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MOSQUITO AND ARBOVIRUS SURVEILLANCE IN CONNECTICUT, 1991–1992

THEODORE G. ANDREADIS,[1] PAUL M. CAPOTOSTO,[2] ROBERT E. SHOPE[3] AND SHIRLEY J. TIRRELL[3]

ABSTRACT. A surveillance program for mosquito-borne arboviruses was conducted in Connecticut following an epizootic of eastern equine encephalitis (EEE) in horses and domestic birds during 1990. Mosquito trapping was done weekly using CO^2-baited miniature light traps at 12 freshwater swamp sites that were located mostly in the southeastern portion of the state. Trapping was conducted from June 27 to October 11, 1991 and from June 2 to September 30, 1992. Totals of 7,435 (1991) and 13,912 (1992) adult female mosquitoes representing 21 species in 7 genera were collected and assayed for arboviruses. Virus isolates were identified by ELISA using reference antibody of California encephalitis, EEE, Highlands J (HJ), Jamestown Canyon (JC), LaCrosse, and St. Louis encephalitis viruses. *Culiseta melanura* was the most common species trapped each year, followed by *Aedes canadensis, Aedes cinereus,* and *Coquillettidia perturbans.* The most abundant univoltine snowmelt species was *Aedes abserratus.* Three isolates positive for JC virus were obtained from *Ae. abserratus, Ae. canadensis* (new state record), and *Ae. cinereus* (new state record) that were collected from 2 different sites in June (1992) and July (1991 and 1992). Six isolates positive for HJ virus were made from *Cs. melanura* and one isolate from *Ae. cinereus* (new host record) collected in mid- to late September, 1992 from 3 locations. Based on repeated virus isolations in this and other studies, high field infection rates, and its relative abundance, *Ae. abserratus* appears to be a principal vector of JC in Connecticut. However, the prevalence and importance of JC as a human disease in the state are unknown. *Culiseta melanura* populations were abundant throughout the summer and early fall, and the availability of this potential mosquito vector does not appear to be a limiting factor for enzootic maintenance and subsequent amplification of EEE virus in presumed foci in southeastern Connecticut.

INTRODUCTION

Knowledge of mosquito-borne arboviruses in Connecticut is limited. Sporadic epizootics of eastern equine encephalitis (EEE) historically have occurred in horses and flocks of domestic pheasants since 1938 (see Andreadis 1993 for review), and at least 2 California group viruses that cause human disease (Jamestown Canyon [JC] and Keystone [KEY]) have been recognized since 1966 (Whitman et al. 1968, Sprance et al. 1978, Main et al. 1979b). However, comprehensive surveillance of mosquito populations and arboviruses has not been conducted since Main et al. (1979a, 1979b) obtained several isolations of EEE, Highlands J (HJ), JC, and KEY viruses from mosquitoes collected from 1969 through 1978.

In October 1990, an epizootic of EEE occurred among horses and commercial flocks of ring-necked pheasants at 6 locations in eastern Connecticut (Andreadis 1993). These were the first confirmed cases of EEE in the state in almost 10 years and occurred coincident with major outbreaks involving humans and horses in neighboring Massachusetts (Edman et al. 1993) and Rhode Island (Gettman 1993). In response to this renewed activity and in an attempt to obtain information on the current status of other mosquito-borne arboviruses in the state, we initiated a 2-year surveillance program. The results of this investigation are reported herein.

MATERIALS AND METHODS

Field collecting techniques: Adult female mosquitoes were collected with dry ice-baited CDC miniature light traps at 12 freshwater swamp sites located in the towns of Chester, Cornwall, Fairfield, Haddam, Killingworth, Ledyard, North Stonington, Salem (1991 only), Southington, Voluntown, Waterford, and Willington (1992 only) (Fig. 1). The majority of these sites were in the southeastern portion of the state where arbovirus activity had been reported previously (Andreadis 1993). These included all but one locale (Canterbury) where the confirmed EEE horse deaths were detected in 1990. The Willington site was added in 1992 following a single fatal EEE horse case in October 1991. Most collection sites were characterized as mature hardwood swamps dominated by red maple *Acer rubrum,* Atlantic white cedar, *Champaecyparis thyoides,* and eastern hemlock *Tsuga canadensis.*

[1] Department of Soil and Water, The Connecticut Agricultural Experiment Station, 123 Huntington Street, P. O. Box 1106, New Haven, CT 06504.

