THE DETERMINATION OF THE POTENTIAL VECTORS OF EASTERN EQUINE ENCEPHALITIS IN MICHIGAN

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ABSTRACT

THE DETERMINATION OF THE POTENTIAL VECTORS OF EASTERN EQUINE ENCEPHALITIS IN MICHIGAN

By

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In the summer of 1974, a study was performed to determine the species of mosquito(es) which may have acted as potential vectors in the Eastern equine encephalitis outbreak that occurred at Camp Ohiyesa, Michigan, in 1973. Mosquito populations were studied using New Jersey light traps, CDC traps, and chicken baited live traps as well as aspirator collections from horse and human baits.

Three mosquito species appeared to be likely potential vectors, *Coquillettidia perturbans* (Walker), *Aedes vexans* (Meigen), and *Culiseta melanura* (Coquillett). *Coquillettidia perturbans* was in greatest abundance and fed readily on horses, humans, and birds. *Aedes vexans* fed preferentially on horses, and *Culiseta melanura* is a known bird feeder.

Possible reasons that the outbreak spread primarily to horses in the camp are discussed.

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INTRODUCTION

Epidemiology

Eastern equine encephalomyelitis (EEE) is the most severe of the various mosquito-borne encephalitides known to exist today. This particular virus, belonging to the group A arboviruses and miscellaneous subgroup c, causes an extremely high mortality in horses, sometimes over 95 percent of those infected. In man, the mortality rate is not as high, but is still severe, averaging 65 percent of those individuals infected. The incubation period is usually three to five days. Symptoms follow a regular pattern, beginning with an exceptionally high fever ranging between 105 and 107°F. Nausea, vomiting, drowsiness, disorientation, difficulty in concentrating, and twitching may develop as the infection progresses. The terminal stages are manifested by severe convulsions and a comatose state. In the most severe cases, death occurs two to three days after the onset of the symptoms, and in others death may result from complications. In children, survivors are often left with mental retardation, convulsions, and paralysis while adult survivors generally recover completely (James and Harwood, 1969). Because the symptoms of EEE are similar to those of other virus infections including lymphocytic choriomeningitis, poliomyelitis, herpes, and mumps (Cockburn, Price, and Rowe, 1951), diagnosis on the basis of symptoms alone is not always possible. Differential diagnosis of these infections requires specialized technique, equipment, and time.

EEE is an arbovirus which follows a definite pattern of transmission from animal to animal via an arthropod vector. The major vectors are believed to be various species of mosquitoes, although infrequent EEE virus isolations have been made from Culicoides, fowl mites, lice, and Simuliidae (James and Harwood, 1969). The normal transmission cycle involves wild birds and mosquitoes. An infected bird may appear asymptomatic even though it has a high virus titer, or it may show varying clinical symptoms, possibly ending in death in three to four days. Some species of birds are highly resistant to infections with this virus. For example, after receiving an experimental subcutaneous injection of 1 cc. virus emulsion containing approximately 10^6 mouse lethal doses of virus, one out of two mourning doves tested had no detectable viremia and no neutralizing antibodies were detected two weeks after inoculation (Davis, 1940). Apparently, only some avian species are susceptible to this virus, and develop high viremias. If a vector mosquito takes an infectious blood meal with an adequate level of viremia, it becomes infective, and is then able to transmit the virus while feeding on various susceptible vertebrates. When humans and animals, other than birds, are infected with EEE virus, they are termed accidental hosts. Humans and horses can be infected with EEE virus and develop clinical disease, but the virus titer does not develop to high enough levels in these hosts to infect mosquitoes feeding on them. This means they thus become dead-end hosts.

History of Eastern Equine Encephalitis

EEE virus has been reported along the eastern seaboard and Gulf Coast of the United States, in Canada, Panama, Brazil, Mexico, Cuba, the Phillipine Islands (Ferguson, 1954), and in the Dominican Republic, and Jamaica (James and Harwood, 1969). The virus has also been reported in areas within Europe and Asia but because these isolations have not been associated with epidemics or epizootics, it is thought the disease is different from that which occurs in the New World areas (James and Harwood, 1969).

Many investigators think that EEE has been in the United States for centuries. It is interesting to note a published report from Dr. Arad Thompson of Middleboro, Massachusetts, a section that extends a few miles north of Boston to about 30 miles south of that city. Dr. Thompson observed a disease in horses from about the first of August to the third week in September, 1831. He reported, "the number of horses that have died may be estimated to be 75. Fifty of this number may have died in the first three weeks. After that the cases became less frequent" (Hanson, 1957). The doctor's description of the symptoms of the disease corresponds closely with the symptoms of EEE as we now know them. Dr. Thompson explained,

> When the horse was first observed to be indisposed, he was dull and moping, the eye was dull and heavy, he ceased to eat, he was inattentive to surrounding objects, he had inaptitude to motion, and when driven or moving voluntarily he was disposed to gyrate in a greater or less circle. The same horse when driven or led by the bridle would continually incline to the right or the left and the same horse always the same way. They all had an expression of suffering pain, or uneasy sensation; some frequently putting the nose to the side. Some when first discovered to be sick were down on the side and unable to remain on the feet. All, as the disease progressed, remained down, and occasionally [made] spasmodically violent motions with the legs and some became, perhaps 24 hours before death, apparently wholly insensible to surrounding objects. Some sooner or later [were] delirious while others seemed to possess all the operations of instinct until their last moments. The bowels in no case within the results of my inquiry presented indications, by the discharges or bloating, that they were diseased (Hanson, 1957).

The above was not the only epizootic of EEE-like equine disease to be recorded in the 1800s in the United States. Equine diseases with symptoms similar to those displayed by both EEE and Western equine encephalitis (WEE), have been recorded for the United States in 1847, 1850, 1867, 1882, and 1897 (Ferguson, 1954). During this time severe outbreaks of what appeared to be EEE-like disease were labeled forage poisoning, cerebrospinal meningitis, staggers, or Kansas Nebraska horse disease.

Lesser outbreaks occurred in the intervening years. In 1900-1901 and again in 1912, a disease with symptoms like those of EEE appeared on the east coast with most of the activity occurring in Maryland and some of the middle and south Atlantic States. It was during November of 1912, the term "equine encephalomyelitis" was first applied to this disease by C. H. Stange when he spoke before the Iowa State Veterinary Medical Association (Ferguson, 1954).

Outbreaks of equine encephalomyelitis recurred during July, August, and September of 1933, in the states of Virginia, Maryland, and Delaware. The morbidity rate on some farms was as high as 25 to 30 percent of the horses and mules, with a mortality of 90 percent or more. It was observed that this disease outbreak reached serious proportions by the middle of August, was still prevalent during all of september, and continued at a reduced rate well into October (Giltner and Shahan, 1933). The symptoms were identical to those of EEE-like disease described earlier, the most convincing being that death was commonly encountered 24-48 hours after the onset of definite symptoms of this disease.

Zwick and Seifried, working on European horses in 1924, reported that the equine disease was caused by a filterable virus. This preceded similar work by Meyer and his co-workers in the United States in 1930 (Shahan and

Giltner, 1945). It was not until 1933 that this virus was first isolated from the brain of horses and called Eastern equine encephalitis (James and Harwood, 1969).

The first recognized epidemic of EEE involving human infections occurred in Massachusetts in 1938 (Goldman and Sussman, 1968). In the 1938 outbreak, 38 humans and 248 horses were stricken (Hayes, LaMotte, and Hess, 1960). Massachusetts also became the scene of later epidemics in 1955, when four cases broke out, and in 1956, when 12 more occurred (Levitt, Lovejoy, and Daniels, 1971).

In the United States until 1938, the two immunologically distinct viruses of EEE and WEE were thought to occur only in geographically separate areas, EEE on the eastern seaboard of the United States and inland, east of the Appalacian Mountains, and WEE west of the Appalacian Mountains. However, in 1939, virus isolations of both viruses were made in Alabama (Mohler, 1940) making the former line of demarcation, the Appalacian Chain, no longer the geographical boundary between the overlapping virus territories (Giltner and Shahan, 1945).

EEE was next isolated in a small number of cases well west of the original line of demarcation during a localized outbreak in April and May of 1941 near Brownsville, Texas. This outbreak spread to include an area several hundred miles inland from the Gulf of Mexico and two strains of virus obtained in this outbreak were identified as EEE (Giltner and Shahan, 1945).

In 1941, WEE was discovered in Michigan. The following year in the same general locality, EEE was isolated (Giltner and Shahan, 1945). These disease outbreaks in Alabama, Texas, and Michigan, showed, conclusively, that WEE co-existed with EEE in an area somewhat centered between those where the two viruses originally were thought to occur.

The largest confirmed EEE epizootic occurred in southern Louisiana and Texas in 1947. An official report from the Bureau of Animal Industry, United States Department of Agriculture, shows 3,815 equine cases for Louisiana and 715 cases for Texas. A report from the Department of Veterinary Science, Louisiana State University, concerning the same epizootic lists 14,334 horse cases with 11,722 deaths for the state of Louisiana. Accompanying this epizootic was a small outbreak in humans. From September 8 to October 23, ten human cases were definitely diagnosed as EEE virus infections. Seven of the ten died, and all infections except one were in children (Beadle, 1952).

During the summer of 1948, a few horses from different parts of Georgia were diagnosed as having encephalomyelitis. The brains from two animals were transferred to the U.S. Public Health Service Virus Laboratory in Montgomery, Alabama, where virus isolates proved to be

Eastern equine encephalitis. At the same time, mosquito collections were made in Burke and Jenkins Counties, where sick horses had previously been reported. Isolates of EEE were made from pools of Mansonia (now called Coquillettidia), perturbans (Walker) taken from farms in the area. C. perturbans is a persistent feeder on warm blooded animals, including horses and chickens, making it a potentially dangerous vector of equine encephalitis viruses (Howitt, Dodge, Bishop, and Gorrie, 1949).

In 1949, at least 1,700 equines in Louisiana and Arkansas experienced an acute fulminating type of encephalitis. The mortality of this epizootic was reported as 98 percent. This outbreak, which began on the coast near the site of the 1947 outbreak, progressed northward to the middle of Arkansas (Beadle, 1952).

