supernatant was mixed with 0.05 ml of antibiotics (20,000 units of penicillin and 40 mcg of streptomycin/ml), held overnight at 4°C and inoculated the next morning into cell culture tubes. These were then observed for signs of cytopathic effects (CPE) for 7 days, with a change of media on the 3rd or 4th day postinoculation.

Virus isolates were initially identified by an indirect immunofluorescent antibody (IFA) procedure. Positive and negative control slides were made from Vero cells infected with CAL serogroup viruses in 75-cm^2 plastic flasks, after the method of Emmons and Riggs (1977). We used the New York strain (7432813) of La Crosse (LAC) and the prototype strains of JC, snowshoe hare (SSH), trivittatus (TVT) and keystone (KEY) viruses. An equal concentration of infected and uninfected cells was diluted to approximately 1×10^6 cells/ml using a hemocytometer, dispensed onto 10-well microscope slides and fixed.

The technique was modified for virus isolates as follows: when CPE (2–3+) were noted, one of the infected culture tubes was frozen at −70°C, quickly thawed and frozen 2 times and centrifuged. The resulting supernatant was separated into aliquots and stored at −70°C as a virus seed for confirmatory tests. The fluid was decanted from the other infected cell culture tube and the monolayer washed several times in sterile phosphate-buffered saline (PBS, pH 7.6). The infected monolayer was wiped off the surface of the tube in 0.25 ml of PBS using a sterile cotton swab. The cell suspension was gently pipetted using a 0.5-ml syringe fitted with a 26-gauge needle to break up any cell clumps, and 1 drop of the infected cell suspension was dispensed onto each well of a 10-well IFA microscope slide. The slides were allowed to air-dry, fixed in cold acetone for 20 min, air-dried again and stored at −70°C for use in IFA tests.

The IFA slides were stained using CAL serogroup (JC, TVT, LAC, SSH, KEY) mouse hyperimmune ascitic fluids produced in the laboratory (Tikasingh et al. 1966) and a goat anti-mouse IgG conjugate. The slides were then examined using a Zeiss fluorescent microscope equipped with an IV FL epifluorescence condenser, 12V/100W halogen tungsten lamp and FITC excitation and barrier filters. Virus identification was determined by a 2-fold or greater difference in the intensity of immunofluorescence (1–4+) from that of heterologous antibodies.

Identification of viruses was confirmed by a serum dilution plaque reduction neutralization method modified after that of Lindsey et al. (1976). In our laboratory, tests were performed in Vero cell monolayers maintained in 25-cm^2 screw cap flasks and Eagle's 2× minimum essential medium with 5% fetal bovine serum and antibiotics (100 units of penicillin and 100 mcg of streptomycin/ml) and 2% Noble Agar. Virus/serum dilution mixtures were incubated at 37°C in a water bath for 1 h. The virus dose ranged from 50–150 plaque-forming units per 0.2 ml of inoculum; a neutral red stain was applied in a final dilution of 1:10,000.

Individual IFA head squashes were performed on JC virus infected mosquitoes using the method of Kuberski and Rosen (1977). Before an individual specimen was processed for virus isolation, the head was squashed and fixed on an IFA microscope slide. The body was triturated in 1 ml of diluent and processed for virus isolation as previously described. The head was assayed by IFA if its corresponding body was found to be infected with JC virus. The positive and negative IFA control slides described above were used as controls.

Statistical methods: A χ^2 contingency table analysis (with 1 df and Yates' correction for continuity) was used to determine if there were significant differences in field infection rates.

RESULTS

A total of 9,116 female mosquitoes (433 pools) was tested for virus isolation in 1988 (Table 1). There were 10 JC virus isolates obtained from 2,187 *Ae. provocans* (Walker) (65 pools) with a seasonal MFIR of 1:219. These isolates were obtained from *Ae. provocans* collected from May 20 to June 15 and daily minimum field infection rates (dMFIR) as high as 1:38 were observed (Table 2). Jamestown Canyon virus was also isolated from a pool of 44 *Ae. punctor* (Kirby) (seasonal MFIR = 1:526) and a pool of 8 *Ae. intrudens* (Dyar) (seasonal MFIR = 1:1642) collected on May 27 and June 21, 1988, respectively (Table 1).

Table 1. Field-collected adult mosquitoes tested for virus from Saratoga County, NY, 1988–89.

	1988	1989		Total
Species	Females (no. pools)	Females (no. pools)	Males (no. pools)	(no. pools)
Aedes				
abserratus	454 (27)	117 (13)	—[1]	571 (40)
abserratus/punctor	20 (8)	0 (0)	—	20 (8)
canadensis	1,591 (47)	555 (16)	332 (11)	2,478 (74)
cinereus	625 (22)	382 (15)	—	1,007 (37)
communis	499 (29)	173 (13)	—	672 (42)
communis group	33 (11)	0	1,240 (26)	1,273 (37)
decticus	54 (17)	0	—	54 (17)
diantaeus	711 (30)	118 (14)	—	829 (44)
intrudens	1,642 (45)	269 (14)	—	1,911 (59)
provocans	2,187 (65)	858 (34)	848 (23)	3,893 (122)
punctor	526 (28)	201 (13)	—	727 (41)
sticticus	0	211 (10)	—	211 (10)
stimulans group	651 (77)	1,466 (38)	2,044 (44)	4,161 (159)
triseriatus/hendersoni	3 (1)	0	0	3 (1)
trivittatus	16 (4)	0	0	16 (4)
vexans	96 (20)	419 (15)	556 (15)	1,071 (50)
Anopheles punctipennis	6 (1)	0	0	6 (1)
Coquillettidia perturbans	2 (1)	0	0	2 (1)
Totals	9,116 (433)	4,769 (195)	5,020 (119)	18,905 (747)

[1] Pooled as *Ae. communis* group males.

