# RECENT ISOLATIONS OF ARTHROPOD-BORNE VIRUSES FROM MOSQUITOES IN EASTERN UNITED STATES

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A number of isolations of arthropod-borne viruses have been made from field-caught mosquitoes in eastern United States in recent years. Howitt's recovery of the virus of eastern encephalitis (EE) from *Mansonia perturbans* in Georgia in 1948 was the first reported (1). This was followed in 1950 by an isolation of EE virus from *Culiseta melanura* collected in a Louisiana swamp (2). Four additional isolations were made from this swamp, one of western encephalitis (WE) from *C. melanura* and two of WE from *Aedes infirmatus* in 1952, and one of EE from *Anopheles crucians* in 1953 (3).

Holden made two (possibly three) isolations of EE virus in 1953 from pools of *Culiseta melanura* collected in a swampy habitat on a pheasant farm in New Jersey (4). In 1955 the virus of St. Louis encephalitis was recovered from engorged *Culex pipiens* in Kentucky (5), and of WE from engorged *C. pipiens* in North Carolina (present report). In 1956 two isolations of EE were made from engorged *Culex salinarius,* and three of EE and one of WE from *Culiseta melanura* collected in New Jersey (present report). In this same year five other isolations of EE virus from *C. melanura* were made in Massachusetts (6), and one isolation each of EE virus from *Aedes mitchellae* and *Anopheles crucians* collected in Georgia (7).

Details concerning the 1955 North Carolina and the 1956 New Jersey isolations are presented here.

METHODS. The mosquitoes were collected alive in the field by aspirator from natural or artificial shelters of various types, in New Jersey light traps. They were from human or animal bait, or were taken killed by chloroform or freezing, sealed in glass tubes, and kept frozen at dry ice temperatures until tested for virus.

In the laboratory the specimens were thawed but kept chilled during identification under a dissecting microscope. They were pooled according to species, status of engorgement, and time and place of collection. The number of mosquitoes per pool ranged from 1 to 70. Each pool was ground in a chilled mortar in 2 ml. of 25 percent normal horse serum-buffered water, pH 7.8, containing 2 mg of streptomycin sulfate and 1,000 units of penicillin G sodium per ml. The suspensions were centrifuged 10 minutes at 2,500 r.p.m. in an angle-head centrifuge, the supernatants decanted, and each inoculated in .03 ml aliquots into six 3-week-old CFW mice via the intracerebral route. Brains of mice dying of infection were used as virus stock for identification by neutralization tests performed in mice. Most of the infected mosquito pools were titrated in mice to approximate the amount of virus the specimens contained.

VIRUS ISOLATIONS AND DISCUSSION. NORTH CAROLINA, 1955. An isolation of WE was made from a pool of 31 recently engorged mosquitoes of the *Culex pipiens-quinquefasciatus* complex collected from a chicken house on a farm in Bladen County, North Carolina. Because of the latitude in which they were captured the specimens were presumed to be *C. pipiens* rather than *C. quinquefasciatus.* The collection was made on October 6, 1955, by Mr. M. D. Bogue during an investigation of a current outbreak of EE in equines and pheasants in that vicinity. A "sick"

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TABLE 1—Mosquitoes collected July 5 to September 25, 1956, in New Jersey and tested for virus

| Mosquito species | Total engorged * | Total non engorged | Total mosquitoes | Total pools tested |
|---|---|---|---|---|
| *Aedes* | | | | |
| *canadensis* | | 4 | 4 | 3 |
| *cantator* | 2 | 96 | 98 | 23 |
| *sollicitans* | 58 | 1390 | 1448 | 98 |
| *taeniorhynchus* | | 5 | 5 | 5 |
| *triseriatus* | 1 | | 1 | 1 |
| *vexans* | 5 | 175 | 180 | 50 |
| *Anopheles* | | | | |
| *crucians* | 5 | 3043 | 3048 | 132 |
| *quadrimaculatus* | 7 | 370 | 377 | 55 |
| *punctipennis* | | 2 | 2 | 2 |
| *Culex* | | | | |
| *pipiens* | 9 | 389 | 398 | 64 |
| *restuans* | 3 | 130 | 133 | 46 |
| *salinarius* | 158 | 4571 | 4729 | 183 |
| *territans* | | 71 | 71 | 37 |
| *Culiseta* | | | | |
| *melanura* | 71 | 1807 | 1878 | 190 |
| *morsitans?* | | 1 | 1 | 1 |
| *Mansonia* | | | | |
| *perturbans* | 14 | 790 | 804 | 84 |
| *Orthopodomyia* | | | | |
| *signifera* | | 1 | 1 | 1 |
| *Psorophora* | | | | |
| *ciliata* | | 1 | 1 | 1 |
| *Uranotaenia* | | | | |
| *sapphirina* | | 381 | 381 | 26 |
| | 333 | 13,227 | 13,560 | 1,002 |

* Containing blood as evidence of recent feeding.

but recovering pony was on the premises at the time.

Taken in the same collection but negative for virus were two engorged *Culiseta melanura* and one unengorged and four engorged *Anopheles crucians*. A total of 22 other collections of mosquitoes, comprising 868 specimens in 16 species, taken resting in outbuildings or biting humans or horses on farms in Bladen, Cumberland, Brunswick, and Columbus counties, North Carolina, were all negative.