At this point, a few words are in order concerning the mosquito species involved in EEE virus transmission. Since EEE infections in vertebrates are prevalent during the insect season, it was hypothesized very early that insects possibly were involved in the transmission of this disease. In 1934, Merrill and his co-workers found that EEE virus could be transmitted in the laboratory by Aedes sollicitans (Walker), A. cantator (Coquillett), and also A. agypti (Linnaeus) (Merrill, Lacaillade, and Ten Broeck, 1934). In 1935, A. taeniorhynchus (Wiedemann) and A. vexans (Meigen) were added to this list (Ten Broeck and

Merrill, 1935). Davis, in 1939, demonstrated transmission of the EEE virus by six species of *Aedes*, including *A. triseriatus* (Say), and many epidemiologists were convinced *Aedes* mosquitoes were the principal vectors of that virus (Davis, 1940). However, Beadle (1952) found no evidence of naturally infected *Aedes*. Therefore, at the date of the writing of the article by Beadle (1952), only two species of mosquitoes were reported as naturally infected with EEE virus. These were *Coquillettidia perturbans*, collected in Georgia, in August 1948, and *Culiseta melanura* (Coquillett), trapped in southeastern Louisiana in August 1950.

Until approximately 1951, there also was confusion on the mechanism of mosquito infection. Consideration was given to both mammals and birds as being the primary hosts and reservoirs of the infection. It was later concluded that birds were the principal reservoir. Studies indicated widespread infections in birds, primarily inapparent infections, with a high virus titer in their blood for a short period of time (Beadle, 1952).

In the summer of 1952, EEE virus was identified, using virus neutralization tests, from the brain of a horse that had died in south central Wisconsin. Of the two arthropod-borne encephalitides, EEE and WEE, only WEE had been known to exist in that area (Hanson, Scott, Ferris, and Upton, 1954).

In 1956, 107 equine cases of EEE were reported from Florida, while in 1957 there were 386, and in 1958, 94 (Animal Morbidity Reports, 1956-1958). These numbers appear quite high but it should be noted that Florida is a point of departure and return for many migratory birds, primary reservoirs of EEE virus, flying to and from Cuba, Central and South America, and Mexico (Lincoln, 1950).

Mosquito collections were made in Florida in 1958, in an attempt to determine the vectors responsible for arthropod transmission of EEE virus. The three most likely suspects were *Culiseta melanura*, *Coquillettidia perturbans*, and *Aedes sollicitans* because these same mosquito species had been considered as vectors elsewhere (Favorite, 1960). Unfortunately, suspensions prepared from pools of the above species and inoculated into mice did not produce any virus isolations. The data obtained in this study revealed that detectable virus in the mosquito population was absent during a period when only a few equine cases were reported (Favorite, 1960).

Maryland has had a number of EEE epizootics. There were 98 equine cases reported in 1938, 70 in 1939, 180 in 1945, 42 in 1955, and 33 in 1956. In 1959, veterinarians reported 17 cases, 7 of which were confirmed by laboratory studies (Bickley and Byrne, 1960). Between 1938 and 1959 the actual number of equine cases of EEE appeared to be tremendously reduced in Maryland. During this 21 year interval, there was a marked decline in the national and state horse population. Actually, the attack rate was higher in 1959 than it was 20 years earlier if consideration is given to the difference in horse population (Bickley and Byrne, 1960).

Newson (1962) showed that five species of mosquitoes in Maryland had feeding habits that made them potential vectors with a sixth as a narrow possibility. They were (1) Aedes sollicitans, (2) A. taeniorhynchus, (3) A. vexans, (4) Psorophora ciliata (Fabricius), (5) P. confinnis (Lynce Arribalzaga), and (6) Culiseta melanura. No virus isolations were attempted from any of the mosquitoes collected.

EEE virus activity has been found in New Jersey horses and birds almost every year since first identified in the state by Ten Broeck and Merrill in 1933 (Hayes, Beadle, Hess, Sussman, and Bonese, 1962). Studies were performed there to determine the arthropod vectors responsible for the transmission of this disease. Three different mosquito species were found to contain the EEE virus. The bird feeding preference of Culiseta melanura implicated it in the transmission of the virus from bird to bird to continue the EEE cycle. Aedes sollicitans also was found to feed on avian hosts, but also fed readily on humans, and to a lesser extent on horses. It was more abundant along the coast than inland. This evidence, along with the virus isolation of EEE, added weight to the hypothesis that, since more human cases were found on the coast than elsewhere in the state, Aedes sollicitans could be considered as a vector which readily transmits EEE to humans and much less frequently to horses. The third species, Aedes vexans, was thought to be the main vector responsible for the equine cases inland. It was found to feed predominantly on horses rather than man and it also had a peak biting activity in the evening after sundown when most of the human population was indoors. Another factor which might explain the high number of equine cases and low number of human cases in the inland areas where maximum A. vexans populations occurred is that these rural areas had relatively high equine populations and relatively low human populations in comparison with the coastal area (Hayes, Beadle, Hess, Sussman, and Bonese, 1962).

In Connecticut, there have been EEE outbreaks in birds (pheasants), and horses. Wallis (1960) isolated EEE virus from Aedes vexans mosquitoes, and he believed that this species was involved in transmitting the virus from birds to horses.

In 1965, there were no recognized extensive human outbreaks of EEE in the United States. There were, however, 8 human cases reported: 3 cases in Florida; 2 in Georgia; 1 in North Carolina; 1 in Maryland; and 1 in New Jersey (USPHS, CDC, 1966). Epizootics of this disease in equines occurred that year all along the Atlantic Coast. North Carolina was hardest hit with 461 reported horse cases and 461 deaths. It was the greatest year of EEE activity among equines in United States since 1949 (USDA, ARS, 1966).

In 1941, WEE in equines broke out in Michigan (Shahan and Giltner, 1945). In 1942, of the 102 cases of EEE reported in Michigan by Hays (U.S. Bureau of Animal Industry), and Clark (state veterinarian), 93 cases, or 91 percent, were fatal (Shahan and Giltner, 1943). All except one case, occurred within four counties (unnamed in the literature) with disease onset between August 15 and late September. Death occurred in over 90 percent of the cases within 16 to 36 hours after the onset of symptoms, including a few horses which were euthanized. The four counties involved contained many small lakes, ponds, and swamps. It was wet and very warm during the outbreak, and the mosquitoes were in great abundance. During this epizootic no human cases were reported (Giltner and Shahan, 1943).

In 1943, western Michigan was again the scene of an epizootic of equine encephalitis in horses. The sera taken from birds and horses in this area had antibodies to WEE and EEE. The major part of the outbreak was confined to three counties: Barry, Kalamazoo, and St. Joseph. A few scattered cases occurred in nearby Allegan, Ingham, Eaton, Cass, Ionia, Calhoun, Branch, and Jackson counties.

The incidence in Michigan that year was 3.9 per 1,000 horses and mules which, with the exception of Utah, California, and Oregon, was the highest of any state in the country. The mortality in Michigan (Beadle, 1952) was 95 percent, compared with 34, 27, and 11 percent for Utah, California, and Oregon, respectively (Brown, 1947).

The part of Michigan, within which that epizootic occurred, was farmland with many small lakes, woodlands, and marshy areas which provide ideal habitats for mosquitoes. In addition, a 1,000 acre bird sanctuary is in the middle of the epizootic area and Gull Lake, located at the center of the sanctuary, supports some form of bird life throughout the year. This area is also involved in the yearly migration of many birds, some flying to the smaller marshy areas surrounding Gull Lake. It was thought that some of these birds acted as inapparent reservoirs for EEE (Brown, 1947).

Since 1943, EEE was not reported in Michigan until the summer of 1973, when it also occurred in Alabama, Florida, Georgia, Louisiana, Mississippi, New Hampshire, North Carolina, South Carolina, Texas, and Virginia (Veterinary Diagnostic Lab, MSU, 1975). Late in the summer, Oakland County, more specifically, Rose Township, was the focus of an epizootic involving 13 horses (Barr, 1976). Seven cases occurred on seven separate farms located along Hickory Ridge Road in western Oakland County, while the other six were at Camp Ohiyesa in southwestern Rose Township, also just off Hickory Ridge Road.

Purpose of the Study

No study has yet been performed to determine the species of mosquitoes responsible for transmitting EEE in Michigan. The epizootic that occurred at Camp Ohiyesa in 1973 was significant enough to warrant investigation since it is known that humans as well as horses can become infected with EEE virus. Since six horses on the horse ranch portion of Camp Ohiyesa became infected and died of EEE, the danger of infection also existed for all of the approximately 500 children and camp personnel that used the camp's facilities each year.

The purpose of this project was to determine which species of mosquito(es) fed on the three major animals in the EEE transmission cycle (birds, humans, and horses) and what involvement they may have had as a vector at Camp Ohiyesa during the summer of 1973.

DESCRIPTION OF THE RESEARCH SITE

Thirteen cases of Eastern equine encephalitis in horses occurred in eight different locations in Michigan in 1973, within Hartland, Highland, and Rose Townships, in Oakland County (Barr, 1976). Figure 1 shows the locations of the individual cases (except case 13 where data was unavailable). Dates of onset of EEE symptoms, where available, also are shown.

Camp Ohiyesa is located on a tract of over 271 acres of varying terrain including a lake (Fish Lake), and one of the few natural bogs in Michigan. Figure 2 details the area of Camp Ohiyesa. It was within the horse ranch area of the camp that the six equine cases of EEE occurred. One animal, a mule, recovered with apparently no after effects of the disease (Figure 3). Whether or not this had something to do with increased tolerance to EEE is unknown. The major aquatic habitat in Camp Ohiyesa is the bog area, approximately 850 feet long by 650 feet wide. It is completely bordered by woods, some of which are quite extensive and thick. Figure 4 shows a part of the bordering woods which completely encircle the bog. Approximately 600 to 700 feet of woods are between the bog

Fig. 1.--Locations of individual cases of EEE in northwest Oakland County in 1973.

*Veterinary Diagnostic Laboratory, Michigan State University. 1975. Unpublished Report.

