

SURVEILLANCE OF ARBOVIRUS ACTIVITY IN IOWA DURING 1972-1975¹

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ABSTRACT. During a 4-year period (1972-1975), mosquitoes were collected, identified, and processed for virus isolation. Laboratory examinations of clinical specimens from suspected encephalitis cases were made to provide etiologic diagnosis. Arbovirus was isolated from 102 pools of mosquitoes (2 LaCrosse, 2 Cache Valley, 10 Flanders, 3 St. Louis

encephalitis, 83 Trivittatus, and 2 Western equine encephalitis viruses). Laboratory results confirmed recent infections with arbovirus in 44 human cases of diseases of the central nervous system. Chronological and geographical data, information on the mosquito species collected, and the viruses isolated are presented.

Considerable interest in arbovirus surveillance activity was generated in Iowa during the 1975 encephalitis season by the simultaneous occurrence of St. Louis encephalitis (SLE), Western equine encephalomyelitis (WEE), and California (LaCrosse) encephalitis (LAC). During August, and September, there was increased demand for entomological and epidemiological data as well as immediate need for laboratory diagnosis of clinically defined cases. Intensification of interest in arbovirus activity during an encephalitis outbreak is expected. However, arbovirus surveillance has been a continuous long-term activity of our laboratories. These surveillance activities for 1965 to 1971 have been reported (Wong et al. 1970, Wong et al. 1973). A statewide mosquito surveillance-arbovirus isolation program was conducted in 1971 and 1972 as a cooperative effort of the State Hygienic Laboratory, University of Iowa, and the Department of Entomology, Iowa State University. The results of the 1971 study have been summarized (Rowley et al. 1973).

In 1973 and 1974, our efforts were directed toward the mosquito *Aedes trivittatus* and its relationship with Trivittatus virus (TVT). The State Forest Nursery in Ames was the principal site for mosquito collection during this period. There have

been fluctuations in our mosquito-arbovirus surveillance because of budget limitations; but diagnostic services for the detection and laboratory confirmation of arbovirus encephalitis have remained available and unrestricted. This report summarizes mosquito-arbovirus research for the period from 1972 to and including 1975.

METHODS AND MATERIALS

Mosquitoes were collected in state parks, cities, recreational areas, and farms, using battery-operated CDC light traps baited with dry ice. Mosquito collection procedures and the processing and handling of mosquitoes were done according to standard techniques (Sudia and Chamberlain 1967). The mosquitoes were processed in pools of 1 to 100. The procedure described in an earlier paper (Wong et al. 1971) was followed for arbovirus isolation with use of suckling mice. The field infection ratio (FIR) for each mosquito species from a collection site was calculated by dividing the total number of specimens of a given species collected each month by the number of virus isolations from that species during the month.

Studies of human cases of encephalitis were initiated upon receipt of clinical specimens for examination from suspected cases of infection of the central nervous system. Sera were tested for LAC, SLE, and WEE complement fixing

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(CF) and neutralizing (N) antibodies. The microtiter LBCF test, using sucrose-acetone extracted infected mouse brain antigens, was used to detect CF antibody (USPHS 1965). N antibodies were determined by means of a microtiter tissue culture system using baby hamster kidney (BHK 21) cells (Lennette and Schmidt 1969). Occasionally, blood clots from young patients were inoculated into suckling mice for arbovirus isolation.

RESULTS AND DISCUSSION

During the 4 years discussed in this report, 136,807 mosquitoes were tested in 2,492 pools for the presence of arbovirus. Table 1 shows the number of mosquitoes and the collection sites for each of the 4 years. Approximately 80% of the total number of mosquitoes were collected and tested in 1972. About 20,000 were collected at the State Nursery in Ames during 1973 and 1974. Collections during these 2 years were made primarily to study host-parasite relationships of *Ae. trivittatus* and TVT virus. In 1975, selection of mosquito collection sites was made after the appearance of human cases of encephalitis.

Tables 2, 3, and 4 show the numbers

and pools of each mosquito species collected by month for 1972, 1973, and 1975. In 1974, 7,933 (27 pools) *Ae. trivittatus* and 1,211 (13 pools) *Ae. vexans* were collected at the State Nursery in Ames. Arbovirus was isolated from 102 pools of mosquitoes collected and examined during the 4-year period. Two LAC isolates, 2 Cache Valley (CV), 10 Flanders (F1), 3 SLE, 83 TVT, and 2 WEE viruses were identified.

