ISOLATION OF JAMESTOWN CANYON VIRUS (CALIFORNIA SEROGROUP) FROM *AEDES* MOSQUITOES IN AN ENZOOTIC FOCUS IN MICHIGAN[1]

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ABSTRACT. Twenty isolates of Jamestown Canyon virus were obtained from adult females of 5 *Aedes* species collected at the Houghton Lake Wildlife Research Area, Missaukee County, in north-central Michigan between 1985 and 1989. Fourteen were from *Aedes provocans*, and 6 were from 4 other snowmelt *Aedes* species. One isolate of trivittatus virus and one Cache Valley-like virus were also obtained. Seasonal succession patterns for numerous mosquito species were recorded over 4 years. The temporal association of adult mosquito emergence, virus isolations, and infection and seroconversion of sentinel deer suggest that *Ae. provocans* is a primary enzootic vector of Jamestown Canyon virus in that focus. We hypothesize that *Ae. provocans* provides an overwintering reservoir for Jamestown Canyon virus at the study site. A large dry ice-baited "tent trap" was the most productive method for collecting numerous aedine and other mosquito species.

INTRODUCTION

Jamestown Canyon (JC) virus is one of the most widely distributed arboviruses in North America with a range extending beyond the distribution of any single species of mosquito or other hematophagous arthropod considered to be a potential vector (Grimstad 1988). The distribution of JC virus appears to result from a patchwork of different vectors. More than 20 species of mosquitoes have yielded isolates (Grimstad 1983, Grimstad 1988), among which *Aedes stimulans* (Walker) is a known vector (Boromisa and Grimstad 1986). *Culiseta inornata* (Williston), from which JC virus was originally isolated (Hammon and Sather 1966), has been listed as a vector in a review article by Turell and LeDuc (1983); however, no experimental data were cited. *Aedes abserratus* (Felt and Young) is suspected of transmitting virus on the East Coast (Main et al. 1979), and arguments have been presented which suggest anophelines might play a role in transmission (Boromisa and Grimstad 1986, Grimstad 1988).

The JC virus transmission season is relatively long. Cases of human illness due to JC virus infection occur between April and October (Grimstad 1988). Infections in white-tailed deer (*Odocoileus virginianus*) in the upper Midwest occur as early as late February/early March and continue into October (Boromisa and Grimstad 1987, Grimstad et al. 1987, Issel 1973, Issel et al. 1972). The concept of multiple vectors helps explain this long transmission season.

This paper reports some of the findings of a multiyear study of vector-virus-host relationships in a JC virus focus in Michigan's Lower Peninsula. This study site is notable for the absence of *Ae. stimulans* and only rare occurrence of *Cs. inornata* (see below). Despite their absence, an intense period of JC virus transmission has been detected in sentinel deer each of 6 years in late spring (Grimstad et al. 1987; Grimstad, unpublished data). Here we present the results of mosquito seasonal succession and virus isolation studies, which implicate *Aedes provocans* (Walker) as a primary vector at the site.

MATERIALS AND METHODS

Study site: All collections were made within the Houghton Lake Wildlife Research Area, Missaukee County, Michigan (Fig. 1). A captive breeding deer herd of approximately 70 animals is maintained there at Porter Ranch, and yearling does have been used as a sentinel herd since 1984 (Grimstad et al. 1987). The research area lies in a transitional region of the state between the northern coniferous and southern deciduous forests and is characterized by areas of deciduous and coniferous forest, dry grasslands, semipermanent marshes, and swamps. Weather records were obtained from a NOAA weather station located on the premises and from published summaries (NOAA 1989).

Mosquito collection and pooling: The collections reported in this study consisted of only adult females; larval collections were periodically made to provide fresh females with corre-

[1] Supported in part by National Institutes of Health Grant AI-19679, AI-07030 and a contribution of Federal Aid in Wildlife Restoration, Michigan Project W-127-R. In conducting the research described (or cited as unpublished data) in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the National Research Council and used facilities accredited by the American Association for the Accreditation of Laboratory Animal Care.