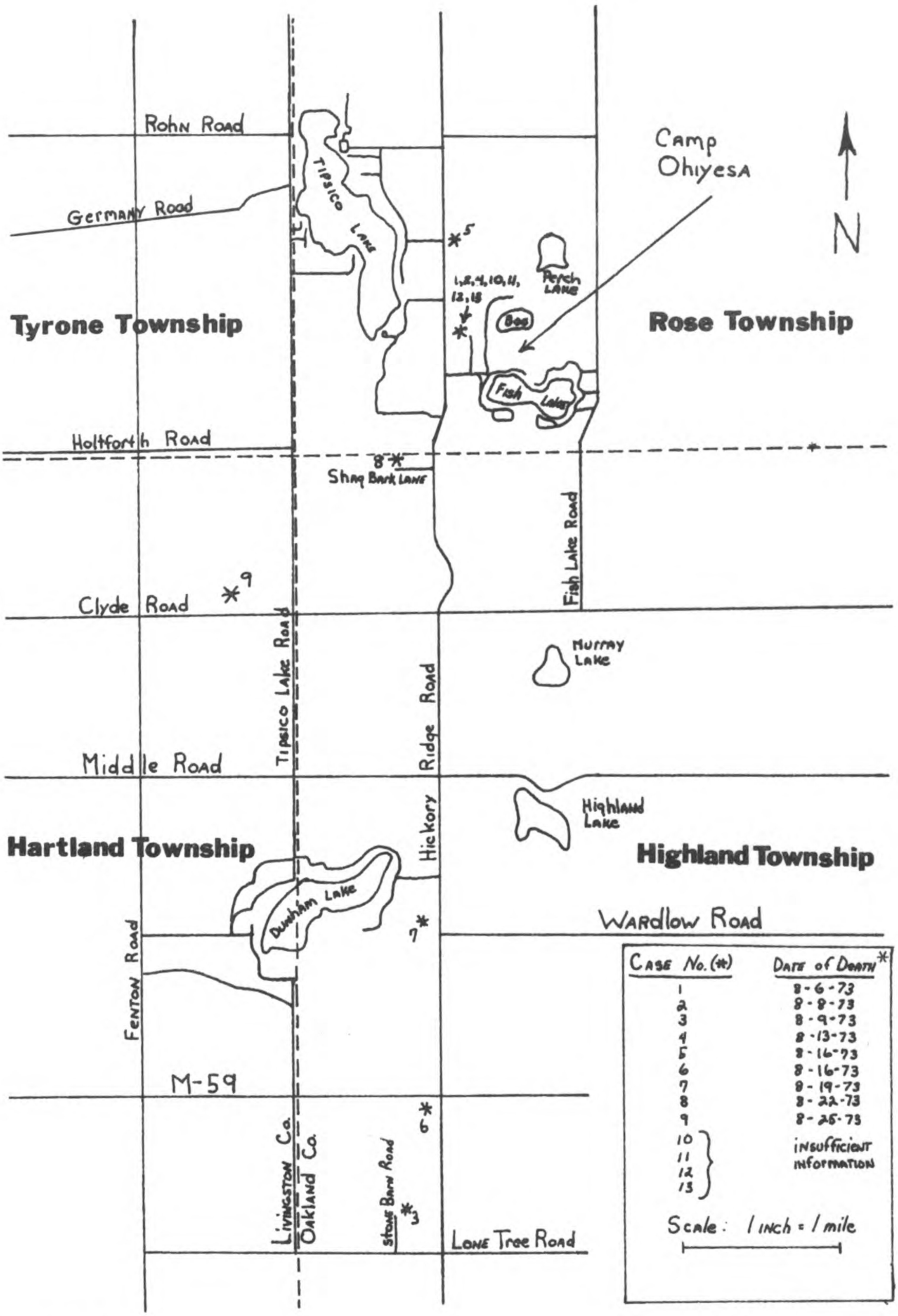
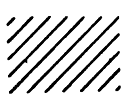
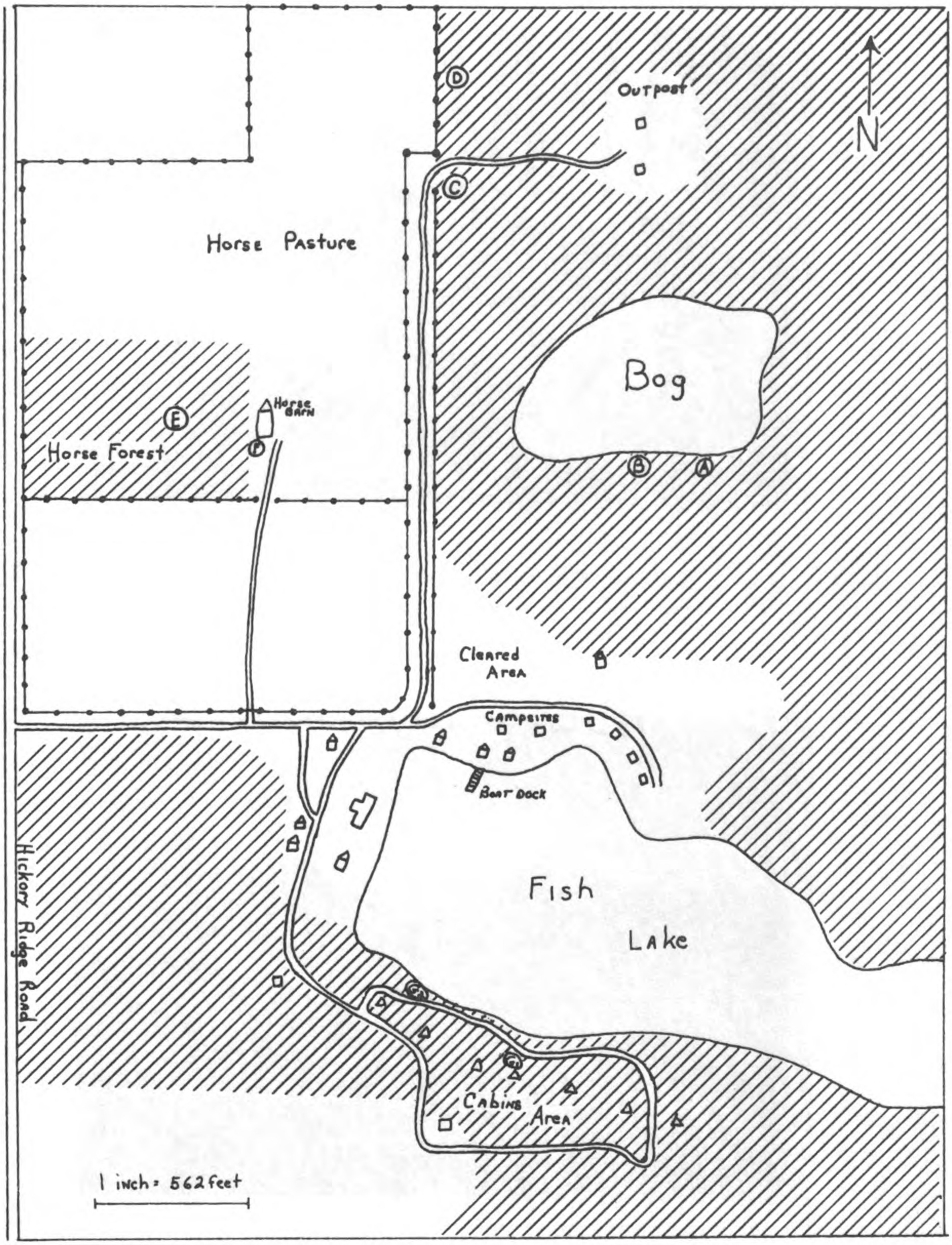

Rohn Road
Germany Road
Tipsico Lake
Camp Ohiyesa
N
Tyrone Township
Rose Township
Perch Lake
Bog
Fish Lake
Holtforth Road
Shag Bark Lane
Fish Lake Road
Clyde Road
Murray Lake
Tipsico Lake Road
Hickory Ridge Road
Middle Road
Highland Lake
Hartland Township
Highland Township
Dunham Lake
Wardlow Road
Fenton Road
M-59
Livingston Co.
Oakland Co.
Stone Barn Road
Lone Tree Road
Case No. (*)
Date of Death
1 8-6-73
2 8-8-73
3 8-9-73
4 8-13-73
5 8-16-73
6 8-16-73
7 8-19-73
8 8-22-73
9 8-25-73
10, 11, 12, 13 insufficient information
Scale: 1 inch = 1 mile

Figure 1.

Fig. 2.--Camp Ohiyesa.

Code	Definition
A	Bog CDC trap
B	Bog chicken baited trap
C	Road's end CDC trap
D	Road's end chicken baited trap
E	Horse forest chicken baited trap
F	Horse Barn New Jersey trap
G_1	Cabin area New Jersey trap - original location
G_2	Relocated cabin area New Jersey trap
Δ	Cabins
▯	Tent foundations
	Buildings
	Fence
	Dirt Road
	Wooded Area



Outpost
N
D
C
Horse Pasture
Bog
Horse Barn
E
F
Horse Forest
B
A
Cleared Area
Campsites
Boat Dock
Hickory Ridge Road
Fish
Lake
Cabins Area
1 inch = 562 feet

Figure 2.

Fig. 3.--Mule which recovered from EEE infection at Camp Ohiyesa.

Fig. 4.--Bog area showing a portion of the woods which completely borders the bog proper.

and the cleared camp area. A nature cabin, located at the southwest extremity of the woods, is the starting point for nature trail hikes through the bog forest area. Fish Lake is approximately 1000 feet south of the bog and is used for boating, swimming, and shoreline walks (Figure 5). The cabin area of the camp borders the south side of Fish Lake in a wooded habitat.

An outpost area, consisting of a pavillion and a few cabins about 400 to 450 feet due north of the bog, once served as an additional accommodation for small groups, but is no longer used except as the endpoint for hikes through the bog.

The horse pasture and adjacent forest, is another major camp land area which provides grass for the horses and is an area in which the horses are allowed to wander. The forest provides a cool retreat on warm summer days and has high mosquito populations during the day. The pasture, situated near the small forest area, is divided into north and south portions by an open gate fence (Figure 6). Figures 7 and 14 are additional photos of the forest habitat. During the summers of 1973 and 1974, there were approximately 30 horses maintained for the purpose of teaching the children how to ride, basically for their enjoyment. In the summer of 1973, when the EEE outbreak took place, all horses had remained on the premises prior

Fig. 5.--Wooded area of recreation hall overlooking Fish Lake.

Fig. 6.--Horse pasture and horse forest area.

to and during the outbreak, eliminating any doubt that they had contracted the disease in another area.