[2] Department of Environmental Protection, Field Services/Boating Division, Wetlands Restoration Unit, 51 Mill Road, Madison, CT 06443.

[3] Yale Arbovirus Research Unit, Department of Epidemiology and Public Health, Yale University School of Medicine, P. O. Box 3333, New Haven, CT 06510.

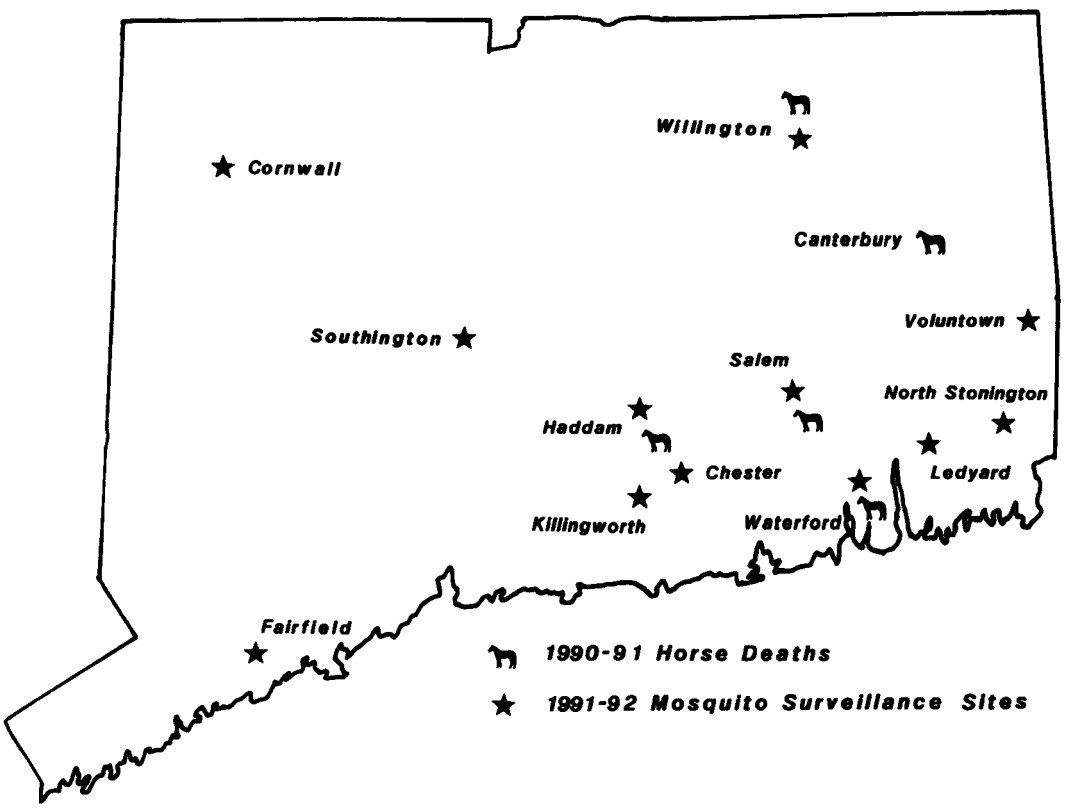

Fig. 1. Distribution of equine cases of eastern equine encephalitis in Connecticut, 1990–91 and location of mosquito and arbovirus surveillance sites, 1991–92.

Trapping was conducted weekly at each site (one trap per site) from June 27 to October 11, 1991 and from June 2 to September 30, 1992. Traps were set at the perimeter of the swamp during the late afternoon and retrieved the following morning. Mosquitoes were transported to the laboratory where they were cold anesthetized on a chill table and promptly identified using the keys of Darsie and Ward (1981) and Means (1979, 1987). Specimens were pooled by species, site, and collection date and then stored for 1–3 days at −60°C pending virus assay. The number of mosquitoes per pool was variable and the maximum number was 100.

