LABORATORY TRANSMISSION OF JAMESTOWN CANYON AND SNOWSHOE HARE VIRUSES (BUNYAVIRIDAE: CALIFORNIA SEROGROUP) BY SEVERAL SPECIES OF MOSQUITOES[1]

PHILIP B. HEARD, MINGBAO ZHANG AND PAUL R. GRIMSTAD

Laboratory for Arbovirus Research and Surveillance, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

ABSTRACT. The ability of 14 species of mosquitoes to biologically transmit Jamestown Canyon virus was tested. Four species not previously described as vectors of that virus transmitted to suckling mice. Among membrane-fed mosquitoes with disseminated infections, field-collected *Aedes canadensis* (1/3), *Anopheles punctipennis* (1/12), *Coquillettidia perturbans* (2/14) and a laboratory strain of *Ae. epactius* (19/67) transmitted virus. Two species were tested for their ability to transmit snowshoe hare virus: field-collected *Ae. provocans* (4/20) and *Ae. abserratus-punctor* (2/20) successfully transmitted to suckling mice. Evidence regarding the role of these species as field vectors is summarized.

INTRODUCTION

Oral transmission of Jamestown Canyon (JC) virus has been described for 3 mosquito species: *Aedes stimulans* (Walker) (Boromisa and Grimstad 1986), *Aedes provocans* (Walker) (Boromisa and Grayson 1991) and *Aedes albopictus* (Skuse) (Grimstad et al. 1989). There is evidence to suggest that *Ae. stimulans* and *Ae. provocans* are natural vectors (Boromisa and Grimstad 1986, Boromisa and Grayson 1990). Jamestown Canyon virus isolations have been made from at least 19 additional species of mosquitoes (Grimstad 1988, Campbell 1990) for which transmission data are lacking. Included among these are *Aedes abserratus* (Felt and Young) and other *Ae. communis* group members that are suspected of transmitting on the basis of high field infection rates (Sudia et al. 1971, Main et al. 1979, Grayson et al. 1983, Campbell 1990). There is also interest in *Anopheles* species, which may be involved in early spring and late fall transmission in the Midwest (DeFoliart et al. 1986).

In an effort to address questions of vector competence for JC virus, we conducted transmission trials with 14 species (18 geographic strains) of mosquitoes. Species were selected on the basis of their apparent potential as vectors and their availability for testing. Nine species were from an enzootic focus of JC virus located in the Lower Peninsula of Michigan (Grimstad et al. 1987). The remaining species/strains came from northern Indiana and other enzootic regions of the United States, with the exception of one laboratory strain that originated from El Salvador.

Large field collections of 2 *communis* group members, *Ae. provocans* and *Ae. abserratus-punctor*, in Michigan in 1989 made it possible to test the vector competence of these species for a second strain of JC virus from Quebec where both mosquito species are found (Darsie and Ward 1981). We also took this opportunity to test for snowshoe hare (SSH) virus (Bunyaviridae: California serogroup) transmission by these species. Snowshoe hare virus has been isolated from numerous mosquito species, including *Ae. punctor* and other members of the *communis* group (Belloncik et al. 1983, Grimstad 1988). Oral transmission of this virus has been demonstrated in *Ae. communis* (De Geer) (McLean 1983). In addition, this virus is relevant to our ongoing studies of multiple California serogroup virus infections of white-tailed deer (*Odocoileus virginianus*), the primary vertebrate host for JC virus (Grimstad 1988), in the upper Midwest.

MATERIALS AND METHODS

Ten species (11 geographic strains) were collected and tested directly from the field and 4 species (7 strains) were colonized. Field-collected mosquitoes were taken as adults from CO_2-baited tent traps at sites in northcentral Michigan and northwestern Indiana (Table 1). Porter Ranch, in the Lower Peninsula of Michigan, lies within an enzootic focus of JC virus (Grimstad et al. 1987, Heard et al. 1990). Snowshoe hare virus is undocumented at the site; however, its range likely includes Michigan's Lower Peninsula (Calisher 1983). All snowmelt *Aedes* species were collected at Porter Ranch in the late spring, within days of their emergence; others species were collected as indicated in Table 1. Potato Creek and Spicer Lake are in northern Indiana in a region where

[1] Supported in part by National Institutes of Health Grants AI-19679 and AI-07030. In conducting the research described 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.

Table 1. Mosquito strains used in transmission trials with Jamestown Canyon and Snowshoe Hare viruses.

