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The vector competence of selected species of Iowa mosquitoes for three strains of western equine encephalomyelitis virus

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THE VECTOR COMPETENCE OF SELECTED SPECIES OF IOWA MOSQUITOES FOR THREE STRAINS OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS

Iowa State University

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The vector competence of selected species of Iowa mosquitoes for three strains of western equine encephalomyelitis virus

by

Richard Graham Andre

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GENERAL INTRODUCTION

Western equine encephalomyelitis (WEE) has been recognized for nearly 50 years as a disease not only of equidae but also of people in the central and western United States. Based upon various morphological, chemical, and physical characteristics, the virus causing WEE is grouped taxonomically in the family Togaviridae and the genus **Alphavirus** (Melnick, 1980). Less specifically, this virus is part of the large and heterogeneous set of arthropod-borne animal viruses, commonly known as the arboviruses.

The family Togaviridae contains four genera—**Alphavirus**, **Flavivirus**, **Pestivirus**, and **Rubivirus**. The main characteristics of the family are as follows: single-stranded, linear RNA, molecular weight about $4 \times 10^6$ daltons, and ether sensitive. Virions have isometric nucleocapsids surrounded by a lipoprotein envelope containing host cell lipid and virus-specified polypeptides. The virions yield infectious RNA (Porterfield et al., 1978).

In the genus **Alphavirus** there are 20 closely related viruses, known previously as group A arboviruses. Alphaviruses are transmitted in nature to their mammalian or avian host by arthropods, mainly mosquitoes. The viruses multiply in both vertebrate and invertebrate hosts, and also will grow in the cellular cytoplasm of a large variety of cell cultures.
at incubation temperatures ranging from 20 to 41°C (Kääriäinen and Söderlund, 1978).

Alphaviruses have been serologically divided into three serocomplexes, which are: the Semiliki Forest complex, the Venezuelan equine encephalomyelitis (VEE) virus complex, and the WEE complex. Within each complex, antigenic variants of individual viruses are recognized which are closely related but serologically distinguishable. The WEE complex contains the registered alphaviruses WEE, Whataroa, Sindbis, and Aura. Also, there are two unregistered isolates, Highlands J (HJ) and Y62-33. Based on differences in serology, virulence, and epidemiology, WEE-related alphaviruses from the eastern United States are referred to as HJ virus strains (Trent and Grant, 1980).

WEE virus is maintained in nature in a wild bird-mosquito transmission cycle. This virus seems to exist endemically in numerous foci in western North America and has been recovered from about 20 species of mammals, including humans and equines (Hess and Holden, 1958).

Severe outbreaks in horses have occurred periodically in the western United States. One of the first epidemics to be described took place in Kansas and Nebraska in the fall of 1912 (Udall, 1913). The total losses were estimated to have numbered more than 35,000 head. In 1931, Meyer et al. reported that over 3,000 horses and mules in the San Joaquin
Valley of California died from encephalomyelitis, and close to 6,000 equine animals developed recognizable symptoms. Meyer (1932) estimated that during 1930 and 1931 over 6,000 horses died in California just from encephalomyelitis. Outbreaks have also been noted in Iowa during 1939 (Biester and Schwarte, 1940), in Kern County, California, from the years 1943 to 1952 (Reeves and Hammon, 1962), in Weld County, Colorado, in 1949 (Cockburn et al., 1957), and in the Red River Valley area of eastern North Dakota and northwestern Minnesota (Potter et al., 1977). Mortality rates in horses due to WEE ranged from 10 to 50% (Hanson, 1973).