The two LAC isolates were from mosquitoes collected in areas where LAC virus had not been found in previous years. The virus was isolated from a pool of *Ae. triseriatus* collected in Black Hawk State Park in Waterloo, Iowa, in August 1972. The other LAC isolate was from mosquitoes collected in August 1975 in Sioux City. Laboratory-confirmed cases of LAC encephalitis have occurred in these areas. The FIR's for LAC virus in *Ae. triseriatus* ranged from 1:10 to 1:86, with isolations from pools of 3 and 19 mosquitoes.

Three isolations of SLE were made from *Culex tarsalis* and *Cx. pipiens* collected during August and September 1975. The calculated FIR's for SLE virus in *Cx. pipiens* ranged from 1:19 at Des Moines to 1:158 at Ames. The FIR was 1:47 in *Cx.*

Table 1. Summary of mosquitoes collected in Iowa for virus isolation, 1972-1975.

| Location | 1972 | 1973 | 1974 | 1975 | Total |
|----------------------------|-----------------------|--------------------|-------------------|-------------------|-----------------------|
| Clive, (Des Moines) | 4,098(92)* | | | 188(9) | 4,286(101) |
| Eldora | 13,250(206) | | | | 13,250(206) |
| Lost Island Lake, Clay Co. | 13,081(181) | | | | 13,081(181) |
| Malvern | 227(57) | | | | 227(57) |
| Yellow River Forest | 909(36) | | | | 909(36) |
| Iowa City | 2,485(58) | | | | 2,485(58) |
| Ventura | 15,705(206) | | | | 15,705(206) |
| Waterloo | 9,215(243) | | | | 9,215(243) |
| Hornick | 5,737(178) | | | 228(22) | 5,965(200) |
| Ames | 43,837(583) | 11,673(203) | 9,144(140) | 5,125(128) | 69,779(1,054) |
| Berne Farm, Allamakee Co. | 218(32) | | | 261(4) | 479(36) |
| Lake MacBride State Park | 297(34) | 56(4) | | | 353(38) |
| Lacey-Keosauqua State Park | 213(26) | | | | 213(26) |
| Sioux City | | | | 611(34) | 611(34) |
| Des Moines | | | | 249(16) | 249(16) |
| TOTAL | 109,272(1,932) | 11,729(207) | 9,144(140) | 6,662(213) | 138,807(2,492) |

* Number of mosquitoes (pools).

Table 2. Mosquito species collected in Iowa during 1972 and number of pools tested for arbovirus.

| Mosquito Species | May | June | July | August | September | October | Total |
|----------------------------------|----------|--------------|--------------|--------------|-------------|------------|-----------------|
| <i>Aedes canadensis</i> | — | 100 (7) | 17 (2) | — | — | — | 117 (9) |
| <i>Ae. cinereus</i> | — | — | 2 (1) | — | — | — | 2 (1) |
| <i>Ae. dorsalis</i> | — | — | 2 (2) | 20 (4) | 11 (3) | — | 33 (9) |
| <i>Ae. flavescens</i> | — | — | 1 (1) | 1 (1) | — | — | 2 (2) |
| <i>Ae. nigromaculis</i> | — | 4 (3) | 86 (9) | 618 (31) | 24 (3) | — | 732 (46) |
| <i>Ae. sticticus</i> | — | 3 (3) | 4 (2) | 5 (2) | — | — | 12 (7) |
| <i>Ae. stimulans</i> | — | 1 (1) | 5 (2) | — | — | — | 6 (3) |
| <i>Ae. triseriatus</i> | — | 33 (3) | 91 (13) | 481 (40) | 44 (10) | — | 649 (66) |
| <i>Ae. trivittatus</i> | 581 (6) | 12,176 (136) | 22,899 (259) | 24,798 (271) | 1,425 (29) | 1,458 (17) | 63,337 (718) |
| <i>Ae. vexans</i> | 334 (4) | 578 (15) | 5,723 (80) | 12,679 (155) | 2,580 (37) | 103 (4) | 21,997 (295) |
| <i>Anopheles punctipennis</i> | 2 (1) | 42 (6) | 248 (23) | 1,158 (70) | 123 (12) | — | 1,573 (112) |
| <i>An. quadrimaculatus</i> | — | — | 2 (2) | 20 (7) | 31 (5) | — | 53 (14) |
| <i>An. walkeri</i> | — | — | 84 (6) | 19 (6) | 59 (7) | — | 162 (19) |
| <i>Culex erraticus</i> | — | — | 6 (4) | 87 (17) | 16 (9) | — | 109 (30) |
| <i>Culex pipiens complex*</i> | 2 (1) | 344 (19) | 1,552 (75) | 2,664 (130) | 348 (27) | 11 (2) | 4,921 (244) |
| <i>Cx. tarsalis</i> | 1 (1) | 11 (2) | 390 (27) | 903 (53) | 116 (9) | — | 1,421 (92) |
| <i>Cx. territans</i> | — | — | — | 4 (3) | 3 (1) | — | 7 (4) |
| <i>Culiseta inornata</i> | 4 (1) | 32 (7) | 12 (4) | 4 (4) | 146 (9) | — | 198 (25) |
| <i>Coquillettidia perturbans</i> | — | 3 (3) | 12,971 (138) | 500 (15) | 7 (3) | — | 13,481 (159) |
| <i>Orthopodomyia signifera</i> | — | — | — | 6 (3) | — | — | 6 (3) |
| <i>Psorophora ciliata</i> | — | — | — | 118 (10) | 1 (1) | — | 119 (11) |
| <i>Ps. confinis</i> | — | — | — | 16 (1) | — | — | 16 (1) |
| <i>Ps. horrida</i> | — | — | 149 (15) | 124 (20) | 15 (7) | 1 (1) | 289 (43) |
| <i>Ps. signipennis</i> | — | — | 4 (2) | 5 (3) | 15 (1) | — | 24 (6) |
| <i>Uranotaenia sapphirina</i> | — | — | — | 1 (1) | 5 (2) | — | 6 (3) |
| Total | 924 (14) | 13,327 (205) | 44,248 (667) | 44,231 (847) | 4,969 (175) | 1,573 (24) | 109,272 (1,932) |