The portion of the state involved was characterized by flat pine woods and cypress swamps. Apparently without exception the premises where cases occurred and the mosquitoes were collected were within a mile or two of swampland. Of additional interest is the fact that a significant increase in mosquito breeding had occurred due to rains associated with fall hurricanes.

Laboratory studies have indicated that the *Culex pipiens-quinquefasciatus* group of mosquitoes are highly resistant or refractory to WE virus (8, 9). It is probable, therefore, that the virus isolated came from recently ingested blood rather than the tissue of any mosquito specimens in the pool. Since the collection was made in a chicken house, the blood was most likely that of chicken, but it is possible that it was from some other bird or mammal instead.

This isolation is the second of WE virus to be made from the Atlantic coast states. The first was from English sparrows in New Jersey in 1953 (10).

NEW JERSEY, 1956. A total of 13,560 mosquitoes were collected in New Jersey from July 5 to September 25, 1956, by Dr. Paul Burbutis. These were captured in 1-cubic-foot red resting boxes or New Jersey light traps in or near swampy habitats of the South River Game Farm (about 5 miles south of May's Landing, New Jersey), and from adjacent areas of similar terrain. A review of ecologic studies on EE, of which these collections were a part, is presented elsewhere (11).

These mosquitoes were shipped frozen on dry ice to the Virus Laboratory, Montgomery, Alabama, where they were identified and tested for virus in 1,002 pools. A list of the mosquitoes tested is given in table 1. Nineteen mosquito species were represented. The most abundant were *Aedes sollicitans, Anopheles crucians, Culex salinarius, Culiseta melanura,* and *Mansonia perturbans.*

Six mosquito pools yielded isolations of virus, as shown in table 2. Two of the pools containing EE virus were freshly engorged *Culex salinarius.* Since this species has been shown to be refractory to EEE infection in the laboratory (8), the virus isolated was presumed to be derived from recently ingested blood rather than the mosquito tissues.

The other four virus isolations were from *Culiseta melanura,* three of EE and one of WE. One of the EE-positive pools was composed of engorged specimens so that true mosquito infection in this instance was also questionable.

All the virus isolations were made from mosquitoes collected by light trap between July 31 and August 28, 1956, a period during which both EE and WE viruses were active in wild birds in the same area (12). The pool of engorged *C. melanura* which yielded EE virus was collected near Woodbine, New Jersey. All the other infected mosquitoes were taken on the South River Game Farm.

The recovery of EE virus from *C. melanura* further substantiates its role as an important vector. The isolation of WE virus is the second on record for this species, the first being from specimens captured in Louisiana in 1952 (3). This finding suggests the possibility that *C. melanura* may be an endemic vector of WE in the eastern part of the country.

SUMMARY. Several isolations of the viruses of eastern and western encephalitis from mosquitoes in the eastern part of the United States are reported. One isolation of WE was made in North Carolina in 1955 from a pool of engorged *Culex pipiens.* In New Jersey in 1956 two isolations of EE were made from engorged *Culex salinarius* and one from engorged *Culiseta melanura.* Pools of non-engorged

TABLE 2—Isolations of virus from mosquitoes collected by light trap in New Jersey, 1956

| Place of collection | Date collected | Mosquito species | No. mosq. in pool | Feeding status | Virus | Approx. mouse $LD_{50}$ of virus in mosq. pool | Titer of virus after mouse brain passage |
|---|---|---|---|---|---|---|---|
| South River Game Farm (SRGF) | 7/31–8/3/56 | *C. salinarius* | 4 | Engorged * | EE | $10^{2.2}$ | $10^{8.8}$ |
| SRGF | 8/7–10/56 | *C. salinarius* | 4 | Engorged | EE | $10^{1.8}$ | $10^{7.5}$ |
| SRGF | 8/16–17/56 | *C. melanura* | 16 | Non-engorged | EE | $10^{4.8}$ | $10^{8.3}$ |
| Woodbine | 8/17/56 | *C. melanura* | 6 | Engorged | EE | $10^{1.8}$ | $10^{7.6}$ |
| SRGF | 8/28/56 | *C. melanura* | 7 | Non-engorged | WE | $10^{3.1}$ | $10^{6.8}$ |
| SRGF | 8/28/56 | *C. melanura* | 20 | Non-engorged | EE | $10^{2.6}$ | $10^{8.5}$ |

* Containing blood as evidence of recent feeding.

*C. melanura* also yielded two isolations of EE and one of WE.

The possible significance of these isolations is discussed briefly.

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# A REVIEW OF THE MOSQUITO LARVAE OF FRANCE

## I. GENERA *Culiseta, Mansonia, Orthopodomyia,* AND *Uranotaenia*

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INTRODUCTION. In recent years the study of the taxonomy and bionomics of the culicine mosquitoes of Western Europe has declined. In fact, no work which includes all of the species found in this region has been published since 1931, when Martini's "Culicidae" in Lindner's, "Die Fliegen der Palaearktischen Region," was published. Marshall's work on the species occurring in Great Britain (1938), and Natvig's book on the species of the Scandinavian countries (1948) are excellent treatises, but they deal with relatively small geographical areas and, therefore, do not include all of the species found in the central and southern portions of the Continent. Séguy (1923) reviewed the species found within the geographical limits of France, and since that time only scattered papers which deal with the mosquitoes of certain specific areas of France have appeared in the literature.

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