

ARBOVIRUS SURVEILLANCE IN CONNECTICUT. III. FLANDERS VIRUS¹

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ABSTRACT. Flanders virus was recovered from 75 pools of mosquitoes—36 *Culiseta melanura*, 8 *Culex pipiens*, 25 *Cx. restuans*, 3 *Cx. salinarius*, 2 *Cx. (Culex)* species, and 1 *Coquillettidia perturbans*—in Connecticut. Virus has been isolated each year from 1969 through 1978. Minimum field infection rates for the 10-year period were higher in 3 ornithophilic species—*Cs. melanura* (1:465), *Cx. pipiens* (1:435), and *Cx. restuans* (1:306)—than in 2 general feeders—*Cx. salinarius* (1:1773) and *Cq. perturbans* (1:26666). Virus was demonstrated in mosquitoes collected during the

months of June, July, August, and September, and isolation rates followed the population curves for *Cs. melanura* and *Culex (Culex)* species.

Flanders virus was not recovered from 84,632 adult female mosquitoes of species other than *Culiseta*, *Culex*, and *Coquillettidia*; from 13,767 adult male mosquitoes including 2912 *Culiseta* spp., 4445 *Culex* spp., and 143 *Cq. perturbans*, from 9791 reared from larvae mosquitoes (mostly *Aedes* spp.), or from 47,034 hematophagous flies other than mosquitoes.

INTRODUCTION

Flanders virus was reported for the first time from *Culiseta melanura*, *Culex pipiens*, and an ovenbird (*Seiurus aurocapillus*) near Peconic Bay, Long Island, New York in 1961 and 1962 (Whitney 1964). Since that time, this virus, or Hart Park, a closely related virus, has been recovered from naturally infected mosquitoes, usually ornithophilic species of the genera *Culiseta* and *Culex*, and from birds throughout much of the United States and parts of Canada, including Alabama, Arkansas, California, Florida, Georgia, Illinois, Iowa, Kentucky, Massachusetts, Missouri, New Jersey, North Carolina, Ohio, Tennessee, Texas, Utah, and Saskatchewan (Whitney 1964, Johnson 1965, Mack et al. 1967, Sudia et al. 1967, Hall et al. 1969, Kokernot et al. 1969, Crane et al. 1970, Boyd 1972, Berge 1975, Wong et al. 1978).

This paper summarizes the results of 75 isolations of Flanders virus from mosquitoes collected in Connecticut during the 10-year period 1969 through 1978.

MATERIALS AND METHODS

The materials and methods used in these studies were described in detail in Part I of the series (Main et al. 1979).

RESULTS

Flanders virus was isolated from 75 pools of mosquitoes in Connecticut from 1969 through 1978 (Table 1). The largest number (36) of virus isolations were from *Cs. melanura*, although the minimum field infection rates were higher in *Cx. pipiens* (1:435) and *Cx. restuans* (1:306) than in *Cs. melanura* (1:465). Extremely low infection rates were observed with *Cx. salinarius* (1:1773) and *Coquillettidia perturbans* (1:26666).

Isolations of this virus were made from mosquitoes in all areas where large numbers of *Culiseta* and *Culex* species were collected. Virus was demonstrated in New Haven (33 strains), Hartford (28),

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Table 1. Adult female mosquitoes from Connecticut tested for the presence of Flanders virus, 1969 through 1978.