* *Culex pipiens complex* = *Cx. pipiens pipiens*, *Cx. pipiens quinquefasciatus*, *Cx. restuans* and *Cx. salinarius*.

Table 3. Mosquito species collected in Iowa during 1973 and number of pools tested for arbovirus.

| Mosquito Species | May | July | August | September | Total |
|----------------------------------|----------|------------|------------|------------|--------------|
| <i>Aedes</i> spp. | — | 32 (1) | — | — | 32 (1) |
| <i>Aedes dorsalis</i> | 8 (1)* | — | — | — | 8 (1) |
| <i>Ae. sticticus</i> | 1 (1) | 3 (1) | — | — | 4 (2) |
| <i>Ae. triseriatus</i> | — | 7 (1) | 7 (2) | 2 (1) | 16 (4) |
| <i>Ae. trivittatus</i> | 4 (1) | 600 (14) | 6,196 (65) | 1,831 (19) | 8,631 (99) |
| <i>Ae. vexans</i> | 152 (3) | 492 (11) | 102 (2) | 86 (2) | 832 (18) |
| <i>Anopheles punctipennis</i> | — | 16 (2) | 52 (2) | 25 (2) | 93 (6) |
| <i>Culex pipiens</i> complex* | 38 (2) | 42 (2) | 1,019 (23) | 358 (5) | 1,457 (32) |
| <i>Cx. tarsalis</i> | 127 (7) | 9 (2) | 9 (2) | 2 (1) | 147 (12) |
| <i>Culiseta inornata</i> | 492 (25) | — | 1 (1) | 9 (2) | 502 (28) |
| <i>Coquillettidia perturbans</i> | — | 3 (2) | — | — | 3 (2) |
| <i>Psorophora ciliata</i> | — | 3 (1) | — | — | 3 (1) |
| <i>Ps. horrida</i> | — | — | 1 (1) | — | 1 (1) |
| Total | 822 (40) | 1,207 (37) | 7,387 (98) | 2,313 (32) | 11,729 (207) |

* *Culex pipiens* complex = *Cx. pipiens*, *Cx. pipiens quinquefasciatus*, *Cx. restuans* and *Cx. salinarius*.

tarsalis collected at Sioux City (Table 5). SLE virus had been isolated 4 years earlier (1971) from a pool of *Cx. tarsalis* collected in Des Moines (Rowley et al. 1973), but no human case was detected at the time. Six confirmed human SLE infections occurred in this general area during 1975.

