AN EPIDEMIOLOGICAL STUDY OF MOSQUITO-BORNE CALIFORNIA ENCEPHALITIS IN A SOUTHERN MICHIGAN SUBDIVISION

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ABSTRACT

AN EPIDEMIOLOGICAL STUDY OF
MOSQUITO - BORNE CALIFORNIA ENCEPHALITIS
IN A SOUTHERN MICHIGAN SUBDIVISION

by

Ralph J. Gorton

The presence of California Group arthropod - borne viruses, and the epidemiological conditions which may maintain these viruses in nature, were investigated in a woodlot - subdivision community in Shiawassee County, Michigan during the late summer and early fall of 1971.

Three clinical cases of encephalitis caused by infections with California Group viruses have occurred in children living in this community; two in 1970 and one in 1971. A serological survey of 30 residents (ages 1 - 39 years) indicated the presence of hemagglutination inhibition antibody titers (La Crosse antigen) of ≧ 1:20 in 23.5% of the susceptible - age group tested (i.e., children under 16 years of age). One of these children had a history of severe encephalitis - like disease in 1967 and a convalescent serum titer (4 years later) of 1:80, which was considered presumptive evidence of infection with California encephalitis virus.

Epidemiological conditions in the community include a large number of susceptible - age children that play in the

woodlots and are exposed to biting insects, 56 species of vertebrates which potentially may serve as mosquito and/or virus hosts, and at least 9 species of man - biting mosquitoes, several of which are proven or suspected vectors of California Group viruses in other North Central states.

Of 312 mosquitoes collected at human bait, 60% were Aedes triseriatus, 17% Ae. vexans, 11% Coquillettidia perturbans, 5% Anopheles quadrimaculatus, 4% Ae. fitchii, 1% Ae. canadensis, < 1% Ae. stimulans, and < 1% An. punctipennis. Culex pipiens was abundant in the woodlot in immature stages, but was not collected at human bait. Only Aedes triseriatus, a proven vector of La Crosse viral subtype in Wisconsin, exhibited a biting pattern which coincided with the daytime play activities of subdivision children.

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INTRODUCTION

Within the last decade there has been a tremendous increase in the number of known arthropod - borne animal viruses (arboviruses) throughout the world. The American Committee on Arthropod - borne Viruses (1970) lists 243 known or presumed arboviruses, but in only about half of these are the vectors known. The others are designated as arboviruses on the basis of biochemical properties and on their ability to multiply in both experimentally infected arthropod and vertebrate tissues (Mattingly, 1969). According to James and Harwood (1969, page 206), "Arthropod - borne viruses are causal agents of some of the most serious and widespread diseases of man and animals. They are by far the most varied and numerous pathogens transmitted by arthropods..." Classified as arboviruses are such well known diseases as dengue, Japanese B encephalitis, urban yellow fever, and Venezuelan equine encephalitis, all of which can occur in epidemic proportions with occasional high mortality to man.

In the United States, arboviruses have been associated with human central nervous system disease since the 1930's, when Eastern equine encephalitis, Western equine encephalitis

and St. Louis encephalitis viruses were shown to cause encephalitis in man (Reeves and Hammon, 1962). These viruses are maintained in essentially mosquito-bird cycles and have been responsible for numerous human epidemics and epizootics in horses and pheasants (James and Harwood, 1969). A fourth arbovirus causing human illness in the United States and Canada is California encephalitis virus, which, unlike the equine and St. Louis encephalitis viruses, seems to be a mammalian virus, transmitted primarily by mosquitoes of the genus Aedes (Mattingly, 1969; Thompson, 1969; Sudia et al., 1971). As presently constituted, there are eight serotypically distinct California encephalitis viruses known to occur in North America, which are called, collectively, the California Group arboviruses.

In 1964 the first epidemic of encephalitis caused by the California Group viruses was reported in North America, and produced 12 cases of central nervous system disease in young children in Indiana (Marshall, 1965). Since 1964 the number of confirmed and presumptive human cases due to this group of viruses has risen to more than 520, primarily in the North Central and Eastern United States (Sudia et al., 1971). In less than a decade this arbovirus, which causes illness primarily in children under the age of 15, has become an important late summer - early fall health problem in the North Central United States (Thompson and Inhorn, 1967; Hanson and Hanson, 1970) and a number of institutions have initiated research programs in an effort to study the

epidemiological and ecological aspects of the natural disease cycle.

The first evidence of California Group virus in Michigan was reported by Newhouse et al. (1963), who found neutralizing antibodies in 8 of 9 snowshoe hares (Lepus americanus) collected at Shingleton (Alger County) in the Upper Peninsula. During the summers 1968 - 1970, the Michigan Department of Public Health Laboratory (MDPH) began selectively testing blood samples submitted by private physicians for routine virus diagnosis in an attempt to determine if California Group viruses were involved in human central nervous system diseases occurring in Michigan. During this period the MDPH selected and tested 254 serum samples which met the following criteria: (1) Patients had illnesses of menigitic and/or encephalitic nature but their sera were negative in tests with routine antigens such as mumps, herpes, measles, poliomyelitis and the equine encephalitides; (2) patients were not more than 15 years of age; and (3) the onset of disease occurred between July 1 and October 1. The paired serum samples were subjected to hemagglutination inhibition tests using the La Crosse subtype antigen obtained from the Center for Disease Control in Atlanta, Georgia. The results of the tests were as follows (Becker and Bail, 1972):

Titer increase of 2-fold or greater	-	6
Titer increase of 1-fold	-	3
Initial elevated titer of 1-20 or more	-	2
Negative	-	243
Total with California virus antibodies	-	11 (4.6%)

Although the number of sera with California Group virus antibody was minimal, Newson (1971) noted in an unpublished report that . . . "no special effort was made to obtain human sera from patients with central nervous system disease and the samples tested were only those routinely submitted to the State Public Health Department Laboratory. Test results reported on human sera, therefore, will in no way be indicative of the total number of human California encephalitis virus infections that may have occurred in Michigan during this three year period." An important result of these MDPH tests was the detection of the state's first known clinical illness resulting from an infection with a California Group virus. During the summer of 1969, a four month old child living in St. Johns, Clinton County, was hospitalized with a clinical illness later shown, through serological tests, to have been caused by California encephalitis virus. Epidemiological evidence indicates that the virus must have been contracted in that community. In the summer of 1970, the MDPH tested sera of small mammals collected in the St. Johns City Park and found hemagglutination inhibition antibodies for California Group viruses in 2 of 12 Fox Squirrels (Sciurus niger) and 1 of 5 raccoons (Procyon lotor) (Becker and Bail, 1972).

During the summer of 1970, in a community approximately 20 miles east of St. Johns, two more clinical human cases of encephalitis were detected. In late August, two young boys from the same neighborhood in Owosso, Shiawassee County,

were hospitalized with high fevers, headaches and convulsions. Both of these cases were also confirmed by MDPH officials as central nervous system diseases caused by California Group viral infections.

In view of the occurrence of these first clinical cases in Michigan and the increasing health problem created by these viruses in neighboring states, a preliminary epidemiological investigation was conducted near the residence of the two Owosso clinical cases with the objectives of: (1) Assessing the extent of human California encephalitis group virus infections in that area; (2) determining the vertebrates in the area which might serve as potential mosquito and/or virus hosts; (3) ascertaining the composition of pest mosquito species during the late summer and early fall; and (4) elucidating the epidemiological importance of the man - biting mosquitoes in the study area as potential vectors of California Group viruses. It is hoped that this study will provide some of the basic data needed for future arbovirus research in the community of Owosso and in the State of Michigan.

HISTORY OF THE CALIFORNIA GROUP ARBOVIRUSES

In 1943 and 1944 an unknown virus was isolated from two species of mosquitoes, Aedes melanimon Dyar and Culex tarsalis Coquillett, in Kern County, California. In a series of publications (Hammon and Reeves, 1952; Hammon et al., 1952; and Reeves and Hammon, 1952) the unknown viral agent was named California encephalitis virus and was successfully transmitted in the laboratory to a rabbit by infected Aedes melanimon. In addition, serological evidence implicated the newly found virus as the causative agent of three human cases of encephalitis in the San Joaquin Valley of California during 1945 (Hammon and Reeves, 1952). After the initial isolation, the virus was not reported again in California for nearly two decades, even though virus isolations were attempted for over 500,000 hematophagous arthropods during that period (Reeves and Hammon, 1962). It was assumed that some unknown ecological changes had resulted in the disappearance of California encephalitis virus from Kern County, although antibodies were found in humans and small mammals in 1963 (Gresikova et al., 1964). During this unexplained 20 year absence in California, other laboratories in the United States began to isolate viruses that were antigenically related to,

but distinct from, the 1943 - 44 California isolate. In 1948, Trivittatus virus was isolated from Aedes trivittatus (Coquillett) in North Dakota by Dr. C.M. Eklund (Hammon et al., 1952). A decade later San Angelo virus was isolated from Anopheles pseudopunctipennis (Say) in Texas (Taylor, 1967) and for the next six years, new antigenically related viruses were isolated almost yearly in North America. During this period related viruses also were being discovered in Trinidad, Czechoslovakia and Africa. Table 1 lists the California Group viruses by the year and locality of their initial isolation. The column "Mosquito Species" lists the number of species from which the virus subtypes have been isolated, and is based on the reported isolations by all researchers in North America from 1943 to 1970 (compiled by Sudia et al., 1971).

The two most recent and comprehensive reviews of this group of arboviruses (Henderson and Coleman, 1971; Sudia et al., 1971) indicate that the classification is still in a state of flux. A tentative classification has been established by the Subcommittee on Immunological Relationships Among Cataloged Arboviruses of the American Committee on Arthropod-borne Viruses (reviewed by Sudia et al., 1971). This places three complexes in the California Group of arboviruses: California, Trivittatus and Melao. The latter two complexes presently contain only one virus type (the original isolated agents), while the California complex now has eight subtypes. In this classification, Lumbo is

Table 1. A chronological summary of the California Group arboviruses of the world

Complex[a]	Subtype	Year of Isolation	Sourse of Isolation	Locality of Isolation	Literature Cited	Mosquito[b] Species
C	Prototype	1943	Aedes melanimon	California	Hammon et al., 1952	7
T	Trivittatus	1948	Aedes trivittatus	North Dakota	Hammon et al., 1952	15
M	Melao	1955	Aedes scapularis	Trinidad	Spence et al., 1962	-
C	San Angelo	1958	Anopheles p. pseudopunctipennis	Texas	Taylor, 1967	3
C	Tahyna	1958	Aedes caspius	Czechoslovakia	Bardos and Danielova, 1959	-
C	(Lumbo)[c]	1959	Aedes pembaensis	Mozambique	Kokernot et al., 1962	-
C	Snowshoe Hare	1959	Snowshoe Hare (Lepus americanus)	Montana	Burgdorfer et al., 1961	12
C	South River	1960	Anopheles crucians	New Jersey	Sudia et al., 1971	3
C	La Crosse	1960	Man (fatal case)	Wisconsin	Thompson et al., 1965	11
C	Jamestown Canyon	1961	Culiseta inornata	Colorado	Sather and Hammon, 1967	11
C	(Jerry Slough)[d]	1963	Culiseta inornata	California	Taylor, 1967	-
C	Keystone	1964	Aedes atlanticus-tormentor	Florida	Bond et al., 1966	10

[a]C=California, T=Trivittatus, M=Melao.
[b]Number of species from which the subtype has been isolated (data from Sudia et al., 1971).
[c]A variant of Tahyna virus. [d]A variant of Jamestown Canyon virus.

considered a variant of Tahyna virus and Jerry Slough a variant of Jamestown Canyon. Henderson and Coleman (1971) present an excellent detailed review of the antigenic structure and classification, the physical and biochemical properties, and *in vitro* and *in vivo* studies that have been made of these viruses.

