# ARBOVIRUS SURVEILLANCE IN CONNECTICUT I. GROUP A VIRUSES[1]

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ABSTRACT. Four strains of eastern equine encephalomyelitis virus were isolated from 16,731 adult female *Culiseta melanura* collected in Connecticut from 1969 through 1978. Two of the isolates were from mosquitoes collected during a widespread epizootic of this virus among equines and domestic birds in the state during 1972.

Western equine encephalomyelitis virus, subtype II, was isolated for the first time in Connecticut in 1972. Three strains were recovered from *Cs. melanura* and one from the blood of a rufous-sided towhee (*Pipilo erythrophthalmus*).

Group A arboviruses were not detected in 140,915 adult female mosquitoes other than *Cs. melanura,* 13,767 adult male mosquitoes including 2,390 *Cs. melanura,* in 9,791 mosquitoes reared from immature stages, or in 47,034 other hematophagous Diptera.

## INTRODUCTION

Human disease caused by indigenous arboviruses has not been recognized in Connecticut, although cases of eastern equine (EEE), St. Louis, and California encephalitis have been reported in neighboring states (Deibel et al. 1975, 1977; Grady et al. 1978). Sporadic epizootics of EEE among equines and domestic birds indicate the presence of the virus in Connecticut and illustrate the need for continued surveillance (Bryant et al. 1973; Wallis and Main 1974). Arbovirus surveillance in the state has been

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a cooperative effort by personnel at the University of Connecticut Department of Pathobiology, the Connecticut Agricultural Experiment Station, the State Department of Health and the Yale University School of Medicine. The present series of papers summarizes the results of 10 years of arbovirus surveillance in arthropods and, to a lesser extent, in birds in Connecticut by the Yale Arbovirus Research Unit Section of Medical Entomology and the State Mosquito Control Section

## MATERIALS AND METHODS

Mosquitoes and other hematophagous flies, and birds were collected and processed by standard arbovirus techniques (Sudia and Chamberlain 1967, Sudia et al. 1970). Most of the arthropods tested were collected with CDC or New Jersey (A. C. and D. C.) light traps supplemented with dry ice and set in strategic locations in southcentral Connecticut. Trapping sites were selected initially because of a history of EEE activity in domestic animals in the area. In 1974, permanent study sites were established along both sides of the Hammonasset River (Fig. 1) where *Culiseta melanura*, *Aedes abserratus*, and *Ae. triseriatus* breeding was known to occur. Other sites were maintained where mosquito populations and/or virus isolations deemed trapping advisable. A 2nd important source of mosquitoes was a series of shelters in Farmington (Hartford County) Connecticut (Fig. 1) where resting *Anopheles*, *Culex*, and *Culiseta* species were collected with aspirators (Wallis 1953, 1957, 1959; Wallis et al. 1958, 1974). Other sources of