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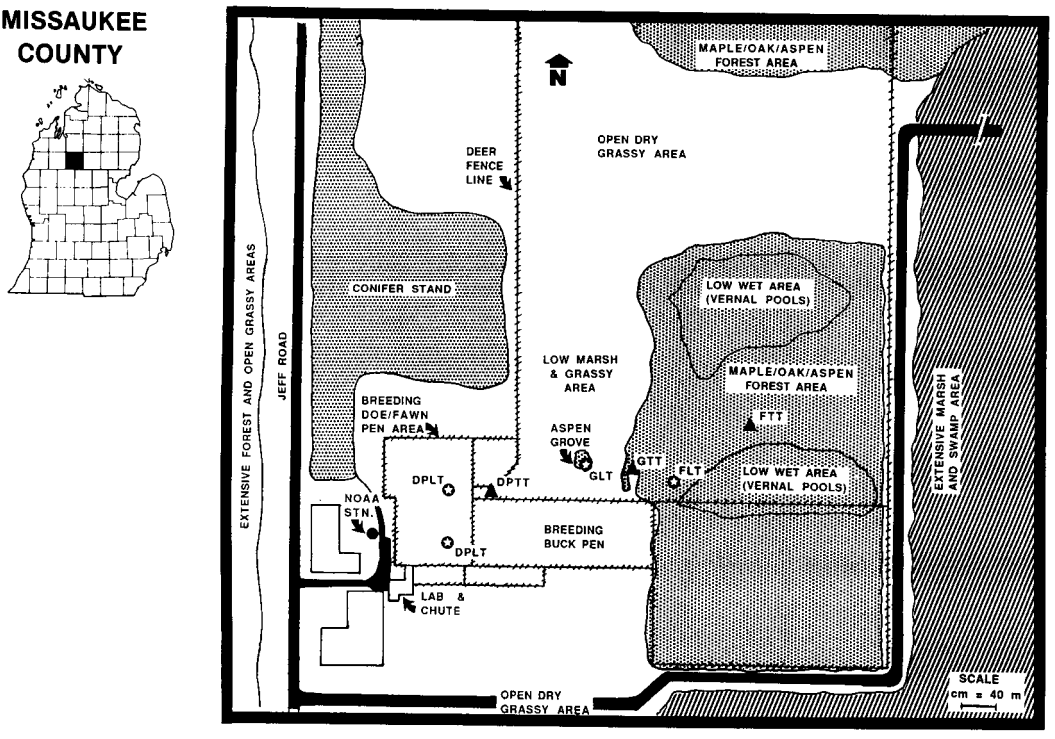

Fig. 1. Houghton Lake Wildlife Research Area-Porter Ranch and trap locations. Abbreviations: DPLT = deer pen light traps, GLT = aspen grove light trap, FLT = forest light trap, DPTT = deer pen tent trap, GTT = forest grove tent trap, FTT = forest tent trap. The DeFoliart-Morris trap was positioned midway between the GTT and the FLT; the aspiration path was a circuitous route from the FLT to the FTT. The breeding doe/fawn pen area consists of 47 separate pens surrounded by 3.1-m high fencing that minimally restricts mosquito flight. All reference to deer seroconversion at Porter Ranch refers to animals held only within the breeding pen area. The "NOAA STN" is an official weather recording station with daily high/low temperature, rainfall and snowfall records.

sponding 4th instar larval skins to aid in identification of trap-collected specimens. Collections were made over a 6-year period between 1984 and 1989 with the exception of 1986. Collections were initiated yearly in early April, several weeks before the appearance of adult snowmelt *Aedes* mosquitoes, and continued on a weekly or biweekly basis, weather permitting, until mid-September. A single collection was made in 1989 (week 21, May 23). A variety of trapping methods were used in the early phases of the study including: 1) man-bait collections with hand-held aspirators, 2) the DeFoliart-Morris trap (DeFoliart and Morris 1967), 3) a large 12-volt DC battery-powered aspirator (Nasci 1981), 4) standard 6-volt DC CDC miniature light traps (Hausherr's Machine Works, Toms River, NJ), and 5) a large screen-tent trap (Sears nylon polyester screenhouse with a 3 × 3.7 m base). All traps were baited with dry ice. The first 3 methods were abandoned after 1–2 years due in part to the relatively few mosquitoes collected compared with tent and light trap collections. Tent traps were used every year, and light traps were used except for 1989. Tent and light traps were set up in the same locations each year (Fig. 1).

Mosquitoes collected from the traps were immobilized in the field on dry ice, then quickly transferred to labeled 50-cc polypropylene screw-cap tubes and returned to dry ice storage. In the laboratory, frozen specimens were sorted on a chill table according to species, sex and evidence of bloodfeeding or gravid state, then pooled in groups of 25 or less for virus isolation and returned to a −70° C freezer. Only nonblood engorged, nongravid females were assayed for virus.