After the three initial human cases of encephalitis in California in 1945, additional evidence of human clinical infections with California Group viruses in the United States did not appear until the early 1960's. In 1962, Thompson *et al*. (1963) recorded the first known evidence of widespread human infections in an area other than California. They found neutralizing antibodies in 51 of 144 wildlife conservation workers tested in Wisconsin. Twenty cases of California Group encephalitis, diagnosed from a retrospective study of 351 patients with central nervous system disease, occurred in Wisconsin from 1960 through 1964 (Thompson and Evans, 1965). During August and September of 1964, the first epidemic of California Group virus occurred in the United States in southeastern Indiana (Marshall, 1965). The epidemic included twelve serologically confirmed cases, all associated with children under 16 years of age. In the same year cases of California Group encephalitis in children were also reported in Ohio (32 cases), Wisconsin (4 cases) and North Carolina (1 case) (Beadle, 1966).

The first documented human fatality due to these viruses occurred in La Crosse, Wisconsin in 1960. In

September of that year a 4-year-old girl died with meningoencephalitis, but it was not until 1964, when virus isolation procedures were conducted on frozen brain and spinal cord tissues, that a California Group viral agent (now called the La Crosse subtype) was isolated (Thompson et al., 1965).

As diagnostic laboratories throughout the United States began to include California virus tests in their routine surveillance of central nervous system illnesses, the number of reported cases began to increase, particularly in the North Central United States. Sudia et al. (1971) indicates that 519 confirmed and presumptive cases were diagnosed in the United States during the period 1945 - 1970. Of these, 466 (90%) were reported from the North Central region. The distribution of these cases, shown in Table 2, emphasizes the increasing incidence of infections with this arbovirus in states bordering Michigan.

Infections in man may be even more widespread than the reported cases indicate since extensive serological surveys have revealed the presence of antibodies in human populations in the absence of reported clinical disease, in many parts of North America. A recent review of the existing data from these surveys (Henderson and Coleman, 1971) points out several important correlations: Antibodies are most prevalent in rural populations and in certain subgroups within these populations (e.g., wildlife

Table 2. Confirmed and presumptive human cases of California encephalitis in the North Central United States (excluding Michigan), 1945 - 1970.

State	Number of cases by year[a]								Totals
	1945-1963	1964	1965	1966	1967	1968	1969	1970[b]	
Illinois	-	-	-	1	1	-	-	-	2
Indiana	-	12	7	2	2	1	-	-	24
Iowa	-	-	6	-	10	1	6	14	37
Minnesota	1	2	1	13	9	7	16	4	53
Ohio	-	35	33	38	25	43	30	39	243
Wisconsin	16	4	12	12	6	11	20	26	107
Totals	17	53	59	66	53	63	72	83	466

[a]From Sudia et al., 1971.
[b]Not complete (cases pending diagnosis not included).

conservation workers, Seminole and Miccosukee Indians in Flordia, and Wisconsin cranberry workers); and virus activity seems to be endemic in certain localities, as shown by an increase in prevalence of antibody in older age groups.

Clinical studies (Thompson and Evans, 1965; Cramblett et al., 1966; Young, 1966; Thompson and Inhorn, 1967; Chun et al., 1968; and Johnson et al., 1968) indicate that illnesses produced by infections with California Group viruses are most prevalent in children under the age of 15, particularly in the 5 - 9 age group, and occur predominantly during the months of July, August and September. Infections in adults are generally subclinical, although Vianna et al. (1971) report a case of California encephalitis in a 33-year-old male in southeastern New York. Patients typically experience a 5 to 10 day febrile period, headache, vomiting or nausea, a lethargic feeling and, in severe cases, convulsions and coma. The initial clinical diagnosis in such cases is often aseptic meningitis or meningo-encephalitis (Chun et al., 1968). There are few studies investigating the subsequent effects of acute central nervous system disease caused by the California Group viruses in children, but it appears that major sequelae are uncommon (Chun et al., 1968; Matthews et al., 1968).

With the exception of the original three cases in 1945, associated with California encephalitis prototype virus

infections, most of the virus isolates from human cases have been the La Crosse subtype. Recent studies have indicated the possibility of human disease related to infections with Trivittatus subtype in Minnesota (Monath et al., 1970) and Jamestown Canyon in Wisconsin (Sudia et al., 1971). Human deaths from clinical disease are extremely rare; only three deaths have thus far been reported in North America (Thompson et al., 1965; Parson, 1967).

The viruses in this group are transmitted to humans and other susceptible vertebrates by the bite of hematophagous arthropods. In North America, California Group viruses have been isolated from two species of Tabanidae (Diptera), two species of ticks (Acarina: Ixodidae) and at least 38 species of mosquitoes (Culicidae). Wright et al. (1970), who succeeded in isolating La Crosse subtype from three specimens of the horse fly Hybomitra lasiophthalma (Macq.), speculated as to whether these tabanids were mechanically or biologically infected. None of the specimens pooled together for testing had contained full or even partial blood meals, and it may be that these were true infections (i.e., multiplication of the virus within the insect's tissue) and not residual ingested virus. DeFoliart et al. (1969) isolated Jamestown Canyon subtype from both H. lasiophthalma and the deer fly Chrysops cincticornis (Walk.). California Group viruses have been found in ticks in only one instance. In western Montana, Newhouse et al.

(1963) obtained two isolations from the wood tick Dermacentor andersoni Stiles (collected from a chipmunk and a golden-mantled ground squirrel) and one isolation from a rabbit tick Haemaphysalis leporispalustris (Packard) (collected from a snowshoe hare). Based on preliminary laboratory experiments conducted on other specimens of these two species which indicated that all traces of the virus were lost within 48 hours after infectious feedings, Newhouse felt that ticks are of a very minimal, if any, importance in the maintenance of California viruses in nature. This is substantiated by Burgdorfer et al. (1961), who were unable to isolate virus from a pool of 131 D. andersoni which had been collected from a viremic snowshoe hare in western Montana. Their attempts to infect other species of ticks, Ornithodoros parkeri Cooley and O. turicata Duges (Argasidae) proved unsuccessful. McLean et al. (1972) failed to isolate virus from H. leporispalustris collected in the Yukon.

The majority of the virus isolations have been from mosquitoes, primarily those of the genus Aedes. Sudia et al. (1971) report that California Group viruses have been isolated in North America from 38 species of mosquitoes: 21 species of Aedes; 5 species of Psorophora; 4 species of Anopheles; 4 species of Culex; 3 species of Culiseta; and Coquillettidia perturbans (Walker). These data clearly establish that mosquitoes are the major vectors of California Group viruses, but the significance of

tabanids and ticks in the transmission cycle presently is uncertain. It is generally thought that these viruses are harbored in small mammals such as hares, rodents and raccoons, and that they are transmitted by mosquito vectors. Humans do not appear to be involved in the natural cycle, but may become infected when their activities encroach upon the natural mosquito - wild mammal cycle.

In order for mosquito - born viruses to be maintained in nature, a set of complex interactions must take place. A blood - feeding female mosquito must be attracted to, and ingest blood from, a vertebrate in which the virus is circulating in the peripheral blood stream in sufficiently high quantities to infect the mosquito. Obviously, the relationship between the vertebrate and the virus must be such that the virus does not kill its host before an infective level of viremia develops. The virus, once ingested by the mosquito, must then be capable of multiplying within the tissues of the insect. It is at this point that many mosquito species are eliminated as true vectors of a particular virus, for the virus may be unable to multipy to an infectious level. Therefore, only unengorged mosquitoes should be used when conducting virus isolation studies in order to differentiate between actual vectors and those species which may harbor the virus for a short period while the blood is being digested. Once an arthropod vector is infected with the virus, it remains so for life (Reeves and Hammon, 1952; Reeves, 1965).

It is essential for a vector to acquire at least two blood meals during its adult life if transmission of the virus is to occur. The virus must first be acquired from a viremic vertebrate and then, after a proper incubation period in which the virus multiplies and infects the mosquito's tissues, be introduced into a susceptible vertebrate at a subsequent feeding. This multiple feeding must occur in a significant proportion of a vector population before the virus can be transmitted and maintained in the environment. In this manner a natural cycle of mosquito to wild vertebrates to mosquito can exist in an area. Man or his domesticated animals normally become infected as a result of their encroachment upon this natural cycle.

The viremia in vertebrates is of relatively short duration so the maintenance of arboviruses becomes more complex in temperate regions since mosquito populations are not active throughout the year and some type of over - wintering mechanism is required. No over - wintering mechanism has as yet been substantiated for the California Group viruses (Rosicky, 1969), although an annual introduction by migrating birds might be possible, since neutralizing antibodies for the Tahyna subtype have been reported from a number of migratory bird species in Europe (Bardos et al., 1960).

METHODS

Description of the Study Area

Epidemiological studies were conducted in a 70 acre woodlot - subdivision complex located 500 yards west of the residence of the two children who had contracted infections with California Group virus during the late summer of 1970. This study area is situated on the eastern edge of the city of Owosso, Michigan (1970 population: 18,500) and slightly northwest of Corunna (1970 population: 3,000). The Shiawassee River flows in an east-west direction one half mile south of the study area, and a small airport lies between the river and the woodlot. Figure 1 shows the basic topography of the region, which is primarily residential-business south and west of the study area and rural-agricultural north and east of the study area. Details of the woodlot - subdivision are shown in Figure 2. Construction of the subdivision disturbed little of the woodlot, and except for the paved roadways and the modest lawns around the houses, no woods were cleared. At the time of this study there were ten homes in the subdivision, with three more being built (Figure 2). Children living in the subdivision played in all parts of the woodlot, but particularly in the northwest corner where they had

Figure 1. Topography of the Owosso, Michigan study area and the region surrounding it.