	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	Total
<i>Culiseta melanura</i>	1165(2)*	1401(4)	1275(2)	2893(13)	862(1)	357(1)	1667(6)	2696(2)	851	3564(5)	16731(36)
<i>Culiseta morsitans</i>	2063	856	706	170	93	48	159	449	142	229	4915
<i>Culex pipiens</i>	0	0	1369(2)	811(1)	143	397	201(4)	239	55	262(1)	3477(8)
<i>Culex restuans</i>	0	10	48	541(2)	273	1029(2)	775(6)	3361(5)	359(2)	1259(8)	7655(25)
<i>Culex salinarius</i>	0	0	3	19	556(2)	711	1419(1)	1124	155	1331	5318(3)
<i>Culex (Culex) spp.^a</i>	5365	1580(2)	54	48	364	0	0	0	0	0	7411(2)
<i>Culex territans</i>	447	65	9	67	12	21	60	138	22	0	841
<i>Coquillettidia perturbans</i>	1	214	6	12	1165	1587	2519	19197(1)	1963	2	26666(1)
<i>Anopheles</i> species ^b	743	703	94	808	48	120	260	635	649	1	4061
<i>Aedes</i> species ^c	5494	3549	3492	651	5797	3353	11639	29846	9230	5369	78420
<i>Psorophora</i> species ^d	130	0	0	0	3	0	35	75	0	0	243
<i>Uranotartaria sapphirina</i>	331	125	154	59	36	218	313	546	122	0	1904
<i>Orthopodomyia signifera</i>	0	0	0	0	2	0	1	1	0	0	4
Total Mosquitoes	15739(2)	8503(6)	7210(4)	6079(16)	9354(3)	7841(3)	19048(17)	58307(8)	13548(2)	12017(14)	157647(75)

^a Number of specimens tested (number of positive pools in parenthesis).

^b Includes *pipiens*, *restuans*, and *salinarius*.

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

^d Includes 6564 *aberratus*, 615 *aurifer*, 22392 *canadensis*, 8047 *cantator*, 5344 *cinerus*, 15 *communis*, 9 *intrudens*, 1475 *sollittians*, 8 *sticticus*, 3023 *stimulans* group, 1 *provocans*, 4877 *triseriatus*, 628 *trivittatus*, 25353 *veans*, and 69 unidentified *Aedes*.

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

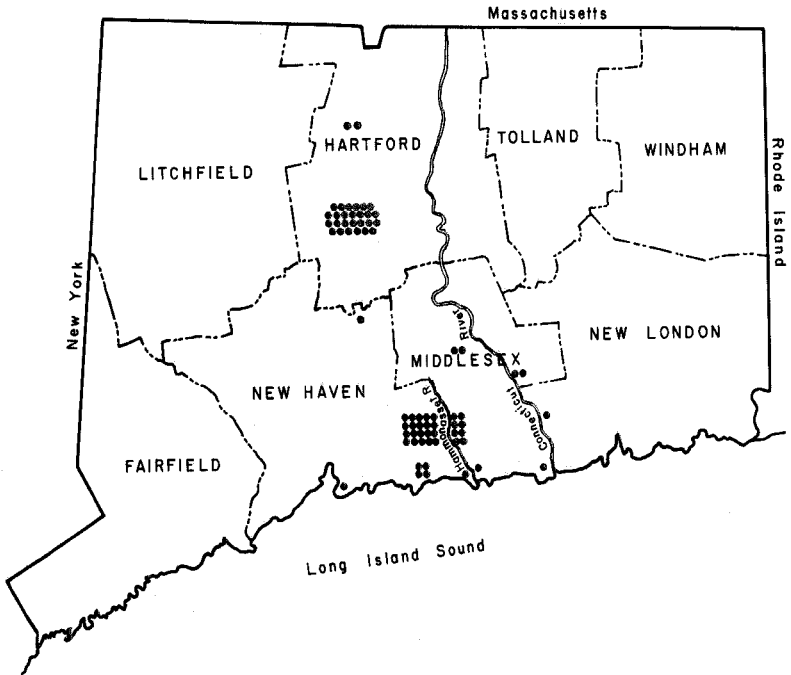


Figure 1. The locations of isolations of Flanders virus from mosquitoes in Connecticut, 1969 through 1978.

Legend • Flanders virus isolation.

Middlesex (13), and New London (1) Counties (Fig. 1). The largest number of isolations (28) was from mosquitoes taken in light traps in the Hammonasset River Basin study sites in Madison and Killingworth, but nearly as many (26) were from resting collections in natural shelters in Farmington.