Virus isolation: Isolation attempts employed 6-well (9.6-cm^2) tissue culture plates with Vero cell (African green monkey kidney) monolayers. A 0.3-ml aliquot of supernatant from homogenized pools (Grimstad and Haramis 1983) was added to each well and an overlay applied. During 1984 a double agar overlay with neutral red dye was used (Hayes et al. 1976). In subsequent years a single overlay was used, consisting of cell culture media supplemented with 0.8% gum

tragacanth (as final concentration) (Sigma Chemicals, St. Louis, MO). Plates were incubated at 37° C in a 5% CO_2 atmosphere for 4–5 days then fixed and stained with a formalin-crystal violet solution to allow visualization of viral plaques. Plaque-positive pools were passaged to either suckling mouse brain (ICR strain, Harlan Sprague Dawley, Indianapolis, IN) or Vero cells to expand the working stock of virus (Sudia and Chamberlain 1967, Boromisa and Grimstad 1986).

Virus identification: Virus isolates were typed using the serum-dilution neutralization test in 96-well microtiter plates in a manner similar to that of Calisher et al. (1981), Lindsey et al. (1976) and Pantuwatana et al. (1972). Samples were run in duplicate. Type-specific mouse hyperimmune ascitic fluids (MHAF) (from the reference bank at the Centers for Disease Control, Ft. Collins, CO) were used as the immune sera for typing. We used MHAF to the prototype strains of JC, trivittatus (TVT), snowshoe hare (SSH), Cache Valley (CV) and western equine encephalitis (WEE) viruses. Control viruses included an Indiana isolate of JC virus (800245, Boromisa and Grimstad 1986) and prototype CV, SSH, TVT and WEE viruses. Median tissue culture infective doses were calculated using the method of Reed and Muench (1938).

RESULTS

Weather records: Precipitation records collected at Porter Ranch showed that the area was dry to very dry most years prior to the annual emergence of adult spring *Aedes* in May and early June. For example, in 1985 and 1988 during that period, precipitation fell to 60% of normal (based on precipitation records for 1951–80; NOAA 1989); 1987 saw a period of 6 months (January–June) when precipitation averaged < 60% of normal.

Table 1. Total number of female mosquitoes of each species pooled for virus isolation that were collected at the Houghton Lake Wildlife Research Area.[1]

Species	Year collected					Total collected
	1984	1985	1987	1988	1989	
Aedes						
abserratus/punctor	228	1,112	21	896	2,613	4,870
aurifer	37	24	0	360	0	421
canadensis	1,060	1,202	16	1,131	1	3,410
cinereus	2,574	960	16	721	3	4,274
decticus	0	18	0	0	0	18
dorsalis	0	0	0	3	0	3
excrucians	8	114	17	1,145	8	1,292
fitchii	33	95	13	387	6	534
leucomelas[2]	2	6	0	4	0	12
intrudens	40	164	1	121	96	422
provocans	58	225	27	959	5,713	6,982
sticticus	0	0	1	0	0	1
triseriatus	1	1	8	11	0	21
trivittatus	1	0	0	0	0	1
vexans	288	491	1,779	405	0	2,963
Anopheles						
punctipennis	21	110	90	171	2	394
quadrimaculatus	8	10	7	5	0	30
walkeri	75	123	33	109	0	340
Coquillettidia						
perturbans	1,803	14,466	11,877	1,428	0	29,574
Culex						
pipiens/restuans	51	98	30	191	0	370
salinarius	0	0	2	1	0	3
tarsalis	0	0	0	2	0	2
territans	8	3	1	1	0	13
Culiseta						
impatiens	1	1	0	0	0	2
inornata	0	0	0	20	9	29
minnesotae	6	18	255	19	0	298
morsitans	67	126	4	4	0	201
Psorophora						
ferox	0	0	1	0	0	1
Totals	6,370	19,367	14,199	8,094	8,451	56,481

[1] Specimens that could not be reliably identified to species were not pooled.
[2] This species was formerly known as *Aedes implicatus*.