Epidemics of human cases of western encephalomyelitis have appeared sporadically over the past 40 years in a number of areas in North America. Wallis and Hayes (1976) stated that an outbreak involving over 3,000 cases occurred in North Dakota, Minnesota, and adjacent areas of Canada during the summer of 1941. In this epidemic the case fatality rate ranged from eight to 15%. Outbreaks of western encephalomyelitis in humans have also occurred in Weld County, Colorado, in 1949 (Cockburn et al., 1957), in Kern County, California, in 1952 (Reeves and Hammon, 1962), in Iowa during 1960 to 1963 (Zymet et al., 1966), and in Hale County, Texas, in 1965 (Hayes et al., 1967). Hess et al. (1963) mapped out the distribution of recorded human outbreaks of western encephalomyelitis and showed that most were located at or north of the
Factors which affect the epidemic potential and enzootic maintenance of WEE virus have been extensively studied. They include temperature, precipitation, viremic rates and population sizes of basic vertebrate hosts, size of and immune status of equine and human populations, vector abundance, vector longevity, host preference of the vector, and viral infection rates in vectors (Olson et al., 1979).

Many studies on WEE have been designed to determine which mosquitoes are capable of transmitting the virus in nature. Although Kelser (1933) first reported success in transmitting equine encephalomyelitis virus to guinea pigs using Aedes (Stegomyia) aegypti (Linnaeus), not until 1941 was WEE virus isolated from wild caught mosquitoes, when Hammon et al. (1941) collected infected Culex (Culex) tarsalis Coquillett in the Yakima Valley, Washington. Hammon et al. (1942b) found that one of every 386 Cx. tarsalis captured in this area was infected, and Hammon et al. (1942a) therefore concluded that Cx. tarsalis was the most important vector of WEE virus in the Yakima Valley. In early August of 1943, Hammon et al. (1945) collected large numbers of mosquitoes in eastern Nebraska. WEE virus was isolated from one pool of 67 Cx. tarsalis out of a total of 631 caught.

During subsequent years, isolations of WEE virus have
been made from mosquitoes of other species. Hess and Holden (1958) list the following species from which isolations have been made: *Culex (Culex) pipiens* Linnaeus, *Culex (Culex) pipiens quinquefasciatus* Say, *Culex (Culex) restuans* Theobald, *Culex (Culex) peus* Speiser, *Aedes (Ochlerotatus) melanimum* Dyar, *Aedes (Ochlerotatus) nigromaculis* (Ludlow), *Aedes (Ochlerotatus) infirmatus* Dyar and Knab, *Aedes (Aedimorphus) vexans* (Meigen), *Anopheles (Anopheles) freeborni* Aitken, *Culiseta (Culiseta) inornata* (Williston), and *Culiseta (Climacura) melanura* (Coquillett). Species other than *Cx. tarsalis* were, however, considered to be of secondary importance in the transmission cycle of WEE virus (Hess and Holden, 1958). Later investigations have shown, though, that species such as *Cs. inornata* (McLintock et al., 1970), *Cs. melanura* (Williams et al., 1972, 1974), *Ae. melanimum* (Hardy and Bruen, 1974), and *Aedes (Ochlerotatus) trivittatus* (Coquillett) (Rowley et al., 1973; Dorsey et al., 1978) may play a significant role in transmission of the virus in certain ecological situations.

Barnett (1960) listed four criteria for the incrimination of mosquitoes as vectors of vertebrate viruses. They were as follows: (1) demonstration that the suspected mosquito species feeds upon the host, (2) demonstration of a convincing biological association in time and/or space of the suspected mosquito species and the occurrence of clinical or subclinical
infection in the host, (3) repeated demonstration that the suspected mosquito species, collected under natural conditions, harbors the completely identified infectious virus, and (4) demonstration of transmission of the completely identified infectious virus by the suspected mosquito species to suitable susceptible hosts under controlled conditions. In addition, Reeves (1971) stated that the following three characteristics of vector populations are most critical in successful virus transmission: numerical abundance, longevity at temperatures that favor completion of extrinsic incubation of the virus, and affinity of the vector for a vertebrate species that can circulate the virus in its blood in an infective dose for the vector.