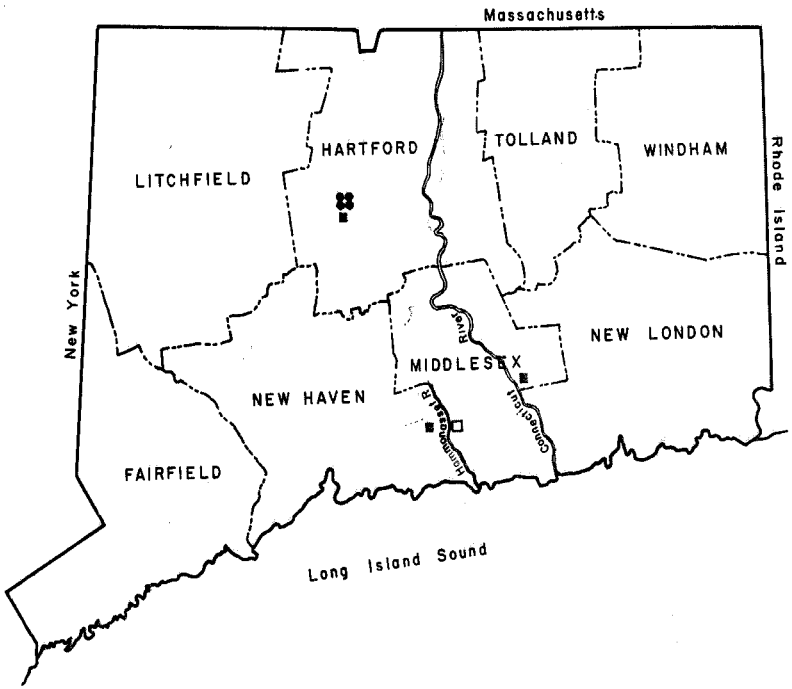


Figure 1. The locations of group A arbovirus isolations from mosquitoes and wild birds in Connecticut, 1969 through 1978.

Legend: • isolation of eastern equine encephalomyelitis virus from *Culiseta melanura*.
■ isolation of western equine encephalomyelitis virus from *Culiseta melanura*.
□ isolation of western equine encephalomyelitis virus from *Pipilo erythrophthalmus*.

arthropods included biting and landing collections, natural and artificial shelter collections, and bait traps. Immature stages were brought into the laboratory where they were reared to the adult stage for testing.

Insects were lightly anesthetized with chloroform and identified to species under a stereoscopic microscope. They were pooled by species, sex, location, and date of collection and frozen at −70°C. Pools of one to 100 specimens were triturated in sterile mortars containing alundum, as an abrasive, and 2 ml of either 0.75% bovine albumin or 10% fetal calf serum in phosphate-buffered saline (pH 7.2) with penicillin and streptomycin. Suspensions were centrifuged at 1600 X g for 20 min and 0.02 ml of the supernatant injected intracerebrally into each of eight 1- to 4-day old Swiss mice (Sudia and Chamberlain 1967).

In 1974, birds were captured in Japanese mist nets set in the Hammonasset River basin. Blood samples were collected from the jugular vein (Sudia et al. 1970) and diluted 1:2 or 1:5 with 0.75% bovine albumin containing 0.025 mg/ml heparin. Samples were centrifuged and most of the serum stored at −20°C for serology; the cells and remaining serum were rediluted and frozen at −70°C for virus isolation attempts in suckling mice.

Virus isolations were identified by complement-fixation, hemagglutination-inhibition (HI), and/or neutralization tests. Complement-fixation tests were done on microtiter plates using 0.025 ml each of either sucrose-acetone extracted antigens or infected mouse brain in veronal buffer (2-fold serial dilutions), guinea pig complement (2 units), and immune mouse ascitic fluids (2-fold serial dilutions). Plates were incubated 18 hr at 4°C before 0.025 ml of hemolysin (1:800) and sheep cells (1:25) were added. Hemagglutination-inhibition tests were also done in microtiter plates using 0.025 ml of 4 to 8 units of sucrose-acetone extracted antigen and an equal volume of serial 2-fold dilutions of acetone-extracted sera. The plates were incubated at 4°C for 18 hr before 0.05 ml of goose cells diluted 1:24 in the appropriate pH adjusting diluents was added (Clarke and Casals 1958). Neutralization tests were assayed in suckling mice using equal volumes of serum and serial 10-fold virus dilutions incubated 1 hr at 37°C.

## RESULTS

A total of 157,646 adult female mosquitoes were tested during the 10-year period covered by this study (1969 through 1978); group A arboviruses were isolated only from *Cs. melanura* (Table 1). Of the 16,731 female *Cs. melanura* tested, half were from the shelters in Farmington and half from light trap collections; EEE virus was recovered from 4 pools and western equine encephalitis (WEE) virus from three. All of the EEE positive mosquitoes were from the resting collections in Farmington during September (1970, 1972, and 1973) and October (1972).