Mosquito collections: A total of 56,481 adult female mosquitoes were collected, representing 30 species in 6 genera (Table 1). *Coquillettidia perturbans* (Walker) was the most common species, followed by certain snowmelt *Aedes* species. *Aedes abserratus* and *Ae. punctor* (Kirby), and *Culex pipiens* Linn. and *Cx. restuans* Theobald could not be reliably separated and were grouped under the dual names as indicated in Table 1. Very few males entered any of the CO_2-baited traps and none were pooled.

Figure 2 illustrates the yearly seasonal succession of those mosquito species whose total collections for all years exceeded 350. Considerable variation was seen in the date of first collection and duration of some species in the field. The year-to-year differences were greatest for *Anopheles punctipennis* (Say) and *Cx. pipiens/restuans*; overwintering adults of these species were the earliest collected any year. *Aedes vexans* (Meigen), *An. punctipennis* and *Cx. pipens/restuans* usually remained longest into the late summer and early fall.

The univoltine snowmelt *Aedes* adults emerged between weeks 18 and 24 (approximately late April through mid-June) and were present for 6–11 weeks. *Aedes intrudens* Dyar, *Ae. provocans* and *Ae. abserratus/punctor* had the earliest emergences among the aedine species. First collection dates for any one species varied from year to year by 4 weeks; one exception was *Aedes excrucians* (Walker) whose first collection dates spanned 9 weeks (Fig. 2).

Fifteen species were collected by tent trap, 14 by light trap, 13 by aspiration, 7 in man-bait collections and 6 by the DeFoliart-Morris trap. Carbon dioxide-baited tent traps were very useful for collecting large numbers of most *Aedes* species, although aspiration collections were quite productive for *Aedes cinereus* Meigen, *Ae. canadensis* (Theobald), and *Ae. abserratus/punctor*. All *Aedes* species listed in Table 1 were collected by tent trap, by aspiration and, with the exception of *Ae. fitchii* (Felt and Young), by light trap. The tent traps collected 99% of the *Ae. excrucians*, 95% of the *Ae. fitchii*, 93% of the *Ae. provocans*, 78% of the *Ae. vexans* and 93% of the *An. punctipennis* (Say). However, only 55% of the *Ae. abserratus/punctor* were collected in the tent traps as were 60% of the *Ae. aurifer* (Coquillett), 69% of the *Ae. canadensis*, 42% of the *Ae. cinereus*, 54% of the *Cq. perturbans* and 50% of the *Cx. pipiens/restuans*. Total collections for all species in all years were greater in the grove tent trap (GTT; 12,677 specimens), located at the forest edge, than in the forest tent trap (FTT; 3,053 specimens), located approximately 50 m into the forest (Fig. 1).

More *Ae. cinereus* (49%) were taken by aspiration than by any other method; this was not true for any other species. The light trap collections accounted for 46% of the *Cx. pipiens/restuans* and 45% of the *Cq. perturbans*; all other species were taken in much smaller proportions by this method. *Aedes stimulans* was not detected although tent traps were shown to be effective for collecting that species in Indiana (Boromisa and Grimstad 1986). *Culiseta inornata* was only rarely collected (29 specimens over 5 years), despite the proven value of light traps for collecting that species (Barr 1958).

Virus isolation: Twenty JC virus isolates were made during the study, along with one TVT virus and one CV-like virus isolate, all from nonblood engorged females (Table 2). Table 3 details the cross-reactivity among the control viruses and the MHAFs used for viral typing. Fourteen isolates were obtained from *Ae. provocans*, representing all years except 1984. All isolates of *Ae. provocans* were obtained within the first few days to 2 weeks after the emergence of the spring brood. Eight isolates were taken from this species in the first week of their appearance in 1989; no other species yielded an isolate that year. The JC virus isolates from *Ae. intrudens* and *Ae. abserratus/punctor* were also made within 2 weeks of the emergence of these species, while the isolates from *Aedes aurifer* and *Ae. canadensis* were obtained more than 2 weeks following emergence. The isolation of TVT virus from *Ae. excrucians* was made in mid-July, well after that mosquito's initial emergence, as was the late July isolation of a Cache Valley-like virus from *Ae. fitchii*.

Minimum field infection rates (MFIR) for JC virus ranged from 1:27 for *Ae. provocans* to 1:1,131 for *Ae. canadensis*. The MFIRs for *Ae. provocans* varied widely, with a mean of 1:495 (Table 4).

