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Isolations of Jamestown Canyon Virus (Bunyaviridae: *Orthobunyavirus*) from Field-Collected Mosquitoes (Diptera: Culicidae) in Connecticut, USA: A Ten-Year Analysis, 1997–2006

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ABSTRACT

Jamestown Canyon virus (JCV) (Bunyaviridae: *Orthobunyavirus*) is a mosquito-borne zoonosis belonging to the California serogroup. It has a wide geographic distribution, occurring throughout much of temperate North America. White-tailed deer, *Odocoileus virginianus* are the principal amplification hosts, and boreal *Aedes* and *Ochlerotatus* mosquitoes are the primary vectors. A 10-year study was undertaken to identify potential mosquito vectors in Connecticut, quantify seasonal prevalence rates of infection, and define the geographic distribution of JCV in the state as a function of land use and white-tailed deer populations, which have increased substantially over this period. Jamestown Canyon virus was isolated from 22 mosquito species. Five of them, *Ochlerotatus canadensis, Oc. cantator, Anopheles punctipennis, Coquillettidia perturbans,* and *Oc. abserratus* were incriminated as the most likely vectors, based on yearly isolation frequencies and the spatial geographic distribution of infected mosquitoes. Jamestown Canyon virus was isolated from *Oc. canadensis* more consistently and from a greater range of collection sites than any other species. Frequent virus isolations were also made from *Aedes cinereus, Aedes vexans,* and *Oc. sticticus,* and new North American isolation records were established for *Anopheles walkeri, Culex restuans, Culiseta morsitans, Oc. sticticus, Oc. taeniorhynchus,* and *Psorophora ferox*. Other species from which JCV was isolated included *C. melanura, Oc. aurifer, Oc. communis, Oc. excrucians, Oc. provocans, Oc. sollicitans, Oc. stimulans, Oc. triseriatus,* and *Oc. trivittatus.* Jamestown Canyon virus was widely distributed throughout Connecticut and found to consistently circulate in a diverse array of mosquito vectors. Infected mosquitoes were collected from June through September, and peak infection rates paralleled mosquito abundance from mid-June through mid-July. Infection rates in mosquitoes were consistent from year to year, and overall virus activity was directly related to local mosquito abundance. Infected mosquitoes were equally distributed throughout the state, irrespective of land use, and infection rates were not directly associated with the abundance of white-tailed deer, possibly because of their saturation throughout the region. Key words: Jamestown Canyon virus—*Orthobunyavirus*—Mosquitoes—Vectors—*Ochlerotatus canadensis*—Spatial geographic distribution.

INTRODUCTION

Jamestown Canyon virus (JCV) (Bunyaviridae: *Orthobunyavirus*) is a mosquito-borne zoonosis belonging to the California serogroup. It has a wide geographic distribution, occurring throughout much of temperate North America, and in humans it causes mild febrile illness with acute central nervous system infection including meningitis and encephalitis and fre-

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quent respiratory system involvement (Grimstad 2001). White-tailed deer, *Odocoileus virginianus* are generally recognized as the principal amplification hosts, but other free-ranging ungulates including moose (*Alces alces*), elk (*Cervus elaphus*), bison (*Bison bison*), and mule deer (*Od. hemionus*) may participate in the maintenance cycle (Grimstad 1988). The virus has been isolated from at least 26 species of mosquitoes and 3 species of tabanids (Table 1), but species of boreal *Aedes* and *Ochlerotatus* appear to be the primary vectors, depending on geography. The virus overwinters in mosquito eggs as a result of transovarial transmission (Boromisa and Grimstad 1986, Grimstad 2001).

Jamestown Canyon virus was first detected in Connecticut in 1966 when it was isolated from a pool of *Ochlerotatus abserratus* collected from a suburban landscape site in central Connecticut during June (Whitman et al. 1968). Subsequent surveillance activities resulted in the isolation of JCV from five additional mosquito species, *Aedes cinereus, Ae. vexans, Coquillettidia perturbans, Oc. canadensis*, and *Oc. cantator* collected from a limited number of sites in the south central, coastal region of the state (Sprance et al. 1978, Main et al. 1979, Andreadis et al. 1994). A seroprevalence rate of 21% was found in an analysis of sera from hunter-killed white-tailed deer collected in the state in 1993 (Zamparo et al. 1997), and a corresponding survey of human sera from blood donations made in 1990 and 1995 (Mayo et al. 2001) revealed seroprevalence rates ranging from 3.9% to 10.1%. In 2001, the first confirmed human case of JCV infection was diagnosed from a hospitalized teenage patient with illness onset in late August (Nelson et al. 2002).

Connecticut, like other states in the northeastern United States, has experienced a substantial rise in white-tailed deer populations in recent years, with an estimated increase of nearly 50% over the 10-year period 1993–2003 (Michael Gregonis, Connecticut Department of Environmental Protection, personal communication). Coincident with this has been the documentation of at least one confirmed human case of JCV, as noted above. Knowledge of the mosquito vectors and their respective roles in enzootic transmission of JCV to deer and epidemic transmission to humans is largely unknown. The present study was undertaken to (1) identify potential mosquito vectors of JCV in Connecticut, (2) quantify and temporally evaluate natural mosquito infection rates throughout the season, and (3) define the geographic distribution of JCV in the state as a function of land use and white-tailed deer populations.

MATERIALS AND METHODS

Collection site descriptions

Mosquito trapping was conducted from June through October at 36 fixed collection sites in 1997 and 1998, and at 91 fixed collection sites (including the original 36) from 1999 through 2006 (Andreadis et al. 2004). Approximately one-third of the sites were located in densely populated residential locales along an urban/suburban corridor in the coastal southeastern corner of the state that also extended up through the Connecticut River Valley (Fig. 1). Trap sites typically included parks, greenways, golf courses, undeveloped wood lots, sewage treatment plants, dumping stations, and temporary wetlands associated with waterways. Trapping locations in the other regions of the state were established in more sparsely populated rural settings that included permanent fresh-water swamps (red maple/white cedar) and bogs, coastal salt marshes, horse stables, and swamp-forest border environs.