Many studies have been carried out to determine if a particular mosquito species feeds upon vertebrate species that are known to be capable of circulating an infective dose of WEE virus. The isolation of WEE virus from wild specimens of Cx. tarsalis caught in the Yakima Valley made it desirable to learn more about this mosquito's feeding habits (Bang and Reeves, 1942). Collections of wild-caught engorged mosquitoes in this area showed that Cx. tarsalis fed frequently on domestic fowl and included most of the common domestic animals and man in its feeding range (Reeves and Hammon, 1944). Later studies (Dow et al., 1957; Blackmore and Dow, 1958) demonstrated that Cx. tarsalis feeds on a wide range of wild
avian and mammalian species, and that nestling birds (the main amplifying hosts for WEE virus) were preferred over adult birds. Utilizing precipitating antisera with a high degree of specificity to mammalian and avian bloods, Reeves et al. (1963) analyzed the bloodmeals of 3,310 Cx. tarsalis collected in Kern County, California. They found that 84% of feedings were on birds, including chickens, doves, and passerine species; and 14% of feedings were on mammals, including cattle, dog, horse, rabbit, and cat. Andersen et al. (1967) determined that there was a correlation between the host preference of Cx. tarsalis in Utah and host availability. The feeding habits of Cx. tarsalis have favored the spread of WEE virus from avian hosts to mammalian hosts by this vector (Reeves, 1971).

The intimate relationship between the mosquito vector and the virus begins when the mosquito acquires the virus. Establishment of an arbovirus infection in a mosquito following ingestion of virus is dependent, among other factors, on the amount of virus ingested by the mosquito and on susceptibility of the mosquito to infection by the virus. Some mosquito species require only low concentrations of a specific virus to become infected whereas others require much higher concentrations. These infection levels are known as infection thresholds, and they vary for different arboviruses and different mosquito vectors (McLintock, 1978).
Many experiments have been carried out to determine the minimum infection threshold for various arboviruses in suspected mosquito vectors. However, as seen below, published results of these susceptibility experiments have been difficult to compare because of the wide variety of techniques used to titer the viruses and calculate the infection thresholds. Also, in some experiments mosquitoes were allowed to feed on infected vertebrate hosts; in others, mosquitoes were fed on cotton pledgets soaked in a blood-virus suspension. Lastly, some mosquitoes were allowed to feed through membranes of various sorts on infected blood or blood suspensions, using methods described by Bishop and Gilchrist (1946).

Merrill and TenBroeck (1934) first infected *Ae. aegypti* females with equine encephalomyelitis virus (WEE) by allowing them to feed on a blood-virus suspension. Unfortunately, the titer of the blood-virus suspension was not calculated. When Thomas (1963) investigated the growth of WEE virus in *Cx. tarsalis*, he found that 50% of the mosquitoes became infected after having fed on viremic chicks or canaries with titers of $10^{4.5}$ chick LD$_{50}$/0.05 ml. Hardy et al. (1979) evaluated the susceptibility of 45 field collections made in 1972 and 1973 of *Cx. tarsalis* to infection with WEE virus by pledget feeding. He determined that the median infectious dose fifties (ID$_{50}$s) were $10^{4.7}$ and $10^{4.2}$ in 1972 and 1973, respectively. The log$_{10}$ of the ID$_{50}$s obtained for different
populations ranged from 3.3 to 5.8. Viral titers were measured by plaquing serial tenfold dilutions in duck embryonal cell cultures.