Mosquito species	Strain tested	Origin and collection date	Laboratory generation tested
Aedes			
canadensis	PORTER RANCH	Missaukee Co., MI, May 1988	0[1]
cinereus	PORTER RANCH	Missaukee Co., MI, May 1988	0
excrucians	PORTER RANCH	Missaukee Co., MI, May 1988	0
fitchii	PORTER RANCH	Missaukee Co., MI, May 1988	0
provocans	PORTER RANCH	Missaukee Co., MI, May 1988	0
provocans	PORTER RANCH	Missaukee Co., MI, May 1989	0
intrudens	PORTER RANCH	Missaukee Co., MI, May 1989	0
abserratus/punctor	PORTER RANCH	Missaukee Co., MI, May 1989	0
triseriatus	VERO	Indian River Co., FL, 1977	30+
triseriatus	SALADO	Bexar Co., TX, 1972	40+
triseriatus	ALA	Alabama, prior to 1958	75+
stimulans	POTATO CREEK	St. Joseph Co., IN, June 1988	0
hendersoni	U-VILLAGE	St. Joseph Co., IN, 1981	20+
epactius	SANSAL	Matapan, El Salvador, 1968	40+
epactius	GRAND	Grand Co., Utah, 1968	40+
Anopheles			
punctipennis	PORTER RANCH	Missaukee Co., MI, April 1988	0
punctipennis	PORTER RANCH	Missaukee Co., MI, August 1988	0
quadrimaculatus	SAVANNA	Savanna, GA, acquired in 1971[2]	100+
Coquillettidia			
perturbans	PORTER RANCH	Missaukee Co., MI, June 1988	0
perturbans	SPICER LAKE	St. Joseph Co., IN, July 1988	0

[1] These strains were tested as field-collected, newly emerged females.

[2] This strain was acquired as colony stock in 1971. All other strains were originally obtained as field stock and maintained in the Vector Biology Laboratory from the year of collection listed until the time of use in this study.

a high prevalence of antibody to JC virus occurs in all surveyed deer populations (Boromisa and Grimstad 1987). Specimens were carried from the field in ice-cooled boxes and housed at 19°C in 3.8-liter plastic buckets. Additional handling measures were described by Grimstad et al. (1989).

Laboratory-reared mosquitoes were taken from colonies at the University of Notre Dame (Table 1). Larvae were reared according to Grimstad et al. (1989), and adults were maintained in the same manner as wild-caught mosquitoes, with the exception that the *Aedes triseriatus* (Say) VERO strain was kept at 27°C.

Viruses used in the trials were as follows: 1) JC virus strain 800245, passage 3 in suckling mouse brain (SMB), was isolated from a pool of *Ae. stimulans* males in Indiana and is horizontally transmitted by that species (Boromisa and Grimstad 1986); 2) JC virus strain 136A3, passage 3 in Vero cells, passage 1 in SMB, was isolated from mosquitoes in Quebec, Canada (H. Artsob, personal communication); and 3) SSH virus, "original" strain, was supplied in lyophilized form, passage 20 in SMB, from the Centers for Disease Control.

Mosquitoes were orally infected at a membrane feeder and individually tested for transmission to newborn mice as described by Grimstad et al. (1989). Extrinsic incubation was at 19°C. Brain extracts from sick and moribund mice were tested for the presence of virus. Plaque assays for the detection of virus in mosquitoes and mouse brain tissue were modified from Grimstad and Haramis (1983). The Vero cell cultures used for this purpose were maintained in medium 199 with a 10% serum supplement (Seru-Max 2, Sigma Chemical Co., St. Louis, MO), 2.2 g $NaHCO_3$ per liter, 75.0 mg gentamicin per liter, and 125.0 mg amphotericin B per liter. For mosquito assays, the concentrations of antibiotics were increased 2 and 4 times, respectively. Samples were applied to Vero cell monolayers in 6-well plates, and replaced, after a 1-h incubation at 37°C and 5% CO_2, with an overlay consisting of culture medium supplemented with 0.8% gum tragacanth (Sigma Chemical Co.). After 3–4 additional days of incubation, the plates were fixed and stained with a crystal violet-formalin solution and examined for plaques.

Infectious blood meal (IBM) titers were determined from observations of cytopathic effects in Vero cells grown in 96-well plates as described by Grimstad and Haramis (1983). Median tissue culture infectious doses ($TCID_{50}$) were calculated using the method of Reed and Muench (1938). The values in Table 2 represent an average of titers taken at the beginning and end of the feeding period.

Table 2. Summary of *in vivo* transmission trials with Jamestown Canyon (JC) and Snowshoe Hare (SSH) viruses.