Two of the WEE isolates were from light trap collections (East Haddam, September 1972; Madison, September and October 1978) and 1 from the Farmington shelters (August 1973). This virus was also recovered from the blood of an adult female rufous-sided towhee (*Pipilo erythrophthalmus*) netted on 9 September 1974 in Killingworth (Fig. 1). The 1st mosquito isolate (72666) and the bird isolate (B-8-74) were examined serologically and found to be WEE subtype II.

Group A arboviruses were not recovered from 140,915 adult female mosquitoes other than *Cs. melanura,* 13,767 adult male mosquitoes including at least 2,390 *Cs. melanura,* or from 9,791 mosquitoes reared from immature stage (Table 2). Evidence of group A arboviruses was not detected in 4,545 Tabanidae, 1,999 Simuliidae, 39,825 Ceratopogonidae (*Culicoides* spp.), or 665 Rhagionidae (*Symphoromyia* spp.) (Table 2).

Although both EEE and WEE HI antibodies were demonstrated in birds netted along the Hammonasset River during 1974 (Table 3), there was no

Table 1. Adult female mosquitoes from Connecticut, 1969 through 1978, tested for the presence of group A arboviruses.

| Mosquitoes | YEAR 1969 | 1970 | 1971 | 1972 | 1973 | 1974 | 1975 | 1976 | 1977 | 1978 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Culiseta melanura* | | | | | | | | | | | |
| resting collections | 1149 | 1314[E] | 1273 | 2830[EE] | 797[EW] | 183 | 134 | 404 | 126 | 176 | 8386[EEEEW] |
| light trap collections | 16 | 87 | 2 | 63[W] | 65 | 174 | 1533 | 2292 | 724 | 3388[W] | 8345[WW] |
| *Culiseta morsitans* | 2063 | 856 | 706 | 170 | 93 | 48 | 159 | 449 | 142 | 229 | 4915 |
| *Culex* spp.* | 5812 | 1655 | 1483 | 1486 | 1348 | 2158 | 2455 | 4862 | 591 | 2852 | 24702 |
| *Coquillettidia perturbans* | 1 | 214 | 6 | 12 | 1165 | 1587 | 2519 | 19197 | 1963 | 2 | 26666 |
| *Orthopodomyia signifera* | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 | 4 |
| *Uranotaenia sapphirina* | 331 | 125 | 154 | 59 | 36 | 218 | 313 | 546 | 122 | 0 | 1904 |
| *Psorophora* spp.** | 130 | 0 | 0 | 0 | 3 | 0 | 35 | 75 | 0 | 0 | 243 |
| *Aedes* spp.*** | 5494 | 3549 | 3492 | 651 | 5797 | 3353 | 11639 | 29846 | 9230 | 5369 | 78420 |
| *Anopheles* spp.**** | 743 | 703 | 94 | 808 | 48 | 120 | 260 | 635 | 649 | 1 | 4061 |
| Total Mosquitoes | 15739 | 8503[E] | 7210 | 6079[EEW] | 9354[EW] | 7841 | 19048 | 58307 | 13548 | 12017[W] | 157646[EEEEWWW] |

E=eastern equine encephalomyelitis virus isolate; W=western equine encephalomyelitis virus isolate.

* Includes 3477 *pipiens*, 7655 *restuans*, 5318 *salinarius*, 841 *territans*, and 7411 unidentified *Culex*.

** Includes 1 *ciliata* and 242 *ferox*.

*** Includes 6564 *abserratus*, 615 *aurifer*, 22392 *canadensis*, 8047 *cantator*, 5344 *cinereus*, 15 *communis*, 9 *intrudens*, 1475 *sollicitans*, 8 *sticticus*, 3023 *stimulans* group, 1 *provocans*, 4877 *triseriatus*, 628 *trivittatus*, 25353 *vexans*, and 69 unidentified *Aedes*.