Land cover characterization was obtained for each collection site from digital Landstat satellite imagery data published for each municipality by the University of Connecticut's Center for Land Use Education and Research (CLEAR) (http://clear.uconn.edu/projects/landscape/local/town.asp). The 11 initial categories were reduced into five major classes for analysis following the Anderson Level I classification system (Anderson 1976). These included: agriculture/soil/grass, developed, forest (deciduous and coniferous), wetland (forested, non-forested, and tidal), and deep water (Fig. 1). Detailed descriptions of each category can be found online at: http://clear.uconn.edu/projects/landscape/category_description.htm.

Potential associations between JCV activity and specific land use classes were assessed us-

Table 1. Isolations of Jamestown Canyon Virus from Field-Collected North American Mosquitoes and Tabanids[a]

Species	*Locale*	*Reference(s)*
Culicidae		
Aedes		
Ae. albopictus (Skuse)	TN	Gottfried et al. (2002)
Ae. cinereus Meigen	CT, NY	Grayson et al. (1983), Andreadis et al. (1994), this study
Ae. vexans (Meigen)	CT, NY, WI	Sudia et al. (1971b), Sprance et al. (1978), Main et al. (1979), Grayson et al. (1983), this study
Anopheles		
An. crucians Weidemann	NJ, SC	Sudia et al. (1971b), Wozniak et al. (2001)
An. punctipennis (Say)	CT, NY, OH	Berry et al. (1983), Grayson et al. (1983), this study
An. quadrimaculatus Say	OH	Berry et al. (1983)
An. walkeri Theobald	CT	This study
Coquillettidia perturbans (Walker)	CT, NY	Main et al. (1979), Grayson et al. (1983), this study
Culex restuans Theobald	CT	This study
Culiseta		
Cs. inornata (Williston)	AZ, CA, CO, UT	Sudia et al. (1971b), Elbel and Crane (1977), Jozan and Work (1983), Reeves et al. (1983), Reeves (1990)
Cs. melanura (Coquillett)	CT, NJ	Sudia et al. (1971b), this study
Cs. morsitans (Theobald)	CT	This study
Ochlerotatus		
Oc. abserratus (Felt & Young)	CT, MA, MI, NY, Newfoundland	Whitman et al. (1968), Sprance et al. (1978), Main et al. (1979), Grayson et al. (1983), Mokry et al. (1984), Heard et al. (1990), Walker et al. (1993), Andreadis et al. (1994), this study
Oc. aurifer (Coquillett)	CT, MI, NY	Grayson et al. (1983), Heard et al. (1990), this study
Oc. canadensis (Theobald)	CT, MD, MI, NY	LeDuc et al. (1975), Grayson et al. (1983), Howard et al. (1988), Heard et al. (1990), Andreadis et al. (1994), this study
Oc. cantator (Coquillett)	CT, NY, RI	Sprance et al. (1978), Main et al. (1979), Grayson et al. (1983), Takeda et al. (2003), this study
Oc. cataphylla Dyar	CA	Campbell et al. (1991), Hardy et al. (1993)
Oc. communis (De Geer)	CA, CT, NY, WI, Alberta	DeFoliart et al. (1969), Iverson et al. (1969), Sudia et al. (1971b), Grayson et al. (1983), Boromisa and Grayson (1990), Campbell et al. (1991), this study
Oc. dorsalis (Meigen)	CA, UT	Fulhorst et al. (1966), Crane and Elbel (1977)
Oc. excrucians (Walker)	CT, NY	Grayson et al. (1983), this study
Oc. hexodontus Dyar	CA	Campbell et al. (1991), Hardy et al. (1993)
Oc. intrudens Dyar	MA, MI, NY	Boromisa and Grayson (1990), Heard et al. (1990), Walker et al. (1993)
Oc. melanimon (Dyar)	CA	Sudia et al. (1971a, 1971b)
Oc. provocans (Walker)	CT, MI, NY	Boromisa and Grayson (1990), Heard et al. (1990), this study
Oc. punctor (Kirby)	MA, MI, NY	Grayson et al. (1983), Boromisa and Grayson (1990), Heard et al. (1990), Walker et al. (1993)
Oc. sollictans (Walker)	CT, NJ, NY, TX	Sudia et al. (1971b), Grayson et al. (1983), this study
Oc. sticticus (Meigen)	CT	This study
Oc. stimulans (Walker)	CT, IN, NY, WI	DeFoliart et al. (1969), Sudia et al. (1971b), Grayson et al. (1983), Boromisa and Grimstad (1986), Howard et al. (1988), this study
Oc. taeniorhynchus (Wiedemann)	CT	This study
Oc. tahoensis (Dyar)	CA	Hardy et al. (1993)
Oc. thelcter (Dyar)	TX	Sudia et al. (1971b)
Oc. triseriatus (Coquillett)	CT, NY, OH	Berry et al. (1977, 1983), Grayson et al. (1983), this study
Oc. trivittatus (Coquillett)	CT, NY, WI	Sudia et al. (1971b), Grayson et al. (1983), this study
Psorophora		
Ps. columbiae (Dyar & Knab)	TX	Sudia et al. (1971b)
Ps. discolor (Coquillett)	TX	Sudia et al. (1971b)
Ps. ferox (von Humboldt)	CT	This study
Tabanidae		
Chrysops cincticornis (Walker)	WI	DeFoliart et al. (1969)
Hybomitra		
H. lasiophthalma (Macquart)	WI	DeFoliart et al. (1969)
H. nuda (McDunnough)	WI	Sudia et al. (1971b)

[a]Includes isolations of Jerry Slough and South River viruses, which are now recognized as JCV (Karabatsos 1985).