MATERIALS AND METHODS

Mosquito collections were made with New Jersey light traps, Communicable Disease Center (CDC) traps, chicken baited traps, and an aspirator. Weather Data recorded in the nearby city of Milford was also used.

New Jersey Light Traps

It was hoped that by comparing the weather factors in 1974 and 1973, and by analyzing the mosquito species collected in 1974, one might make some speculations concerning the EEE transmission cycles that occurred in 1973.

Each of two New Jersey traps were operated for one 24 hour period every week. Each 24 hour period was divided into two separate collection times, a day period and a night period. The traps usually were started just before dusk on Friday night (at approximately six o'clock), and operated for 12 hours. At six o'clock the following morning, the night collections were removed and a new collection bottle inserted to collect mosquitoes that were active through the following day. The traps were turned off, just before dusk (about six o'clock) the following evening.

These two New Jersey traps were located at the horse barn and in the cabin area of the camp where a source of electrical current was available, one in each location. The one at the horse barn (Figure 2 letter F, and Figure 7) was hung from the overhanging roof on the southwest side of the barn, seven feet above ground level, to attract the mosquitoes west of the horse barn and from the horse pasture, southeast of the trap. The one in the cabin area (Figure 2 letter G_1, and Figure 8), originally was in a wooded area midway between the cabins and Fish Lake, about five feet from the ground in a small clump of trees. On September 5, it was moved west 1000 feet, closer to the shore of the lake because the original source of electricity was shut off for the season (Figure 2 letter G_2, and Figure 9).

CDC Traps

CDC traps are compact and lightweight, designed especially for collecting mosquitoes in areas without a source of electrical power. The trap's power supply is a six volt battery which supplies the energy necessary to light a small light bulb and turn a small fan for approximately 12 hours. Two CDC traps were operated concomitantly with the New Jersey traps, for two consecutive 12 hour periods. Early in the study, there was considerable difficulty in keeping the traps running for longer than three or hour hours. It was later discovered that a few drops of

Fig. 7.--New Jersey light trap hung from horse barn next to horse forest.

Fig. 8.--New Jersey light trap position in the highly wooded cabin area.

Fig. 9.--Second location of New Jersey light trap in cabin area.

oil applied at the beginning of each 24 hour period assured continuous operation. A Shell "No Pest Strip" containing Vapona® in the collection container of the trap killed the mosquitoes that went into the trap, minimizing damage or destruction to necessary taxonomic features.

The locations of these traps are shown in Figure 2 (A and C). One was placed about 20 feet from the waterline near the south side of the bog, five feet above ground level in a small tree at the edge of the wooded area which completely surrounded the bog (Figure 10). The second was placed at the northwest end of the extreme outer edge of the wooded area which surrounds the bog. It was approximately 600 feet from the northwest border of the bog proper (Figure 11).

Chicken-Baited Traps

The five chicken baited traps used were a very necessary part of the study, as they were needed to capture the mosquitoes that normally feed on birds. The trap construction was based on the design of Hayes (1961), and Taylor, Meadows, and Baughman (1966). They were made of thin sheet metal and were 12 inches in diameter by 24 inches in length (Figure 12). Each end had a detachable screen mesh funnel 12 inches in diameter and 4.5 inches deep pointing into the trap interior. The entrance holes at the vortex were one inch in diameter. A compartment was built into the center of the trap, to accommodate the

Fig. 10.--CDC trap position in bog area of Camp Ohiyesa.

Fig. 11.--CDC trap at extreme outer edge of woods surrounding bog.

Fig. 12.--The position of chicken baited trap in the bog area.

Fig. 13.--Chicken baited trap located between woods of bog and horse pasture.

chicken bait, with screened sides on each end to keep the mosquitoes away from the chicken and confined to the outer compartments of the trap. This prevented the chickens from feeding on the mosquitoes.

The traps, each with an adult New Hampshire chicken as bait, were hung two to five feet above the ground and were operated for two consecutive 12 hour periods, the same as the New Jersey and CDC traps. To retrieve the captured mosquitoes, the funnel entrance holes were plugged with wads of cotton and the chicken was removed through a door in the trap center. The trap was next positioned vertically on the ground and a large wad of cotton soaked with chloroform was placed on top of the uppermost funnel, over which a plastic bag was positioned. In approximately five minutes the anesthetized mosquitoes were removed and placed in a container to await identification.

The three chicken baited traps were positioned as marked in Figure 2, letters B, D, and E. The first trap was located within the wooded area surrounding the bog about 30 feet from shore, and about 100 feet west of the CDC trap. This chicken baited trap was placed one and one half feet above ground level (Figure 12). The second was located about 125 feet north of the second CDC trap on the outer edge of the wooded area at the west end of the bog area, approximately 600 feet northwest of the bog. It was positioned three and one half feet above the ground

(Figure 13). The third trap (Figure 14) was located in the horse forest, about 150 feet northwest of the New Jersey trap, five feet above ground level to keep the horses from trampling it.

Resting Traps

Resting traps were used to capture species of mosquitoes present in the area that might not be attracted to one of the other three types of traps. The resting traps were constructed from cans of ten gallon capacity, 12 inches in diameter by 15 inches in length. One half of one end of each can was cut out to provide an opening for the entering mosquitoes. The traps were then painted a flat black color, inside and out, to provide the dark space necessary for a good resting place. However, these traps did not perform adequately and were abandoned after three weeks of use.

Aspirator Collections

A portable mouth operated aspirator (Figure 15), was used to collect mosquitoes from the skin of horses and humans to determine which mosquito species feed on these hosts. The aspirators were constructed of a glass tube about three eighths inch in diameter by one foot in length. One end was open; the other end was covered by a small piece of screen. Over this, a rubber tube two to three feet lone was positioned. On occasion a horse was tied in

Fig. 14.--Horse forest chicken baited trap.

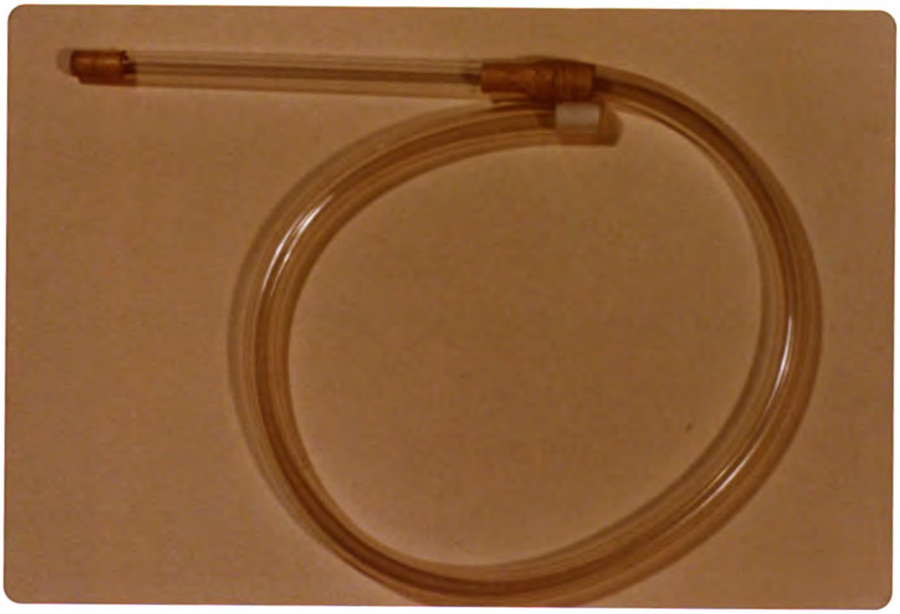


Fig. 15.--Portable mouth operated aspirator.

the middle of the pasture at dusk and mosquitoes were collected from it for a ten minute period. Then, the horse was taken into the adjacent forest and the mosquitoes landing on the horse were collected during a second ten minute period. Human bait collections were made in the bog 20 feet south of the chicken baited trap in medium high grass about 50 feet from the bog shore. A ten minute collection period was also used. Figure 2 indicates the locations of horse and human baited collection sites.

Table 1 indicates the types of traps used and whether or not a collection sample was obtained from the operation of a trap on a particular date.

Table 1.--Mosquito traps and collection dates.

Trap and Location	7-13	7-14*	7-20	7-21*	7-26	7-27*	8-3	8-4*	8-8*	8-9	8-18	8-19*	8-22	8-29	8-29*	9-5	9-5*	9-17	9-17*	9-27
New Jersey Traps:																				
Horse Barn	X	X	X	X	X	X	X	X	X	X	X	X		X				X		X
Cabins	X	X	X	X	X	X			X	X	X	X		X		X		X		
Chicken Baited Traps:																				
Bog			X	X	X		X			X	X									
Road's End	X		X	X	X		X				X			X						
Horse Forest			X	X	X	X	X			X	X			X						
CDC Traps:																				
Bog							X			X	X			X						X
Road's End			X				X			X	X									
Human Bait:																				
Bog						X				X			X							
Horse Pasture																				
Horse Forest										X			X							
Cabins										X										
Horse Bait:																				
Road's End										X			X							
Horse Forest						X				X			X							

* = Day collections. All others are night collections.

RESULTS

The mosquito trap and aspirator collections, indexed by collection dates are shown in Table 2. With the exception of 2 days (July 26 and 27), all traps were operated for two 12 hour shifts, once each week during this study. On July 26 and 27, the CDC motors did not run continuously and were removed from service. The aspirator collections are indicated in Table 2 under "human baited" and "horse baited" headings on July 27, August 8, 9, and 22.

In total, 5,257 mosquitoes were collected, with the largest numbers collected on July 13-14, and 26-27. Sixteen different species of mosquitoes were collected. Specimens that could not be identified to the species level are shown in Table 2 as *Aedes* species, *Anopheles* species, *Culex* species, or *Culiseta* species.

Coquillettidia perturbans (Walker) was in greatest abundance on July 13-14 (1,660), and July 26-27 (1,549). This species comprised the bulk of each collection, except for the weeks of September 17 and 27, and formed 86.7 percent of the total collection. *C. perturbans* was most abundant in the New Jersey and chicken baited traps (Table 3). It was collected in large numbers early in the

Table 2.--Collection of various mosquito species by date and trap type.