WEE virus was isolated from 2 pools of

Cs. tarsalis collected in the Sioux City area (Hornick) during 1972. Laboratory-confirmed human WEE infections occurred in this area in 1975, but the virus was not isolated from mosquitoes at that time. Mosquito field collection efforts for the Sioux City area were limited to a single 2-day trip in late August 1975. Cold and windy evenings limited the

Table 4. Mosquito species collected in Iowa during 1975 and number of pools tested for arbovirus.

| Mosquito Species | May | June | July | August | September | Total |
|----------------------------------|---------|------------|------------|----------|------------|-------------|
| <i>Aedes canadensis</i> | — | — | 1 (1) | — | — | 1 (1) |
| <i>Ae. nigromaculis</i> | — | — | — | 7 (1) | — | 7 (1) |
| <i>Ae. spenceri</i> | — | — | — | 4 (1) | — | 4 (1) |
| <i>Ae. triseriatus</i> | — | — | 3 (1) | 10 (3) | 1 (1) | 14 (5) |
| <i>Ae. trivittatus</i> | — | 550 (11) | 1,413 (29) | 158 (11) | 342 (10) | 2,463 (61) |
| <i>Ae. vexans</i> | — | 1,200 (24) | 421 (10) | 374 (11) | 353 (14) | 2,348 (59) |
| <i>Anopheles punctipennis</i> | — | 303 (7) | 50 (1) | 13 (4) | 270 (11) | 636 (23) |
| <i>Culex pipiens</i> complex* | 261 (4) | 59 (2) | 162 (4) | 179 (11) | 344 (16) | 1,005 (38) |
| <i>Culex tarsalis</i> | — | — | 12 (1) | 90 (12) | 66 (6) | 168 (19) |
| <i>Culiseta inornata</i> | — | — | — | — | 1 (1) | 1 (1) |
| <i>Coquillettidia perturbans</i> | — | — | 11 (2) | — | — | 11 (2) |
| <i>Psorophora ciliata</i> | — | — | — | 4 (2) | — | 4 (2) |
| Total | 261 (4) | 2,112 (44) | 2,073 (50) | 839 (56) | 1,377 (59) | 6,662 (213) |

* *Culex pipiens* complex = *Cx. pipiens*, *Cx. pipiens quinquefasciatus*, *Cx. restuans* and *Cx. salinarius*.

Table 5. Arbovirus field infection ratios (FIR) in mosquitoes for months when non-TVT isolations were made from each species, Iowa 1972-1975.

| Mosquito species | Date | Location | Virus | No. Mosq. Tested | No. Isol. | FIR |
|-----------------------------|------------|------------|-------|------------------|-----------|--------|
| <i>Cx. pipiens</i> complex* | July 1972 | Eldora | F1 | 82 (4) | 2 | 1:41 |
| <i>Cx. pipiens</i> complex | Aug. 1972 | Eldora | F1 | 499 (22) | 3 | 1:166 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Ventura | F1 | 3,816 (39) | 1 | 1:3816 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Waterloo | LAC | 86 (14) | 1 | 1:86 |
| <i>Ae. nigromaculis</i> | Aug. 1972 | Hornick | CV | 570(24) | 1 | 1:570 |
| <i>Cx. tarsalis</i> | Aug. 1972 | Hornick | WEE | 497 (19) | 1 | 1:497 |
| <i>Cx. tarsalis</i> | Aug. 1972 | Hornick | F1 | 497 (19) | 1 | 1:497 |
| <i>Cx. tarsalis</i> | Sept. 1972 | Hornick | WEE | 111 (5) | 1 | 1:111 |
| <i>Cs. inornata</i> | Sept. 1972 | Hornick | CV | 68 (5) | 1 | 1:68 |
| <i>Cx. pipiens</i> complex | July 1972 | Ames | F1 | 891 (37) | 2 | 1:445 |
| <i>Cx. pipiens</i> complex | Aug. 1972 | Ames | F1 | 1,192 (49) | 1 | 1:1192 |
| <i>Ae. triseriatus</i> | Aug. 1975 | Sioux City | LAC | 10 (3) | 1 | 1:10 |
| <i>Cx. tarsalis</i> | Aug. 1975 | Sioux City | SLE | 47 (7) | 1 | 1:47 |
| <i>Cx. pipiens</i> complex | Sept. 1975 | Des Moines | SLE | 19 (1) | 1 | 1:19 |
| <i>Cx. pipiens</i> complex | Sept. 1975 | Ames | SLE | 158 (7) | 1 | 1:158 |

* *Cx. pipiens* complex = *Cx. pipiens pipiens*, *Cx. pipiens quinquefasciatus*, *Cx. restuans* and *Cx. salinarius*.

number collected to a total of 267 *Culex* mosquitoes, which were divided into 25 pools. Only one isolation of SLE virus was made from 1 of the 7 pools of *Cx. tarsalis* totalling 47 specimens.