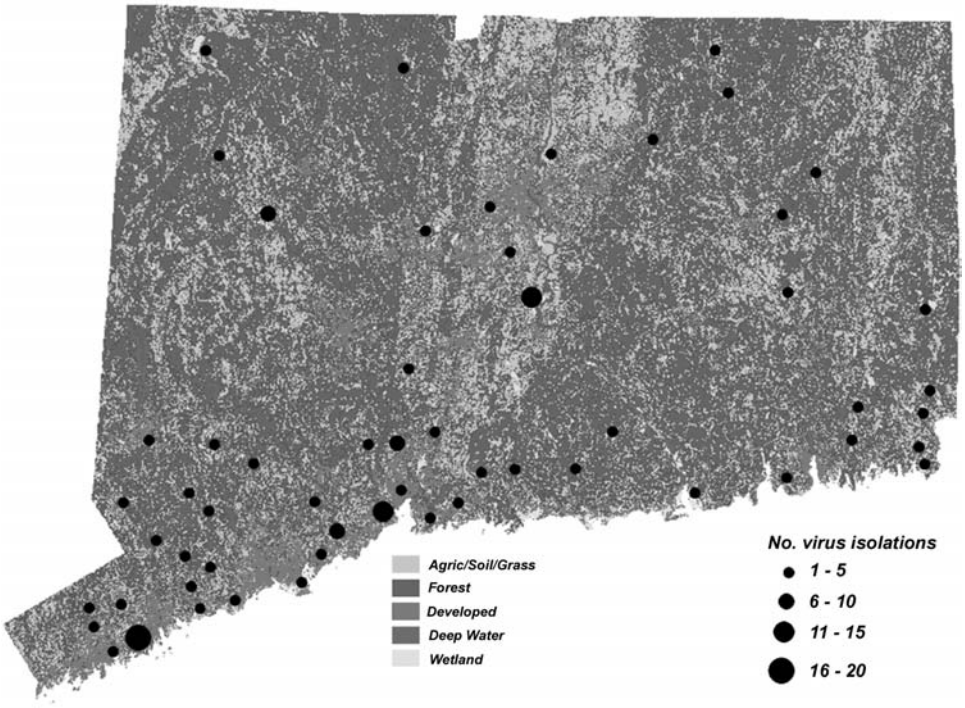

FIG. 1. Land use map of Connecticut showing geographic distribution of Jamestown Canyon virus isolations obtained from mosquitoes collected from 1997 to 2006.

ing Pearson product-moment correlation coefficients (Jandel Corp. 1995), wherein the overall number of virus isolations obtained from field-collected mosquitoes during the 10-year period was compared with the proportion of each of the five land use categories found at each collection site where virus activity was detected.

Mosquito trapping and identification

Mosquito trapping was conducted with CO_2 (dry ice)-baited Centers for Disease Control and Prevention (CDC) miniature light traps equipped with aluminum domes (John W. Hock Co., Gainesville, FL). Traps were suspended from a tree branch at a height of approximately 1.5 m. They were placed in the field in the afternoon, operated overnight, and retrieved the following morning. One trap was used at each site on each trapping occasion. Trapping frequency was variable but was minimally made once every 10 days at each trap site over the course of the entire season. The yearly mean number of trap nights per site was as follows: 1997 and 1998 (14); 1999 (18); 2000 (21); 2001 (23); 2002 and 2003 (31); 2004 (33); 2005 (30); and 2006 (31).

Adult mosquitoes were transported alive to the laboratory each morning in an ice chest lined with cool packs. Mosquitoes were immobilized with dry ice and transferred to chill tables where they were identified to species with the aid of a stereomicroscope (90×) based on the morphological characters and descriptive keys of Darsie and Ward (1981) and Andreadis et al. (2005). Female mosquitoes were pooled in groups of 50 or fewer by species, collection date, and location in 2 mL microcentrifuge tubes containing a copper BB. Mosquitoes were stored at –80°C until processed for virus.

Virus isolation and identification

Pools were processed by adding 1–1.5 mL of PBS-G (phosphate buffered saline, 30% heat-inactivated rabbit serum, 0.5% gelatin, and 1× antibiotic/antimycotic) to each tube. Mosquitoes were homogenized in a Vibration Mill MM300 (Retsch Laboratory, Irvine, CA) set at 25 cycles per second for 4 minutes. Samples were centrifuged at 4°C for 10 minutes at 520*g*, after which 100 μL of the supernatant was inoculated onto a monolayer of Vero cells growing in a 25 cm^2 flask. Cells were maintained at 37°C, 5% CO_2 and monitored daily for cytopathic effect (CPE) from day 3 through day 7 after inoculation. Infected cell supernatants

were harvested and stored at –80° C until further testing.

Viruses were identified by the cell-lysate antigen enzyme-linked immunosorbent assay (ELISA) through 2000, by cross-neutralization tests from 2001 through 2004, and by reverse transcriptase-polymerase chain reaction (RT-PCR) thereafter. In addition, all samples initially typed by ELISA were reconfirmed by RT-PCR to distinguish among closely related viruses of the California and Bunyamwera serogroups (Armstrong et al. 2005). For ELISA testing, antigen was harvested from infected Vero cell cultures and prepared as previously described (Ansari et al. 1993). Viral antigens were titered in twofold dilutions from 1:10 to 1:1280 and identified using a panel of hyperimmune mouse ascitic fluids against JCV, La Crosse virus (LACV), Cache Valley virus (CVV), Highlands J virus (HJV), eastern equine encephalitis virus (EEEV), and West Nile virus (WNV), at a 1:10 dilution. For neutralization tests, virus dilutions from 10^{-4} to 10^{-6} were incubated with a panel of hamster antisera (1:10) directed against JCV, LACV, Snowshoe Hare virus, Trivittatus virus, Keystone virus, CVV, Potosi virus, or hyperimmune mouse ascitic fluids (1:10) against EEEV, HJV, or WNV for 1 hour at 37°C. The virus–antibody mixture was then assayed for neutralizing activity by inoculating Vero cell cultures and screening them for CPE.