Rainfall data were obtained from 7 National Weather Service Climatological Stations (Cockaponset Ranger Station, Falls Village, Groton, Jewett City, Norwich Public Utilities Plant, Shuttle Meadow Reservoir, and Stafford Springs) that were located in the immediate vicinity of the collection sites. Moon phases were not monitored.

Virus assays: Each frozen mosquito pool was homogenized in phosphate-buffered saline containing 0.5% gelatin, 30% rabbit serum, antibiotic, and antimycotic. The homogenate was centrifuged for 10 min at 1,500 rpm to clear the mixture of mosquito debris. A 0.1-ml aliquot of each supernatant was then inoculated into a 25-cm^2 flask containing a monolayer of Vero cells in growth medium and incubated at 37°C in 5% CO_2 for up to 7 days (Tesh et al. 1992). One uninoculated flask was kept as a negative control. The remainder of the supernatant was stored at −70°C. The flasks were examined daily for cytopathic effect. If cytopathic effect was noted, the cells were scraped from the flask and centrifuged for 5 min at 8,000 rpm. A cell lysate antigen was then made from the cell button to use in the cell lysate antigen ELISA (Ansari et al. 1993). The isolates were identified using reference antibodies that were prepared in mice and provided by the WHO Center for Arbovirus Research and Reference. These included California encephalitis (CE) (BFS-283), EEE (Ten Broeck), HJ (B240), JC (61V2235), LaCrosse (LAC) (Prototype), and St. Louis encephalitis (SLE) (Parton) viruses. The antibodies were sufficiently specific by ELISA so that end-point titrations were not necessary to distinguish between related viruses.

Table 1. Field-collected adult female mosquitoes tested for arbovirus from Connecticut, 1991–92.

Species	1991		1992	
	No. mosquitoes	No. pools	No. mosquitoes	No. pools
Aedes				
abserratus	48	18	466	40
aurifer	—	—	57	8
canadensis	1,323	81	2,614	135
cinereus	893	50	1,565	105
excrucians	20	9	29	9
sticticus	1	1	202	38
stimulans	1	1	49	29
triseriatus	267	44	207	43
trivittatus	163	18	427	62
vexans	747	37	682	70
Anopheles				
punctipennis	221	59	197	73
quadrimaculatus	26	17	32	9
walkeri	—	—	43	6
Coquillettidia				
perturbans	713	50	1,249	64
Culex				
pipiens	522	33	232	63
restuans	288	45	698	84
territans	7	6	3	2
Culiseta				
melanura	1,974	122	4,608	216
morsitans	18	15	57	27
Psorphora				
ferox	175	23	467	33
Uranotaenia				
sapphirina	28	16	28	16
Totals	7,435	645	13,912	1,132

RESULTS

Totals of 7,435 (1991) and 13,912 (1992) adult female mosquitoes representing 21 species in 7 genera were collected, identified, and assayed for arboviruses (Table 1). *Culiseta melanura* (Coquillett) was the most common species trapped each year, followed by *Aedes canadensis* (Theobald), *Aedes cinereus* Meigen, and *Coquillettidia perturbans* (Walker). The most abundant univoltine snowmelt species was *Aedes abserratus* (Felt and Young).

Substantially higher rainfall amounts were recorded in June (13.7 cm) and July (10.4 cm) of 1992 than in June (4.1 cm) and July (5.6 cm) of 1991. Average monthly rainfalls for August and September, however, were similar in each year: 25.5 cm and 13.5 cm in 1991 and 21.7 cm and 10.7 cm in 1992, respectively.

The virus ELISA data are summarized in Table 2. Three isolates positive for JC virus were obtained from *Ae. canadensis, Ae. abserratus,* and *Ae. cinereus* collected in June and July from 2 different sites (Waterford and Killingworth). The minimum field infection rate (MFIR) at each site ranged from 1:19 to 1:100. Six isolates positive for HJ virus were obtained from *Cs. melanura* and another from *Ae. cinereus* collected in mid- to late September from 3 locations in 1992 (Ledyard, N. Stonington, and Voluntown). The MFIR for *Ae. cinereus* at the Voluntown site was 1:25, whereas the MFIRs for *Cs. melanura* ranged from

Table 2. Isolates of Jamestown Canyon (JC) and Highlands J (HJ) viruses from Connecticut mosquitoes in 1991–92.