DISCUSSION

Our study suggests that *Ae. provocans* is a primary enzootic vector of JC virus at the study site. Although they were well within their reported ranges (Darsie and Ward 1981), populations of *Ae. stimulans* and *Cs. inornata* were too low at the site for these species to have a significant role in JC virus transmission.

Previous work with sentinel deer at Porter Ranch (Grimstad et al. 1987) has demonstrated the perennial transmission of JC virus, indicating the existence of a stable enzootic focus. In an average year, virtually 100% of the captive yearling deer seroconvert to JC virus in the late spring-early summer period. This change in antibody status occurs over a 2- to 10-week period (Grimstad et al. 1987; Grimstad, unpublished data). The dates of earliest spring *Aedes* adult emergences shown in this study (weeks 18–21) are routinely 1–2 weeks ahead of the initial seroconversions in yearling deer. *Aedes provo-*

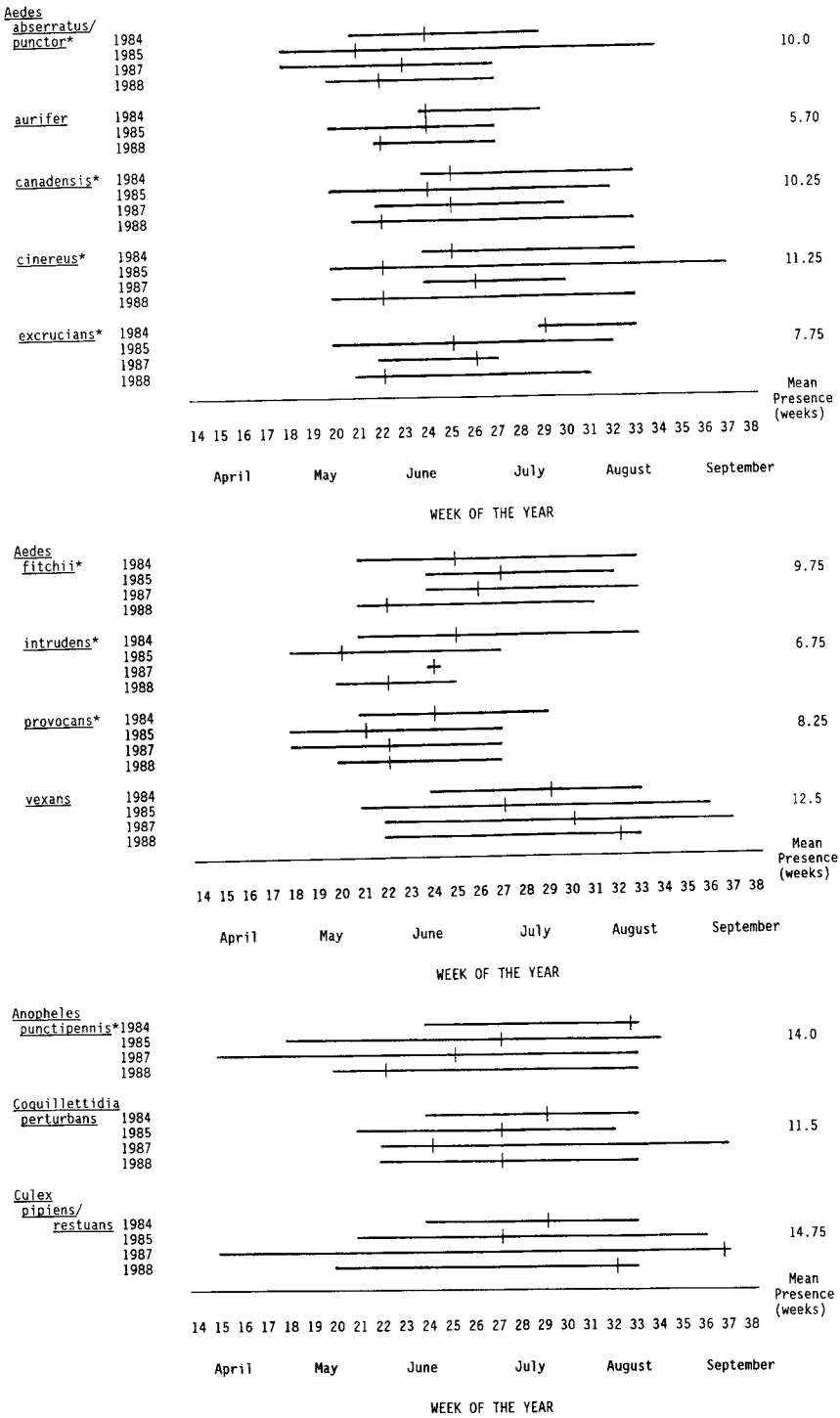

Fig. 2. Seasonal succession of select mosquito species collected at the Houghton Lake Wildlife Research Area in 1984–88. The species listed here are those for which a minimum of 350 were collected in the 4 years of the study. Collections were made only during the 21st week of 1989 and species collected that week are designated with an * following the name. The horizontal line indicates the period of time each year the given species was collected. The vertical bar indicates the week when the median number of individuals of that species was reached (see Table 1 for collection totals). No specimens of *Ae. aurifer* were collected in 1987.

Table 2. Isolations of Jamestown Canyon virus from mosquitoes at the Houghton Lake Wildlife Research Area in 1985–89.

Mosquito species	Isolate no.[1]	Pool size	Reciprocal serum dilution neutralization titer[2]				Week collected[3]
			JCV	SSHV	TVTV	CVV	
Aedes							
abserratus/punctor	88-0201	25	640	40	80	—[4]	22
	88-0203	25	320	20	80	—	22
aurifer	88-0341	1	40	10	—	—	25
canadensis	88-0281	25	320	20	40	—	24
excrucians	88-0440	8	—	—	40	—	29
fitchii	85-0708	19	—	—	—	20	30
intrudens	85-0169	2	160	10	20	—	22
	85-0186	23	80	10	20	—	22
Aedes							
provocans	85-0130	11	160	20	20	—	21
	85-0217	7	80	10	20	—	22
	87-0042	15	40	10	10	—	22
	88-0120	25	160	20	20	—	22
	88-0205	25	160	20	40	—	22
	88-0299	25	320	40	80	—	24
	89-0039	25	160	—	40	—	21
	89-0056	25	640	10	20	—	21
	89-0067	25	640	10	20	—	21
	89-0077	25	640	10	40	—	21
	89-0138	25	320	10	20	—	21
	89-0224	25	320	10	20	—	21
	89-0258	25	640	10	20	—	21
	89-0357	25	640	10	40	—	21