Virus strain	Mosquito species	Strain tested	Range of IBM titers[1]	Days of EIP[2]	Percent positive (no. positive/no. tested)			
					Total infected	Disseminated infection	Transmission rates[3] (modified)	Transmission rates[3] (population)
JC (800245)	*Aedes*							
	abserratus-punctor	PORTER RANCH	5.8–7.1	13–14	52 (30/58)	12 (7/58)	0 (0/4)	0 (0/27)
	canadensis	PORTER RANCH	6.0–6.2	21	100 (7/7)	57 (4/7)	33 (1/3)	33 (1/3)
	cinereus	PORTER RANCH	6.2	21	60 (1/5)	40 (2/5)	0 (0/2)	0 (0/4)
	excrucians	PORTER RANCH	6.0–6.2	21	47 (9/19)	26 (5/19)	0 (0/5)	0 (0/19)
	fitchii	PORTER RANCH	6.0–6.1	21	42 (5/12)	17 (2/12)	0 (0/2)	0 (0/12)
	intrudens	PORTER RANCH	5.8–7.1	14	75 (3/4)	0 (0/4)	—[4]	0 (0/2)
	provocans	PORTER RANCH 1988	6.1	14	100 (15/15)	20 (3/15)	—	0 (0/6)
	provocans	PORTER RANCH 1989	5.8–7.1	13–14	97 (198/204)	25 (52/204)	0 (0/29)	0 (0/110)
	triseriatus	VERO[5]	7.2–7.5	20–21	19 (7/37)	0 (0/37)	—	0 (0/37)
	triseriatus	SALADO[5]	7.2–7.5	20–21	4 (2/51)	0 (0/51)	—	0 (0/50)
	triseriatus	ALABAMA[5]	7.5	21	3 (1/34)	0 (0/34)	—	0 (0/34)
	triseriatus	WALTON[6]	6.0	14–21	11 (10/90)	1 (1/90)	0 (0/1)	0 (0/90)
	stimulans	POTATO CREEK	6.9	21	36 (15/42)	24 (10/42)	30 (3/10)	7 (3/42)
	stimulans	KINGSBURY[7]	5.3–6.0	21	44 (19/43)	16 (7/43)	71 (5/7)	12 (5/43)
	hendersoni	U-VILLAGE[5]	6.0–6.3	20–21	19 (8/52)	2 (1/52)	—	0 (0/40)
	epactius	SANSAL[5]	5.2	21	88 (36/41)	83 (34/41)	9 (3/32)	9 (3/32)
	epactius	SANSAL[5]	7.1–7.6	14–21	100 (60/60)	100 (60/60)	46 (16/35)	46 (16/35)
	epactius	GRAND[5]	5.2	21	14 (1/7)	0 (0/7)	—	0 (0/4)
	epactius	GRAND[5]	6.6	15	80 (8/10)	50 (5/10)	0 (0/5)	0 (0/10)
	epactius	GRAND[5]	6.2–6.6	14–21	54 (21/39)	28 (11/39)	0 (0/11)	0 (0/39)
	Anopheles							
	punctipennis	PORTER RANCH (April collection)	6.3	21	94 (37/39)	8 (3/39)	33 (1/3)	3 (1/39)
	punctipennis	PORTER RANCH (August collection)	6.9	21	48 (14/29)	31 (9/29)	0 (0/9)	0 (0/29)
	quadrimaculatus	SAVANNA[5]	6.2	22	0 (0/9)	0 (0/9)	—	0 (0/9)

Coquillettidia							
perturbans	PORTER RANCH	6.0–6.2	21	64 (45/70)	4 (3/70)	33 (1/3)	1 (1/70)
perturbans	SPICER LAKE	6.4	21	73 (29/40)	28 (11/40)	9 (1/11)	3 (1/40)
JC (136A3) *Aedes*							
abserratus-punctor	PORTER RANCH	5.0	14	17 (2/12)	8 (1/12)	0 (0/1)	0 (0/11)
provocans	PORTER RANCH	5.0	14	58 (11/19)	5 (1/19)	0 (0/1)	0 (0/18)
SSH (orig.) *Aedes*							
abserratus-punctor	PORTER RANCH	5.2	14	96 (26/27)	78 (21/27)	10 (2/20)	8 (2/25)
provocans	PORTER RANCH	5.2	14	100 (34/34)	97 (33/34)	20 (4/20)	19 (4/21)

[1] Infectious blood meal titers ($\log_{10}$ $TCID_{50}$/ml). The range reflects the different titers fed to multiple groups of the same strain. Where no significant differences were seen in rates of infection and transmission, the data were pooled.
[2] Extrinsic incubation period(s).
[3] The modified transmission rate is based on the number of mosquitoes with disseminated infections that refed on suckling mice. The population transmission rate is based on the total number of mosquitoes refed, regardless of infection status. In cases where the transmission rate denominator is lower than that of the disseminated infection (DI) or total infection rate few females refed, we included those that would not refeed in tests for DI and midgut infection to increase sample size.
[4] No mosquitoes with disseminated infections refed on suckling mice.
[5] Laboratory strains.
[6] Data from Grimstad et al. (1989), presented for purposes of comparison.
[7] Data from Boromisa and Grimstad (1986), presented for purposes of comparison.

Chi-square (χ^2) tests of homogeneity with 1 d.f. were applied to the results for mosquito strains tested under 2 or more combinations of infectious blood meal titer and extrinsic incubation period (EIP) (Sokal and Rohlf 1981). When the frequency in one or more cells was <5, a Fisher's exact test (FET) was used for the pairwise comparison. Similar analyses were performed between selected strains.

RESULTS

Table 2 summarizes the results of the trials with JC and SSH viruses. Jamestown Canyon virus isolate 800245 caused midgut infections (MI) in 13/14 species (17/18 strains), disseminated infections (DI) in 11/14 species (13/18 strains), and oral transmission in 5/14 species (5/18 strains). The JC virus isolate from Quebec, 136A3, caused DIs in both species tested; however, only one individual from each species acquired a DI and neither transmitted virus.