Species	Site	Date	Pool size	MFIR[1]	Cell lysate antigen titer	
					JC	HJ
Aedes						
canadensis	Waterford	7-25-91	1	1:19	1:160	—
abserratus	Killingworth	6-12-92	24	1:40	1:160	—
cinereus	Killingworth	7-10-92	1	1:100	1:160	—
cinereus	Voluntown	9-23-92	9	1:25	—	≥1:160
Culiseta						
melanura	Ledyard	9-17-92	2	1:238	—	≥1:160
melanura	N. Stonington	9-23-92	15	1:120	—	≥1:160
melanura	N. Stonington	9-29-92	19	1:120	—	≥1:160
melanura	Voluntown	9-16-92	15	1:132	—	≥1:160
melanura	Voluntown	9-23-92	11	1:132	—	≥1:160
melanura	Voluntown	9-23-92	23	1:132	—	≥1:160

[1] Site specific minimum field infection rate (MFIR) for that mosquito species based on total cumulative number of mosquitoes collected at the site during the year.

1:120 to 1:238, depending on the site. No isolates positive for CE, EEE, LAC, or SLE viruses were obtained in either year.

The weekly collection data of those mosquito species from which virus isolates were obtained, as well as those that have been incriminated in previous EEE epizootics (*Aedes vexans* (Meigen) and *Cq. perturbans*), are shown in Figs. 2 and 3. *Culiseta melanura* was consistently collected throughout the entire sampling period of both years (Fig. 2). There were 2 peaks of adult abundance in 1991. The first was observed in late June and the second in mid- to late September. The latter followed major flooding that was associated with hurricane "Bob" on August 18. Adult populations were generally higher during the midsummer months (July and August) of 1992, but 2 peaks of abundance again were observed. These occurred in mid-July and late August. The HJ virus isolates all were obtained from *Cs. melanura* mosquitoes collected during the last 3 weeks of September, 1992.

Identical patterns of adult *Cq. perturbans* abundance were observed each year (Fig. 2). Substantial numbers were collected throughout July with peak populations occurring during the 2nd and 3rd weeks. By mid-August, however, very few adults were collected and none was detected after September 9 and 15, 1991 and 1992, respectively.

Collections of the floodwater mosquito *Ae. vexans* generally were limited to late August and early September (Fig. 2). The largest catches were obtained during the first week of September 1991, 2 weeks after the hurricane.

Large numbers of the univoltine mosquito *Ae. abserratus* were collected during early June 1992 (Fig. 3). The isolate of JC virus was obtained from a pool of 24 females collected during the 2nd week. A steady decline in adult abundance was observed thereafter. No valid comparisons could be made with 1991 collections as trapping was not initiated until the end of June in that year.

Adults of the multivoltine mosquitoes *Ae. canadensis* and *Ae. cinereus* generally were found throughout the season (Fig. 3). Two periods of peak abundance were observed for both species, mid-July and early to mid-September. Both species were particularly abundant during September. The 2 isolates of JC virus from each mosquito were made in mid-July and the isolate of HJ virus from *Ae. cinereus* in late September.

DISCUSSION

The isolates of JC virus from adult female populations of *Ae. abserratus, Ae. canadensis,* and *cinereus* reaffirm the presence of this virus in southern Connecticut. The first isolation of JC virus in Connecticut was made from a pool of 20 adult female *Ae. abserratus* collected in the central portion of the state during late June 1966 (Whitman et al. 1968). The virus was isolated again in July 1973 from a salt marsh species, *Aedes cantator* (Coquillett) (Sprance et al. 1978), and each year thereafter from 1975 through 1978 (*Ae. abserratus* = 15, *Ae. cantator* = 1, *Ae. vexans* = 1, *Cq. perturbans* = 1) Main et al. 1979a). The majority of these isolations were made from