In studies on eastern equine encephalomyelitis (EEE) with *Culex melanura*, Howard and Wallis (1974) determined the minimal mosquito infective dose to be $10^4$ suckling mouse intracerebral LD$_{50}$/0.02 ml of blood. The 50% dose was a log higher. Jupp (1976) compared the susceptibility of *Culex pipiens*, *Culex quinquefasciatus*, *Cx. pipiens* (Culex) univittatus Theobald, and *Culex (Culex) theileri* Theobald to West Nile and Sindbis viruses. He calculated a 10% infection threshold and found that all four mosquito species were much more susceptible to West Nile virus. While studying the growth of Japanese B encephalitis virus in the organs of *Culex p. quinquefasciatus*, LaMotte (1960) was able to infect 100% of his test mosquitoes by feeding them on viremic chicks with titers of $10^{3.5}$ mouse LD$_{50}$/0.003 ml. Recently, Corner et al. (1980) conducted experiments to examine the dynamics of Cache Valley virus in *Culex inornata* and were able to infect 32 out of 36 mosquitoes that had fed on a viremic mouse with a titer of $10^4$ suckling mouse intracerebral LD$_{50}$/0.02 ml.

Mosquitoes have been shown to be susceptible to a broad spectrum of arboviruses by ingestion, and this spectrum was broadened still further when the midgut was bypassed by parenteral inoculation of the virus into the mosquito.
McLintock (1978) concluded that therefore mosquito susceptibility to arbovirus infection resides primarily in the midgut. He based his conclusion on studies such as McLean's (1955), who compared the multiplication of Murray Valley encephalitis (MVE) virus in mosquitoes following feeding or injection into the body cavity of the mosquito. From his results, he hypothesized that what determines whether or not an encephalitis virus is capable of undergoing a biological cycle in a given mosquito is the capacity of the cells of the gut to allow multiplication of the virus. This hypothesis was further supported by Carley et al. (1973), who found that 28 of 30 arboviruses isolated in Australia multiplied when inoculated into mosquitoes. All of 17 viruses multiplied in *Cx. p. quinquefasciatus* after intrathoracic inoculation, while only two of 12 viruses multiplied after feeding.

The fact that any mosquito can acquire a virus when it ingests the infected blood of its host has been proven, but the question has arisen as to why some mosquitoes can function as biological hosts for the virus and other mosquitoes cannot. Five hypotheses have been proposed (McLintock, 1978) to explain the mechanism of the gut barrier in mosquitoes: (1) virus inactivation by digestive fluids, (2) impermeability of the peritrophic membrane, (3) variations in the permeability of gut cell membranes, (4) limited number of specific virus receptor sites on the gut cell, and (5) surface type defense
mechanisms. Despite much effort, none of these five hypothe-
ses has been shown to account for all the variability in vec-
tor susceptibility (McLintock, 1978). Following field studies
on the vector competence of Cx. tarsalis with WEE virus,
Hardy et al. (1975) suggested that susceptibility is geneti-
cally inherited, but they did not elaborate on which of the
above five hypotheses might be under genetic control.

Following ingestion of an arbovirus by a mosquito, where
the infecting dosage surpassed the infection threshold, the
course of the infection in the mosquito has been well docu-
mented (McLintock, 1978). Replication of the virus has been
found to be rapid (after an eclipse phase of one or two days),
and maximal titers have been attained within four to six days
(McLean, 1955). The virus moved from one tissue to another
and infected the salivary glands towards the last. However,
the level of infection reached in the salivary glands was
found to be higher than in other organs. The arboviruses
also exhibited a neurotropism in mosquitoes, a feature in
common with their infections in vertebrates (McLintock, 1978).
Rate of movement of the virus to the salivary glands and its
replication in the glands was first found to be temperature
dependent by Davis (1932), who infected Ae. aegypti with yel-
low fever virus in the laboratory. Chamberlain et al. (1954)
later determined the extrinsic incubation periods for EEE and
WEE viruses in Ae. aegypti and Aedes (Protomacleaya)
triseriatus (Say).

The salivary glands have been found to be a site of replication and storage of virus before its transmission in mosquito saliva to another vertebrate host (McLintock, 1978). Chamberlain and Sudia (1961) reviewed the two mechanisms of transmission of viruses by mosquitoes, mechanical transmission and biological transmission. Although in the laboratory Ae. triseriatus has mechanically transmitted EEE virus and Cx. tarsalis has mechanically transmitted WEE virus, Chamberlain and Sudia (1961) stated that this mechanism of transmission is of little epidemiological significance. Therefore, most research on the transmission of arboviruses has dealt with the biological mechanisms.