For the RT-PCR procedure, samples were tested for JCV using the universal RT-PCR protocol for arboviruses with the Titan RT-PCR kit (Roche Diagnostics, Indianapolis, IN) and primers: JCS63C (5′-CCTGGTTGATATGGGAGATTTGGTTTTC-3′) and JCS667V (5′-TCTTCTGCGCCATCCACTTCTCTG-3′) (Kuno 1998). Primers were designed to specifically target the S-segment of JCV yielding amplification products of 605 bp in length (Kuno et al. 1996). RNA was isolated from infected cell supernatants with the QIAamp viral RNA kit (Qiagen, Valencia, CA). For each RT-PCR reaction, 2 μL of RNA was added to a tube containing 500 μM of each nucleotide, 12.5 μM DTT, and 1 μM of each primer for a final volume of 20 μL. This preparation was heated to 85°C for 5 minutes and then rapidly cooled on ice to denature RNA. A second master mix was added to each tube containing 1× RT-PCR buffer and 1 μL of Titan enzyme mix for a final volume of 50 μL. Amplification was performed as follows: 1 cycle of 50°C for 30 minutes and 94°C for 30 seconds, 10 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 68°C for 2 minutes, followed by 25 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 68°C for 2 minutes + 5 seconds per cycle, and 1 cycle of 68°C for 7 minutes. Amplification products were separated on a 2% agarose gel and visualized by staining with ethidium bromide.

Minimum field infection rates for estimating JCV infection per thousand pooled mosquitoes were calculated individually for each species and collectively for each year and week of the trapping season using the bias-corrected maximum likelihood estimation (MLE) methodology of Biggerstaff (2006). Chi-square analysis using the Yates correction for continuity was applied to compare infection rates for each species, and the number of virus isolations as a function of the number of mosquitoes collected each year over the 10-year collection period was evaluated by regression analysis (Jandel Corp. 1995).

White-tailed deer data and analysis

Data on white-tailed deer populations were obtained from projected deer densities in Connecticut's 12 deer-management zones (LaBonte et al. 2007) based on an aerial deer survey conducted between January and March 2003 (Michael Gregonis, Connecticut Department of Environmental Protection, personal communication). Jamestown Canyon virus MLEs were calculated based on virus isolations obtained from mosquitoes collected in 10 of the 12 deer-management zones where trap sites were located. Relationships between deer density and JCV MLEs were analyzed by regression analysis and Pearson product moment correlation (Jandel Corp. 1995).

RESULTS

Mosquito collection and virus isolation data

The mosquito collection and virus isolation data for the 10-year period 1997–2006 are summarized for each species in Table 2 and graphically depicted in Figure 2. Collection data for

TABLE 2. ISOLATIONS OF JAMESTOWN CANYON VIRUS FROM CONNECTICUT MOSQUITOES 1997–2006

Mosquito species	*Total no. mosquitoes (pools)*	*No. virus isolations*	*MLE (95% CI)*	*No. years detected*	*No. sites detected*
Aedes					
Ae. cinereus	95,688 (7015)	8	0.08 (0.04–0.16)	5	7
Ae. vexans	122,646 (7789)	7	0.06 (0.03–0.11)	5	6
Anopheles					
An. punctipennis	18,953 (4510)	13	0.69 (0.38–1.15)	8	10
An. walkeri	7716 (11,001)	1	0.13 (0.01–0.63)	1	1
Coquillettidia					
Cq. perturbans	210,863 (8112)	9	0.04 (0.02–0.08)	7	9
Culex					
Cx. restuans	43,875 (5514)	1	0.02 (0.00–0.11)	1	1
Culiseta					
Cs. melanura	65,703 (5742)	1	0.02 (0.00–0.07)	1	1
Cs. morsitans	2495 (760)	1	0.40 (0.02–1.94)	1	1
Ochlerotatus					
Oc. abserratus	19,132 (1145)	16	0.84 (0.50–1.34)	6	10
Oc. aurifer	16,602 (1058)	11	0.67 (0.35–1.17)	3	2
Oc. canadensis	177,983 (7784)	40	0.23 (0.16–0.30)	9	24
Oc. cantator	26,398 (2342)	30	1.16 (0.80–1.64)	6	15
Oc. communis	612 (67)	1	1.66 (0.10–8.23)	1	1
Oc. excrucians	6549 (1084)	6	0.92 (0.38–1.91)	4	6
Oc. provocans	226 (21)	1	4.57 (0.26–23.21)	1	1
Oc. sollictans	15,137 (811)	5	0.33 (0.12–0.73)	2	1
Oc. sticticus	52,831 (2399)	12	0.23 (0.12–0.39)	5	9
Oc. stimulans	9437 (1301)	2	0.21 (0.04–0.69)	2	2
Oc. taeniorhynchus	31,892 (1069)	6	0.19 (0.08–0.39)	3	1
Oc. triseriatus	21,012 (4364)	2	0.10 (0.02–0.31)	2	2
Oc. trivittatus	99,136 (4947)	5	0.05 (0.02–0.11)	4	4
Psorophora					
Ps. ferox	25,695 (2206)	1	0.04 (0.00–0.19)	1	1

species from which no virus isolations were made ($n = 19$) are not included. A total of 1,070,581 specimens processed as 71,041 pools resulted in 179 isolations of JCV from 22 different species of mosquitoes in seven genera. Multiple virus isolations were made from 15 of these species that included mostly *Aedes, Anopheles, Coquillettidia,* and *Ochlerotatus*;