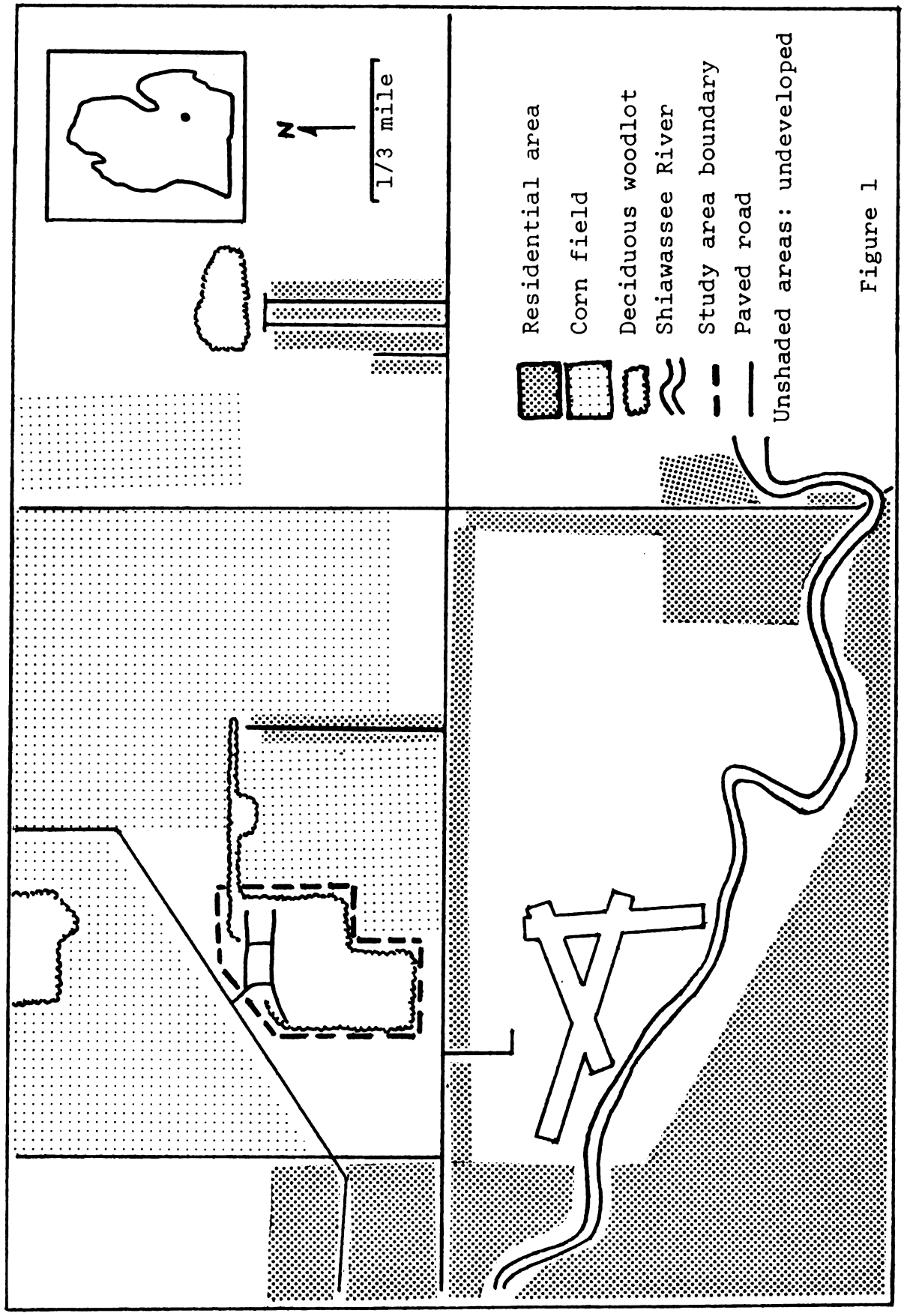

N
1/3 mile
Residential area
Corn field
Deciduous woodlot
Shiawassee River
Study area boundary
Paved road
Unshaded areas: undeveloped

Figure 1

Figure 2. Map of the 1971 woodlot - subdivision study area, Owosso, Michigan.

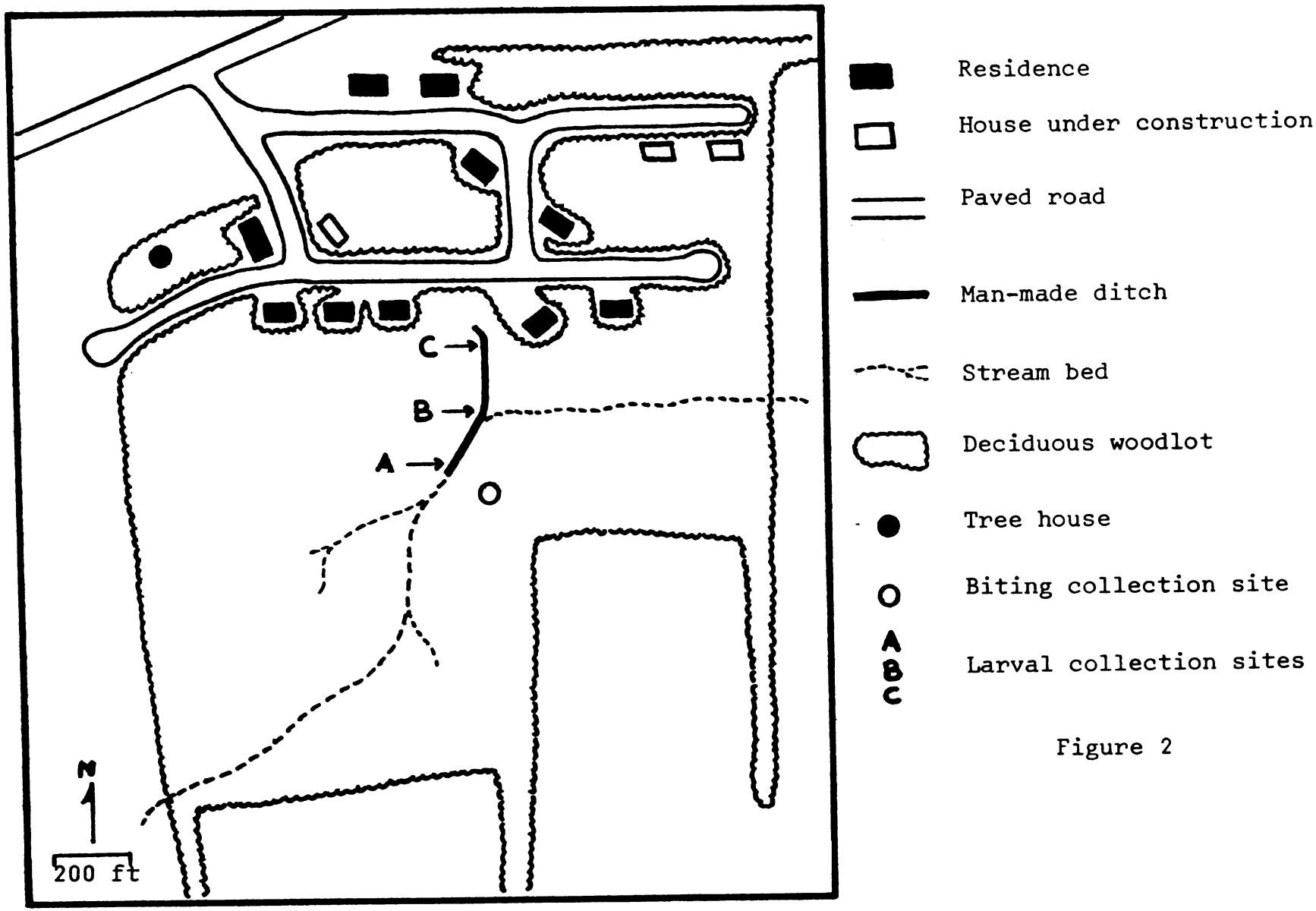

C
B
A
O
N
200 ft
Residence
House under construction
Paved road
Man-made ditch
Stream bed
Deciduous woodlot
Tree house
Biting collection site
Larval collection sites
A
B
C

Figure 2

constructed an elaborate 25-feet high tree-house. In the western half of the woods a number of trails had been made by children riding bicycles and motorscooters, and these trails were frequently used by local and non-subdivision children.

The 50 acre woodlot proper was a Beech-Maple-Oak-Hickory association. Shagbark Hickory (Carya ovata) predominated in the eastern half of the woodlot and the undergrowth there was not as thick as in the central and western portions. Two shallow stream beds, running east and south, contained snow-melt water in early spring, but were generally dry by mid-May. The only permanent aquatic habitat found in the woodlot was a one-meter wide, man-made ditch extending 400 feet into the woods, and was fed by rains, ground water and sewage runoff. A large amount of leaf litter and other organic matter lined the bottom of the ditch and extensive algal growths covered this substrate. During the latter half of June, 1971, a large portion of this ditch dried up and subsequent rains in July created conditions favorable for the larvae of Culex pipiens Linneaus.

Weather data for the Owosso area (see Appendix I - IV) were recorded by the United States Department of Commerce at a weather station located at the Owosso Wastewater Plant, 3 miles west northwest of the study area. Information recorded at this station was obtained from the Environmental Data Service in East Lansing, Michigan.

Epidemiological Procedures

Preliminary data concerning encephalitis - like illnesses in children living in the study area were obtained through personal interviews with the parents of the children. Information such as the age and sex of the child, areas in which the child frequently played, date of onset of the illness, activities for several weeks prior to onset, description of the symptoms observed at onset, the child's playmates, and the name of the family physician was obtained in this manner. Details of the clinical symptomology were obtained from patients' personal folders at Memorial Hospital in Owosso. Records of encephalitis - like illnesses in other Owosso area residents were also obtained by screening the diagnostic records at the hospital.

The serological survey for antibody to California Group viruses was conducted on October 18, 1971. Thirty residents of the subdivision being studied were bled (1 serum sample per person) by a physician from the Michigan Department of Public Health (MDPH). Blood samples were collected in disposable vacutainers, allowed to clot, and transported to the MDPH Laboratory in Lansing for hemagglutination inhibition tests, utilizing goose red blood cells and La Crosse subtype antigen (obtained from the Center for Disease Control in Atlanta, Georgia). Sera with California encephalitis virus antibody titers of 1:20

or greater were considered positive and indicative of prior infection with this agent (Bail, 1971).

Vertebrate Survey Procedures

Field observations were made of the vertebrates in the study area in an effort to establish the spectrum of potential sources of mosquito blood meals, and thus the available hosts for California Group viruses. Tally sheets which listed common species were used for recording observations. Mammals, reptiles and amphibians present were noted while conducting mosquito studies in the woodlot and surrounding areas. Several small, nocturnal species of rodents were found drowned in the steep - sided portion of a ditch leading into the woods. Road killed animals near the study area also were identified. In early fall, 20 live traps ("Have-a-Heart") were operated in the woodlot for several days in an attempt to obtain vertebrate serum samples, but with little success. To avoid repetitious observations of birds due to nesting pairs and singing males, only the presence of a species, rather than the number of individuals, was noted in order to obtain the frequency of species in the study area.

Mosquito Survey Procedures

Human - bait biting collections were made while sitting on a camp stool near the center of the study area (Figure 2). During each collection period an equal amount of skin was exposed. Both trouser legs were rolled above the knees and the left shirt sleeve was rolled above the elbow. By placing the left hand on top of the head, mosquitoes which would normally land on the face, and be difficult to catch, would land on the back of the hand and arm, and by slowly lowering the arm they could be seen and captured. A chloroform killing tube equipped with a paper cone in the mouth to prevent mosquitoes from tumbling out on subsequent captures was manipulated with the right hand. For collecting after dark, a flashlight with red cellophane shading the lens was used to locate landing mosquitoes.