While confirming Kelser’s (1933) work on the transmission of equine encephalomyelitis of the western type by Ae. aegypti, Merrill and TenBroeck (1935) learned that mosquitoes must be fed virus of high titer to insure transmission success. They found that a period of only four to five days must elapse after feeding before the mosquitoes are capable of transmitting the virus. Hammon and Reeves (1943) successfully demonstrated laboratory transmission of WEE virus when they fed infected Cx. tarsalis, Cs. inornata, and Culiseta (Culiseta) incidens (Thompson) on guinea pigs. They determined the minimum extrinsic incubation period at 25°C to be eight days for the
Culiseta and ten days for Cx. *tarsalis*. Barnett (1956) calculated a minimum extrinsic incubation period of only four days for Cx. *tarsalis* at 24°C during his studies on the transmission of WEE virus. He did find, however, that the rate of transmission to chickens and canaries steadily increased from 10% on the fourth day to 84% after the 13th day following the infective bloodmeal. During a year-long study, Bellamy et al. (1967) demonstrated that Cx. *tarsalis* could still transmit WEE virus to susceptible chicks six months after being infected. They stated, however, that even though Cx. *tarsalis* could carry WEE virus through the winter period under experimental conditions, extensive evidence has shown this to be a rare phenomenon under field conditions.

Arbovirus transmission rates at or near 100% have rarely been encountered. Chamberlain and Sudia (1961) attributed these failures to transmit viruses to susceptible hosts to either an absence of gland infection in the vector or a low level of virus in the mosquito's saliva. They presented the hypothesis that the absence of salivary gland infection might be caused by failure of virus to reach the salivary glands or, once reaching them, being unable to penetrate the glandular tissue to establish infection. However, they also stated that an inadequate viremia in the mosquito despite substantial virus growth in the gut could account for an unsuccessful
gland infection just as certainly as insusceptibility of the glands themselves. Subsequent research since 1961 has helped to explain the lower transmission rates.

Takahashi (1976) studied the effects of environmental and physiological conditions of *Culex* (Culex) *tritaeniorhynchus* Giles on the pattern of transmission of Japanese B encephalitis virus. He found that the interval between virus infection and initiation of virus secretion in mosquitoes was not influenced by mosquito age, concentration of sugar given to adults, or history of blood engorgement in advance of or following an infective bloodmeal. However, virus secretion was delayed and suppressed when infected mosquitoes were kept at 20°C. He suspected that this suppression was a consequence of the relatively slow physiological activity of mosquito tissues at low temperature rather than of a delay in the velocity of virus multiplication.

After conducting laboratory studies on the transmission of West Nile virus by *Cx. univittatus*, Jupp (1974) reported that a reduction in temperature for the postinfection period from 26 to 18°C caused a drop in the transmission rate from 97 to 48%. He also found that a reduction in the infecting titer from 5.0 to 2.6 logs in mosquitoes held at 26°C caused a decrease in transmission rate from 89 to 33%. In experiments designed to elucidate the vector competence of *Cx. tarsalis* for WEE virus, Hardy et al. (1979) found that the
ability of a vector to transmit virus may be under genetic control. Their colonized strain of Cx. tarsalis had the ability to transmit virus 100% of the time; whereas, Cx. tarsalis from the Poso Creek area of California rarely transmitted the virus.