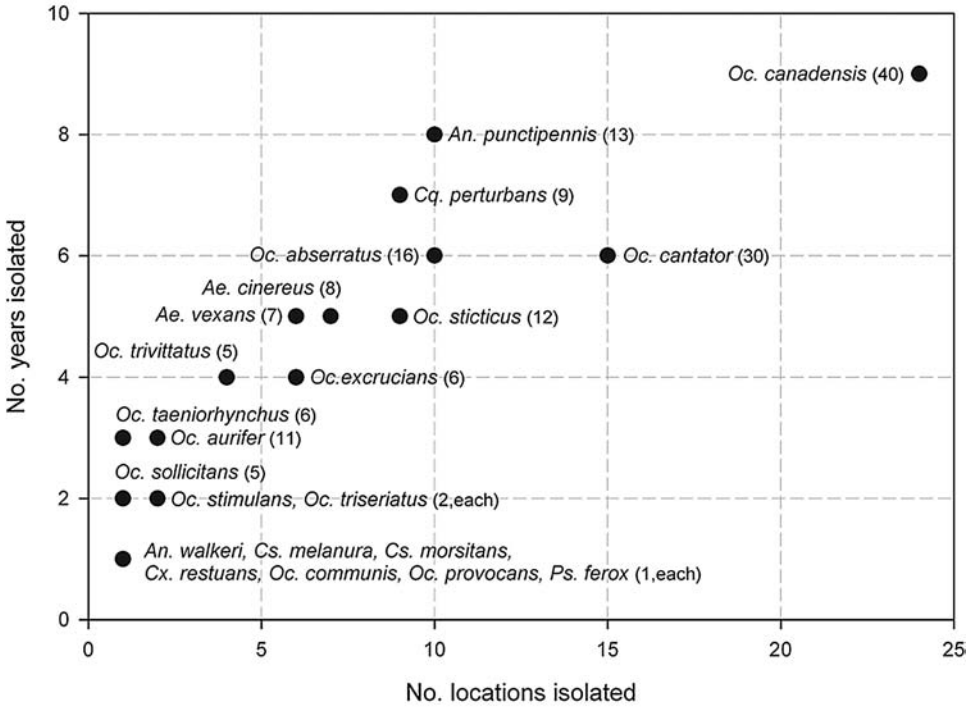

FIG. 2. Cluster graph depicting the distribution and prevalence of Jamestown Canyon virus isolations among 22 species of mosquitoes as a function of number of years and locations virus was isolated in each species. The total number of virus isolations is given in parentheses.

whereas single isolations were made from seven species, mostly *Culiseta, Culex,* and *Psorophora*. New North American records for the isolation of JCV were established for *Anopheles walkeri, Culex restuans, Cu. morsitans, Oc. sticticus, Oc. taeniorhynchus,* and *Psorophora ferox.*

Jamestown Canyon virus MLEs among the 22 species for the 10-year period ranged from 0.02 to 4.57, with an overall combined mean = 0.58 and median = 0.22. The largest numbers of virus isolations were obtained from *Oc. canadensis* ($n = 40$, MLE = 0.23), *Oc. cantator* ($n = 30$, MLE = 1.16), *Oc. abserratus* ($n = 16$, MLE = 0.84), *Anopheles punctipennis* ($n = 13$, MLE = 0.69), *Oc. sticticus* ($n = 12$, MLE = 0.23), and *Oc. aurifer* ($n = 11$, MLE = 0.67).

Jamestown Canyon virus was isolated from *Oc. canadensis* more consistently and from a greater range of collection sites than any other species (Fig. 2). It was detected in 9 of 10 years and from 40 of 91 (44%) trapping locations. *Oc. canadensis* was also among the most widely distributed and frequently collected mosquito in the light traps, representing 16.6% of the total collection. Other species exhibiting frequent temporal and broad spatial geographic patterns of virus isolation included *Oc. cantator, An. punctipennis, Cq. perturbans,* and *Oc. abserratus.*

Spatial geographic distribution of virus and relationship to white-tailed deer populations

The spatial geographic distribution of JCV isolations from mosquitoes as a function of land use is shown in Figure 1. Jamestown Canyon virus was isolated from mosquitoes collected from 57 of 91 (62.6%) collection sites. Locations were widely distributed throughout the state irrespective of land use. No significant associations were demonstrated between the number of virus isolations made from mosquitoes, and any of the five land cover categories that were analyzed using Pearson product moment correlation analysis: agriculture/soil/grass ($r = 0.18$, $p = 0.18$), developed ($r = 0.17$, $p = 0.21$), forest ($r = 0.21$, $p = 0.12$), wetland ($r = 0.10$, $p = 0.45$), and open water ($r = 0.02$, $p = 0.87$) where $n = 57$ collection sites.

The relationships between estimated white-tailed deer populations in 10 of 12 deer-

management zones within the state and the number of JCV isolations and MLEs from mosquitoes trapped in those zones are shown in Figure 3. Average deer densities within the zones ranged from 9.0 to 30.0 deer/mi^2, whereas the overall number of corresponding virus isolations ranged from 2 to 65 and MLEs ranged from 0.05 to 0.54. However, no significant relationships or associations between any pair of variables were found by regression analysis ($r = 0.45$, $p = 0.20$ for MLE, and $r = 0.45$, $p = 0.19$ for number of virus isolations), or Pearson product moment correlation analysis ($r = 0.20$, $p > 0.05$ for MLE and number of virus isolations), respectively.

Yearly and seasonal prevalence

The number of JCV isolations made from mosquitoes varied yearly and ranged from 5 to 56 (mean = 17.9 ± 4.8 SE; median = 14.0) (Fig. 4). However, this variability was a function of overall mosquito abundance and was positively correlated with the number of mosquitoes trapped and tested each year ($r = 0.97$, $p < 0.0001$, regression analysis). This was reflected in the MLEs, which averaged 0.27 ± 0.02 SE (median = 0.28) and varied little from year to year (range = 0.12 to 0.37).