Date	Trap and Location[1]	Numbers of Mosquito Species Collected: Aedes species	Anopheles species	Culex species	Culiseta species	Aedes canadensis	Aedes cinereus	Aedes fitchii-stimulans	Aedes triseriatus	Aedes vexans	Anopheles earlei	Anopheles punctipennis	Anopheles quadrimaculatus	Anopheles walkeri	Coquillettidia perturbans	Culex erraticus	Culex pipiens	Culex salinarius	Culiseta inornata	Culiseta melanura	Culiseta morsitans	Trap Subtotals
7-13-74	N.J. Horse barn		10										26	11	817					1		864
	N.J. Cabins	1	2										2		145							150
	C.B. Road's end									2				18	237							257
7-14*	N.J. Horse barn	4								3		1	7	20	371							406
	N.J. Cabins									2			3		90							95
	Subtotal	5	12							7		1	38	49	1660					1		1773
7-20	N.J. Horse barn									11			17	23	111							162
	N.J. Cabins	1													5							6
	CDC Road's end														4		1					5
	C.B. Bog							1							196							197
	C.B. Road's end														26							26
	C.B. Horse forest														36							36
7-21*	N.J. Horse barn														6					1		7
	C.B. Bog														27							27
	C.B. Road's end														5							5
	C.B. Horse forest														5							5
	Subtotal	1						1		11			17	23	421		1			1		476
7-26	N.J. Horse barn									3			42	22	1062							1129
	N.J. Cabins												1	6	123							130
	C.B. Bog														147							147
	C.B. Road's end														92		1					93
	C.B. Horse forest														68							68
7-27*	N.J. Horse barn											1		1	19							21
	H.B. Bog					3	4								9							16
	X.B. Horse forest									8					29							37
	Subtotal					3	4			11		1	43	29	1549		1					1641
8-3	N.J. Horse barn					1				8			16	35	298		1				2	361
	CDC Bog										1		3	1	7					2		14
	CDC Road's end									1			8	8	25					1		43
	C.B. Horse forest														44		1					45
	C.B. Road's end														31		6					37
	C.B. Bog														48		4					52
8-4*	N.J. Horse barn														5							5
	Subtotals					1				9	1		27	44	458		12			3	2	557
8-8*	N.J. Horse barn												5		3							8
	N.J. Cabins												5	1								6
	H.B. Bog												1		4							5
	H.B. Forest									1					9							10
	H.B. Cabins									2					5							7
8-9	N.J. Horse barn	1	1							3		2	27	14	64	1						113
	N.J. Cabins												45		71							116
	CDC Bog										3		5	3	23				1		4	39
	CDC Road's end												6		1							7
	C.B. Bog														25							25
	C.B. Horse forest														21		3					24
	X.B. Road's end	11				2				11					3							27
	X.B. Horse forest									4			1		52							57
	Subtotals	12	1			2				21	3	2	95	18	281	1	3		1		4	444

8-18	N.J. Horse barn									1	1		18	5	19				1			45
	N.J. Cabins									1			5	1	51		4					62
	CDC Bog				1						6	1	3		11							22
	CDC Road's end												4		2							6
	C.B. Bog														21		2					23
	C.B. Road's end														5							5
	C.B. Horse forest														12		6					18
8-19*	N.J. Horse barn												6									6
	N.J. Cabins														2							2
	Subtotals				1					2	7	1	36	6	123		12		1			189
8-22	H.B. Bog	1							1	5												7
	H.B. Horse pasture												1		17							18
	X.B. Road's end												16		17							33
	X.B. Horse forest												15		1							16
	Subtotals	1							1	5			32		35							74
8-29	N.J. Horse barn			1						2			19	10	6							38
	N.J. Cabins										1		9		10							20
	C.B. Road's end														3		1					4
	C.B. Horse forest														8		8					16
	CDC Bog												2	3		1				2		8
	Subtotals			1						2	1		30	13	27	1	9			2		86
9-5	N.J. Cabins												2		1							3
	Subtotals												2		1							3
9-17	N.J. Horse barn											1	4		1							6
	N.J. Cabins																1					1
	Subtotals											1	4		1		1					7
9-27	N.J. Horse Barn									1					1							2
	CDC Bog													1	1			2				4
	Subtotals									1				1	2			2				6
July	Monthly subtotals	6	12			3	4	1		29		2	98	101	3630		2			2		3890
August	Monthly subtotals	13	2	1	1	3			1	39	12	3	220	81	924	2	36		2	5	6	1351
September	Monthly subtotals									1		1	6	1	4		1	2				16
	Season Totals	19	14	1	1	6	4	1	1	69	12	6	324	183	4558	2	39	2	2	7	6	5257

[1] N.J. = New Jersey light trap; C.B. = Chicken baited trap; X.B. = Horse bait trapping method; H.B. = Human bait trapping method.

*Daytime collection.

Table 3.--Seasonal totals per trap type (N = night, D = day).

Species Collected	Trap Types: New Jersey		CDC		Chicken Baited		Human Baited		Horse Baited		Species Subtotals		Species Totals
	N	D	N	D	N	D	N	D	N	D	N	D	
Aedes species	3	4					1		11		15	4	19
Aedes canadensis	1							3	2		3	3	6
Aedes cinerius								4				4	4
Aedes fitchii-stimulans					1						1		1
Aedes triseriatus							1				1		1
Aedes vexans	30	5	1		2		5	3	15	8	53	16	69
Anopheles species	13								1		14		14
Anopheles earlei	2		10								12		12
Anopheles punctipenis	3	2	1								4	2	6
Anopheles quadrimaculatus	233	26	31				1	1	32		297	27	324
Anopheles walkeri	127	22	16		18						161	22	183
Coquillettidia perturbans	2785	496	74		1020	37	17	27	73	29	3969	589	4558
Culex species	1										1		1
Culex erraticus	1		1								2		2
Culex pipiens	6		1		32						39		39
Culex salinarius			2								2		2
Culiseta species			1								1		1
Culiseta inordinata	1		1								2		2
Culiseta melanura	1	1	5								6	1	7
Culiseta morsitans	2		4								6		6
Trap Totals	3209	556	148		1073	37	25	38	134	37	4589	668	5257

summer season in a chicken baited trap in the bog area. As the season progressed, however, more of this species were collected in the horse ranch area than the bog area or road's end area. Most of the C. perturbans collected during the summer season were taken from the horse ranch area in the New Jersey trap (2,783 specimens).

Aedes vexans were collected mainly from the horse ranch New Jersey trap (32 specimens), as opposed to the cabin area New Jersey trap (3 specimens). Thirty three percent of the total A. vexans collected were taken from horses in the horse ranch area of the camp.

Two specimens of Culiseta melanura were taken from the horse barn New Jersey trap and none from the cabin area New Jersey trap. One C. melanura was taken from the road's end CDC trap, and four specimens were taken by the bog area CDC trap.

A further breakdown was necessary to determine which habitat was more favorable to a particular species (Table 4). The relative numbers of a given species collected each week at a specific trap location, indicates when it reached peak levels. The results show that the two most favorable habitats for Coquillettidia perturbans, Aedes vexans, and Culiseta melanura are the bog area and the horse forest area.

Table 4.--Numbers and species of mosquitoes collected per trap type during the summer of 1974.

Traps, Location, and Species Collected	Dates of Mosquito Collection: 7-13 Night	7-14 Day	7-20 Night	7-21 Day	7-26 Night	7-27 Day	8-3 Night	8-4 Day	8-9 Night	8-8 Day	8-18 Night	8-19 Day	8-22 Dusk	8-29 Night	9-5 Night	9-17 Night	9-27 Night	Species Subtotals per Trap Type: Night	Day	Subtotals
I. New Jersey Traps																				
A. Horse Barn Area																				
1. Aedes sp.		4							1									1	4	5
2. Aedes canadensis							1											1		1
3. Aedes vexans		3	11		3		8		3		1			2			1	29	3	32
4. Anopheles sp.	10								1									11		11
5. Anopheles earlei											1							1		1
6. Anopheles punctipenis		1				1			2							1		3	2	5
7. Anopheles quadrimaculatus	26	7	17		42		16		27	5	18	6		19		4		169	18	187
8. Anopheles walkeri	11	20	23		22	1	35		14		5			10				120	21	141
9. Coquilletidia perturbans	817	371	111	6	1062	19	298	5	64	3	19			6		1	1	2379	404	2783
10. Culex sp.														1				1		1
11. Culex erraticus									1									1		1
12. Culex pipiens							1											1		1
13. Culiseta inordinata											1							1		1
14. Culiseta melanura	1			1														1	1	2
15. Culiseta morsitans							2											2		2
Trap Subtotals	865	406	162	7	1129	21	361	5	113	8	45	6		38		6	2	2721	453	3174
B. Cabins Area																				
1. Aedes sp.	1		1															2		2
2. Aedes vexans		2									1							1	2	3
3. Anopheles sp.	2																	2		2
4. Anopheles earlei														1				1		1
5. Anopheles quadrimaculatus	2	3			1				45	5	5			9	2			64	8	72
6. Anopheles walkeri					6					1	1							7	1	8
7. Coquilletidia perturbans	145	90	5		123				71		51	2		10	1			406	92	498
8. Culex pipiens											4					1		5		5
Trap Subtotals	150	95	6		130				116	6	62	2		20	3	1		488	103	591
Trap Totals	1015	501	168	7	1259	21	361	5	229	14	107	8		58	3	7	2	3209	556	3765
II. CDC Traps																				
A. Bog Area																				
1. Anopheles earlei							1		3		6							10		10
2. Anopheles punctipenis											1							1		1
3. Anopheles quadrimaculatus							3		5		3			2				13		13
4. Anopheles walkeri							1		3					3			1	8		8
5. Coquilletidia perturbans							7		23		11						1	42		42
6. Culex erraticus														1				1		1
7. Culex salinarius																	2	2		2
8. Culiseta species											1							1		1
9. Culiseta inordinata									1									1		1
10. Culiseta melanura							2							2				4		4
11. Culiseta morsitans									4									4		4
Trap Subtotals							14		39		22			8			4	87		87
B. Road's End																				
1. Aedes vexans							1											1		1
2. Anopheles quadrimaculatus							8		6		4							18		18
3. Anopheles walkeri							8											8		8
4. Coquilletidia perturbans			4				25		1		2							32		32
5. Culex pipiens			1															1		1
6. Culiseta melanura							1											1		1
Trap Subtotals			5				43		7		6							61		61
Trap Totals			5				57		46		28			8			4	148		148
III. Chicken Baited Traps																				
A. Bog Area																				
1. Aedes fitchii-stimulans			1															1		1
2. Coquilletidia perturbans			196	27	147		48		25		21							437	27	464
3. Culex pipiens							4				2							6		6
Trap Subtotals			197	27	147		52		25		23							444	27	471