When possible, collections were conducted for at least one hour. The longest consecutive collection was 3 hours, 20 minutes. The catches were segregated at 20-minute intervals by transferring the contents of the killing tube to prelabeled field tins containing tissue paper. Several drops of chloroform were pipetted onto the tissue to ensure the death of all mosquitoes. The field tins were transported to the laboratory where the mosquitoes were mounted on card points, labeled and identified to species according to keys in Carpenter and LaCasse (1955).

In an effort to supplement data provided by the biting study, collections of immature mosquitoes were made at various times throughout the summer. A standard one pint, white enamel dipper was used to sample ditches and mud puddles in the study area. The contents of the dipper were poured through an open plastic tube with cheese cloth covering one end, and the larvae and pupae remaining in the cloth were transferred to labeled shell vials containing 70% ethyl alcohol. A basting pipette was used to sample certain tree holes.

RESULTS AND DISCUSSION

Because of the lack of epidemiological studies in the state, the natural transmission cycle of California Group viruses in Michigan and the subtype(s) involved are unknown. Most of the knowledge concerning this arbovirus has been accumulated in other North Central states where environmental conditions, vertebrate fauna and mosquito populations may vary considerably from those of Michigan. The vectors and vertebrate hosts of the viruses in Michigan will remain unknown until state-wide virus isolation studies are completed. The true incidence of human California encephalitis in Michigan will also remain unknown until a comprehensive effort is made to obtain and test acute and convalescent serum samples from suspect patients throughout the state. This study is an attempt to gain at least a partial understanding of the epidemiological conditions that exist in a Michigan community where human California encephalitis is known to have occurred. With this understanding, it may be possible to more accurately evaluate the feasibility of pursuing in-depth, state-wide studies of this arbovirus.

Clinical Cases of California Encephalitis

The two cases of California encephalitis in Owosso which prompted this investigation occurred in the late summer of 1970. These cases were particularly significant in that they were the second and third human cases reported for the State of Michigan, the first case having occurred in St. Johns in 1969. The histories of the two Owosso cases, based on hospital records and interviews with the parents, were as follows: One case involved a 10-year old boy (T.T.), who was admitted to Memorial Hospital in Owosso on August 31, 1970 with a severe headache, abdominal pains, and a sore throat. He experienced headaches for two days prior to admission, and two hours after being brought to the hospital went into convulsions and remained comatose for a time. His temperature peaked at 103.5°F. and began to decline after the seizures. His speech remained garbled for several days, but then improved steadily and he was discharged on September 9, 1970. The hemagglutination inhibition (HI) antibody titer to La Crosse antigen was 1:1280 in his acute-phase serum and 1:320 in convalescent serum tested on December 4, 1970. Follow-up examinations of T.T. by a physician revealed no abnormal developments and his recovery was apparently complete and uneventful. The other case of California encephalitis involved a 13-year old boy (R.T.) who was admitted to the hospital on September 3, 1970.

Five days previous to this, he had developed headaches, fever and vomiting. Upon admission he was incoherent and having seizures, with a peak temperature of 105°F. The patient was fed intravenously for three days and according to hospital reports, "thrashes about wildly in bed with somewhat sunken eyes. Does not recognize people or faces, and restraints and pains seem to make him wilder." About the third day after admission his sensorium cleared, and he was released four days later. The HI antibody titer in his acute serum was 1:40, whereas the titer of the convalescent serum tested on September 25, 1970, was 1:640.

Several aspects of these case histories seem to be of special epidemiological interest. T.T. and R.T. were unrelated playmates, living next door to one another in a residential area 3/8 mile east of the woodlot - subdivision. Both boys spent a considerable amount of time together in this woodlot during July and August, 1970 and were frequently exposed to biting insects while playing there. They had traveled with their families to other parts of Michigan in the week previous to the onset of symptoms, but the families did not visit the same areas and the parents reported that the boys had not spent any appreciable amount of time outdoors during the trips because of heavy rains. Thus, while there is the possibility that the boys were bitten by infected mosquitoes in separate areas of the state, it seems likely that they became infected with the virus while playing together near their homes.

In addition to these two confirmed California encephalitis illnesses in 1970, there is also evidence that California virus transmission may have occurred near the same area in 1971. In January, 1972, the Michigan Department of Public Health reported the results of serological tests made on the serum of an Owosso child who had had a central nervous system disease during the early fall of 1971. The patient, a 2-year old girl (C.H.), had been admitted to Memorial Hospital on September 9, 1971. She had experience a fever of 101°F. for two days prior to admission. At the hospital her fever rose to 104°F. and she had seizures similar to those of R.T. and T.T. She was released ten days later and recovered uneventfully. Her HI antibody titer to La Crosse antigen, 1:80 in her acute-phase serum and 1:320 in convalescent serum, indicates an infection with California Group viruses. The home address of C.H. was obtained from hospital records. She lived one mile east of the residences of the two 1970 cases in a 96-house subdivision with a Beech-Maple-Oak woodlot along the northern boundary (see Figure 3). The home of C.H. was situated at the edge of this woodlot, and her mother stated in an interview that C.H., and many other children in the subdivision, played in this woodlot throughout the summer of 1971. Bicycles, swings, and toys were seen in many of the yards in this subdivision, indicating that many families had one or more young children. The

close proximity of this subdivision to the study area and to other deciduous woodlots, and the presence of a large number of children in a susceptible age group, would make this an important area for future epidemiological studies.

Human Serological Survey

As a result of interviews with subdivision residents of the study site throughout the spring and summer of 1971, it was learned that three children in the immediate area had previously been hospitalized for illnesses which, based upon descriptions by the parents, appear to have been of encephalitis-like nature. A girl (M.G.) who had lived in the subdivision since 1965 was hospitalized on September 28, 1967, (age 6 when hospitalized) with a headache, sore neck and high fever. She suffered convulsions, was unable to talk coherently for several days, and reacted violently to both hospital personnel and members of her family. She was more alert by October 5, but complained of a constant headache. Her recovery was uneventful and she apparently suffered no ill effects. A boy (B.B.) who had lived in the subdivision since early 1967 was also hospitalized in September of 1967 with similar symptoms, although no convulsions occurred (age 9 when hospitalized). These two children lived within 700 feet of one another and played frequently in the woodlot around their homes. Neither child

had traveled to other parts of Michigan for several weeks prior to the onset of symptoms. In late August of 1969, a third child, a boy (M.M.) who had lived in the subdivision since 1965, suffered from headache, high fever and vomiting. These symptoms began on August 26 and he was admitted to the hospital on August 31, 1969 with a diagnosis of meningitis. During his hospitalization (age 9 at the time) he was sleepy and irritable but made an uneventful recovery. Two weeks prior to the onset of symptoms he had returned from a summer camp in northern Michigan where he spent several weeks.

According to records at Memorial Hospital in Owosso, where the three children had been admitted for central nervous system disorders, there had been no diagnostic tests for arboviruses conducted at the time of illness. It was important, therefore, to determine if these three children had had any previous experience with the California Group viruses.

Results of the serological survey, which included members from 8 of the 10 subdivision families, are presented in Table 3. Of the 30 individuals tested, four children (13.3%) exhibited significantly high hemagglutination inhibition (HI) antibodies (i.e., titer $\geq$ 1:20) to La Crosse antigen, which indicates that they had had previous exposure to California Group viruses. More importantly, when only the number of individuals (17) in the age group most susceptible

Table 3. Results of a serological survey for hemagglutination inhibition antibody to California Group arbovirus (La Crosse antigen) in the Owosso woodlot - subdivision residents.

Age Group (years)	Sex	Number* Tested	Number Positive	Titer Level	Subject Code
0 - 4	M	0			
	F	2	0		
5 - 9	M	4	1	1:20	R.G.
	F	5	1	1:80	R.R.
10 - 14	M	3	0		
	F	2	2	1:20 1:80	L.R. M.G.
15 - 19	M	2	0		
	F	1	0		
20 - 29	M	0			
	F	1	0		
30 - 39	M	5	0		
	F	5	0		
	Total:	30	4 (13.3%)		

* Date of serum collection was October 18, 1971.

to California virus infections (i.e., children under 15 years of age) is considered, the percentage of positive reactions is 23.5% (4/17). Antibodies were not found in any individual over 14 years of age. One of the four seropositive individuals was M.G., a 10-year old girl whose 1967 encephalitis-like illness was described above. Because no acute-phase serum had been obtained for subsequent comparative testing, and because no California Group virus isolation was attempted at the time of her illness, it is impossible to definitely attribute her encephalitis to this virus. However, her antibody titer was 1:80, which is presumptive evidence of a past infection with this arbovirus. Sera from the other two subdivision children with encephalitis-like disease histories (B.B. and M.M.) exhibited titers of 1:10 and < 1:10, respectively. These levels cannot be considered indicative of previous California virus infections.

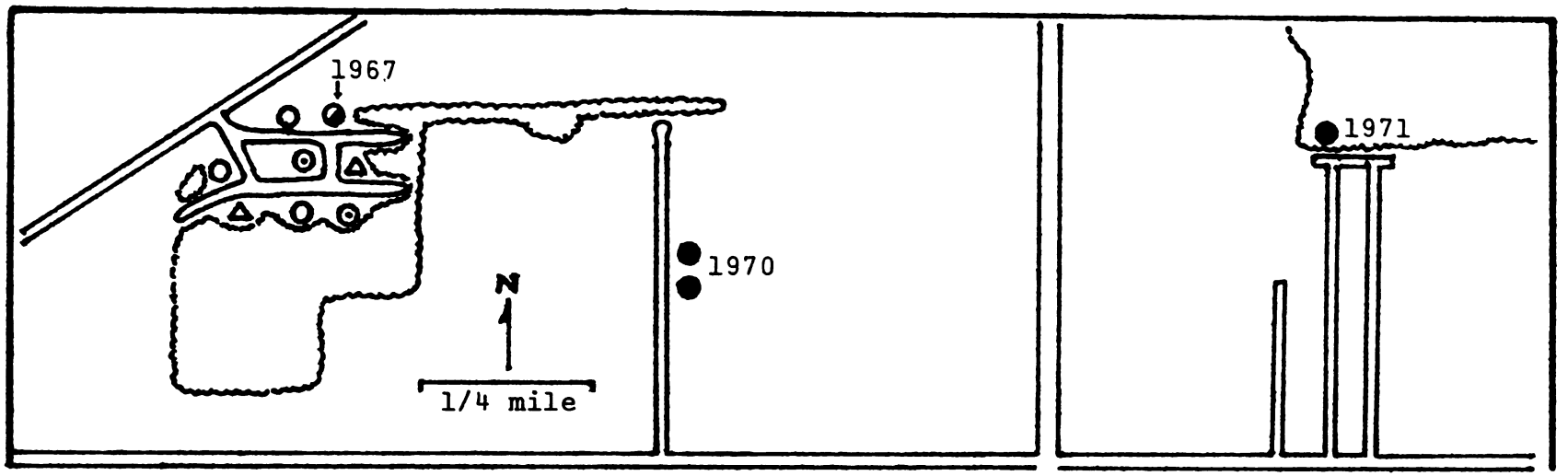
In addition to the high California virus antibody titer of M.G., significant HI antibody levels were present in three other children: R.G., a 9-year old boy who had lived in the subdivision since 1967 had an HI titer of 1:20; L.R., a 12-year old girl who had lived there since 1966 had an HI titer of 1:20; and R.R., an 8-year old sister of L.R. had a titer of 1:80. None of these three children have a record of any clinical central nervous system disease, although R.R., whose high titer strongly

indicates an infection with this arbovirus, had experienced a non-encephalitic illness during the late summer of 1970.