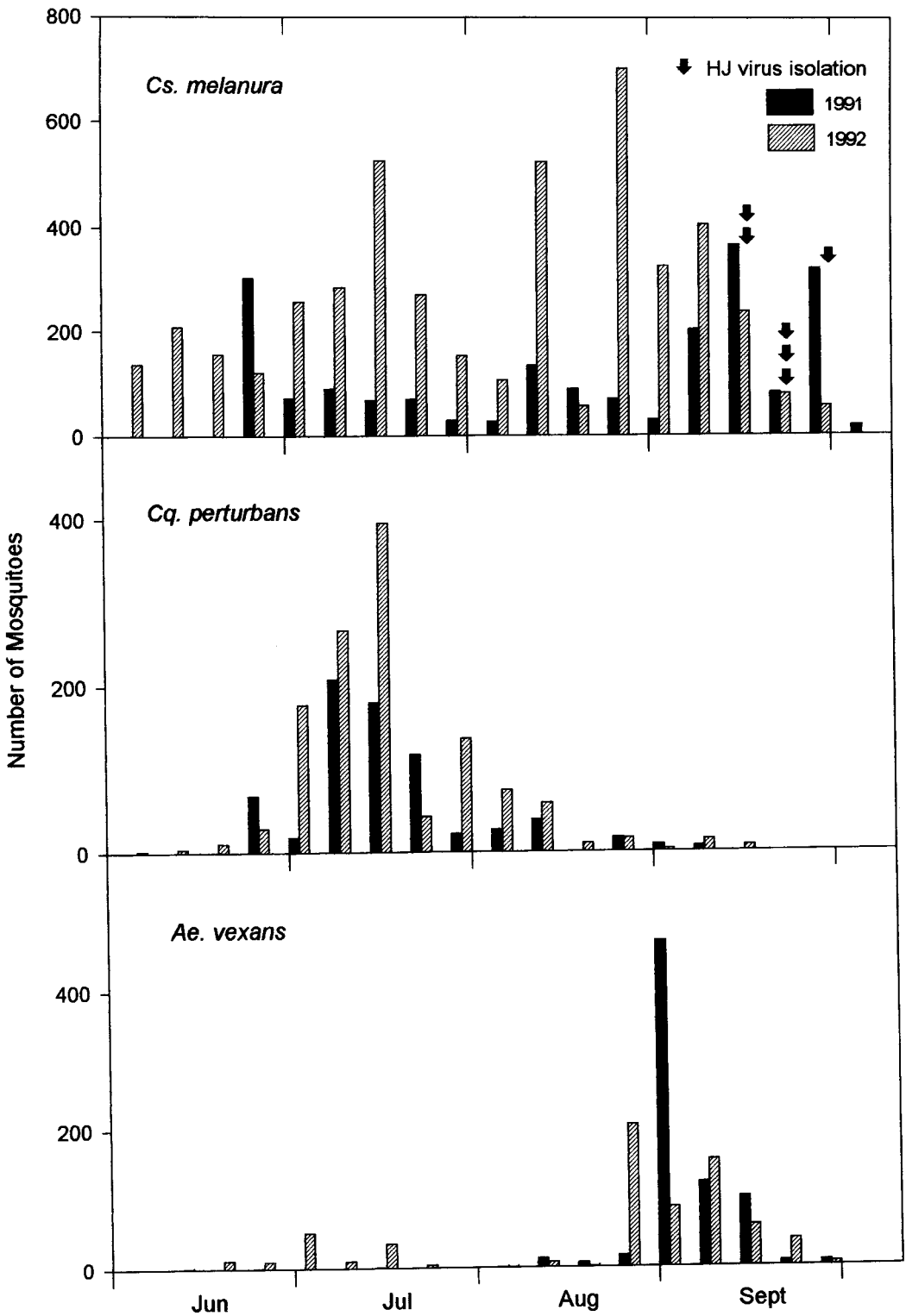

Fig. 2. Total weekly collections (all traps) of *Culiseta melanura, Coquillettida perturbans,* and *Aedes vexans* and isolates of Highlands J (HJ) virus in Connecticut, 1991–92.