The earliest isolations of the year were from *Cx. restuans* collected in June (1972, 1978); the majority were from mosquitoes taken in July and August and the latest, from specimens captured in September.

Flanders virus was not detected in 84,632 adult female mosquitoes of species other than *Culiseta*, *Culex*, and *Coquillettidia* (Table 1), from 13,767 adult male mosquitoes including 2912 *Culiseta* spp., 4445 *Culex* spp., and 143 *Cq. perturbans* (Table 2), from 9791 mosquitoes reared

from field collected larvae (mostly *Aedes* spp.) (Table 2), or from 47,034 hematophagous flies other than mosquitoes (Table 3).

Three strains of virus—1 from *Cs. melanura* (Ar 274-74) and 2 from *Cx. restuans* (Ar-228-74; Ar-379-74)—isolated from Connecticut mosquitoes in 1974 were selected for comparison by complement-fixation tests with the prototype strains of Flanders (61-7484) and Hart Park (Ar 70). All three isolates were identical to each other and to the prototype Flanders, but clearly distinct from the Hart Park prototype (Table 4).

DISCUSSION

Members of the Flanders/Hart Park virus complex are found throughout

Table 2. Immature and adult male mosquitoes from Connecticut tested for Flanders virus,* 1974-1978.

	Immatures**						Males			
	1974	1975	1976	1977	1978	Total	1976	1977	1978	Total
<i>Culiseta melanura</i>	0	0	0	0	0	0	2238	139	13	2390
<i>Culiseta morsitans</i>	2	0	11	0	0	13	258	59	24	341
<i>Culiseta</i> species unknown	0	0	0	0	0	0	0	181	0	181
<i>Culex pipens</i>	0	0	0	0	0	0	43	0	1	44
<i>Culex restuans</i>	0	0	34	0	0	34	3860	5	2	3867
<i>Culex salinarius</i>	0	0	0	0	0	0	90	1	0	91
<i>Culex territans</i>	0	0	2	0	0	2	291	4	0	295
<i>Culex</i> species unknown	0	0	0	0	0	0	0	148	0	148
<i>Coquillettidia</i> <i>perturbans</i>	0	0	0	0	0	0	93	48	2	143
<i>Aedes</i> species	195	8202	1163	6	176	9742	5459	314	37	5810
<i>Psorophora ferox</i>	0	0	0	0	0	0	3	0	0	3
<i>Uranotaenia sapphirina</i>	0	0	0	0	0	0	310	77	0	387
<i>Anopheles</i> species	0	0	0	0	0	0	38	29	0	67
Total Mosquitoes	197	8202	1210	6	176	9791	12683	1005	79	13767

* All negative.

** Reared in the laboratory and tested as adults.

much of the United States and parts of Canada (Boyd 1972, Berge 1975). Boyd (1972) demonstrated that strains from 12 states east of the Mississippi River and Texas were serologically similar or identical to the prototype Flanders isolate from Long Island, New York, but distinct from the prototype Hart Park from California. Our studies showed similar results by complement-fixation tests with 3 isolates from Connecticut, although this was not surprising because the infected mosquitoes were collected less than 100 km from the type locality of Flanders virus (Whitney 1964).

Flanders virus was prevalent in Connecticut and was isolated from mosquitoes each year of this study (1969-1978). It was recovered wherever large numbers of *Cs. melanura*, *Cx. pipiens*, or *Cx. restuans* were collected. Infection rates were highest in these 3 ornithophilic species, indicating that birds are probably important vertebrate hosts. This hypothesis is supported by the large number of isolations from ornithophilic mosquito species in other parts of the country (Boyd 1972, Berge 1975), and virus isola-