**** Includes 10 *crucians*, 3305 *punctipennis*, 680 *quadrimaculatus*, 63 *walkeri*, and 3 unidentified *Anopheles*.

Table 2. Connecticut Diptera tested for group A arboviruses, 1969 through 1978.

| Genera | No. of Species | Immatures | Adult Males | Adult Females |
|---|---|---|---|---|
| CULICIDAE | | | | |
| *Anopheles* | 4 | 0 | 67 | 4061 |
| *Aedes* | 16 | 9742 | 5810 | 78420 |
| *Coquillettidia* | 1 | 0 | 143 | 26666 |
| *Culex* | 4 | 36 | 4445 | 24702 |
| *Culiseta* | 2 | 13 | 2912 | 21646* |
| *Orthopodomyia* | 1 | 0 | 0 | 4 |
| *Psorophora* | 2 | 0 | 3 | 243 |
| *Uranotaenia* | 1 | 0 | 387 | 1904 |
| TABANIDAE | | | | |
| *Chrysops* | 22 | 0 | 3 | 4255 |
| *Hybomitra* | 4 | 0 | 0 | 179 |
| *Tabanus* | 9 | 0 | 23 | 85 |
| SIMULIIDAE | | | | |
| *Cnephia* | 1 | 0 | 0 | 21 |
| *Prosimulium* | 3 | 0 | 0 | 1124 |
| *Simulium* | 6 | 0 | 0 | 854 |
| CERATOPOGONIDAE | | | | |
| *Culicoides* | 11 | 152 | 0 | 39673 |
| RHAGIONIDAE | | | | |
| *Symphoromyia* | 1 | 0 | 0 | 665 |

* Includes 4 isolates of eastern equine encephalomyelitis virus and 3 isolates of western equine encephalomyelitis virus from adult female *Culiseta melanura*.

evidence—by virus isolation, seroconversions, or antibody in immature birds—of concurrent transmission of EEE virus. However, in addition to the virus isolation, WEE antibody was detected in 4 of 13 (30.8%) immature towhees indicating current virus transmission during 1974 (Table 3). WEE antibody rates were 28.8% in permanent resident species and 25.8% in summer resident species while antibody was not detected in fall migrants or winter residents suggesting that birds were being infected in Connecticut, not farther north. Nearly twice as many positive sera were found using a local WEE antigen (72666 or B-8-74) than with a prototype strain (McMillan); in addition, geometric mean titers were more than 8-fold greater (Table 4).

The etiologic agent of a nonfatal case of WEE in an adult male Connecticut resident was serologically diagnosed as Subtype I (Table 5). These results, plus a travel history, suggested that the patient was infected in western United States (F.J. Bia, unpublished).

## DISCUSSION

Except for a widespread epizootic of EEE virus among domestic animals during 1972, there was very little overt activity of group A arboviruses in Connecticut during the 10-year period covered by this study. The 1st evidence of the 1972 outbreak occurred in a pheasant flock in Meriden, Connecticut in late August; by the end of the mosquito season in late October, EEE virus had been demonstrated in at least 8 horses, 24 pheasant flocks, plus other birds including turkeys, quails, doves, and house sparrows throughout much of the central and northeastern parts of the state (Bryant et al. 1973). Prior to the epizootic, an increase in the *Cs. melanura* population was noted in Farmington (Wallis et al. 1974). Populations of *Cs. melanura* increased rapidly in July reaching a peak during the 3rd week to a level 12 times greater than the average number collected for that week during the 4 years prior to 1972. This peak of *Cs. melanura*

Table 3. Hemagglutination-inhibition antibody in wild birds caught along the Hammonasset River, Connecticut, fall 1974.