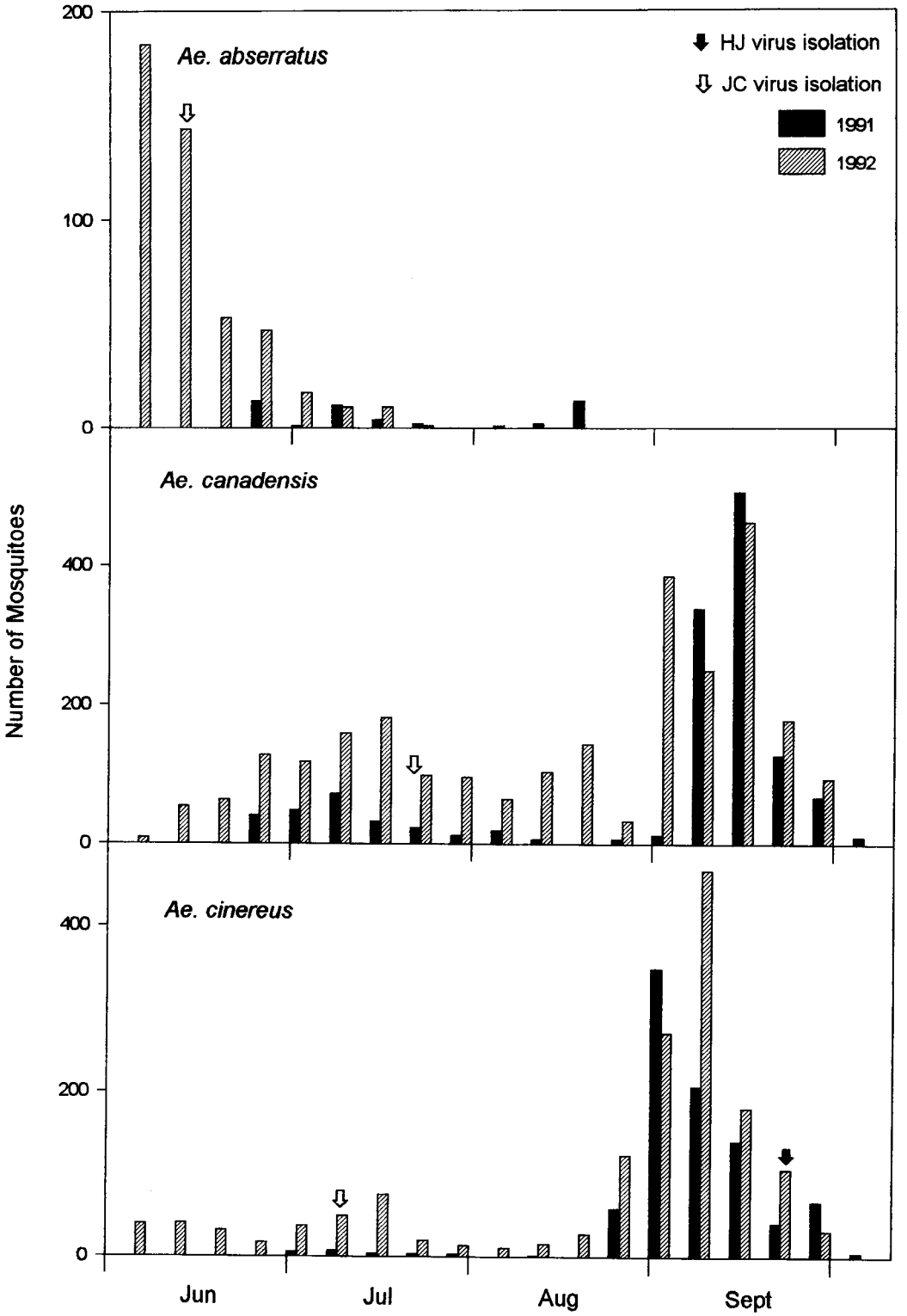

Fig. 3. Total weekly collections (all traps) of *Aedes abserratus, Aedes canadensis,* and *Aedes cinereus* and isolates of Highlands J (HJ) and Jamestown Canyon (JC) viruses in Connecticut, 1991–92.

mosquitoes collected in June, and all were collected in the same general vicinity (Killingworth and Madison) as the present isolations. Our isolates from *Ae. canadensis* and *Ae. cinereus* represent new host records for JC virus in Connecticut and add to previous isolations from *Ae. cinereus* in New York (Grayson et al. 1983) and *Ae. canadensis* in Maryland, Michigan, and New York (LeDuc et al. 1975, Grayson et al. 1983, Howard et al. 1988, Heard et al. 1990). Jamestown Canyon virus has been isolated from *Ae. abserratus/punctor* in Massachusetts (Walker et al. 1993), Michigan (Heard et al. 1990), and Newfoundland (Mokry et al. 1984), in addition to Connecticut.