The geographical distribution of the homes of the confirmed clinical cases of encephalitis and the seropositive individuals, as shown in Figure 3, indicates the close association with deciduous woodlots where exposure to biting insects is likely. The number of susceptible individuals living in this narrow strip was augmented by visiting playmates and children who lived within bicycling distance. On numerous occasions during the summer, non-subdivision children were seen walking, bicycling or riding motor scooters on the trails in the study woods. Many local children utilized this woodlot for extended daytime play activities, as was evident by their elaborate tree-house, underground huts and well-worn trails. The large number of children playing in this area is an important factor which would tend to increase the potential for transmission of California encephalitis virus.

In an effort to locate possible encephalitis cases in other Owosso localities, the diagnostic records at Owosso Memorial Hospital were screened for the months of June - November, 1965 - 1971. All "suspect-diagnoses" of aseptic meningitis, meningo - encephalitis, or encephalitis of probable viral etiology, which are often the initial diagnoses of what are actually found to be infections with California Group viruses (Cramblett et al., 1966), were

Figure 3. The geographical distribution of the homes of the confirmed cases of California encephalitis and the families involved in the serological survey, Owosso, Michigan.



● Confirmed case of California encephalitis

◐ Presumptive case of California encephalitis

⊙ Significant antibody (≧1:20), but no encephalitis-like illness

○ Antibody level not significant (<1:20)

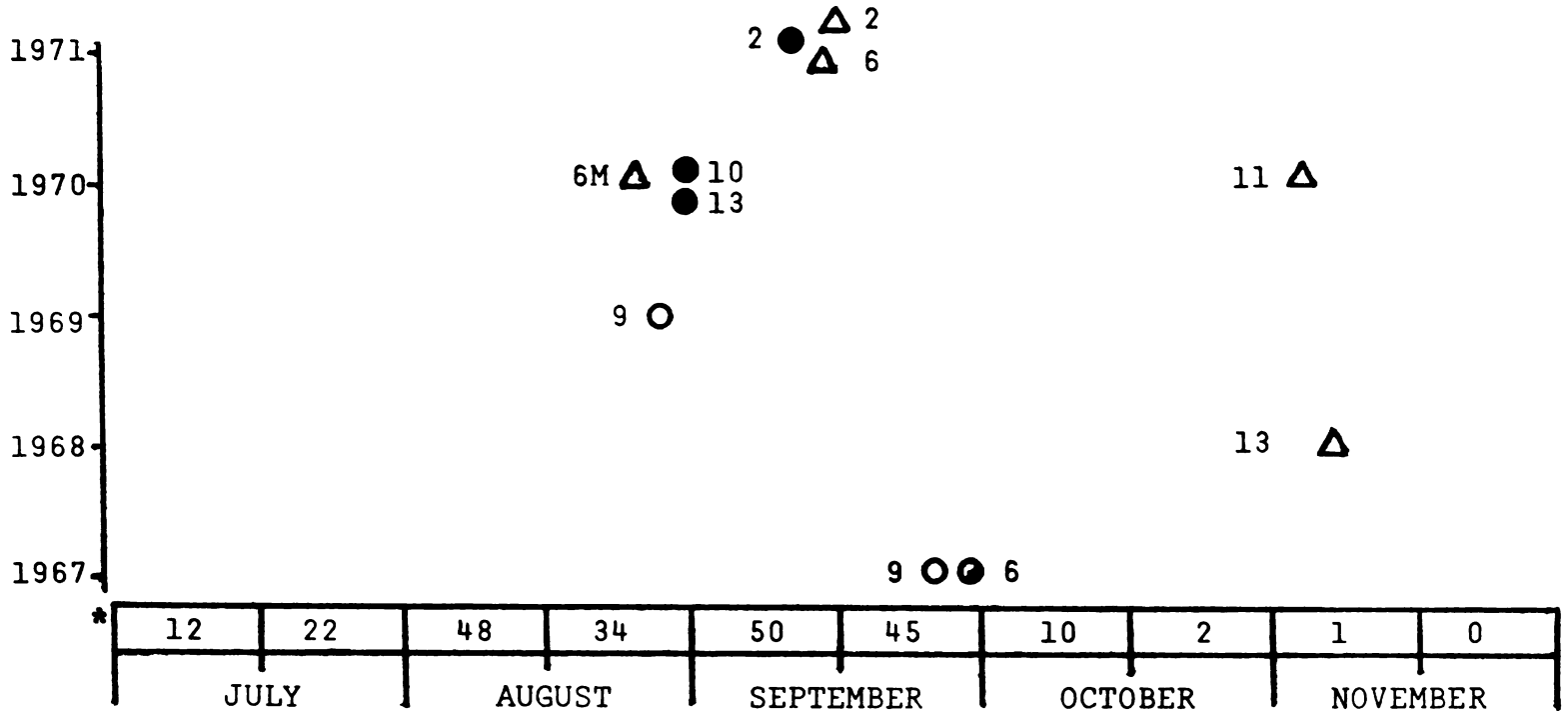
△ Antibody level insignificant (<1:20), with history of encephalitis-like illness

Figure 3

noted and the corresponding patients' folders were then checked for age, sex, date of admission to the hospital, and general symptoms of the illness. In addition to the six patients whose illnesses were already known and have been described earlier (patients R.T., T.T., C.H., B.B., M.G., and M.M.), seven other patients were found to have had central nervous system diseases of "suspect-diagnosis" during the months of June - November, 1967 - 1971. Two of these patients were over 30 years of age, and are omitted from the discussion since infections with California viruses are extremely uncommon in age groups above 20 years (Henderson and Coleman, 1971).

Figure 4 compares the seasonal distribution (1967 - 1971) of both California encephalitis cases and encephalitis-like illnesses of unknown origin in the Owosso area to the distribution dates of 234 cases of California encephalitis reported from North America, 1963 - 1968 (Henderson and Coleman, 1971). Although the two early November illnesses of "suspect-diagnosis" are questionable as being California Group virus induced diseases, the two mid-September (1971) illnesses, as well as the one in late August (1970), would perhaps indicate more extensive virus activity, since the latter three cases occurred at times when other cases of confirmed California encephalitis were reported in Owosso. According to addresses obtained from the patients' folders, the "suspect-diagnosis" children lived in Owosso, with the exception of the mid-September 2-year old who lived in

Figure 4. Seasonal distribution of California encephalitis and encephalitis - like illnesses in children admitted to Memorial Hospital, Owosso, Michigan (according to dates of onset, 1967 - 1971) compared with seasonal distribution of 234 cases reported in the literature.



CIRCLES indicate known clinical cases in Owosso and children living in the study area.
- ● Confirmed case of California encephalitis
- ◐ Presumptive case of California encephalitis
- ○ Encephalitis - like disease of unknown origin

TRIANGLES indicate illnesses located only through hospital diagnostic records.
- △ Encephalitis - like disease of unknown origin

NUMBERS next to symbols indicate the age of the patient in years.

* Seasonal distribution of 234 cases of California encephalitis in North America with known dates of onset, 1963 - 1968 (Henderson and Coleman, 1971).

Figure 4

Ovid, Michigan, eight miles west of Owosso. In any future arbovirus study in the Owosso community, a detailed serological follow-up of these "suspect-diagnosis" patients would be necessary, since these five illnesses indicate the possibility of virus activity extending beyond the immediate study area.

Mosquito Fauna

The primary objective of the mosquito survey was to ascertain which human-biting species were active during the late summer and early fall, and might be regarded as potential vectors of California Group viruses in the study area. All collections from human bait were made at a single point in the woodlot, approximately 900 feet from the home of the 1967 presumptive case of California encephalitis and 1/2 mile from the homes of the two 1970 confirmed cases.

Residents of the woodlot - subdivision stated that the density of mosquitoes throughout the late spring and summer of 1971 was abnormally low. They reported that in some years mosquitoes were unbearable in the subdivision and made backyard picnics, parties and other prolonged outdoor activities extremely unpleasant. The low density of mosquitoes during 1971 may have been associated with the unusually low amount of rainfall in May, June and August, which would lower the number of available breeding sites. The monthly

rainfall for May, June and August was 2.21, 2.41 and 1.24 inches below a 30-year norm, respectively (see Appendix III for comparisons with 1967 - 1970). Human-bait biting collections conducted between July 20 and September 7, 1971, yielded only 312 female mosquitoes, including 5 species of Aedes, 2 species of Anopheles, and Coquillettidia perturbans (Walker). Table 4 indicates the frequency of collections for each of the 8 species during 56 20-minute collection periods. Aedes triseriatus (Say), the most widely distributed treehole-breeding mosquito in North America, constituted 60% of the total catch at human bait.

The biting patterns for the three major pest species encountered in the study area between 12 noon and 9 p.m. are shown in Figure 5. This period corresponds to the time when many children in the subdivision were actively playing in the woodlot. Adult residents were also engaged in outdoor activities during this period and several families had frequent late afternoon and early evening barbeques and lawn parties for large groups of people throughout the summer. Female mosquitoes seeking blood meals during this period of time could come in contact with many human hosts in the subdivision and, as discussed earlier, visiting friends and playmates of the residents could increase the number of available human hosts.

As shown in Figure 5, Aedes triseriatus was attracted to, and fed upon, human bait throughout the afternoon and early evening, but terminated its feeding soon after sunset.

Table 4. Frequency of eight species of mosquitoes collected at human bait between July 20 - September 7, 1971, in the Owosso woodlot - subdivision.

Species	Number Caught	% of Total Catch
Aedes triseriatus	188	60
Aedes vexans	54	17
Coquillettidia perturbans	35	11
Anopheles quadrimaculatus	17	5
Aedes fitchii	13	4
Aedes canadensis	3	1
Aedes stimulans	1	<1
Anopheles punctipennis	1	<1
Total	312	100%

Figure 5. Time of biting of the major pest species of mosquitoes in the Owosso study area, based on 43 twenty-minute collections at human bait, July 20 - September 7, 1971.