TVT was the most frequently encountered virus during the 1972-1975 period, with 83 isolations. The FIR's calculated for TVT in mosquito species yielding isolates ranged from 1:17 to 1:11,611 (Table 6). The high FIR for TVT in *Coquillettidia perturbans* may be due to a single isolation from a small number of this species collected at Eldora in July 1972. TVT was not found in any of the 113 pools of this mosquito, totalling 11,213 specimens, collected at Lost Island Lake during the same period. Each year, this virus was isolated from *Ae. trivittatus* collected in several regions of Iowa during the months of June to October. Because of the prevalence and widespread distribution of *Ae. trivittatus* and TVT in Iowa, studies on the host-parasite relationship are underway.

CV virus was isolated from a pool of 25 *Ae. nigromaculis* and a pool of 2 *Culiseta inornata* mosquitoes, both collected in the Sioux City area during 1972. This is our

first isolation of CV virus from *Ae. nigromaculis*. Previous isolations of this virus have been obtained from *Anopheles punctipennis* and *Cq. perturbans* (Wong et al. 1973).

One F1 isolate was from *Ae. trivittatus* and the other nine isolates were from *Culex* mosquitoes. Although F1 virus has been isolated from many species of mosquitoes, this is the first isolate from an *Aedes* mosquito in Iowa. This isolation is of particular interest because *Ae. trivittatus* is reputed to be mammalophilic; whereas the vertebrate hosts of F1 virus are thought to be birds.

There were 20 cases (19 confirmed and 1 presumptive) of LAC encephalitis in Iowa during the 4-year period from 1972 to 1975. Most of these cases (12) occurred during the summer of 1975. Whether these data represent an absolute increase in annual case incidence or are the result of an increased interest in diagnosis of CNS infections during a year of increased awareness of arbovirus infections remains to be determined. The 10 male and 9 female confirmed cases were mostly under 16 years of age, and 14 were under 7 years of age. They lived in 12 Iowa

Table 6. Field infection ratios (FIR) for trivittatus virus in mosquitoes for the months and locations they were isolated from each species, Iowa 1972-1975.

| Mosquito species | Date | Location | No. Mosq. Tested | No. Isol. | FIR |
|------------------------|------------|------------|---------------------|-----------|---------|
| <i>Ae. trivittatus</i> | July 1972 | Des Moines | 827 (17) | 8 | 1:103 |
| <i>Ae. trivittatus</i> | Sept. 1972 | Des Moines | 269 (3) | 1 | 1:269 |
| <i>Ae. trivittatus</i> | July 1972 | Eldora | 2,834 (30) | 4 | 1:709 |
| <i>Cq. perturbans</i> | July 1972 | Eldora | 141 (2) | 1 | 1:141 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Eldora | 7,776 (80) | 5 | 1:1555 |
| <i>Ae. trivittatus</i> | July 1972 | Ventura | 1,551 (16) | 1 | 1:1551 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Ventura | 3,816 (39) | 19 | 1:200 |
| <i>Ae. trivittatus</i> | Sept. 1972 | Ventura | 147 (2) | 1 | 1:147 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Waterloo | 2,437 (33) | 1 | 1:2437 |
| <i>Ae. trivittatus</i> | Sept. 1972 | Waterloo | 450 (10) | 2 | 1:225 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Hornick | 2,022 (23) | 3 | 1:674 |
| <i>Ae. trivittatus</i> | June 1972 | Ames | 11,611 (119) | 1 | 1:11611 |
| <i>Ae. trivittatus</i> | Aug. 1972 | Ames | 7,191 (73) | 1 | 1:7191 |
| <i>Ae. trivittatus</i> | Oct. 1972 | Ames | 1,458 (17) | 1 | 1:1458 |
| <i>Ae. trivittatus</i> | July 1973 | Ames | 569 (13) | 2 | 1:285 |
| <i>Ae. trivittatus</i> | Aug. 1973 | Ames | 6,196 (65) | 5 | 1:1239 |
| <i>Ae. trivittatus</i> | Sept. 1973 | Ames | 1,831 (19) | 1 | 1:1831 |
| <i>Ae. trivittatus</i> | June 1974 | Ames | 7,933 (127) | 19 | 1:417 |
| <i>Ae. vexans</i> | June 1974 | Ames | 1,200 (24) | 1 | 1:1200 |
| <i>Ae. trivittatus</i> | June 1975 | Ames | 550 (11) | 1 | 1:550 |
| <i>Ae. trivittatus</i> | July 1975 | Ames | 1,413 (29) | 4 | 1:353 |
| <i>Ae. trivittatus</i> | Sept. 1975 | Des Moines | 17 (1) | 1 | 1:17 |