Individual *Ae. provocans* ($n = 394$) were tested for virus to obtain a more accurate JC virus seasonal FIR. Of 44 individual *Ae. provocans* collected on May 27, two JC virus isolates were obtained, yielding a dFIR of 1:22, which was not significantly different ($\chi^2 = 0.53$; $P > 0.25$) from the dMFIR of 1:68 collected on that same day (Table 2). An additional JC virus isolate was obtained from 71 individually tested *Ae. provocans* collected on June 9 (dFIR = 1:71), which was also not significantly different ($\chi^2 = 0.0035$; $P > 0.95$) from the dMFIR of 1:38 collected on that same day (Table 2). No other JC virus isolates were obtained by testing individual specimens. As a group, the individual specimens yielded a seasonal FIR of 1:131. There was no significant difference ($\chi^2 = 0.16$; $P > 0.50$) between this seasonal FIR and the seasonal MFIR of 1:219. None of the heads from these three JC virus infected mosquitoes were positive for antigen as demonstrated by the IFA head squash procedure.

In 1989, from the 9,789 mosquitoes in 314 pools processed for virus isolation, 2 JC virus isolates were obtained from 858 female *Ae. provocans* (34 pools) tested for virus. This group had a seasonal MFIR of 1:429 (Table 1). Both of these positive pools (dMFIR = 1:70) were collected on June 1 (Table 2). There was no significant difference ($\chi^2 = 0.32$; $P > 0.50$) in seasonal MFIRs for *Ae. provocans* between 1988 and 1989. No individual *Ae. provocans* were processed for virus isolation in 1989. Jamestown Canyon virus was also isolated from one pool of 17 female *Ae. communis* mosquitoes collected on June 1 yielding a seasonal MFIR of 1:173 (Table 1).

Table 2. Daily minimum field infection rates (dMFIR) of Jamestown Canyon virus in field-collected female *Aedes provocans* from Saratoga County, NY, 1988–89.

Date of collection	Number collected	dMFIR[1]
May 20, 1988	312	1:312
May 27, 1988	205	1:68
June 1, 1988	191	1:191
June 7, 1988	231	1:231
June 9, 1988	76	1:38
June 15, 1988	164	1:82
June 1, 1989	140	1:70

[1] No. of isolates/no. collected on this date.

In the spring of 1989, 4,985 adult *Aedes* mosquitoes, comprising 735 females (42 pools) and 4,250 males (101 pools), collected at the study site between May 1 and May 9 were reared in the insectary from larvae and pupae (Table 3). *Aedes provocans* comprised 85% of these mosquitoes. The remainder of the collection was comprised of *Ae. canadensis* (Theobald), *Ae. stimulans* group, and other members of the *Ae. communis* group. Jamestown Canyon virus was isolated from 2 pools each of 50 adult male *Ae.*

Table 3. Insectary-reared adult mosquitoes tested for virus from Saratoga County, NY, 1989.

Species	Female (no. pools)	Male (no. pools)	Total (no. pools)
Ae. abserratus	14 (3)	—[1]	14 (3)
abserratus/punctor	—	47 (4)	47 (4)
canadensis	6 (2)	12 (3)	18 (5)
cinereus	2 (1)	—[2]	2 (1)
communis	4 (2)	—[2]	4 (2)
communis group	—	381 (8)	381 (8)
intrudens	174 (5)	—[2]	174 (5)
provocans	506 (24)	3,714 (82)[3]	4,220 (106)
punctor	19 (4)	—[1]	19 (4)
stimulans group	10 (1)	96 (4)	106 (5)
Totals	735 (42)	4,250 (101)	4,985 (143)

[1] Pooled as *Ae. abserratus/punctor* males.
[2] Pooled as *Ae. communis* group males.
[3] Two JC isolates.

provocans collected as pupae on May 4 (dMFIR = 1:1,144) and May 9 (dMFIR = 1:1,728). The MFIR for the entire collection of insectary-reared male *Ae. provocans* at the study site was 1:1,857.

The identification of all isolates preliminarily characterized as JC virus by the IFA technique was confirmed by the serum dilution plaque reduction neutralization method; in each case, JC titers were at least 1:80-1:160 whereas LAC, SSH, TVT and KEY titers were less than 1:40.