Within a given strain, changes in IBM titer and EIP usually had no significant effect on the proportion of individuals with MI or DI or on the proportion of individuals transmitting virus. Therefore, the results of most trials were pooled (Table 2). Significantly higher proportions of DI and transmission were noted for *Aedes epactius* Dyar and Knab SANSAL after IBMs of 7.2–7.5 versus 5.2 $\log_{10}$ $TCID_{50}$/ml (FET, $P = 0.002$–0.043). Higher proportions of both MI and DI were noted for *Ae. epactius* GRAND after an IBM of 6.6 $\log_{10}$ $TCID_{50}$/ml and EIP of 15 days compared with an IBM of 5.2 $\log_{10}$ $TCID_{50}$/ml and EIP of 21 days (FET, $P = 0.044$ and 0.015, respectively) (Table 2).

Among field-collected species exposed to JC virus isolate 800245, MI was detected in 36–100% and DI in 0–57%. The highest proportions appeared in *Aedes canadensis* (Theobald), the only springtime *Aedes* from Porter Ranch to transmit virus (1/3 mosquitoes with DIs). Neither JC virus isolate was transmitted by *Ae. provocans* (total number of mosquitoes with DIs tested = 30), despite the well-documented vector potential of this species in New York (Boromisa and Grayson 1990, 1991).

Jamestown Canyon virus was transmitted by 1/12 field-collected *Anopheles punctipennis* (Say) (April and August collections combined) and 2/14 field-collected *Coquillettidia perturbans* (Walker) (PORTER RANCH and SPICER LAKE strains combined). The *An. punctipennis* collected in April were fed an IBM of 6.3 $\log_{10}$ $TCID_{50}$/ml and transmitted virus (1/3) despite evidence of a midgut-escape barrier (MI without DI) in 92% of infected individuals. No transmission was detected in the females tested from the August collection (0/9; IBM of 6.9 $\log_{10}$ $TCID_{50}$/

ml), although midgut-escape barriers occurred in only 36% of the August-collected females. Transmission was demonstrated for *Cq. perturbans* from both Michigan (1/3) and Indiana (1/11); the proportion with DIs was significantly higher in the latter strain (χ^2, $P < 0.001$); IBM titers were comparable for the 2 strains.

Among colonized species tested with JC virus, 46% (16/35) of *Ae. epactius* SANSAL transmitted whereas 100% (60/60) of these females had DIs. No *Ae. epactius* GRAND transmitted, and DIs occurred in only 29% overall, despite IBM titers equal to and sometimes greater than those used to infect the SANSAL strain. The remaining colonized strains, representing *Ae. triseriatus*, *Aedes hendersoni* (Cockerell) and *Anopheles quadrimaculatus* Say, were resistant to infection; only 0–19% became infected at the midgut, whereas only 0–2% developed disseminated infections.

Snowshoe hare virus was readily infectious for *Ae. abserratus-punctor* and *Ae. provocans* and was transmitted by both species: 20% (4/20) of the *Ae. provocans* and 10% (2/20) of the *Ae. abserratus-punctor* transmitted. Significantly higher proportions of females had SSH virus DIs (FET, $P < 0.001$) than JC virus DIs at equal, if not lower, IBM titers (Table 2).

DISCUSSION

This survey provides evidence of laboratory transmission of JC virus by 4 additional species: *Ae. canadensis*, *Ae. epactius*, *An. punctipennis* and *Cq. perturbans*. Evidence is also provided for the laboratory transmission of SSH virus by *Ae. provocans* and *Ae. abserratus-punctor*. Conclusive proof of vector status is difficult to achieve and cannot be claimed for any of these species in light of the limitations of these trials and general lack of field data. However, in paragraphs below we summarize what is known from published field studies regarding JC and SSH viruses as it relates to the results of these trials.

The recorded IBM titers in these trials (5.0–7.6 $\log_{10}$ $TCID_{50}$/ml) were generally higher than the levels of viremia reported for the amplifying hosts of JC and SSH viruses. White-tailed deer develop peak viremias of 3.4–5.0 $\log_{10}$ suckling mouse intracerebral median lethal dose ($SMICLD_{50}$) per ml after experimental inoculation (Issel et al. 1972, Watts et al. 1979). No other vertebrate species has yet been shown to have demonstrable viremia in the field (Grimstad 1988). Snowshoe hares (*Lepus americanus*), the natural vertebrate host of SSH virus (Grimstad 1988), develop peak viremias of 2.3–3.9 $\log_{10}$ suckling mouse intraperitoneal median lethal dose per ml after *experimental inoculation* (Newhouse et al. 1971). Unpublished studies (M. Zhang) indicate that $SMICLD_{50}$ titers of JC virus are within 0.2–0.7 $\log_{10}$ in either direction of the corresponding $TCID_{50}$ titers. It is recognized that differences in mosquito infection rates can occur between vertebrate blood meals and artificial blood meals of equal titer. Thus, it is unclear whether the effective IBM titers employed in these trials overlapped the range of titers found in *naturally infected* wild vertebrates.