tions from the spleen of an ovenbird and the liver of a starling (*Sturnus vulgaris*) in New York State (Whitney 1964, Berge 1975) and from the blood of house sparrows (*Passer domesticus*) and red-winged blackbirds (*Agelaius phoeniceus*) in Illinois (Kokernot et al. 1969). Hart Park virus was recovered from the blood of a yellow-headed blackbird (*Xanthocephalus xanthocephalus*), a tricolored blackbird (*A. tricolor*), a house finch (*Carpodacus mexicanus*) and house sparrows in California (Johnson in Berge 1975). The isolations of Flanders virus in Connecticut from 3 pools of *Cx. salinarius* and 1 pool of *Cq. perturbans* probably represents spillover from an avian cycle. Both species are general feeders that do engorge on birds although they appear to prefer mammals (Hayes 1961, Crans 1964, Edman 1971, 1975; Magnarelli 1977). The minimum field infection rates for both of these species were low. However, while field isolations do suggest a bird-*Culiseta* or *Culex*-bird cycle, laboratory studies are necessary in order to demonstrate that *Cs. melanura*, *Cx. pipiens*, and *Cx. restuans* transmit the virus.

Table 3. Biting flies other than mosquitoes from Connecticut, 1972-1978, tested for Flanders virus infections.*

	1972	1973	1974	1975	1976	1977	1978	Total
Females								
Tabanidae								
<i>Chrysops</i> species	0	0	0	2590	1639	25	1	4255
<i>Hybomitra</i> species	0	0	0	47	38	0	0	85
<i>Tabanus</i> species	0	0	0	15	152	11	1	79
Simuliidae								
<i>Cnephia</i> species	0	0	0	0	20	1	0	21
<i>Prosimulium</i> species	0	0	0	0	1103	21	0	1124
<i>Simulium</i> species	0	0	0	339	488	27	0	854
Ceratopogonidae								
<i>Culicoides</i> species	0	0	0	0	39477	196	0	39673
Rhagionidae								
<i>Symphoromyia</i> species	2	0	0	0	663	0	0	665
Males								
Tabanidae								
<i>Chrysops</i> species	0	0	0	0	0	3	0	3
<i>Tabanus</i> species	0	0	0	0	23	0	0	23
Larvae								
Ceratopogonidae								
<i>Culicoides</i> species	0	0	0	152	0	0	0	152

* All negative.

Seasonal prevalence of virus isolations from adult female mosquitoes followed the population curves of *Cs. melanura* and *Culex* mosquitoes of the subgenus *Culex* in southern New England. Populations of host-seeking female mosquitoes of these species increase in late spring and early summer, peak in July and August, and decline in the autumn (Wallis 1960, Main et al. 1968). We have no data for virus maintenance cycles during other times of the year. In New England, these species

overwinter as larvae (*Cs. melanura*) or as inseminated adult females (*Culex* species). We did not find evidence of transovarial transmission by testing adult male mosquitoes with the same techniques by which the females were assayed. The lack of virus isolations from males may reflect the numbers tested; however, during 1976, 2238 male *Cs. melanura* and 3860 male *Cx. restuans* were examined; minimum infection rates for female mosquitoes taken in the same traps during

Table 4. Serologic identification of virus strains Ar-228-74, Ar-274-74, & Ar-379-74 from Connecticut mosquitoes.

Antigen	Hyperimmune Mouse Ascitic Fluid				
	Hart Park (Ar 70)	Flanders (61-7484)	Ar-228-74	Ar-274-74	Ar-379-74
Hart Park (Ar 70)	128/64*	32/64	128/64	128/64	128/64
Flanders (61-7484)	32/32	256/64	512/64	256/64	512/128
Ar-228-74	16/64	256/64	512/64	256/64	256/128
Ar-274-74	16/32	256/64	512/64	256/64	512/128
Ar-379-74	32/32	256/64	1024/64	512/64	512/64

* Reciprocal of serum titer/Reciprocal of antigen titer.

that year were 1:1348 and 1:672, respectively.

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