B. Road's End																				
1. Aedes vexans	2																	2		2
2. Anopheles walkeri	18																	18		18
3. Coquilletidia perturbans	237		26	5	92		31				5			3				394	5	394
4. Culex pipiens					1		6							1				8		8
Trap Subtotals	257		26	5	93		37				5			4				422	5	422
C. Horse Forest																				
1. Coquilletidia perturbans			36	5	68		44		21		12			8				189	5	194
2. Culex pipiens							1		3		6			8				18		18
Trap Subtotals			36	5	68		45		24		18			16				207	5	212
Trap Totals	257		259	37	308		134		49		46			20				1073	37	1105
IV. Human Baited																				
A. Bog Area																				
1. Aedes sp.													1					1		1
2. Aedes canadensis						3													3	3
3. Aedes cinerius						4													4	4
4. Aedes triseriarus													1					1		1
5. Aedes vexans													5					5		5
6. Anopheles quadrimaculatus										1									1	1
7. Coquilletidia perturbans						9				4									13	13
Trap Subtotals						16				5			7					7	21	28
B. Horse Pasture																				
1. Anopheles quadrimaculatus													1					1		1
2. Coquilletidia perturbans													17					17		17
Trap Subtotal													18					18		18
C. Horse Forest																				
1. Aedes vexans										1									1	1
2. Coquilletidia perturbans										9									9	9
Trap Subtotal										10									10	10
D. Cabin Area																				
1. Aedes vexans										2									2	2
2. Coquilletidia perturbans										5									5	5
Trap Subtotal										7									7	7
Trap Totals						16				22			25					25	38	63
V. Horse Baited																				
A. Road's End																				
1. Aedes sp.									11									11		11
2. Aedes canadensis									2									2		2
3. Aedes vexans									11									11		11
4. Anopheles quadrimaculatus													16					16		16
5. Coquilletidia perturbans									3				17					20		20
Trap Subtotals									27				33					60		60
B. Horse Forest																				
1. Aedes vexans						8			4									4	8	12
2. Anopheles sp.													1					1		1
3. Anopheles quadrimaculatus									1				15					16		16
4. Coquilletidia perturbans						29			52				1					53	29	82
Trap Subtotals						37			57				17					74	37	111
Trap Totals						37			84				50					134	37	171
Mosquito Daily Totals	1272	501	432	44	1567	74	552	5	408	36	181	8	75	84	3	7	6	4589	668	5257

B. Road's End																				
1. Aedes vexans	2																	2		2
2. Anopheles walkeri	18																	18		18
3. Coquilletidia perturbans	237		26	5	92		31				5			3				394	5	394
4. Culex pipiens					1		6							1				8		8
Trap Subtotals	257		26	5	93		37				5			4				422	5	422
C. Horse Forest																				
1. Coquilletidia perturbans			36	5	68		44		21		12			8				189	5	194
2. Culex pipiens							1		3		6			8				18		18
Trap Subtotals			36	5	68		45		24		18			16				207	5	212
Trap Totals	257		259	37	308		134		49		46			20				1073	37	1105
IV. Human Baited																				
A. Bog Area																				
1. Aedes sp.													1					1		1
2. Aedes canadensis						3													3	3
3. Aedes cinerius						4													4	4
4. Aedes triseriarus													1					1		1
5. Aedes vexans													5					5		5
6. Anopheles quadrimaculatus										1									1	1
7. Coquilletidia perturbans						9				4									13	13
Trap Subtotals						16				5			7					7	21	28
B. Horse Pasture																				
1. Anopheles quadrimaculatus													1					1		1
2. Coquilletidia perturbans													17					17		17
Trap Subtotal													18					18		18
C. Horse Forest																				
1. Aedes vexans										1									1	1
2. Coquilletidia perturbans										9									9	9
Trap Subtotal										10									10	10
D. Cabin Area																				
1. Aedes vexans										2									2	2
2. Coquilletidia perturbans										5									5	5
Trap Subtotal										7									7	7
Trap Totals						16				22			25					25	38	63
V. Horse Baited																				
A. Road's End																				
1. Aedes sp.									11									11		11
2. Aedes canadensis									2									2		2
3. Aedes vexans									11									11		11
4. Anopheles quadrimaculatus													16					16		16
5. Coquilletidia perturbans									3				17					20		20
Trap Subtotals									27				33					60		60
B. Horse Forest																				
1. Aedes vexans						8			4									4	8	12
2. Anopheles sp.													1					1		1
3. Anopheles quadrimaculatus									1				15					16		16
4. Coquilletidia perturbans						29			52				1					53	29	82
Trap Subtotals						37			57				17					74	37	111
Trap Totals						37			84				50					134	37	171
Mosquito Daily Totals	1272	501	432	44	1567	74	552	5	408	36	181	8	75	86	3	7	6	4589	668	5257

DISCUSSION

Chamberlain *et al*. (1954), and Davis (1940) sought to determine infection and transmission thresholds in certain mosquitoes with an end to determining vector potentials of EEE in virus foci. Of the mosquito species they tested, seven were found to exist at Camp Ohiyesa (Table 5). It was with these seven species the highest probability of EEE virus transmission existed at Camp Ohiyesa.

Criteria Used for Vector Incrimination

Barnett (1960) developed four basic criteria used to incriminate an arthropod of being a disease vector. The researcher must:

1. Demonstrate that the arthropod feeds upon or has other effective contact with the host under natural conditions;
2. Demonstrate that a biological association exists between the suspected arthropod species and a clinical or subclinical infection of the host;
3. Make repeated demonstrations that show the arthropod, under natural conditions, harbors an

Table 5.--Vector potential ratings of selected mosquitoes.

	Species	Isolation and Location	% Lab infected with EEE[1]	% Lab Transmitting EEE[1]	EEE Vector Potential Rating
1.	*Aedes triseriatus*	0	100%	56%	(a) Excellent[1] (b) +[8]
2.	*Aedes vexans*	1 - Connecticut[2]	63%	13%	(a) Fair[1] (b) +[8]
3.	*Anopheles quadrimaculatus*	0	79%	0%	(a) Poor[1] (b) +[9]
4.	*Coquillettidia perturbans*	1 isol. - Burke & Jenkins Co., Georgia[3]	94%	20%	Good[1]
5.	*Culex erraticus*	0	43%	14%	Fair[1]
6.	*Culex salinarius*	2 isol. - South River Game Farm, New Jersey[4]	3.3%	0%	Poor[1]
7.	*Culiseta melanura*	(a) 2 isol. - Manchal Swamp, Ponchtoula, Louisiana[5] (b) 3 isol. - Pheasant farms, New Jersey[6] (c) 3 isol. - South River Game Farm, New Jersey[4] (d) 3 isol. - Massachusetts[7]	13%*	13%	+[10]

[1]Chamberlain, Sikes, Nelson, and Sudia (1954).

[2]Wallis, R. (1960).

[3]Howitt, Dodge, Bishop, and Gorrie (1949).

[4]Chamberlain, Sudia, Burbutis, and Bogue (1958).

[5]Chamberlain, Rubin, Kissling, and Eidson (1951).

[6]Holden, Miller, and Jobbins (1953).

[7]Alexander and Murray (1957).

[8]Davis (1940).

[10]Wallis et al. (1958).

*A virus suspension of only $10^{6.5}$ was used to infect C. melanura a low titer suspension. All other chick blood virus titers used for infection were $10^{8.0}$ - $10^{8.5}$

infectious agent (pathogen), in the infectious stage; and

4. Show that transmission of the infectious agent can be made under controlled conditions.

If all four conditions are met, the suspected arthropod(s) may be incriminated as the vector of an infectious agent, in this case, of EEE virus. Inherent limitations in this study precluded the demonstration of all four of these elements of proof. It was therefore, necessary to combine a literature review with data from the study to incriminate some mosquito species as potential vectors of EEE at Camp Ohiyesa. However, unless viremic studies are performed in the area of infection during the time of the epizootic or epidemic, a suspected carrier can never be proved to be a true vector.

Arthropod Feeding Upon the Host

According to Barnett's first criterion, it has to be shown that the mosquito has effective contact with birds, the primary host of EEE, under natural conditions. Birds have been shown to act as a reservoir for the virus. Specific species of birds are involved in maintaining the virus in an infective state, some for very short periods. According to Schaeffer and Arnold (1954), small marsh birds and songbirds (red-winged blackbirds, cardinals, and sparrows), manifest exceptionally high virus titers, but the majority succumb to a fulminating disease within two to three days. Other species (e.g., ibis and egret) develop few or no signs of infection and exhibit a lower level of viremia. Purple grackles were found to exhibit high long lasting viremias, but did not exhibit clinical signs of infection. The virus was almost always fatal in certain other bird species tested (Kissling, Chamberlain, Sikes, and Eidson, 1954).

Bird collections were made in the Camp Ohiyesa area (Scott Askins, personal communication), in the summer of 1974. The birds collected are listed in Table 6. A number of red-winged blackbirds were also seen, but were not collected, in the bog and forests of the camp in 1974. According to Kissling, Chamberlain, Sikes, and Eidson (1954), the white ibis, American egret, snowy egret, purple grackle, red-winged blackbird, cardinal, sparrow, and cedar waxwing were all susceptible to EEE infection. At Camp Ohiyesa, the five types of sparrows, the cardinal, and cedar waxwing collected by Askins, and red-winged blackbirds observed were listed by Kissling and his co-workers as being susceptible to EEE infection. Eight species of birds were present at Camp Ohiyesa in 1974 (which probably were also present in 1973), that could have provided an infection source of EEE virus for transmission to other birds, horses, and man, should the vector mosquito species have been present.

Table 6.--A 1974 list of the bird species at Camp Ohiyesa.