Aedes canadensis has been a source of JC virus isolates in Maryland (LeDuc et al. 1975), New York (Grayson et al. 1983) and Michigan (Heard et al. 1990). In those studies, minimum field infection rates (MFIR) were 1:1,131 at the Porter Ranch site in Michigan, 1:27,403 in New York and 1:35,217 in Maryland. Blood hosts of this species include deer (Wright and DeFoliart 1970, Boromisa and Grimstad 1986), as well as bovines, horses and humans, among others (Nasci and Edman 1981). On the basis of limited field isolations and low field infection rates, Eldridge (1990) excluded *Ae. canadensis* and all nonaedine species from the list of mosquito species possessing a significant biological association with JC virus. *Aedes canadensis*, indeed, does not appear to play a significant role in JC virus maintenance at the Porter Ranch site in Michigan (Heard et al. 1990). This appears to be due in part to the fact that it emerges at a time when seasonal JC virus transmission to deer is usually well underway. The role of ecological/epidemiological factors as predominant determinants of vectorial capacity is an argument against the coevolution of virus and vector and, in turn, against the likelihood of a significant role for this species in the maintenance cycle (Eldridge 1990).

Anopheles punctipennis has yielded JC virus isolates in New York (MFIR 1:16,975, Grayson et al. 1983) and Ohio (MFIRs of 1:36,549 in statewide collections, Berry et al. 1983; 1:128 and 1:733 in localized foci, R. L. Berry in Grimstad 1988). Blood host and other incidental studies in North America indicate that *An. punctipennis* will feed on cattle, horses, humans and numerous other vertebrates (Edman and Downe 1964, Suyemoto et al. 1973, Nasci and Edman 1981), although deer have not been specifically recorded. Although early spring seroconversions and anamnestic responses in penned deer at the Porter Ranch site in Michigan (Grimstad et al. 1987) suggest a vector role for a nonaedine species such as *An. punctipennis* (DeFoliart et al. 1986), no JC virus isolations have been made from the relatively few *Anopheles* collected at this site to date (Heard et al. 1990).

Coquillettidia perturbans has been a source of

JC virus in Connecticut (MFIR 1:26,666, Main et al. 1979) and New York (MFIR 1:117,677, Grayson et al. 1983). No isolates have come from this species in either Michigan or Indiana. Its blood hosts are numerous and include deer, large domestic animals and man (Wright and DeFoliart 1970, Suyemoto et al. 1973, Nasci and Edman 1981, Boromisa and Grimstad 1986). Like *An. punctipennis* and *Culiseta inornata* (Williston), transmission by *Cq. perturbans* might explain JC virus transmission in the absence of *Aedes* species.

The failure of *Ae. provocans* PORTER RANCH to transmit either strain of JC virus was unexpected. Over a 4-year period, *Ae. provocans* yielded 14/20 JC virus isolates from the study site in Michigan (Heard et al. 1990). These isolates were obtained there each year from adult *Ae. provocans* females collected before or soon after the first seroconversions in sentinel deer. *Aedes provocans* is typically a large-mammal feeder and is known to feed on deer (Means 1979, in reference to the synonymized species, *Ae. trichuris*). Furthermore, Boromisa and Grayson (1990, 1991) demonstrated the vectorial capacity of *Ae. provocans* in New York and reported a transmission rate of 50% (9/18) among field-collected specimens. In comparing the results from our trials and those of Boromisa and Grayson with similar EIPs (14 days) and IBM titers (5.8 versus 5.6 $\log_{10}$ $TCID_{50}$/ml), the population of *Ae. provocans* from New York (Boromisa and Grayson 1991) had an identical proportion of individuals with MI (FET, $P = 1.000$) but a significantly higher proportion with DI (χ^2, $P = 0.010$) relative to the Michigan population. The lower extrinsic incubation temperature in our study (19 versus 21°C) may account for differences in DI and/or rates of transmission as may the choice of virus strain, and other intrinsic factors. Mortality of the specimens prevented us from testing beyond a 14-day EIP, a problem also encountered by Boromisa and Grayson (1991).

Eldridge (1990) concluded that *Ae. provocans* (and 4 other species) has a significant biological association with JC virus, achieved in part through coevolution of their respective ancestors. If the inability of the PORTER RANCH population of *Ae. provocans* to transmit JC virus is borne-out in future trials, it would suggest that this vector-virus association is an unpatterned one and must be analyzed on a regional, if not smaller, geographic scale. Variability in vector competence among geographic strains is well-known (Gubler and Rosen 1976, Tesh et al. 1976, Grimstad et al. 1977, Boromisa et al. 1987). Vertical transmission is also evidence of a significant biological association (Eldridge 1990). Indeed, vertical transmission of JC virus has been demonstrated in *Ae. provocans* collected in New York (Boromisa and Grayson 1990), and is believed to occur at Porter Ranch based on the fact that many of the 14 JC virus isolates obtained from *Ae. provocans* at that site were from newly emerged, nonbloodfed females collected before the first seroconversions in sentinel deer (Heard et al. 1990). It is conceivable that orally infected *Ae. provocans* at Porter Ranch contribute to the pool of transovarially infected individuals and play little or no role in horizontal amplification, a role left to transovarially infected progeny. A phenomenon of this sort would explain the discrepancies between our field and laboratory studies. It may be relevant to the *Ae. provocans* results that transovarial transmission in the absence of oral transmission has been demonstrated in over 30% of individually colonized *Ae. albopictus* females orally infected with La Crosse (LAC) virus (Bunyaviridae: California serogroup) (T. Streit, personal communication).