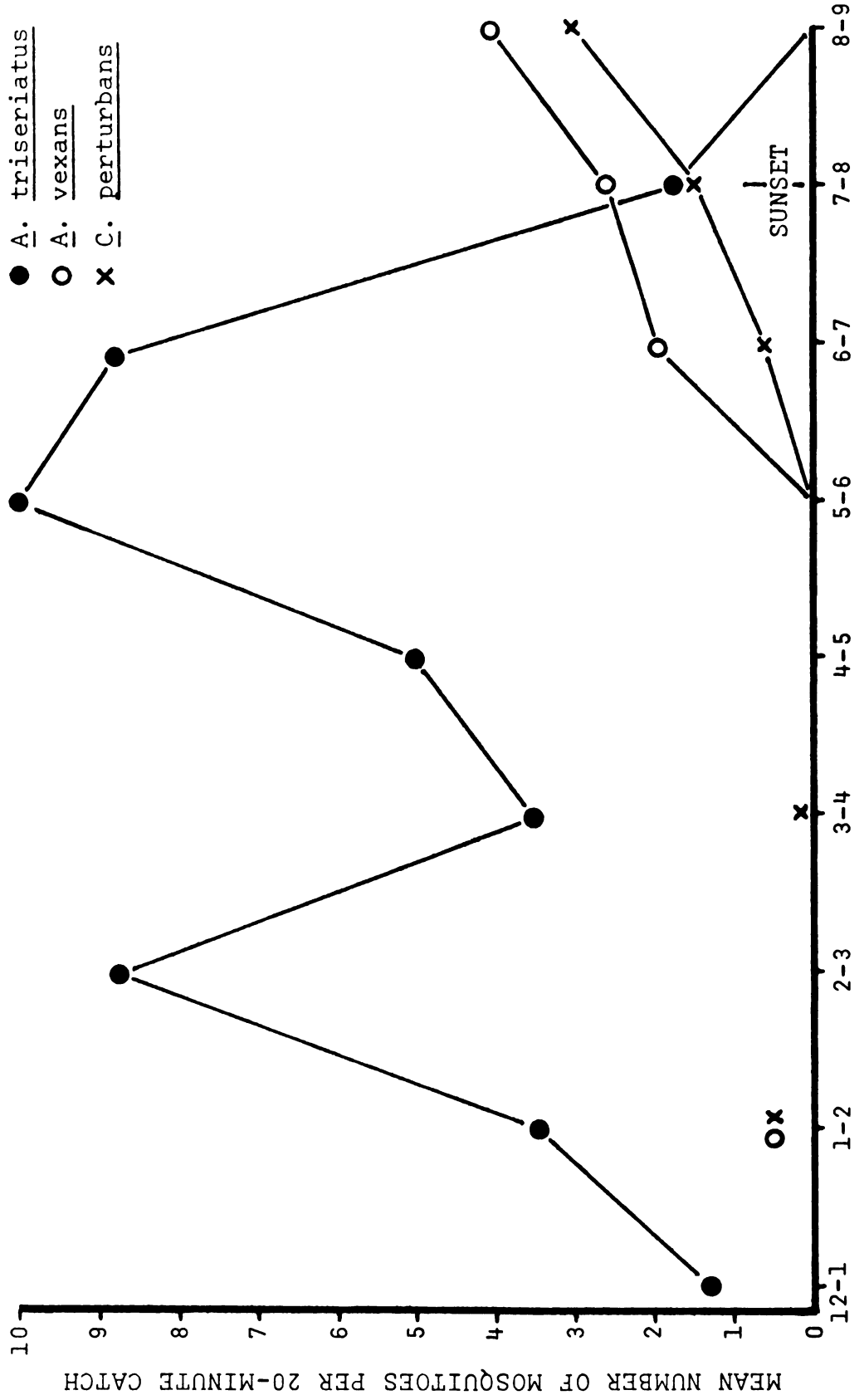

A. triseriatus
A. vexans
C. perturbans
MEAN NUMBER OF MOSQUITOES PER 20-MINUTE CATCH
0
1
2
3
4
5
6
7
8
9
10
12-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
SUNSET
AM
PM
TIME IN 1-HOUR PERIODS

Figure 5

Feeding occurred most frequently between 5 p.m. and 7 p.m., and dropped off considerably after sunset. These data agree with A. triseriatus studies by Loor and DeFoliart (1970), who also noted that biting was very rare after 8 p.m. The latest collection of this species was made between 10:10 and 10:30 p.m. on August 17, when a single female was caught at human bait in the tree house at a height of 25 feet. Aedes vexans Meigen and Coquillettidia perturbans rarely appeared at human bait during the day but became more numerous from one hour before sunset until 9 p.m., when most collecting was terminated. A single A. vexans was collected between 10:10 and 10:30 p.m. on August 17 in the tree house. The other five species attracted to human bait were Anopheles punctipennis (Say), An. quadrimaculatus Say, Aedes fitchii (Felt and Young), Ae. stimulans (Walker), and Ae. canadensis (Theob.), but they were collected too infrequently to determine any biting pattern.

A survey of summer breeding sites in the study area resulted in the collection of immature stages of Aedes triseriatus, Anopheles punctipennis and Culex pipiens Linn. Despite the frequency of adult Ae. triseriatus in the woodlot, only 17 larvae were collected during the study. All 17 were first instar larvae collected on July 2, 1971 in a small depression in the trunk of a partially uprooted maple tree. On the previous day the area had received 0.62 inches of rain, the first substantial rainfall since June 13 (see Appendix IV), which most likely stimulated the hatching of

the eggs. No other tree holes which could be sampled were found to contain water long enough to allow mosquito larval development. Larvae of Anopheles punctipennis were found in a mud puddle in the northeastern corner of the subdivision. A total of 34 larvae, taken in a series of dips made on September 15 and 18, were reared to adults in the laboratory to confirm the identification. Several smaller puddles were checked but no other immatures were found. Adult An. quadrimaculatus were collected in the woodlot but larvae of this species could not be found at any breeding site in the study area. Immature stages of Culex pipiens were collected in several habitats in the area: A child's plastic swimming pool containing leaf litter; a discarded tar bucket; the flooded basements of two houses under construction; and the man-made ditch described earlier which extended 400 feet into the woodlot. This ditch was dry earlier in the summer of 1971 but contained rather high densities of C. pipiens larvae in mid-July after rains earlier in the month (Appendix IV) provided sufficient water levels for their development. This drying - flooding condition removed most of the algae which grew heavily in the ditch in May and June. A total of 2283 larvae and pupae of C. pipiens were collected from the ditch from July 28 - September 7, 1971 (Table 5). Three sites, designated A, B, and C and approximately 200 feet apart (Figure 2), were sampled periodically. Each larval collection consisted of

Table 5. The relative abundance of immature Culex pipiens in the Owosso woodlot - subdivision between July 28 - September 7, 1971.

Date	Collecting Station	Water Temp. °C	Instars I and II	Instars III and IV	Pupae
	A	17.0	66	34	3
JUL 28	B	17.0	120	124	43
	C	17.0	9	26	1
	A	21.0	42	40	4
AUG 2	B	19.5	24	48	2
	C	20.0	4	3	3
	A	18.0	30	66	4
AUG 4	B	18.5	27	49	8
	C	18.5	14	34	0
	A	dry	-	-	-
AUG 10	B	23.5	16	4	0
	C	22.0	16	10	3
	A	20.0	8	2	0
AUG 12	B	19.5	35	15	0
	C	22.0	11	10	1
	A	21.0	5	1	0
AUG 16	B	21.0	31	1	4
	C	20.0	46	56	4
	A	22.5	0	3	0
AUG 19	B	23.0	4	4	0
	C	23.0	7	0	0
	A	21.0	39	18	1
AUG 25	B	21.0	49	39	0
	C	20.5	2	30	0
	A	18.0	27	111	2
AUG 31	B	-	-	-	-
	C	-	-	-	-
	A	24.0	10	40	0
SEP 7	B	22.5	15	71	1
	C	22.0	211	334	158
		Total:	868	1173	242
		Ave/Dip:	16.1	21.7	4.5

Total number of immatures collected: 2283
Average number of immatures/Dip: 42.3

two dips taken near the center of the ditch at each site. Recorded water temperatures were taken 1 inch below the surface.

The effects of dry periods of weather on available larval habitats are reflected in the data for August 10 and 19 (Table 5). On August 10, after 12 days of essentially no rain, site A was completely dry and the water at site B was only several inches deep. Similarly, the few larvae at the three sampling sites on August 19 followed 7 rainless days. Despite these periods of partial drying there were always some portions of the ditch containing sufficient water to which the larval population could move. Immature Culex pipiens, averaging 42.3 per dip, was the only mosquito species taken from the ditch. No females of this species were taken at human bait although it is known to feed on man (Carpenter and LaCasse, 1955).

A total of 9 species of mosquitoes were obtained in biting and larval collections in the Owosso woodlot-subdivison during the summer and early fall of 1971. Since these species are known to feed on man, and are the ones that are most likely to contact children in the subdivision, it is important to consider their role as potential vectors of California Group virus in this area. The most comprehensive review of the species of mosquitoes which may be involved in the transmission of California Group viruses is that of Sudia et al. (1971). They have reviewed all of the published

literature concerning virus isolations from North American mosquitoes between 1943 - 1970, as well as much unpublished data from laboratories active in arbovirus research. Table 6 lists the 9 species of mosquitoes present in the Owosso study area and summarizes the published accounts of the isolations of California Group viruses from these species. As shown in this table, there are several species of mosquitoes which could serve as potential vectors in the study area. Certainly the most suspect mosquito is Aedes triseriatus, which is the proven vector of La Crosse virus in the North Central United States. According to Sudia et al. (1971), this is the only species which has fulfilled the four basic criteria considered necessary to prove vector status, namely: (1) The virus must be isolated from mosquitoes collected in the field; (2) there must be field evidence to confirm an association of the infected mosquito species with a vertebrate population in which the infection is occurring; (3) the mosquito must experimentally be infected by feeding upon a viremic vertebrate; and (4) the mosquito must be capable, in laboratory experiments, of transmitting the virus to a susceptible vertebrate host by bite after a period of extrinsic incubation of the virus.

Aedes triseriatus, which comprised 60% of the total number of mosquitoes collected at human bait in the study area, was a persistent feeder throughout the afternoon and early evening and was likely to be encountered by

Table 6. The mosquito species of the Owosso woodlot - subdivision and the virus subtypes isolated from them in North America, 1943 - 1970.[a]

Species	Virus Subtype[b]	Number of Isolations	Breakdown by States
Anopheles punctipennis	LAC*	1	Minnesota
Anopheles quadrimaculatus		0	
Coquillettidia perturbans	TVT*	1	Iowa
	UNT	1	Florida
Aedes fitchii	SSH	5	Montana
Aedes stimulans	JC*	3	Wisconsin
	SSH	1	Alberta, Canada
Aedes canadensis	KEY	2	Maryland
	LAC	16	2-Georgia; 14-Ohio
	SSH	2	1[c]-British Columbia; 1-Massachusetts
	UNT	6	1-Massachusetts; 1-New York; 4-Ohio
Aedes triseriatus	KEY	1	Louisiana
	LAC	34	4-Iowa; 5-Minnesota; 8-Ohio; 17-Wisconsin
	UNT	1	Ohio
Aedes vexans	CE*	2	New Mexico
	JC	1	Wisconsin
	KEY	2	1-Maryland; 1-Mississippi
	LAC	6	1-New Mexico; 4-Ohio; 1-Wisconsin
	SSH	2	1[c]-British Columbia; 1-Wisconsin
	TVT	13	Iowa
	UNT	15	13-Alaska; 1-California; 1-Utah
Culex pipiens	LAC	1[d]	Wisconsin
	TVT	1	Iowa

[a]Based on the data of Sudia et al. (1971).
[b]CE-California encephalitis; JC-Jamestown Canyon; KEY-Keystone; LAC-La Crosse; SSH-Snowshoe Hare; TVT-Trivittatus; UNT-untyped.
[c]From mixed pool of Aedes vexans - canadensis.
[d]From mixed pool of Culex pipiens - restuans (data from Thompson et al., 1972).
*Subtype implicated in human disease.

children playing in the woodlot. No other mosquito species collected during the late summer and early fall exhibited a biting pattern which would seem to coincide with the daytime outdoor activities of local children (Figure 5).