The relative importance of each of these mosquito species in the epizootiology of JC virus in Connecticut is unknown. The numerous isolations from *Ae. abserratus* and relatively high field infection rates (1:438 for a 10-year period, 1969–78 [Main et al. 1979a] and 1:514 [overall] from 1991–92 in the present study) suggest that this species may be an important vector. This view is consistent with its feeding preference (Magnarelli 1977, Boromisa and Grimstad 1986) for white-tailed deer *Odocoileus virginianus,* the primary vertebrate host of the virus (Grimstad 1983), and its close biological association with the virus throughout the upper midwest and northeastern United States (Eldridge 1990). In a recently completed study, Walker et al. (1993) also implicated *Ae. abserratus/punctor* as a likely epizootic vector of JC virus in western Massachusetts. On the other hand, Heard et al. (1991) were unable to demonstrate laboratory transmission of JC virus by orally infected *Ae. abserratus/punctor* that were field collected from an enzootic focus located in Michigan. Although vertical transmission of JC virus has not been demonstrated for *Ae. abserratus,* we also cannot rule out the possibility that this mosquito may serve as an overwintering reservoir for the virus, as has been suggested for *Aedes provocans* (Walker) (Heard et al. 1990) and *Aedes stimulans* (Walker) (Boromisa and Grimstad 1986).

Aedes canadensis and *Ae. cinereus* also are known to feed on deer (Magnarelli 1977, Boromisa and Grimstad 1986), and our study demonstrates that both species are multivoltine and omnipresent in freshwater swamps in Connecticut throughout the spring, summer, and early fall. *Aedes canadensis* was among the most dominant species, and horizontal laboratory transmission of JC virus has been demonstrated for this mosquito (Heard et al. 1991). However, *Ae. canadensis* does not appear to play an important role in JC virus transmission in northeastern New York (Boromisa and Grayson 1990) or Michigan (Heard et al. 1990), and based on limited field isolations and low infection rates in these areas, Eldridge (1990) has excluded *Ae. canadensis* from his list of mosquito species possessing a significant biological association with JC virus. According to Eldridge (1990), there is evidence of a close association between JC virus and *Ae. cinereus;* however, virus transmission has not been demonstrated with this species (Heard et al. 1991), and our results indicate that although it is almost always present it is not normally abundant during the late spring and early summer when JC virus apparently is active.

The importance of JC virus as a human disease in Connecticut is unknown. To our knowledge, there have been no serological investigations of humans, deer, or other potential vertebrate hosts in this state. It has been suggested that JC virus in an emerging disease that may be expanding with the exploding deer populations in many regions of North America (Deibel et al. 1983, Grimstad 1983, DeFoliart et al. 1986). Widespread human infection with JC virus occurs endemically in Michigan and exhibits a distribution pattern that closely parallels the estimated distribution of white-tailed deer in that state (Grimstad et al. 1986). Deer populations have increased substantially in southeastern Connecticut during the last decade, and this growth has been concurrent with an increase in the abundance of the black-legged tick *Ixodes scapularis* Say (formerly the deer tick *I. dammini* Spielman, Clifford, Piesman, and Corwin) and the incidence of Lyme Disease (Anderson and Magnarelli 1980). Whether or not JC virus also is expanding in this region is subject to speculation. Our findings suggest that JC virus is enzootic in these regions; thus we feel further epidemiological studies are warranted.