Aedes abserratus-punctor and *Ae. intrudens* Dyar represent other examples of aedine species that appear to be potential vectors of JC virus, but for which laboratory evidence remains inconclusive. Jamestown Canyon virus has been isolated from either *Ae. abserratus* or *Ae. punctor* in Newfoundland (Mokry et al. 1984), Connecticut (Sprance et al. 1978, Main et al. 1979), New York (Boromisa and Grayson 1991) and Michigan (Heard et al. 1990). Deer may be used as blood hosts (Wright and DeFoliart 1970). *Aedes intrudens* has yielded isolates in New York (Boromisa and Grayson 1990) and in northcentral Michigan (Heard et al. 1990).

Earlier reports implicated *Ae. stimulans* as an enzootic vector in northern Indiana (see Table 2, KINGSBURY) (Boromisa and Grimstad 1986). This was corroborated with the POTATO CREEK population of *Ae. stimulans*, collected from an adjoining county in Indiana. In comparing the 2 populations, which were tested by different workers using different IBM titers, the proportions of infected and transmitting females were not significantly different (FET, $P > 0.15$). These populations of *Ae. stimulans* appear to have similar capabilities as laboratory vectors of JC virus.

The high proportions of *Ae. epactius* infected and transmitting in this study are unsupported in the literature. No JC virus isolates have been reported from *Ae. epactius*, nor has the existence of JC virus in El Salvador and Central America been reported. However, JC virus does exist within the range of North American populations of *Ae. epactius* (Calisher 1983, Darsie and Ward 1981). Based on limited sample sizes, there appear to be dose-related midgut escape barriers in *Ae. epactius* SANSAL and GRAND.

The susceptibility of *Ae. triseriatus* in these trials was consistently low; however, this species has been a source of isolates in New York (Grayson et al. 1983) and Ohio (Berry et al. 1977, 1983) and is known to feed readily on deer (Nasci 1982). Infection rates were also low for the sibling species, *Ae. hendersoni*, suggesting that neither species is likely to be a significant vector. The potential effect of long-term colonization on the biological transmission of viruses by these and other mosquito species must be kept in mind. Grimstad et al. (1977) noted that laboratory colonization had diverse and unpredictable effects in trials with LAC virus and *Ae. triseriatus*. The same may be true for JC virus and the colonized species in this study.

Sympatry for JC and LAC viruses (Calisher 1983, Grimstad 1988) is probably achieved in part by the absence or near absence of shared vector species. Based on the analysis by Eldridge (1990), there are no species that have a reasonable chance of contributing to the maintenance of both viruses. Our data suggest that *Ae. triseriatus* does not contribute to the JC virus cycle; this species appears to be strictly a natural host of LAC virus. *Aedes canadensis* has been a source of field isolates of both JC and LAC viruses and will transmit them in the laboratory (Berry et al. 1983, Boromisa and Grayson 1991, Watts et al. 1973). Although *Ae. canadensis* appears to be a secondary vector of LAC virus in Ohio (Berry et al. 1983), there is no conclusive evidence that it is important in transmission of JC virus.

The trials with SSH virus underscore the importance of the *communis* group in the epidemiology of this virus. Both *Ae. provocans* and *Ae. abserratus-punctor* were readily infected and capable of transmission to suckling mice. Snowshoe hare virus has been isolated from *Aedes punctor* (Kirby) in Quebec (Belloncik et al. 1983), Saskatchewan (Iverson et al. 1973) and Alaska (Sudia et al. 1971, Ritter and Feltz 1974), and from *communis* group mosquitoes in Alberta, Wisconsin, Alaska (Sudia et al. 1971), Montana (Hoff et al. 1971), the Yukon Territories (McLean 1983) and the Northwest Territories (Wagner et al. 1975). Vertebrate isolates have come from snowshoe hares and other small mammals (Grimstad 1988). McLean (1983) reported that blood meal titers of 0.1 mouse LD_{50} were sufficient to infect *Ae. communis* and that comparably low titers enabled transmission to occur. Whether *Ae. provocans* and *Ae. abserratus-punctor* are susceptible at such low titers is unknown. Definitive evidence for the use of snowshoe hares as blood hosts by these species is not available, although the isolation of SSH virus from *Ae. punctor* would suggest as much for this species. *Aedes punctor, Ae. abserratus* and *Ae. provocans* reportedly feed on small mammals, the former 2 on rabbits (Downe 1960, Means 1968, Wright and DeFoliart 1970). These results underscore the parallels between SSH and JC viruses drawn by Eldridge (1990). Further studies are needed to confirm these findings with JC and SSH viruses and define their relevance in the natural virus cycles.

ACKNOWLEDGMENTS

We thank H. Artsob and N. Karabatsos for providing stocks of JC virus (Quebec strain) and SSH virus, respectively, T. Streit for unpublished data, R. Copeland, S. Hanson and M. Niebylski for their participation in some of the transmission trials and M. Kern for excellent technical assistance.