The second most frequent mosquito species collected at human bait in the study area was Aedes vexans, but its numbers were low and may have been well below the densities of other years. As shown in Table 6, six subtypes of California Group viruses have been isolated from A. vexans in North America, including those subtypes known to cause human illness, but this species has not as yet met the four vector criteria for any of the California Group viruses. The biting pattern of Aedes vexans in the study area was such that any outdoor activity on the part of subdivision residents after 5 or 6 p.m. would bring them in contact with this species. Several children slept infrequently in the tree house during warm summer nights and may have encountered Aedes vexans in this manner.

Arbovirus studies in Ohio suggest that Aedes canadensis may also be a vector of La Crosse subtype, since 14 isolations have been made from this species in that state (Table 6). In the present study, A. canadensis was collected only three times at human bait during the late summer - early fall season, although larvae and pupae were found in several snow pools in the woodlot during late April and early May, 1971. According to Carpenter and LaCasse

(1955), the adults of this species generally emerge in April, May and early June and the females, which appear to be long - lived, persist in small numbers in woodlands until late summer. The feeding habits of this species are varied. It is attracted to, and feeds readily upon, turtles, birds, deer, raccoon, rabbits, and other small mammals (Means, 1968; Wright and DeFoliart, 1970). Because of this wide spectrum of vertebrate hosts and the demonstrated association with La Crosse virus in Ohio, it is possible that A. canadensis in Michigan could serve to amplify the virus in indigenous mammal populations during the spring and early summer and produce a greater number of viremic mammals later in the season.

In the woodlot - subdivision small numbers of five other culicids were collected from human bait: Anopheles punctipennis (1/312); An. quadrimaculatus (17/312); Coquillettidia perturbans (35/312); Aedes fitchii (13/312); and Ae. stimulans (1/312). As indicated in Table 6, none of these species have as yet been consistently associated with any of the subtypes of the California Group, although DeFoliart (1972) feels that Aedes stimulans is one of the vectors in the typical transmission cycle of Jamestown Canyon subtype in Wisconsin.

Culex pipiens, which was not collected at human bait but was nevertheless abundant in the study area in the immature stages, has a very slight association with

California Group viruses and could be tentatively ruled out as a vector in the Owosso area (Henderson and Coleman, 1971). Studies of the feeding habits of C. pipiens indicate that it is primarily a bird feeder, but does occasionally feed on mammals (Tempelis and Washino, 1967; and Wright and DeFoliart, 1970). This preference for avian hosts may exclude C. pipiens from the small mammal - mosquito cycle which typifies the California Group viruses but could possibly involve it with transmission of these viruses to certain avian species. The possibility of birds serving as hosts for California viruses is discussed in the next section.

Vertebrate Fauna

The species of mammals, reptiles and amphibians which were observed in and around the study area are listed in Table 7. Live trapping attempts in the woodlot to obtain blood samples for serological tests had limited success; only two Peromyscus and one Eastern Cottontail (Sylvilagus floridanus) were trapped and one of the mice died before being bled. The absence of chipmunks and red squirrels throughout the summer was particularly noticeable, since the woodlot appeared to be a suitable habitat for both of these species. Forty one species of birds were observed in the woodlot - subdivision from April 29 to

Table 7. A list of the mammals, reptiles and amphibians observed in the Owosso woodlot - subdivision between April 29 - September 14, 1971.

Species	Number Observed
MAMMALIA*	
Order Insectivora	
Starnose Mole (Condylura cristata)	1 (dead)
Shorttail Shrew (Blarina brevicauda)	1 (dead)
Order Carnivora	
Raccoon (Procyon lotor)	3+tracks in cornfields
Domestic Dog (Canis familiaris)	5 seen daily
Domestic Cat (Felis domesticus)	1
Order Rodentia	
Woodchuck (Marmota monax)	2 (1 dead)
Eastern Fox Squirrel (Sciurus niger)	no actual count (1 or 2 seen almost daily)
White-footed Mouse (Peromyscus leucopus)	3 (2 trapped)
Jumping Mouse (Zapus or Napaeozapus)	1 (dead)
Order Lagomorpha	
Eastern Cottontail (Sylvilagus floridanus)	6 (1 trapped)
Order Artiodactyla	
Whitetail Deer (Odocoileus virginianus)	2+tracks in cornfields
REPTILIA**	
Garter Snake (Thamnophis sirtalis)	3
AMPHIBIA**	
American Toad (Bufo americanus)	usually seen daily
Spring Peeper (Hyla crucifer)	heard infrequently
Green Frog (Rana clamitans melanota)	1 or 2 seen daily

* Taxonomy follows Burt (1957)

** Taxonomy follows Conant (1958)

September 14, 1971, and these are listed in Appendix V. Table 8 lists the 13 species which were most frequently observed during the study. These frequencies are based on the number of days a particular species of bird was seen or heard during 38 days of field observation. As an indication of the prevalence of tree hole situations in the woodlot and therefore breeding sites for certain species of mosquitoes, 7 of these 13 frequent species utilize tree holes as nesting sites.

Of the 11 species of mammals recorded in the study area (Table 7), 6 have been reported in the literature as having antibody for California Group viruses: Raccoon (Newhouse et al., 1964; Bond et al., 1966; and Whitney et al., 1969); Woodchuck (Whitney et al., 1969); Fox Squirrel (Moulton and Thompson, 1971); Peromyscus (Gresikova et al., 1964); Cottontail (Whitney et al., 1969; Moulton and Thompson, 1971) and Whitetail Deer (Cook et al., 1965; Emmons, 1968; and Whitney et al., 1969). Tests for California virus antibodies in sera from two mammals trapped in the study area (1 Peromyscus, 1 Cottontail) were negative. Serological surveys of vertebrates in other areas of the state have yielded evidence of California Group virus infections in a number of mammals and birds.

Serum samples from various species of mammals and birds have been collected in other areas of Michigan by personnel of the Rose Lake Wildlife Pathology Laboratory

Table 8. Most frequently observed birds in the Owosso woodlot - subdivision between April 29 - September 14, 1971 (based on data in Appendix V).

Species	Observation* Frequency	Rank
Blue Jay	74%	1
Tufted Titmouse**	71%	2
Red-headed Woodpecker**	61%	3
Red-tailed Hawk	53%	4
White-breasted Nuthatch**	53%	4
Robin	47%	5
Yellow-shafted Flicker**	45%	6
Red-eyed Vireo	40%	7
Crested Flycatcher**	34%	8
Common Grackle	34%	8
Black-capped Chickadee**	29%	9
Starling**	29%	9
Cardinal	29%	9

* Number of days observed/38 field observation days

** Indicates the species nests in tree holes

and tested for HI antibody to La Crosse antigen by the Michigan Department of Public Health. Sera were considered positive if they exhibited a titer of 1:20 or greater. The results of these tests (Becker and Bail, 1972) were as follows (number positive/total tested for each species): Snowshoe Hare (27/160); Opossum (Didelphis marsupialis) (0/6); Raccoon (1/8); Fox Squirrel (2/12); Chipmunk (Tamias striatus) (5/12); Woodchuck (0/1); Cottontail (0/55); Striped Skunk (Mephitis mephitis) (0/1); Thirteen-lined Ground Squirrel (Citellus tridecimlineatus) (0/1); Peromyscus sp. (0/2); Whitetail Deer (3/132); Blue Jay (Cyanocitta cristata) (3/21); Woodcock (Philohela minor) (0/2); Cowbird (Molothrus ater) (0/1); Robin (Turdus migratorius) (0/1); and Ring-necked Pheasant (Phasianus colchicus) (2/2). These data suggest that a wide spectrum of vertebrates may play a role in the natural transmission cycle of California Group viruses in Michigan although further studies are needed to ascertain the specific hosts of the virus.

Of particular interest are the finding of seropositive birds, which are not considered likely hosts of these viruses. The three positive Blue Jays, which were collected during May, 1972, on High Island in Lake Michigan, had titer levels of 1:20, 1:40 and 1:80, which would strongly indicate previous infection with California Group viruses. Blue Jays were the most frequently observed birds in the Owosso woodlot - subdivision (Table 8), but no attempt was made to

obtain serum samples from them. The two seropositive pheasants were collected in 1970 near Wacousta, Clinton County, Michigan. These birds, found in widely separated areas of Michigan, may indicate the need for extensive and intensive surveys of bird populations in areas suspected of California Group virus activity.

The role of birds in natural cycles of the California Group viruses has been adequately investigated only in Europe in connection with the Tahyna subtype. Bardos et al. (1960) reported neutralizing antibodies in the sera of 31 species of migratory and nonmigratory birds in Czechoslovakia, five of which are species which occur commonly throughout North America: Coot (Fulica atra), Barn Swallow (Hirundo rustica), Bank Swallow (Riparia riparia), Starling (Sturnus vulgaris), and House Sparrow (Passer domesticus). Malkova and Marhoul (1966) failed to experimentally infect pheasants with Tahyna virus, but Chernesky (1968) did succeed in transmitting an untyped California virus to newly hatched white Leghorn chickens by the bite of infected Aedes aegypti (L.). Although published North American studies have not reported seropositive wild birds (Henderson and Coleman, 1971), the numbers of birds tested thus far do not seem to be an adequate sample. Studies in this country regarding the susceptibility of birds to California Group viruses are as yet insufficient to establish their importance as natural hosts.

SUMMARY AND CONCLUSIONS

1. In the summer of 1971 a study was conducted to assess the epidemiological conditions existing in a woodlot - subdivision near Owosso, Michigan, where clinical California encephalitis in children had occurred in 1970.
2. Three clinical cases of California encephalitis in subdivision children are reported. Two cases occurred in late August, 1970, in male neighborhood playmates, ages 10 and 13. The other, involving a 2-year old girl, occurred in early September, 1971, one mile east of the homes of the 1970 cases. In each infection the patient experienced headache, high fever and seizures, and recovery was uneventful. Epidemiological evidence for each patient suggests that virus transmission occurred in the vicinity of their home.
3. A serological survey of 30 residents of the woodlot - subdivision revealed significant hemagglutination inhibition antibody titers to La Crosse subtype antigen ($\geq$ 1:20) in 4 individuals (13.3%). These seropositive individuals were children between the ages of 8 and 12 years, all of whom had lived in the subdivision since 1967 or earlier. One of these children, a 10-year old

girl whose titer was 1:80, had experienced an encephalitis - like illness in 1967 and can be considered a presumptive case of California encephalitis.