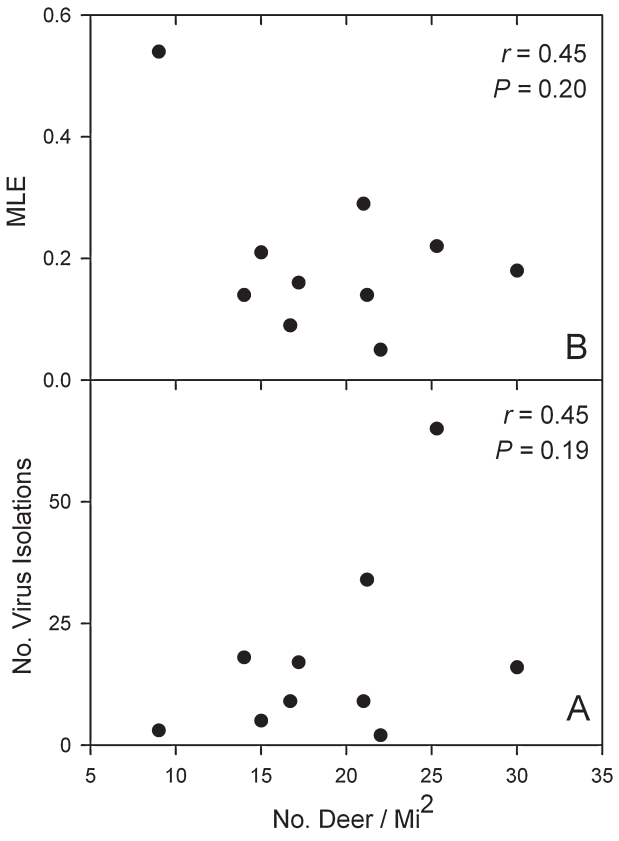

FIG. 3. Relationships between estimated white-tailed deer populations in ten deer-management zones in Connecticut and the number of JCV isolations made from mosquitoes (**A**) and calculated viral minimum field infection rates (MLEs) (**B**) from mosquitoes trapped in those zones from 1999 to 2006.

A composite graph summarizing total weekly JCV activity in all mosquitoes and mosquito abundance as measured by the mean number of mosquitoes trapped and tested during the 10-year period (JCV positive mosquitoes only) is shown in Figure 5. Weekly JCV isolation summaries for each of the 22 mosquito species are shown in Figure 6. With the exception of a few weeks in late August and early September, mosquitoes infected with JCV were detected throughout the collection period, June through September. However, the number of virus isolations closely paralleled overall mosquito abundance, and the greatest number of virus isolations was made in mid-June to mid-July when mosquito collections were at their peak. Correspondingly high MLEs were also calculated during the same weeks (highest = 0.39) and largely remained at or above 0.2 throughout July, thereafter declining markedly to less than 0.1 in August and September.

An examination of weekly JCV isolation summaries for each of the 22 mosquito species (Fig. 6) showed virus activity in all of the woodland univoltine species of *Ochlerotatus* (*Oc. abserratus, Oc. aurifer, Oc. communis, Oc. excrucians, Oc. provocans,* and *Oc. stimulans*) to be largely restricted to June to mid-July, consistent with their abundance in the spring and the natural adult lifespan. Similar weekly virus isolation results were noted with two multivoltine, coastal saltmarsh-inhabiting species, *Oc. sollicitans* and *Oc. taeniorhynchus,* despite their abundance in August and September. This contrasted sharply with virus activity in another multivoltine, coastal saltmarsh-inhabiting species, *Oc. cantator,* from which virus isolations were consistently made throughout June and July (8 of 9 weeks) and into late September. An almost identical pattern of virus activity was seen with a woodland species, *Oc. canadensis,* which is most abundant in the spring but may reappear

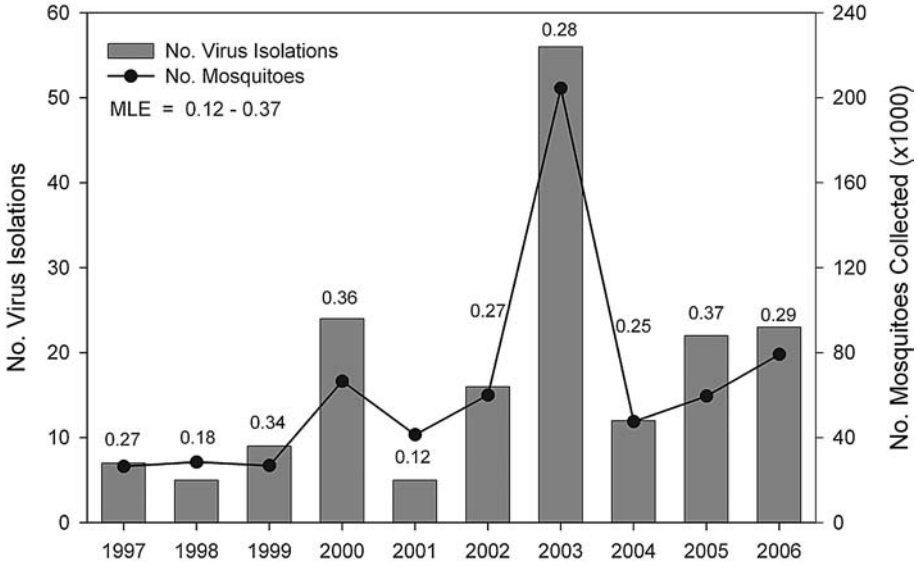

FIG. 4. Yearly number of Jamestown Canyon virus isolations from mosquitoes and overall bias corrected MLEs in relation to number of mosquitoes trapped and tested 1997–2006.

in late summer and early fall if heavy rains reflood the habitat. Virus isolations from *Ae. cinereus*, which exhibits behavior similar to *Oc. canadensis*, and *Ae. vexans*, a multivoltine flood water species, were notably sporadic but generally extended throughout the season, whereas infected *Cq. perturbans* and *Oc. sticticus* were detected from July to early August. The virus isolation data from *An. punctipennis* was notable in that the species was consistently detected throughout July and was the only one from which virus was found through most of August.