| Species Name | Immatures | | Unknown or Adult | | Total | |
|---|---|---|---|---|---|---|
| | EEE | WEE | EEE | WEE | EEE | WEE |
| 1) PERMANENT RESIDENTS | | | | | | |
| Yellow-shafted Flicker | | | 0/2* | 0/2 | 0/2 | 0/2 |
| Hairy Woodpecker | | | 0/1 | 0/1 | 0/1 | 0/1 |
| Downy Woodpecker | | | 0/3 | 0/3 | 0/3 | 0/3 |
| Blue Jay | | | 5/12 | 6/12 | 5/12 | 6/12 |
| Black-capped Chickadee | 0/1 | 0/1 | 1/20 | 8/20 | 1/21 | 8/21 |
| Tufted Titmouse | | | 0/2 | 0/2 | 0/2 | 0/2 |
| White-breasted Nuthatch | | | 0/2 | 1/2 | 0/2 | 1/2 |
| Brown Creeper | | | 0/1 | 1/1 | 0/1 | 1/1 |
| Rufous-sided Towhee | 0/13 | 4/13 | 3/19 | 11/19 | 3/32 | 15/32 |
| Hermit Thrush | | | 0/1 | 0/1 | 0/1 | 0/1 |
| Field Sparrow | | | 0/7 | 0/7 | 0/7 | 0/7 |
| Song Sparrow | 0/1 | 0/1 | 1/14 | 2/14 | 1/15 | 2/15 |
| Swamp Sparrow | 0/5 | 0/5 | 0/15 | 0/15 | 0/20 | 0/20 |
| 2) SUMMER RESIDENTS | | | | | | |
| Eastern Phoebe | | | 0/9 | 1/9 | 0/9 | 1/9 |
| Catbird | | | 3/10 | 4/10 | 3/10 | 4/10 |
| Wood Thrush | | | 0/4 | 3/4 | 0/4 | 3/4 |
| Nashville Warbler | | | 0/1 | 0/1 | 0/1 | 0/1 |
| Ovenbird | 0/2 | 0/2 | 0/1 | 0/1 | 0/3 | 0/3 |
| Canada Warbler | 0/1 | 0/1 | 0/3 | 0/3 | 0/4 | 0/4 |
| 3) MIGRANTS | | | | | | |
| Swainson's Thrush | | | 0/9 | 0/9 | 0/9 | 0/9 |
| Magnolia Warbler | 0/1 | 0/1 | | | 0/1 | 0/1 |
| Myrtle Warbler | | | 0/100 | 0/100 | 0/100 | 0/100 |
| 4) WINTER RESIDENTS | | | | | | |
| Slate colored Junco | | | 0/5 | 0/5 | 0/5 | 0/5 |
| White throated Sparrow | 0/2 | 0/2 | 0/23 | 0/23 | 0/25 | 0/25 |
| TOTALS | | | | | | |
| 1) Permanent residents | 0/20 | 4/20 | 10/99 | 30/99 | 10/119 | 34/119 |
| Percent | 0.0 | 20.0 | 10.1 | 30.3 | 8.4 | 28.8 |
| 2) Summer residents | 0/3 | 0/3 | 3/28 | 8/28 | 3/31 | 8/31 |
| Percent | 0.0 | 0.0 | 10.7 | 28.6 | 9.7 | 25.8 |
| 3) Migrating birds | 0/1 | 0/1 | 0/109 | 0/109 | 0/110 | 0/110 |
| Percent | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 4) Winter residents | 0/2 | 0/2 | 0/28 | 0/28 | 0/30 | 0/30 |
| Percent | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total | 0/26 | 4/26 | 13/264 | 38/264 | 13/290 | 42/290 |
| Percent | 0.0 | 15.4 | 4.9 | 14.4 | 4.5 | 14.5 |

* Number positive/number tested.
EEE=eastern equine encephalomyelitis antibody.
WEE=Western equine encephalomyelitis antibody.

occurred 4 weeks prior to the initial case of EEE in pheasants. Virus was recovered from the *Cs. melanura* in Farmington later in the season, and it was suggested that monitoring the populations of this species early in the summer might be effective in predicting virus activity.