[1] The prefix (e.g., 88-) indicates year of isolation (1988) and the 4 digit suffix indicates the pool number.
[2] Titer obtained using hyperimmune ascitic fluid prepared against the homologous virus (see text). All isolates were tested against WEE virus and all titered <10.
[3] The week indicated is for the year of the collection (see Fig. 2 for the relative time period).
[4] —denotes titer <10.

cans and *Ae. abserratus/punctor* are the only species to be consistently counted among the earliest *Aedes* collections. More importantly, they are the only species whose emergence dates consistently precede the onset of seroconversion in deer. Finally, *Ae. provocans* yielded virus isolates in 4 years of the study, each isolate appearing within 2 weeks of earliest emergence (Table 2, Fig. 2). No other species yielded isolates in more than 1 year. Because of the close association of emergence dates, virus isolations and vertebrate infection, it appears that *Ae. provocans* is an important late spring vector of JC virus at Porter Ranch.

Blood host studies and virus transmission trials will be useful in supporting or rejecting this hypothesis. Blood feeding patterns for *Ae. provocans* are reportedly more a matter of availability than of preference, with the list including deer, domestic ungulates, dogs, pigs, raccoons, rodents, birds and humans (Means 1979). Means' list used *Ae. trichurus* (Dyar); that name is now synonymized with *Ae. provocans* (Wood 1977); however, biological data for *Ae. trichurus* are still relevant. While man-bait collections were very limited at the site due to the risk of infection for workers, 5.8% of all *Ae. provocans* collected in 1985 (Table 1) were taken in just 10 min of man-bait collecting using a single host (PRG).

The temporal variation in emergence dates, collection sizes and adult duration in the field can be partially explained by the precipitation patterns described above. With rainfall < 60% of normal in late April and May some years, numerous breeding sites dried up prior to adult emergence. This was especially true in 1987. This pattern of reduced precipitation and the subsequent loss of immature mosquitoes in breeding sites appeared to be a major factor affecting adult numbers in our study.

The CO_2-baited tent traps facilitated large collections of most *Aedes* species. All isolations of JC virus came from specimens collected in tent traps. This method was formerly used to make large collections of *Ae. stimulans* and other *Aedes* species in northern Indiana; all JC virus isolations there came from tent trap-collected *Ae. stimulans* (Boromisa and Grimstad 1986).