DISCUSSION

Our study has shown that JCV is widely distributed throughout Connecticut and can be consistently isolated from a diverse array of

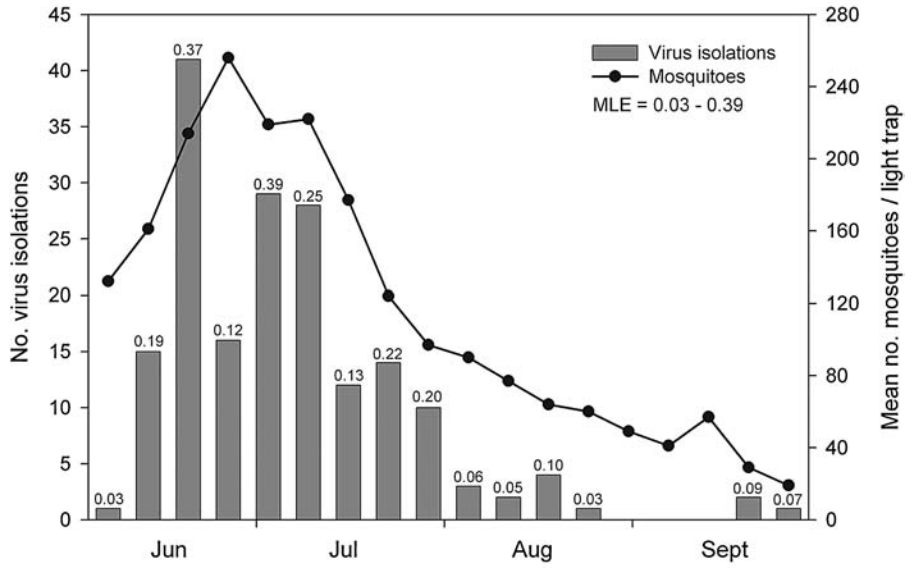

FIG. 5. Weekly isolations and bias-corrected minimum field infection rates (MLE) of Jamestown Canyon virus from field-collected mosquitoes in Connecticut in relation to overall mosquito abundance, 1997–2006.

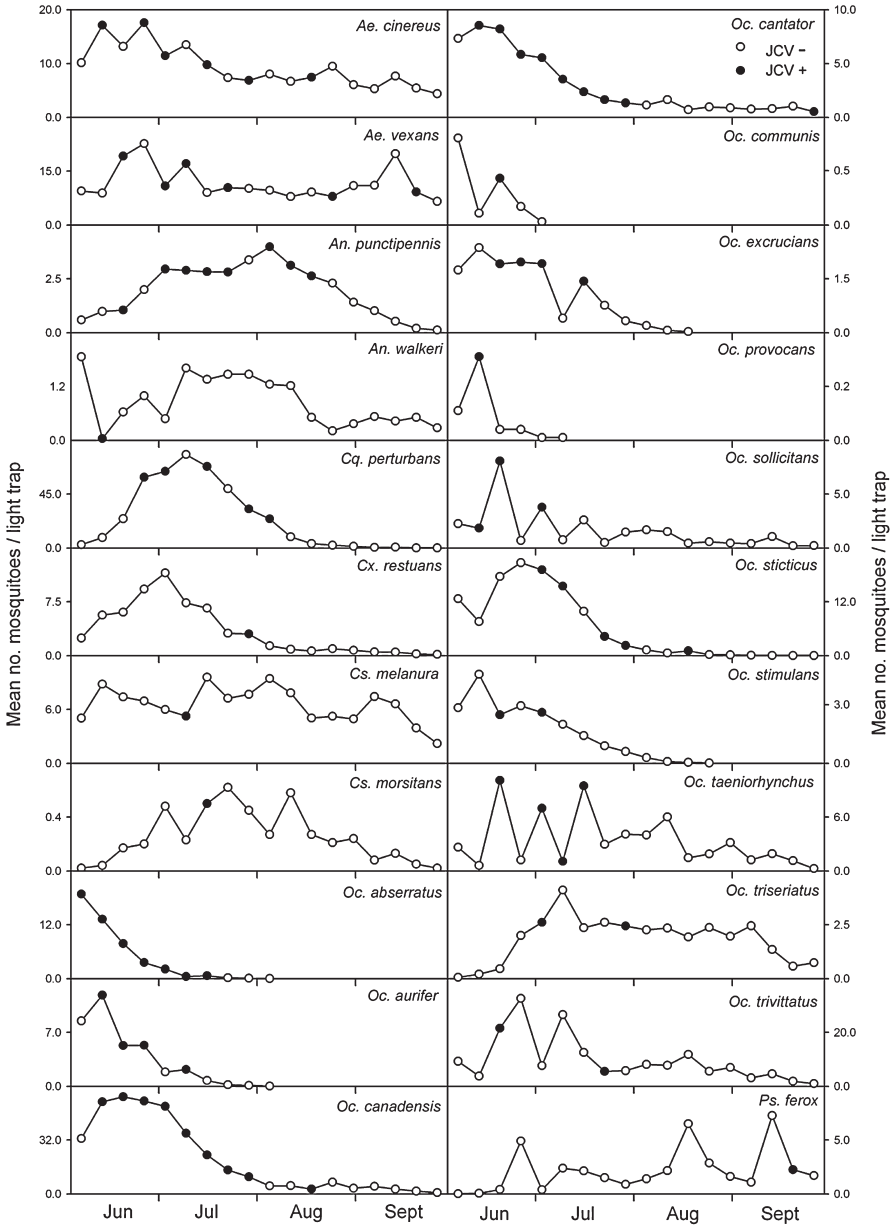

FIG. 6. Weekly summaries of Jamestown Canyon virus isolations from 22 mosquito species in relation to mosquito abundance, 1997–2006. Closed circles, JCV detected; open circles, JCV not detected.