REFERENCES CITED

Belloncik, S., A. Aubin, A. Marie, J. Boisvert, R. Gagnon, C. Th'ng, C. Trudel and H. Artsob. 1983. Arbovirus studies in the Trois-Rivières area, Province of Quebec, Canada. Mosq. News 43:426–431.

Berry, R. L., B. J. Lalonde Weigert, C. H. Calisher, M. A. Parsons and G. T. Bear. 1977. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. Mosq. News 37:494–496.

Berry, R. L., M. A. Parsons, R. A. Restifo, E. D. Peterson, S. W. Gordon, M. R. Reed, C. H. Calisher, G. T. Bear and T. J. Halpin. 1983. California serogroup virus infections in Ohio: an 18-year retrospective summary. pp. 215–223. *In:* C. H. Calisher and W. H. Thompson (eds.), California serogroup viruses. Alan R. Liss, Inc., New York, NY.

Boromisa, R. D. and M. A. Grayson. 1990. Incrimination of *Aedes provocans* as a vector of Jamestown Canyon virus in an enzootic focus of northeastern New York. J. Am. Mosq. Control Assoc. 6:504–509.

Boromisa, R. D. and M. A. Grayson. 1991. Oral transmission of Jamestown Canyon virus by *Aedes provocans* mosquitoes from northeastern New York. J. Am. Mosq. Control Assoc. 7:42–47.

Boromisa, R. D. and P. R. Grimstad. 1986. Virus-vector-host relationships of *Aedes stimulans* and Jamestown Canyon virus in a northern Indiana enzootic focus. Am. J. Trop. Med. Hyg. 35:1285–1295.

Boromisa, R. D. and P. R. Grimstad. 1987. Seroconversion rates to Jamestown Canyon virus among six populations of white-tailed deer (*Odocoileus virginianus*) in Indiana. J. Wildl. Dis. 23:23–33.

Boromisa, R. D., K. S. Rai and P. R. Grimstad. 1987. Variation in the vector competence of geographic strains of *Aedes albopictus* for Dengue 1 virus. J. Am. Mosq. Control Assoc. 3:378–386.

Calisher, C. H. 1983. Taxonomy, classification, and geographic distribution of California serogroup bunyaviruses. pp. 1–16. *In:* C. H. Calisher and W. H. Thompson (eds.), California serogroup viruses. Alan R. Liss, Inc., New York, NY.

Campbell, G. L. 1990. Epidemiology of California and

Bunyamwera serogroup viruses in mountainous and other areas of California. Doctoral dissertation, University of California, Berkeley.

Darsie, R. F., Jr. and R. A. Ward. 1981. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Mosq. Syst. Suppl. 1.

DeFoliart, G. R., D. M. Watts and P. R. Grimstad. 1986. Changing patterns in mosquito-borne arboviruses. J. Am. Mosq. Control Assoc. 2:437–455.

Downe, A. E. R. 1960. Blood-meal sources and notes on host preferences of some *Aedes* mosquitoes (Diptera: Culicidae). Can. J. Zool. 38:689–699.

Edman, J. D. and A. E. R. Downe. 1964. Host-blood sources and multiple-feeding habits of mosquitoes in Kansas. Mosq. News 24:154–160.

Eldridge, B. F. 1990. Evolutionary relationships among California serogroup viruses (Bunyaviridae) and *Aedes* mosquitoes (Diptera: Culicidae). J. Med. Entomol. 27:738–749.

Grayson, M. A., S. Srihongse and C. H. Calisher. 1983. California serogroup viruses in New York State: a retrospective analysis of subtype distribution patterns and their epidemiologic significance, 1965–1981. pp. 257–267. *In:* C. H. Calisher and W. H. Thompson (eds.), California serogroup viruses. Alan R. Liss, Inc., New York, NY.

Grimstad, P. R. 1988. California group virus disease. pp. 99–136. *In:* T. P. Monath (ed.), The arboviruses: epidemiology and ecology, vol. 2. CRC Press, Inc., Boca Raton, FL.

Grimstad, P. R. and L. D. Haramis. 1983. Evaluation of immunofluorescence and cell culture techniques for detecting La Crosse virus infection in individual *Aedes triseriatus* (Diptera: Culicidae). J. Med. Entomol. 20:458–460.

Grimstad, P. R., G. B. Craig, Jr., Q. E. Ross and T. M. Yuill. 1977. *Aedes triseriatus* and La Crosse virus: geographic variation in vector susceptibility and ability to transmit. Am. J. Trop. Med. Hyg. 26:990–996.

Grimstad, P. R., J. F. Kobayashi, M. Zhang and G. B. Craig, Jr. 1989. Recently introduced *Aedes albopictus* in the United States: potential vector of La Crosse virus (Bunyaviridae: California serogroup). J. Am. Mosq. Control Assoc. 5:422–427.

Grimstad, P. R., D. G. Williams and S. M. Schmitt. 1987. Infection of white-tailed deer (*Odocoileus virginianus*) in Michigan with Jamestown Canyon virus (California serogroup) and the importance of maternal antibody in viral maintenance. J. Wildl. Dis. 23:12–22.

Gubler, D. J. and L. Rosen. 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses. Am. J. Trop. Med. Hyg. 25:318–325.