4. Encephalitis-like diseases of unknown origin occurred in 2 Owosso children and 1 Ovid child at times when confirmed clinical California encephalitis was present in Owosso children. This suggests the need for further epidemiological investigations in these communities.
5. Frequencies of mosquitoes collected at human bait between July 20 - September 7, 1971 were: Aedes triseriatus (60%), Aedes vexans (17%), Coquillettidia perturbans (11%), Anopheles quadrimaculatus (5%), Aedes fitchii (4%), Aedes canadensis (1%), Aedes stimulans (<1%), and Anopheles punctipennis (<1%).
6. Culex pipiens, which was never collected at human bait, bred abundantly in the study area. Immature stages of C. pipiens averaged 42.3 per dip.
7. Potential vectors of California Group viruses collected during the late summer and early fall included Aedes triseriatus, the proven vector of La Crosse subtype in other states, as well as Aedes vexans, the source of six different subtype isolates in North America, Aedes canadensis, and Aedes stimulans. Only Aedes triseriatus exhibited a biting pattern which coincided with the daytime play activities of subdivision children.

8. Eleven species of mammals, 1 snake, 3 species of amphibians, and 41 species of birds were recorded in the woodlot - subdivision, indicating a wide spectrum of possible mosquito and/or virus hosts. Six of the 11 mammal species are known to develop antibody to California Group viruses in nature, while 3 species (Fox Squirrel, Raccoon, and White-tail Deer) have been found with significant antibody levels (≧1:20) in Michigan serological surveys. Seropositive Pheasants and Blue Jays (a species which was frequently observed in the study area) have also been found in Michigan.
9. It is concluded that the rural subdivision communities of Owosso may be natural foci of California Group arboviruses and would serve as ideal "field laboratories" for acquiring a more comprehensive understanding of the transmission cycle of these viruses in Michigan. Existing epidemiological conditions in these subdivision include: Large numbers of susceptible - aged children residing and playing in close proximity to deciduous woodlands; a wide variety of vertebrates available as potential hosts for mosquitoes and California Group viruses; and the presence of potential late summer - early fall vectors such as Aedes triseriatus and Aedes vexans. The increasing incidence and importance of California encephalitis infections in Ohio and Wisconsin

indicates the need for determining the specific viral subtypes, vertebrate hosts and mosquito vectors in Michigan.

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APPENDICES

APPENDIX I

Average monthly temperatures for Owosso, Michigan, 1967 - 1971 (May - September).

	MAY	JUN	JUL	AUG	SEP
1971	55.3	70.7	68.6	67.4	64.2
1970	60.2	66.6	71.5	69.8	62.3
1969	56.9	63.0	70.4	70.8	63.0
1968	53.9	66.2	69.8	70.0	63.3
1967	51.8	69.5	68.6	65.7	59.5
*Mean:	56.6	66.9	70.8	69.1	61.9

*Monthly mean for period 1940 - 1969.

APPENDIX II

Daily temperatures for Owosso, Michigan,
May - September, 1971.

Date	MAY	JUN	JUL	AUG	SEP
1	58-39*	61-45	83-66	79-53	79-54
2	50-33	71-48	77-56	82-58	84-63
3	57-29	80-51	83-46	71-50	82-66
4	62-29	88-53	86-58	73-50	85-68
5	70-42	90-59	81-66	79-43	90-68
6	67-43	84-62	86-59	84-46	83-64
7	68-33	87-61	89-57	87-49	85-63
8	73-42	81-49	82-66	88-55	90-65
9	73-45	69-38	85-63	88-58	79-55
10	77-40	76-36	83-55	91-65	86-57
11	70-53	84-46	81-59	72-56	71-53
12	61-31	87-62	82-45	79-47	69-56
13	69-28	83-62	86-68	86-59	73-52
14	69-34	79-61	80-53	84-66	82-47
15	80-43	82-52	78-51	74-53	71-56
16	78-54	85-53	84-55	81-45	72-46
17	81-39	87-60	79-51	86-49	71-50
18	86-63	88-60	80-44	87-50	68-43
19	82-59	91-65	74-57	88-56	63-50
20	68-44	91-70	80-47	88-65	63-52
21	62-41	83-59	82-52	87-64	69-39
22	66-32	82-49	88-65	90-52	71-44
23	73-36	85-56	82-66	71-46	65-49
24	78-53	88-54	79-63	76-40	65-35
25	61-48	86-67	86-58	84-59	57-37
26	51-43	84-54	81-65	74-57	60-47
27	64-39	96-65	77-46	68-57	72-54
28	69-34	98-69	71-56	77-57	83-64
29	71-39	96-71	75-48	83-51	77-58
30	81-42	93-67	76-55	85-53	79-51
31	73-48		73-47	69-55	
AVE: MAX:	69.3	84.5	80.9	81.0	74.8
AVE: MIN:	41.2	56.8	56.2	53.7	53.6

* Maximum - Minimum (Temperature in °F)

APPENDIX III

Rainfall recorded for
Owosso, Michigan, 1967 - 1971 (May - September).

	MAY	JUN	JUL	AUG	SEP
1971	1.01* (-2.21)**	1.31 (-2.41)	2.99 (+0.30)	1.68 (-1.24)	3.19 (+0.49)
1970	3.34 (+0.12)	2.88 (-0.84)	3.60 (+0.91)	3.56 (+0.64)	3.56 (+1.09)
1969	3.97 (+0.75)	3.49 (-0.23)	4.30 (+1.61)	0.64 (-2.28)	0.95 (-1.75)
1968	3.60 (+0.38)	6.11 (+2.39)	2.64 (-0.05)	4.43 (+1.51)	4.60 (+1.90)
1967	1.13 (-2.09)	4.86 (+1.14)	1.75 (-0.94)	2.32 (-0.60)	2.25 (-0.45)

* Precipitation values in inches.

** Departure from the monthly norm (based on records of 30 years).

APPENDIX IV

Daily rainfall recorded for Owosso, Michigan,
May - September, 1971.

Date	MAY	JUN	JUL	AUG	SEP
1	.12	---	.62	---	---
2	---	.05	---	---	---
3	---	.10	---	.08	.08
4	---	---	---	---	.09
5	---	---	.14	---	.02
6	---	---	.98	---	.14
7	---	.29	---	---	.38
8	---	T*	---	---	---
9	---	T	.03	---	---
10	---	---	---	---	---
11	---	---	---	.74	.02
12	.16	.22	---	---	.04
13	---	.34	.01	---	.01
14	---	.10	---	---	---
15	---	---	T	---	---
16	.05	.01	.15	---	---
17	---	---	.18	---	.06
18	---	---	---	---	---
19	---	---	.14	---	.02
20	.07	---	.01	.01	.73
21	---	---	---	T	.06
22	---	---	---	---	---
23	---	.01	---	.09	---
24	.14	---	.14	---	---
25	.42	.19	---	.06	---
26	---	---	.01	.21	.72
27	.05	---	---	.32	.81
28	---	---	---	.17	.01
29	---	---	.58	---	---
30	---	---	---	---	---
31	---		T	---	
Total:	1.01	1.31	2.99	1.68	3.19
**DFN:	-2.45	-2.13	+0.43	-1.20	+0.42

* T=Trace of rain
** DFN=Departure from the monthly norm (based on records of 30 years)

Precipitation values in tenths of an inch

APPENDIX V

Birds recorded in the Owosso study area,
based on 38 field observation days
(April 29, - September 14, 1971).

		A	M	J	J	A	S	Total	%
	Number of Observation Days ----	1	4	3	16	9	5	38	
		Number of Days Bird Observed							
ACCIPITRIDAE									
Buteo jamaicensis	Red-tailed Hawk	1	3	2	11	3	-	20	53
COLUMBIDAE									
Zenaidura macroura	Mourning Dove	-	1	-	1	-	-	2	5
STRIGIDAE									
Otus asio	Screech Owl	-	-	-	-	1	-	1	3
APODIDAE									
Chaetura pelagica	Chimney Swift	-	-	-	1	-	-	1	3
PICIDAE									
Colaptes auratus	Yellow-shafted Flicker	1	3	1	7	2	3	17	45
Centurus carolinus	Red-bellied Woodpecker	1	1	-	3	1	1	7	18
Melanerpes erythrocephalus	Red-headed Woodpecker	1	4	2	9	5	2	23	61
Dendrocopos villosus	Hairy Woodpecker	-	1	-	3	-	-	4	11
D. pubescens	Downy Woodpecker	-	1	1	2	3	2	9	24
TYRANNIDAE									
Tyrannus tyrannus	Eastern Kingbird	-	-	1	-	-	-	1	3
Myiarchus crinitus	Crested Flycatcher	-	2	-	6	4	1	13	34
Contopus virens	Wood Pewee	-	1	-	-	-	-	1	3
HIRUNDINIDAE									
Iridoprocne bicolor	Tree Swallow	-	1	-	-	-	-	1	3
Progne subis	Purple Martin	-	-	-	2	1	-	3	8
CORVIDAE									
Cyanocitta cristata	Blue Jay	1	3	2	11	7	4	28	74
Corvus brachyrhynchos	Common Crow	-	1	1	1	-	2	5	13
PARIDAE									
Parus atricapillus	Black-capped Chickadee	-	2	1	5	1	2	11	29
P. bicolor	Tufted Titmouse	1	4	2	10	7	3	27	71
SITTIDAE									
Sitta carolinensis	White-breasted Nuthatch	1	2	2	7	4	4	20	53
CERTHIIDAE									
Certhia familiaris	Brown Creeper	-	-	-	-	-	1	1	3
TROGLODYTIDAE									
Troglodytes aedon	House Wren	-	2	2	3	-	-	7	18
MIMIDAE									
Dumetella carolinensis	Catbird	-	1	-	1	-	-	2	5

		Month ----	A	M	J	J	A	S	Total	%
		Number of Observation Days ----	1	4	3	16	9	5	38	
		Number of Days Birds Observed								
TURDIDAE										
Turdus migratorius	Robin		-	3	1	7	3	4	18	47
Hylocichla mustelina	Wood Thrush		-	3	1	5	-	-	9	24
STURNIDAE										
Sturnis vulgaris	Starling		1	3	3	3	1	-	11	29
VIREONIDAE										
Vireo flayifrons	Yellow-throated Vireo		-	-	-	1	-	-	1	3
V. olivaceus	Red-eyed Vireo		-	2	3	10	-	-	15	40
PARULIDAE										
Dendroica petechia	Yellow Warbler		-	1	-	-	-	-	1	3
D. coronata	Myrtle Warbler		-	1	-	-	-	-	1	3
D. pensylvanica	Chestnut-sided Warbler		-	1	-	-	-	-	1	3
Setophaga ruticilla	American Redstart		-	3	3	1	-	1	8	21
ICTERIDAE										
Agelaius phoeniceus	Redwinged Blackbird		-	2	-	-	-	-	2	5
Icterus galbula	Baltimore Oriole		-	2	1	4	2	-	9	24
Quiscalus quiscula	Common Grackle		-	2	-	4	3	4	13	34
Molothrus ater	Brown-headed Cowbird		-	1	-	-	-	-	1	3
FRINGILLIDAE										
Richmondena cardinalis	Cardinal		-	2	1	3	3	2	11	29
Passerina cyanea	Indigo Bunting		-	2	-	-	-	-	2	5
Spinus tristis	American Goldfinch		1	2	-	2	1	-	6	16
Pipilo erythrophthalmus	Rufous-sided Towhee		-	1	-	-	-	-	1	3
Spizella passerina	Chipping Sparrow		-	1	1	-	-	-	2	5
Melospiza melodia	Song Sparrow		1	2	1	3	-	-	7	18

Total: 41 species