Virus isolates from the site were limited to the

Table 3. Homologous virus-mouse hyperimmune ascitic fluid titers and cross-reactive heterologous titers.

Virus	Test dose[1]	Reciprocal serum dilution neutralization titers				
		JCV[2]	SSHV	TVTV	CVV	WEEV
JCV	2.0	640	10	—[3]	—	—
SSHV	2.0	40	160	—	—	—
TVTV	2.4	20	10	160	—	—
CVV	1.9	—	—	—	160	—
WEEV	2.4	—	—	—	—	320

[1] $Log_{10}TCID_{50}$/0.025 ml in Vero cells.
[2] Abbreviations: JCV = Jamestown Canyon virus; SSHV = snowshoe hare virus; TVTV = trivittatus virus; CVV = Cache Valley virus; WEEV = western equine encephalitis virus. CVV and WEEV were included as controls as representative viruses of the Bunyaviridae: Bunyamwera serogroup and Togaviridae, respectively.
[3] — indicates the titer was <10.

Table 4. Minimum field infection rates (MFIRs)[1] for mosquito species from which isolates of Jamestown Canyon virus were obtained in 1985–89 at the Houghton Lake Wildlife Research Area.

Species	Year	MFIRs for virus isolated		
		Jamestown Canyon	Trivittatus	Cache Valley
Aedes				
abserratus/punctor	1988	1:448		
aurifer	1988	1:360		
canadensis	1988	1:1,131		
excrucians	1988		1:1,145	
fitchii	1985			1:95
intrudens	1985	1:82		
provocans	1985	1:112		
	1987	1:27		
	1988	1:320		
	1989	1:714		

[1] The minimum field infection rate represents the ratio of the total number of mosquitoes pooled for that species divided by the total number of isolates from that species and assumes a minimum of 1 infected female per pool (in our study, a maximum pool size of 25).

univoltine species of *Aedes* which emerged annually between late April and mid-June. *Aedes intrudens* and *Ae. abserratus/punctor* were both early sources of JC virus (1985 and 1988, respectively) in years when they emerged concurrently with *Ae. provocans*. Thus, they may potentially be vectors of JC virus. *Aedes abserratus* has been implicated as a vector of JC virus on the east coast with an average MFIR of 1:505 for that species (Main et al. 1979). That MFIR is quite similar to the average of 1:495 we calculated for *Ae. provocans* in our study (Table 4). *Aedes canadensis* and *Ae. aurifer* may also transmit virus in the late spring, although isolates of these viruses at our site (1988) were made after the first isolates from *Ae. provocans* and after the first seroconversion among the sentinel deer (Grimstad et al. 1987; Grimstad, unpublished data).

In some instances we isolated JC virus from *Ae. provocans* a full week prior to the first seroconversions in deer. Given the fact that deer are the only known vertebrate source of JC virus (Grimstad 1988), these early isolates suggest that JC virus is passed transovarially in *Ae. provocans*. We hypothesize that this species provides the overwintering reservoir for JC virus in this area. Indeed, workers in New York have documented (in this issue) evidence for transovarial transmission of JC virus in *Ae. provocans* by isolating virus from males collected as larvae in the field (Boromisa and Grayson 1990). Transovarial transmission of JC virus by *Ae. stimulans* in Indiana has also been documented (Boromisa and Grimstad 1986).

The combination of our 14 isolates from Michigan *Ae. provocans* and the 17 JC virus isolates made from male and female New York *Ae. provocans* (Boromisa and Grayson 1990) suggest that this species may be a primary regional vector (along with *Ae. stimulans* in some areas), and, potentially, throughout its entire range. However, the relative contributions of *Ae. provocans*, *Ae. stimulans* and other potential vectors within the vast geographic range of JC virus remains a matter of speculation.

ACKNOWLEDGMENTS

We thank the personnel of the Michigan Department of Natural Resources, especially John Nellist, property manager at Porter Ranch, for their assistance every year of the study and making the property always accessible to us. We also thank Robert Boromisa, Robert Copeland, John Kobayashi, Jeff Maggioli, Michael Mandracchia and David Murphy for assisting in field collections and mosquito pooling, Marcia Kern and Marsha Janota for assisting in processing pools, and Jack Cully and William Hawley for helpful comments made during the preparation of this paper.

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