Heard, P. B., M. Zhang and P. R. Grimstad. 1990. Isolation of Jamestown Canyon virus (California serogroup) from *Aedes* mosquitoes in an enzootic focus in Michigan. J. Am. Mosq. Control Assoc. 6:461–468.

Hoff, G. L., R. O. Anslow, J. Spalatin and R. P. Hanson. 1971. Isolation of Montana showshoe hare serotype of California encephalitis virus group from a snowshoe hare and *Aedes* mosquitoes. J. Wildl. Dis. 7:28–34.

Issel, C. J., D. O. Trainer and W. H. Thompson. 1972. Experimental studies with white-tailed deer and four California group arboviruses (La Crosse, trivittatus, Snowshoe Hare, and Jamestown Canyon). Am. J. Trop. Med. Hyg. 21:979–984.

Iverson, J. O., R. J. Wagner, C. DeJong and J. McLintock. 1973. California encephalitis virus in Saskatchewan: isolation from boreal *Aedes* mosquitoes. Can. J. Public Health 64:590–594.

LeDuc, J. W., W. Suyemoto, T. J. Keefe, J. F. Burger, B. F. Eldridge and P. K. Russell. 1975. Ecology of California encephalitis viruses on the Del Mar Va Peninsula. I. Virus isolations from Mosquitoes. Am. J. Trop. Med. Hyg. 24:118–123.

Main, A. J., S. E. Brown and R. C. Wallis. 1979. Arbovirus surveillance in Connecticut. II. California serogroup. Mosq. News 39:552–559.

McLean, D. M. 1983. Yukon isolates of snowshoe hare virus, 1972–1982. pp. 247–256. *In:* C. H. Calisher and W. H. Thompson (eds.), California serogroup viruses. Alan R. Liss, Inc., New York, NY.

Means, R. G. 1968. Host preferences of mosquitoes (Diptera: Culicidae) in Suffolk County, New York. Ann. Entomol. Soc. Am. 61:116–120.

Means, R. G. 1979. Mosquitoes of New York. Part I. The genus *Aedes* Meigen. N. Y. State Mus. Bull. 430a.

Mokry, J., H. Artsob and R. Butler. 1984. Studies on California serogroup virus activity in Newfoundland, Canada, 1980–83. Mosq. News 44:310–314.

Nasci, R. S. 1982. Differences in host choice between the sibling species of tree hole mosquitoes *Aedes triseriatus* and *Aedes hendersoni*. Am. J. Trop. Med. Hyg. 31:411–415.

Nasci, R. S. and J. D. Edman. 1981. Blood-feeding patterns of *Culiseta melanura* (Diptera: Culicidae) and associated sylvan mosquitoes in southeastern Massachusetts eastern equine encephalitis enzootic foci. J. Med. Entomol. 18:493–500.

Newhouse, V. F., W. Burgdorfer and D. Corwin. 1971. Field and laboratory studies on the hosts and vectors of the snowshoe hare strain of California virus. Mosq. News 31:401–408.

Reed, L. J. and H. A. Muench. 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27:493–497.

Ritter, D. G. and E. T. Feltz. 1974. On the natural occurrence of California encephalitis virus and other arboviruses in Alaska. Can. J. Microbiol. 20:1359–1366.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry, 2nd edition. W. H. Freeman and Co., San Francisco, CA.

Sprance, H. E., A. J. Main, R. C. Wallis and J. Elston. 1978. Jamestown Canyon virus in Connecticut. Mosq. News 38:392–395.

Sudia, W. D., V. F. Newhouse, C. H. Calisher and R. W. Chamberlain. 1971. California group arboviruses: isolations from mosquitoes in North America. Mosq. News 31:576–600.

Suyemoto, W., B. A. Schiefer and B. F. Eldridge. 1973. Precipitin tests of blood-fed mosquitoes collected during the VEE surveillance survey in the southern United States in 1971. Mosq. News 33:392–395.

Tesh, R. B., D. J. Gubler and L. Rosen. 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with chikungunya virus.

Am. J. Trop. Med. Hyg. 25:326–335.
Wagner, R. J., C. DeJong, M. K. Leung, J. McLintock and J. C. Iverson. 1975. Isolation of California encephalitis virus from tundra mosquitoes. Can. J. Microbiol. 21:574–576.
Watts, D. M., P. R. Grimstad, G. R. DeFoliart, T. M. Yuill and R. P. Hanson. 1973. Laboratory transmission of La Crosse encephalitis virus by several species of mosquitoes. J. Med. Entomol. 10:583–586.
Watts, D. M., R. F. Tammariello, J. M. Dalrymple, B. F. Eldridge, P. K. Russell and F. H. Top, Jr. 1979. Experimental infection of vertebrates of the Pocomoke Cypress Swamp, Maryland, with Keystone and Jamestown Canyon viruses. Am. J. Trop. Med. Hyg. 28:344–350.
Wright, R. E. and G. R. DeFoliart. 1970. Associations of Wisconsin mosquitoes and woodland vertebrate hosts. Ann. Entomol. Soc. Am. 63:777–786.