counties, with 2 from out-of-state. Iowa counties reporting cases of LAC encephalitis are located in the eastern half of the state, with most cases occurring in the northeastern counties. The distribution of human arbovirus encephalitis in Iowa for 1972-1975 is illustrated in Figure 1. Most of the LAC encephalitis cases occurred during the month of August. The first cases appeared as early as the 2nd week of July; and the onset of illness of the last case was in the 3rd week in September.

In 1975, there were 5 confirmed cases of WEE in Iowa. One case occurred during the last week of July—the other 4 cases in August. These cases all occurred in the Sioux City area where WEE virus activity has been noted for several years (Rowley et al. 1973). Mosquitoes infected with WEE virus have been collected consistently in western Iowa during these studies. Four of the cases occurred in the 1 to 19 age group, and one was in the 50 to 59 age group.

Nineteen SLE cases occurred in 11 counties throughout the state between the 2nd week in August and the end of September 1975. Six cases occurred in the Des Moines area and 3 in the Sioux City area. There were 10 males and 9 females, and the cases occurred in all age groups from 1 to 70. The appearance of these cases caused considerable concern among state public health officials. In response to local concerns, mosquitoes were collected on an extremely limited basis in 3 cities (Ames, Des Moines, and Sioux City). The isolation of SLE virus from small numbers of *Culex* mosquitoes in each of these cities suggests considerable SLE virus activity in mosquitoes during the early fall and the virus was widespread in Iowa in 1975.

The extent of SLE virus activity in *Cx. tarsalis* and *Cx. pipiens* during 1975 in Iowa can be seen by comparing the 3 isolates in 1975 from only 1,173 *Culex* mosquitoes with the single isolate from 40,657 *Culex* mosquitoes collected and tested for

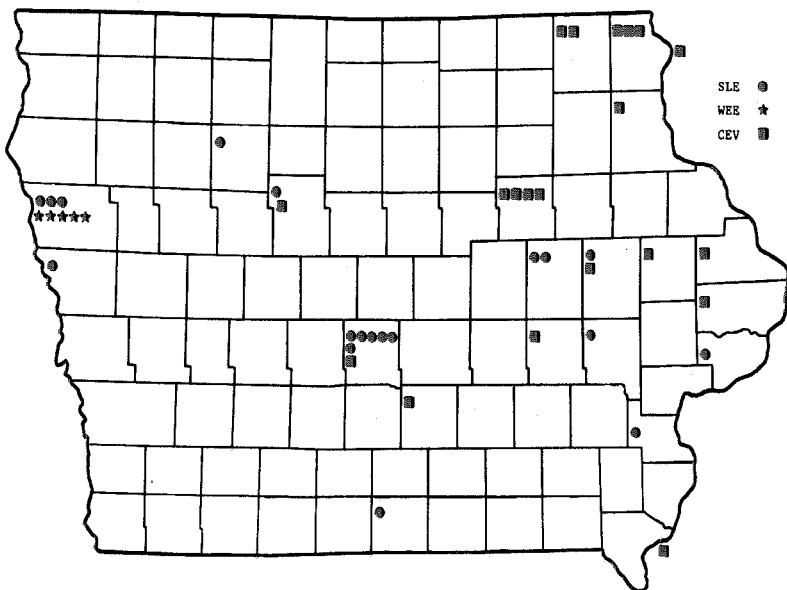


Figure 1. Distribution of mosquito borne encephalitis cases diagnosed in Iowa 1972-1975 (including 2 cases in patients residing outside of Iowa).

mosquito-borne virus from 1966 through 1972 (Wong et al 1971, Wong et al. 1973, Rowley et al. 1973). Unseasonably cool and dry weather in much of the state during early September 1975 may have reduced virus transmission from infected mosquitoes to man and probably abated an overt outbreak of mosquito-borne encephalitis in Iowa.

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