Very little EEE virus activity was detected in mosquitoes from Connecticut in other years when human cases were

Table 4. Comparison of western equine encephalitis antigens (McMillan and B–8–74 or 72666) in hemagglutination-inhibition tests with sera from 291 birds netted in Killingworth, Connecticut in 1974. Only positive sera are presented here.

| Specimen No. | McMillan (California) | USA B–8–74/USA 72666 (Connecticut) | Difference |
|---|---|---|---|
| B–6–74 | 1/10 | 1/40 | 4 × |
| 7 | 1/10 | 1/160 | 16 × |
| 9 | <1/10 | 1/80 | >8 × |
| 10 | <1/10 | 1/20 | >2 × |
| 11 | 1/10 | 1/20 | 2 × |
| 12 | 1/10 | 1/20 | 2 × |
| 13 | 1/10 | 1/80 | 8 × |
| 16 | <1/10 | 1/20 | >2 × |
| 21 | <1/10 | 1/20 | >2 × |
| 22 | <1/10 | 1/80 | >8 × |
| 23 | 1/10 | 1/80 | 8 × |
| 25 | 1/40 | >1/320 | >8 × |
| 26 | 1/10 | 1/80 | 8 × |
| 29 | <1/10 | 1/160 | >16 × |
| 32 | <1/10 | 1/10 | >1 × |
| 33 | 1/10 | >1/320 | >32 × |
| 36 | <1/10 | 1/10 | >1 × |
| 40 | <1/10 | 1/40 | >4 × |
| 44 | 1/10 | 1/40 | 4 × |
| 52 | 1/20 | >1/80 | >4 × |
| 53 | 1/10 | >1/80 | >4 × |
| 55 | <1/10 | >1/80 | >8 × |
| 76 | <1/10 | 1/10 | >1 × |
| 78 | <1/10 | 1/10 | >1 × |
| 80 | <1/10 | 1/20 | >2 × |
| 90 | <1/10 | 1/40 | >4 × |
| 91 | <1/10 | >1/80 | >8 × |
| 92 | 1/20 | 1/80 | 4 × |
| 99 | 1/20 | >1/80 | >4 × |
| 104 | 1/10 | >1/80 | >8 × |
| 106 | <1/10 | 1/40 | >4 × |
| 107 | 1/40 | >1/80 | >2 × |
| 108 | <1/10 | 1/20 | >2 × |
| 147 | 1/20 | >1/80 | >4 × |
| 154 | 1/20 | >1/80 | >4 × |
| 201 | 1/10 | >1/80 | >8 × |
| 218 | 1/10 | >1/80 | >8 × |
| 224 | 1/20 | >1/80 | >4 × |
| 225 | <1/10 | >1/80 | >8 × |
| 294 | <1/10 | >1/80 | >8 × |
| 295 | 1/10 | 1/80 | 8 × |
| 183 | 1/10 | >1/40 | >4 × |
| Total positive | 23 | 42 | |
| Total negative | 19 | 0 | |
| Total | 42 | 42 | |
| Geometric mean titer | 8.6 | 72.9 | 8.4 |

<=less than.
>=equal to or greater than.

Table 5. Complement-fixation (CF) and hemagglutination-inhibition (HI) titers of acute and convalescent sera from an adult male with encephalitis.

| Antigen | Acute Serum 12.VIII.77 CF | HI | Convalescent Serum 2.IX.77 | |
|---|---|---|---|---|
| Western Equine Encephalomyelitis | | | | |
| Connecticut strain | <1:4 | 1:160 | <1:4 | 1:320 |
| California strain | 1:32 | ≥1:1280 | 1:512 | ≥1:1280 |
| Eastern Equine Encephalomyelitis | <1/4 | <1:10 | <1:4 | <1:10 |
| Venezuelan Equine Encephalitis | <1:4 | NT | <1:4 | NT |

NT=not tested.

occurring in Massachusetts (Grady et al. 1978).

WEE virus subtype II has not been associated with disease in man or equines (Hayes and Wallis 1977). This virus was isolated for the first time in Connecticut during 1972. Serological studies with avian and human sera demonstrated the distant antigenic relationships between WEE subtypes I and II and illustrated the need for using local antigens in serosurveys.

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