The 6 isolates of HJ virus from *Cs. melanura* collected in mid- to late September are consistent with the ecology of this virus in the northeastern United States. Highlands J virus is maintained naturally in an enzootic cycle involving mosquitoes and wild birds that inhabit wooded freshwater swamps. Peak periods of activity generally occur during the late summer and early fall (Williams et al. 1972, Main et al. 1979b, Srihongse et al. 1980). Most isolations of the virus have been made from *Cs. melanura,* and this ornithophilic mosquito has been incriminated as the primary enzootic vector (Hayes and Wallis 1977). Although the virus has on occasion been the cause of mild equine encephalitis in Florida (Jennings et al. 1966, Hoff et al. 1978), all virus-positive horse cases in Connecticut have been identified as EEE (plaque reduction neutralization tests), and HJ virus has not been recognized as a public or veterinary health problem in this region (Hayes and Wallis 1977).

Highlands J virus was first isolated in Connecticut in 1972 from a pool of *Cs. melanura* mosquitoes collected in East Haddam during September (Main et al. 1979b). The virus was subsequently recovered from 2 additional pools of *Cs. melanura* collected in August 1973 (Farmington) and October 1978 (Madison) during the 10-year period from 1969 to 1978 in which 16,731 adult female *Cs. melanura* were collected and assayed for arboviruses (Main et al. 1979b). No additional isolations of HJ virus were obtained from 140,915 adult female mosquitoes, representing 28 species in 8 genera, in that same study. This included more than 5,000 *Ae. cinereus*. We believe our single isolation of HJ virus from 1,565 adult female *Ae. cinereus* collected in 1992 represents a new mosquito host record in the eastern United States. *Aedes cinereus* is known to feed on birds as well as mammals (Magnarelli 1977); however, its vector potential for HJ virus would appear to be limited based upon the low number of virus isolations.

Although EEE and HJ viruses can be active at the same time in the same location, HJ virus is usually more prevalent that EEE virus and appears in mosquitoes earlier in the season in Massachusetts swamps (Edman et al. 1993). In upstate New York, there appears to be an unexplained alternation of the 2 viruses in succeeding years (Howard et al. 1988). Thus the detection of HJ virus in *Cs. melanura* populations can, at times, be useful in predicting subsequent EEE virus activity in some areas (Edman et al. 1993). Long-term surveillance would be required to determine whether or not HJ would be a reliable predictor of impending EEE activity in areas of southeastern Connecticut.

The lack of EEE virus isolations from *Cs. melanura* or other recognized mosquito vectors in the present investigation was not surprising, even though the survey was conducted during a time when the virus presumably was active (i.e., confirmed horse deaths occurred during 1990 and 1991). Although widespread epizootics of EEE virus have occurred historically among horses and domestic birds within the state since 1938 (see Andreadis 1993), there have been no confirmed cases of EEE in humans. Furthermore, despite extensive surveillance during epizootic periods of the mid-1950s (Wallis et al. 1958a, 1958b, 1960) and early (Main et al. 1979b), only 5 isolations of EEE virus have been obtained from mosquitoes collected in Connecticut, 4 from *Cs. melanura* (Main et al. 1979b) and one from *Ae. vexans* (Wallis et al. 1960). In other regions of the northeast (i.e., Massachusetts and New York) where EEE virus occurs consistently, isolation of the virus from mosquitoes is very common during epizootics but much less so during inter-epizootic periods (Morris 1988).

The absence of EEE virus in mosquitoes found in presumed foci in southeastern Connecticut is not fully understood. Our data clearly show that *Cs. melanura* populations are abundant throughout the summer and early fall and that other potential epizootic vectors from which EEE virus isolations have been made, specifically *Ae. canadensis* and *Ae. vexans*, also are locally abundant during September when EEE virus activity typically occurs. Thus, the availability of a suitable mosquito vector does not appear to be a limiting factor. In this regard, *Cq. perturbans* does not appear to be a likely vector of EEE in these regions of Connecticut because of its low abundance from mid-August through September. It has been suggested (Wallis and Main 1974) that Connecticut's numerous suburban forests and woodlands, which comprise more than 60% of the state (Andreadis 1993), provide a sylvan swampland ecology in which wild birds hosts and mosquito vectors are widely dispersed. Consequently, there may be an insufficient focus of infected birds during most years to provide for an annual build-up of infection to detectable levels in local mosquito populations. However, whether EEE is reintroduced from time to time by migrating birds or overwinters as a chronic infection in resident passeriforms remains unknown.

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