mosquito vectors on a yearly basis. This conclusion is in agreement with an antibody prevalence rate of 21% in white-tailed deer populations (Zamparo et al. 1997) and human seroprevalence rates ranging from 3.9% to 10.1% (Mayo et al. 2001) in Connecticut. Virus infection in mosquitoes occurs throughout the season, from June through September, and it closely parallels mosquito abundance, with peak infection rates extending from mid-June through mid-July. Jamestown Canyon virus MLEs are consistent from year to year, and overall virus activity, as measured by the number of virus isolations from mosquitoes, is directly related to the number of mosquitoes trapped and tested and, presumably, to local mosquito abundance. Infected mosquitoes appear to be equally distributed throughout urban, suburban, and rural locales, and infection rates are not directly associated with the abundance of white-tailed deer, the most likely amplification host in this region. Our findings are

largely consistent with an earlier study by Grayson et al. (1983), who reported the detection of JCV from 13 or more species of mosquitoes collected in 19 counties in New York State in every year but one from 1972 to 1980. However, these authors noted a prominent increase in the prevalence of infected mosquitoes from 1978 to 1980 that was correlated with a series of unseasonably mild winters which resulted in a population explosion of white-tailed deer. We detected no such increase in the prevalence of infected mosquitoes despite a substantial increase in estimated deer populations from approximately 54,000 to 75,000 from 1996 to 2003 (Michael Gregonis, Connecticut Department of Environmental Protection, personal communication). This observation and the absence of any demonstrable association between the spatial abundance of white-tailed deer and JCV activity in mosquitoes in our investigation may reflect the overall abundance and saturation of white-tailed deer throughout the region.

In our study we isolated JCV from nearly half (22 of 50 = 45%) of the mosquito species known to occur in the State of Connecticut (Andreadis et al. 2005), establishing new virus isolation records for six species: *An. walkeri, Cx. restuans, Cs. morsitans, Oc. sticticus, Oc. taeniorhynchus,* and *Ps. ferox*. However, based on yearly isolation frequencies and the spatial geographic distribution of infected mosquitoes, five species were incriminated as the most consistent and likely vectors in this region: *Oc. canadensis, Oc. cantator, An. punctipennis, Cq. perturbans,* and *Oc. abserratus*.

Laboratory transmission of JCV has been confirmed for *Oc. canadensis, An. punctipennis,* and *Cq. perturbans* (Heard et al. 1991), but vector competencies for *Oc. abserratus* and *Oc. cantator* have not been established. However, JCV has been repeatedly isolated from field-collected *Oc. abserratus* and *Oc. cantator* throughout the northeastern United States and upper midwestern United States, and from Newfoundland (see Table 1), thus inferring that both species possess a significant biological association with JCV (Eldridge 1990) and are likely involved in the natural transmission cycle. *Oc. abserratus* and *Oc. cantator* are also largely mammalophilic species that readily feed on deer (Magnarelli 1977b, Boromisa and Grimstad 1986; G. Molaei personal communication).

Multiple virus isolations were also made from *Ae. cinereus, Ae. vexans, Oc. sticticus,* and *Oc. aurifer,* but on notably fewer occasions and from a lesser number of locales. The early-season isolations from *Oc. abserratus, Oc. aurifer, Oc. communis, Oc. excrucians, Oc. provocans,* and *Oc. stimulans* are consistent with their potential role as overwintering reservoirs (Boromisa and Grimstad 1986, Heard et al. 1990).

Our observations on the relative importance of *Oc. canadensis* as one of the more dominant mosquito vectors of JCV in Connecticut appear to be unique to this region. Although JCV has been isolated on occasion from this species in Maryland (LeDuc et al. 1975), New York (Grayson et al. 1983; Howard et al. 1988), and Michigan (Heard et al. 1990), it does not appear to play a significant role in transmission of JCV in those areas. To the contrary, species incriminated as predominate vectors in these and other regions include several species from which we isolated JCV, but to a far lesser degree: *Oc. provocans* in northeastern New York (Boromisa and Grayson, 1990) and in Michigan (Heard et al. 1990), *Ochlerotatus intrudens* and *Oc. abserratus/punctor* in western Massachusetts (Walker et al. 1993), and *Oc. stimulans* in northern Indiana (Boromisa and Grimstad 1986).

Oc. canadensis is the most commonly trapped species in Connecticut and is among the most widely distributed, occurring through almost all regions of the state (Andreadis et al. 2004, 2005). Larvae develop in a variety of freshwater habitats, especially temporary leaf-lined pools in wooded areas, but they are also found in roadside ditches, vernal pools in open fields, permanent swamps, and acid water sphagnum bogs (Crans 2004, Andreadis et al. 2005). The species is considered univoltine (Crans 2004), but a second brood may occur if heavy rains re-flood the habitat in late summer and early fall (Magnarelli 1977a). It is not certain if this phenomenon is due to delayed hatching of eggs or true multivotinism, but in either case it is wholly consistent with our increased collections of adult females in August through September. Local populations of *Oc. canadensis* are known to feed on a broad range of animals, in-

cluding humans, large and small mammals, birds, amphibians, and reptiles (Magnarelli 1977b), and in a recently completed investigation of blooded females collected in the same locales as the present study, *Oc. canadensis* was shown to exhibit a very strong preference (94.6% of 186 blood meals) for white-tailed deer (G. Molaei, personal communication).

It has been suggested (Grimstad 1988, 2001) that JCV may be transmitted in a dual cycle involving early-spring, vertically infected *Aedes* and *Ochlerotatus* mosquitoes with subsequent horizontal amplification in white-tailed deer populations in June and July, followed by a late-summer, early-autumn amplification cycle primarily involving *Anopheles* mosquitoes, most notably *An. punctipennis* and *An. quadrimaculatus.* Grimstad (2001) has also noted that the majority of human cases of JCV in the northeastern United States and southern Canada have onset in late summer to early fall, coincident with late-season feeding by these same species. Our observations on the detection of JCV in field-collected mosquitoes in mid- to late September following 3 weeks of apparent inactivity, and consistent isolation of virus from *An. punctipennis* throughout most of August, as well as the late-August onset of symptoms in the human case of JCV reported from Connecticut (Nelson 2002), support this hypothesis, in part.

However, we also note the occasional late-September isolations of virus from *Ae. vexans, Oc. cantator,* and to a lesser degree *Ps. ferox,* which also feed on deer and humans (Magnarelli 1977b, Molaei and Andreadis 2006) and could represent potential bridge vectors as well.

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