Species Collected*	No. Col.	Susceptible to EEE infection[1]
Robin (Turdus migratorius)	5	
Indigo bunting (Passerina cyanea)	4	
Catbird (Dumetella corolinensis)	1	
Starling (Sturnus vulgaris vulgaris)	7	
Field sparrow (Spizella pusilla pusilla)	4	X
House sparrow (Passer domesticus domesticus)	1	X
Savannah sparrow (Passerculus sandwichensis)	1	X
Song sparrow (Melospiza melodia)	1	X
Swamp sparrow (Melospiza georgina)	1	X
Rose-breasted grosbeak (Pheucticus ludovicianus)	1	
Cardinal (Richmondena cardinalis)	1	X
Cedar waxwing (Bombycilla cedrorum)	1	X
Oven bird (Seiurus aurocapillus)	1	
Red-headed woodpecker (Melanerpes erythrocephalus erythrocephalus)	1	
White breasted nuthatch (Sita carolinensis)	1	
Red-winged blackbird (Agelaius phoeniceus)[2]	0	X

*S. Askins, Unpublished Report (1975).

[1]Kissling, Chamberlain, Sikes, and Eidson (1954).

[2]Observed in 1974.

All of the mosquito species listed in Table 5 were induced by Chamberlain, Sikes, Nelson, and Sudia (1954), to feed on baby chicks. Individual specimens of each species were placed in small cages made of pint sized ice cream cartons. Half day old chicks were mounted in cloth slings to prevent movement and the plucked breast was held against the end of the cage by hand. If that failed, the chickens were constrained into position for a six to twelve hour period. The purpose was both to attempt to infect the mosquitoes with virus from infected chicks, and also to later transmit the virus from mosquito to normal chicks. The results of this experiment indicate birds may be host for all seven of the mosquitoes listed in Table 5.

Data collected on the species of mosquitoes taken from the chicken baited traps at Camp Ohiyesa indicate that, of the seven species listed in Table 5, only *Coquillettidia perturbans* and *Aedes vexans* were collected in large enough numbers to show they have a natural appetite for avian hosts. Four other species were collected, *Aedes triseriatus* (1), *Culex erraticus* (Dyar and Knab) (2), *Culex salinarius* (Coquillett) (2), and *Culiseta melanura* (7), but not in chicken baited traps. It is presumed their absence in chicken baited traps to be due to the low numbers present in the area, since they have been shown to feed on birds in other areas. *Aedes triseriatus* feeds upon the blood of fowl (Wallis *et al*., 1958); *Culex erraticus* is a bird feeder (Carpenter and LaCasse, 1955); Culex salinarius according to Siverly (1972), will take avian blood meals; and Culiseta melanura prefers avian blood meals over mammalian blood meals (Hayes, 1961).

Since only those mosquito species that naturally feed on birds need be considered as suspected vectors of EEE, Anopheles quadrimaculatus may be deleted from the list of species in Table 5 as a suspect EEE vector. A. quadrimaculatus was collected in large numbers for the whole season but never from a chicken baited trap (Table 3). Carpenter and LaCasse (1954), as well as Siverly (1972), indicate that this mosquito primarily feeds upon a wide variety of mammalian hosts, but not birds.

Laboratory Induced Infection

The susceptibility of the species listed in Table 5 to biological infection of EEE was determined by Chamberlain, Sikes, Nelson, and Sudia (1954). This test relates to Barnett's criterion number 2. Column three of Table 5 gives the susceptibility of mosquitoes to biological infection (the number of species infected, divided by the number of species tested). The number of specimens for each species tested in this study was quite variable, and ranged from 74 specimens of Coquillettidia perturbans to only eight specimens used to test Aedes vexans. Only four species had high rates of susceptibility to biological infection, Aedes triseriatus (100%), Coquillettidia perturbans (94%), Anopheles quadrimaculatus (79%), and Aedes vexans (63%). This shows that should these four species ingest EEE virus, they are likely to become infective. However, three of the other species tested had only low rates of susceptibility to infection, Culex erraticus (43%), C. salinarius (3.3%), and Culiseta melanura (13%). C. melanura was infected with a lower titer virus suspension ($10^{6.5}$), compared to the other species which were infected by feeding on a chick with a blood virus titer of $10^{8.0}$ to $10^{8.5}$. This might account for the low rate of infectivity of C. melanura. The experiments of Chamberlain, Sikes, Nelson, and Sudia (1954), on a number of mosquito species then have shown five species, also collected at Camp Ohiyesa, to be of practical significance as vectors, Aedes triseriatus, A. vexans, Anopheles quadrimaculatus, Coquillettidia perturbans, and Culiseta melanura.

Virus Isolations

Isolations of EEE have been made from four of the species of mosquitoes listed in Table 5 during epidemics and epizootics: (1) Aedes vexans (Wallis, 1960), (2) Coquillettidia perturbans (Howitt, Dodge, Bishop, and Gorrie, 1949), (3) Culex salinarius, from which two disputable isolations have been made (Chamberlain, Sudia, Burbutis, and Bogue, 1958), and (4) Culiseta melanura from which 11 isolations have been made (Alexander and

Murray, 1957; Holder, Miller, and Jobbins, 1953; Chamberlain, Rubin, Kissling, and Eison, 1951; Chamberlain, Sudia, Burbutis, and Bogue, 1958). Although virus was found in *Culex salinarius*, it was thought the engorged mosquitoes included in the mosquito pool contained the virus in a recent blood meal and not in the mosquito tissues. This implies that the virus did not propagate in *C. salinarius* and thus those mosquitoes were not fairly implicated as infective mosquitoes. No EEE isolations have been reported from *Anopheles quadrimaculatus*, *Aedes triseriatus*, and *Culex erraticus* in nature. This gives greater reason to believe that *Aedes vexans*, *Coquillettidia perturbans*, and *Culiseta melanura* are more likely involved in the EEE cycle as vectors than are the other species in Table 5.

Transmission Ability

Barnett's criterion number four requires transmission of EEE from the mosquito to a vertebrate host. Table 5 indicates the ability of each species to transmit EEE virus as verified by Chamberlain, Sikes, Nelson, and Sudia (1954). This is a percentage figure calculated from the number of infected mosquitoes transmitting the virus per the number feeding on 0.5 day old chicks. Chicks were observed for 96 hours, and the brains of those dying were passed in mice to verify virus infection. The last column of Table 5 gives the vector classification of Chamberlain and his co-workers (1954) rated as "excellent," "good," "fair," or "poor." Aedes triseriatus obtained an "excellent" rating, Coquillettidia perturbans was "good," Aedes vexans and Culex erraticus were "fair," and Anopheles quadrimaculatus and Culex salinarius were "poor." Culiseta melanura was tested by Wallis (1960) and was rated with a positive sign indicating positive virus transmission. Only two of the fifteen specimens he tested became infected, although both were able to transmit the virus in the laboratory. Those species that obtained a rating of "excellent," "good," and "fair," or "+" (where the other ratings are not given), are the most strongly implicated in EEE transmission experiments. This implicates Aedes triseriatus, A. vexans, Coquillettidia perturbans, Culex erraticus, and Culiseta melanura as potential vectors at Camp Ohiyesa.

Consideration given to all of Barnett's four criteria seem to incriminate three mosquito species from Camp Ohiyesa as potential vectors of EEE virus. They are Coquillettidia perturbans, Aedes vexans, and Culiseta melanura.

Mosquito Host Preferences

The host animals attacked by mosquitoes are important because they can be the source of an EEE virus infection or the victim of an infection. Culiseta melanura is known primarily as an avid bird feeder and it has been postulated that it is involved in spreading the infection among wild birds (Feemster, 1957). If this mosquito species had been in large numbers during the summer of 1973 at Camp Ohiyesa, it could have been responsible for transmission of the virus from infected birds with high blood virus titer to susceptible uninfected birds. Then the virus might eventually have been transmitted to a large portion of the susceptible indigenous bird population. This would have increased probability of infection for the surrounding population of horses and man, presuming another vector was present which fed on birds and horses or man.

Aedes vexans feeds on avian reservoirs as well as humans, but feeds on horses more frequently than on man (Hayes, Beadle, Hess, Sussman, and Bonese, 1962). This feeding behavior would allow the transmission of the virus from birds to horses in the EEE cycle. This species was collected by the author at Camp Ohiyesa in a chicken baited trap (25 specimens), in a human baited collection (8 specimens), and in horse baited collections (23 specimens). It will definitely feed on all three types on animals. The hypothesis is that if *Aedes vexans* were present in large numbers in 1973, it could conceivably have assisted in the spread of the disease from birds to the horses.

Coquillettidia perturbans is a persistent feeder on warm blooded animals, including horses and chickens (Howitt, Dodge, Bishop, and Gorrie, 1949). In the summer of 1974, C. perturbans were attracted to the chicken baited traps (1,057 specimens), human bait (44 specimens), and horse bait (102 specimens). Of the three mosquito species named as potential vectors through Barnett's criteria, C. perturbans is potentially the most likely vector of equine encephalitis to birds, humans, and horses, since this mosquito feeds readily on many animals.

Mosquito Abundance

A second qualification for a mosquito species to be incriminated as a potential vector is the requirement for the existence of large numbers of individuals. Reeves and Hammon (1962) state that if "all other factors remain constant, there is a critical vector population level below which virus will not be transmitted, and that, as a vector population exceeds this, transmission will be accelerated." Although this critical population level is as yet unknown, a comparative relationship between the abundance of the four species in question above and the disease outbreak in Camp Ohiyesa can be postulated. It can be seen from Table 3 that Coquillettidia perturbans was without doubt the most abundant species in 1974. Aedes vexans followed with 67 specimens, Culiseta melanura with 7, and only one Aedes triseriatus was collected.

Aedes vexans, Culiseta melanura, and Aedes triseriatus were not collected in numbers equal or even close to Coquillettidia perturbans. Assuming that the numbers collected in 1974 were similar to the proportions that existed during the season of the epizootic at Camp Ohiyesa in 1973, the vector potential of *Aedes vexans*, *Culiseta melanura*, and *Aedes triseriatus* would not be as great as that of *Coquillettidia perturbans*. If conditions were favorable for *Culiseta melanura* in 1973, it is possible that their reproduction and survival may have been enhanced so that their numbers could have been greater. According to Carpenter and LaCasse (1955), the larvae of *C. melanura* are found more frequently in small permanent bodies of water, particularly in swamps. Given favorable temperature and precipitation, the bog habitat at Camp Ohiyesa would be quite suitable for oviposition and larval development as well as providing excellent hosts for this bird feeder, as many birds are present in the bog area.