DISCUSSION

Jamestown Canyon virus was first isolated from *Ae. provocans* in 1982; 3 of 4 JC virus isolates from Saratoga County were obtained from that species (J. McKeon, personal communication). In 1986, during our annual arbovirus surveillance in central New York, 4 additional strains of JC virus were isolated from *Ae. trichurus* (=*provocans*) collected in Madison County (M. A. Grayson, unpublished data). In our study a total of 17 JC virus isolates from *Ae. provocans* with seasonal and daily MFIRs as high as 1:219 and 1:38, respectively, suggested an important role for this member of the *Ae. communis* group in the ecology of JC virus in northeastern New York. Although JC virus was isolated from 3 other members of the *Ae. communis* group (*Ae. punctor* and *Ae. intrudens* in 1988 and *Ae. communis* in 1989), the number of isolations was low in comparison with *Ae. provocans* (Table 1). This suggested that these 3 species were less important than *Ae. provocans* in the ecology of JC virus in this locality. Since there were few *Ae. punctor* and *Ae. communis* collected in 1988–1989, the low number of JC virus isolates from these species could be explained by the small number of specimens collected, thus making it difficult to accurately assess their true vector potential. However, since *Ae. intrudens* was very numerous in 1988 and collected during the same period as *Ae. provocans*, it could be concluded that this species of *Ae. communis* group mosquito was not a major vector of JC virus here. Similar results were found in northern Michigan where 14 JC virus isolates were obtained from *Ae. provocans* (seasonal MFIRs of 1:27–1:714) and 2 isolates each from *Ae. abserratus/punctor* and *Ae. intrudens* (Heard et al. 1990). Considering the high JC virus infection rates observed in *Ae. provocans*, especially in 1988, some virus might be expected to "spill over" to other potential vector species, such as *Ae. punctor*, *Ae. intrudens* and *Ae. communis* of the *Ae. communis* group mosquitoes.

Transmission of JC virus in white-tailed deer in the spring has been described from the midwestern U.S. (Issel et al. 1972, Boromisa and Grimstad 1987, Grimstad et al. 1987). Since there is little reason to believe that *Ae. provocans* would not feed on deer (Means 1979), the resulting mosquito/deer virus amplification cycle would increase the chances of encountering a JC virus infected mosquito. Jamestown Canyon virus has been isolated from sentinel white-tailed deer in Michigan as early as one week after the first *Ae. provocans* were collected (Heard et al. 1990). Of 10 cases of JC virus encephalitis from New York State residents with onset of illness occurring from May to September, only 4 of these occurred from May to July (Deibel et al. 1983). Since *Ae. provocans* and other *Ae. communis* group members are univoltine, springtime species, those cases of encephalitis occurring in August and September would be attributable to other mosquito species, notably *Anopheles* spp., whose populations peak later in the year (Boromisa and Grimstad 1986).

This is the first report of transovarial transmission of JC virus from insectary-reared, male *Ae. provocans*. Transovarial transmission of JC virus has been demonstrated in *Ae. triseriatus*

(Say) larvae from Ohio (MFIR = 1:676) (Berry et al. 1977) and male *Ae. stimulans* (Walker) from northern Indiana (MFIR = 1:1,424) (Boromisa and Grimstad 1986). Both species were found at our study site.

The roles of *Ae. canadensis* and the *Ae. stimulans* group must also be further examined in the elucidation of JC virus ecology. There were 14 JC virus isolates from *Ae. stimulans* group mosquitoes (MFIR = 1:4,895) and 4 from *Ae. canadensis* (MFIR = 1:27,403) accounting for 16% and 5% of all JC virus isolates in New York from 1972 to 1980, respectively (Grayson et al. 1983). They also obtained 36 JC virus isolates from *Ae. communis* group mosquitoes (MFIR = 1:1,274). In our study there were no JC virus isolates from *Ae. canadensis* nor *Ae. stimulans* group mosquitoes, even though JC virus was endemic at the study site. Furthermore, no JC virus isolates were obtained from *Ae. stimulans* group mosquitoes even though it was the most abundant species collected. Considering the very low MFIRs for *Ae. canadensis* and *Ae. stimulans* group mosquitoes and the relatively high MFIRs for *Ae. communis* group mosquitoes, reported here and in the earlier New York study, it would appear that *Ae. canadensis* and *Ae. stimulans* group mosquitoes do not play as great a role, if any, as *Ae. provocans* in the transmission of JC virus in northeastern New York.

Aedes stimulans has been shown to be a vector of JC virus in northern Indiana with oral transmission rates of 8–16% and a seasonal MFIR of 1:1260 (Boromisa and Grimstad 1986). However, there were no *Ae. provocans* and very few other members of the *Ae. communis* group in northern Indiana for comparison with northeastern New York. Geographical variation in the ability of a mosquito species to become infected with and transmit an arbovirus has been well documented (Hardy et al. 1983). The possibility exists that JC virus may be transmitted by different mosquito species as well as by different strains of the same species in different geographical locations. These same geographical differences may apply to different strains of JC virus as well.

These findings represent the first report of transovarial transmission of JC virus by *Ae. provocans* and, together with the high field infection rates, implicate *Ae. provocans* as a potentially important vector of this virus in northeastern New York.

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