Only one specimen of *Aedes triseriatus* was collected, during the whole of the 1974 summer season. According to Dr. Siverly (1972), the eggs of this mosquito are laid and the larvae develop in cavities in trees and stumps; it is a treehole mosquito. This means that this mosquito would not normally attain the high numbers as does, for example, a floodwater mosquito such as *Aedes vexans* or *Aedes trivitatus* (Coquillett). So, in addition to never having had EEE isolated from *Aedes triseriatus* in nature, the collection of only one *A. triseriatus* during the whole summer at Camp Ohiyesa was another reason for discounting *Aedes triseriatus* as a vector of EEE at Camp Ohiyesa.

Aedes vexans, on the other hand, is a floodwater mosquito which is multivoltine and prevalent the whole summer season if conditions are favorable. Siverly (1972) says there is practically continual production from early spring to late fall during years when rainfall is plentiful and well distributed. It breeds in roadside puddles, woodland pools, vehicle ruts, borrow pits, and waste lagoons. It develops in polluted water or water low in organic matter and production can be enormous. Thus, the potential of obtaining very high numbers of *A. vexans* is always present.

Coquillettidia perturbans was collected in very large numbers in every trap location, and amounted to 86.7 percent of the total seasonal collection. It lays its eggs on top of permanent bodies of water. After hatching, the larvae, whose air tubes are modified for piercing, obtain oxygen from the roots and stems of submerged plants such as cattail, water hyacinth, and arrowhead. Siverly (1972) indicated that there appears to be only one generation of this species per year in Indiana. Although no samples of larvae were made in the bog area in 1974, the bog habitat in the area of Camp Ohiyesa is a likely place for this species to develop and emerge, after overwintering as larvae, in early summer, i.e., in late June or early July, depending on the temperature. Knowing a large number of C. perturbans were present in 1974, it seems likely that a large population also was present during the EEE outbreak in 1973.

Temperature and Precipitation

Two facets of the weather picture at Camp Ohiyesa are given. They will relate the high temperature and precipitation that existed during the season of 1973, to the emergence of mosquitoes during that period.

A temperature comparison between 1973, 1974, and a 30 year average used as a standard, is given in Table 7. The average monthly highs and lows are given for the period May through September. May and June average monthly temperatures both were higher in 1973 than 1974 by 10.2°F. and 3.4°F., respectively. Compared to the 30 year average, however, only a small variation is observed between the two years and the temperatures experienced in 1973 were not far from the norm.

The cumulative temperature high for the five months of the normal mosquito season was 18.6°F. higher in 1973 than in 1974, and 17.6°F. higher than the cumulative low. The above information indicates a slightly warmer season in 1973 than in 1974.

A second weather factor which plays a significant role in mosquito production is the amount of precipitation.

Table 7.--Average monthly temperatures (°F.) at the Milford General Motors Proving Grounds recording station.[1]

Year		May	June	July	August	September
1973	Ave. High	64.2	79.6	82.5	83.2	74.3
	Ave. Low	46.2	60.9	63.3	63.4	54.0
1974	Ave. High	54.0	76.2	85.4	81.5	68.1
	Ave. Low	45.4	55.7	61.4	60.6	47.1
Average[2]	Ave. High	66.7	77.0	81.1	79.4	71.8
	Ave. Low	45.5	56.2	60.0	58.7	51.5

[1] U.S. Department of Commerce, 1971, 1973, and 1974.

[2] A 30 year average from 1940 through 1969.

Table 8 shows the monthly precipitation for 1973, 1974, and a 30 year average. The 1973 period for May, June, and July was similar to the 30 year average. The same period in 1974 had markedly less rainfall in June and July. June, 1973, had a higher total, by 1.46 inches, than June 1974. July, 1973, records indicate about three times the amount for the same period in July, 1974. Records for May through September, 1973, show 2.03 inches of rainfall greater than the same period in 1974. Although this is .72 inch less than the 30 year average, it supports the contention that there were fewer specimens of *Aedes vexans* collected in 1974 than were thought to exist in 1973. As a result of the additional precipitation combined with the higher temperatures in 1973, *Aedes vexans* and *Culiseta melanura* could have been in higher numbers than in 1974.

Increased precipitation is very favorable to the production and development of mosquitoes that depend on heavy rains to provide adequate habitats for larval development. Larvae of *Culiseta melanura*, a swamp breeder, are found in small holes at ground level that combine three components: earth, wood, and darkness. These cavities are not above ground level, and either the trunks or the roots of trees are in contact with the water containing the larvae. Although *C. melanura* are thought to overwinter as larvae, rains must occur with sufficient frequency to insure the water level is maintained above ground level,

Table 8.--Average monthly precipitation (inches) at the Milford General Motors Proving Grounds recording station.[1]

Year	May	June	July	August	September	Total
1973	3.67	3.69	3.36	2.45	2.29	15.46
1974	4.85	2.23	1.17	3.22	1.96	13.43
Average[2]	3.44	3.64	2.95	3.43	2.72	16.18

[1]U.S. Department of Commerce, 1971, 1973, and 1974.

[2]A 30 year average from 1940 through 1969.

otherwise the larvae burrow into the damp soil and remain until flooding does occur (Siverly, 1972).

Temperature and precipitation are also very important to the production of *Aedes vexans*. These mosquitoes overwinter in the egg stage and are multivoltine. If rains occur in sufficient quantity during the mosquito season, almost continual production of these mosquitoes may be possible (Siverly, 1972). The precipitation for May, 1973, was 1.18 inches less than May, 1974, one and one half times greater for June, 1973, than June, 1974, and three times greater for July, 1973, than July, 1974. The temperature between May and September of 1973 was warmer than during the same period in 1974. This combination of precipitation and temperature shows it may have been possible for the production of a larger number of *Aedes vexans* to have occurred during the mosquito season of 1973 than in 1974.

Coquillettidia perturbans, however, would not be affected by this difference in precipitation between 1973 and 1974 as its development occurs in the deeper waters of the bog and is not dependent on intermittent floodings. Since the temperatures for July, 1973, and July, 1974, are similar, little differences would be expected in emerging numbers of *C. perturbans* in each of these two periods.

The first case of EEE in Camp Ohiyesa occurred on August 6, 1973, so, allowing approximately three to five days for development of the virus to infective levels in the horse from the time it was first infected by the mosquito, and two weeks for the intrinsic incubation period in the mosquito, this would indicate that the mosquito had taken an infective blood meal from a bird about the middle of July, 1973. It may have been possible in 1973, that the months of June and July were critical in the buildup of virus infections in the bird population by providing optimum temperature and precipitation for the production, emergence, and survival of an abundant supply of mosquitoes. There would then have been enough infected birds to serve as a highly concentrated source of infection, and a large supply of mosquitoes to transmit EEE to other animals, such as horses at Camp Ohiyesa.

EEE Affected Only Horses

A question which immediately comes to mind concerning this epizootic is, why were the horses the only accidental or secondary vertebrate hosts that were infected? No human cases of EEE were reported. One possible reason for this is that the period of peak activity for the suspected mosquito species is after daylight hours when most of the human inhabitants of that area are indoors. According to Hayes, Beadle, Hess, Sussman, and Bonese (1962), *Aedes vexans* shows a very high preference for horses and reaches a peak in biting activity in the evening after sundown.

Chamberlain (1958) indicated that one need not be concerned about transmission to man or horse by *Culiseta melanura*. Precipitin tests on specimens engorged with blood when captured have shown that birds are the preferred hosts of *C. melanura*. Epizootics in horses and the occasional cases in man cannot logically be attributed to this species. It would appear that, at least at Camp Ohiyesa, other species are involved in the transmission of the virus from birds to horses and to man.

Coquillettidia perturbans is much more active at night than during the day. Table 3 compares day and night collections of *C. perturbans*. Since the children and personnel at the camp are indoors after dark, they do not have the exposure to *C. perturbans* that birds and horses of that area do. As can be seen from Figure 16, the cabins in which the children sleep are well built, enclosed structures, providing adequate protection for the children from annoying insects, as well as from the elements.

A second possible reason for the children being unaffected and the horses infected with EEE, may be the location of the species under consideration as potential vectors. The New Jersey light trap collections of *Culiseta melanura*, *Aedes vexans*, and *Coquillettidia perturbans* at the horse barn and the cabins (Table 4), indicate a much heavier concentration of these species at the horse ranch than in the cabin area. Since they are

Fig. 16.--Sleeping quarters in the Camp Ohiyesa cabin area.

much more numerous in the horse ranch than in the cabin area, they would be much more likely, if infected with EEE, to transmit the virus to horses rather than humans.

A third point is that some of the birds in Table 6 which may act as EEE carriers were captured on the grounds of the horse ranch section of the camp by Scott Askins (1975). This implies that the mosquito vectors in the horse ranch portion of the camp would have had a ready source of infection from viremic birds present in that location and as a result, transmission of EEE may have been facilitated between the birds and horses in that area.

SUMMARY AND CONCLUSION

A study was performed at Camp Ohiyesa in Northwest Oakland County, Michigan, to determine the potential mosquito vectors of Eastern equine encephalitis. Trap collections for the period from July 13, 1974, to September 27, 1974, totalled 5,257 specimens and included 16 species. Three of these species appeared to be important as vectors. The most likely major vector was *Coquillettidia perturbans*. *Aedes vexans* and *Culiseta melanura* may have assisted *Coquillettidia perturbans* in transmitting the virus to horses and birds. These species were implicated mainly on the basis of Barnett's criteria, but additional evidence of the species numbers collected, host feeding preference, favored breeding habitats of these species, and temperature and precipitation levels in 1973, were considered.

It was hypothesized that only the horses rather than the human inhabitants of the affected area were infected with EEE because of the following: (1) the peak activity of the mosquitoes was at dusk or later when the children tended to be indoors; (2) the major concentration of the three most likely vector species was at the horse ranch area, especially in the horse forest area; and

(3) some of the birds susceptible to EEE infection were captured on the grounds of the horse ranch section, indicating the potential mosquito vectors would have had a ready source of virus to transmit to the horse, a second animal easily fed upon by the vector mosquito.

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