The variation in the susceptibility of different species of mosquitoes to infection with arboviruses and variation of different geographical strains of a single mosquito species to infection by arboviruses have been demonstrated (Gubler and Rosen, 1976; Aitken et al., 1977; Grimstad et al., 1977; Gubler et al., 1979). Experiments in our laboratory with three strains of WEE virus (Green et al., 1980) indicated that vector susceptibility may vary with the strain of virus employed. Variation in the transmission of these three strains by Ae. trivittatus was also noted (Green, 1979), but host susceptibility experiments were not conducted, thus preventing the conclusion that a single mosquito species can vary in its transmission of strains of an arbovirus. The results did however raise the interesting suggestion that a situation analogous to VEE virus (Kramer and Scherer, 1976) might exist for WEE in Ae. trivittatus in Iowa.

Field studies by our laboratory have shown that large populations of Cx. pipiens, Cx. restuans, and Culex (Culex) salinarius Coquillelt may contribute to the amplification of St. Louis encephalitis (SLE) and WEE in Iowa (Wong et al.,
1978). These investigators indicated that the unusually large populations of these mosquitoes in the spring and summer of 1974 and 1975 may have played a role in the epidemic of 1975 in Iowa. However, they did state that the single most important factor was the high numbers of *Cx. tarsalis* in July of 1975.

Studies to compare the vector competency of *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* for Iowa strains of WEE virus have not been done. Elucidation of the epidemiological significance for WEE viral activity in Iowa in these mosquito species is extremely important for future disease prevention and control. Also, the possibility of enzootic and epizootic strains of WEE virus in *Cx. tarsalis* and *Ae. trivittatus* occurring in Iowa needs to be investigated. This dissertation enumerates the results of vector competence studies designed to determine the role the above species play in the transmission cycle of WEE in Iowa.

Explanation of Dissertation Format

The dissertation is composed of four sections. Section I is concerned with the vector competence of *Ae. trivittatus* and *Cx. tarsalis* for three strains of western equine encephalomyelitis (WEE) virus. Part of this section was presented by the senior author at the National Meeting of the American Mosquito Control Association in San Antonio, Texas, during
the spring of 1981. Section II is concerned with the vector competence of Cx. pipiens, Cx. restuans, and Cx. salinarius for the same three strains of WEE virus. Both sections will be submitted to the American Journal of Tropical Medicine and Hygiene as part of a continuing series of articles on the vector competence of Iowa mosquitoes for WEE virus. Section III is concerned with the susceptibility of different chick lines to the three strains of WEE virus. This section will be submitted to the American Journal of Veterinary Research. Section IV is concerned with the surveillance of arbovirus activity in Iowa for the years 1978 to 1980. This last section will be submitted to the Journal of Medical Entomology.

The senior author was responsible for the design and conduct of experiments in the first three sections and in part of the fourth section. Stock viruses were provided by the University Hygienic Laboratory, at the University of Iowa, Iowa City, Iowa. In addition, this laboratory processed all potentially infected specimens generated by the experiments in our laboratory at Iowa State University. Experimental results were assembled, interpreted, and written into manuscript form by the senior author. Interpretations and conclusions expressed in the four sections of this dissertation are those of the senior author.
SECTION I. VECTOR COMPETENCE OF *Aedes trivittatus* AND *Culex tarsalis* FOR THREE STRAINS OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS
Vector Competence of Selected Species of Iowa Mosquitoes
(Diptera: Culicidae) for Three Strains of Western
Equine Encephalomyelitis Virus: I.
*Aedes trivittatus* and *Culex tarsalis*

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INTRODUCTION

Establishment of an arbovirus infection in a mosquito following ingestion of virus is dependent on the amount of virus ingested by the mosquito and on susceptibility of the mosquito to infection by the virus, although other factors can be important (McLintock, 1978). Certain mosquito species are refractory to infection after ingestion of some but not other arboviruses. The susceptibility or nonsusceptibility of a mosquito generally is thought to be associated with a threshold phenomenon, or what has been termed by various workers as a "gut barrier" (Chamberlain and Sudia, 1961) or "mesenteronal infection barrier" (Kramer et al., 1981). A highly susceptible vector has a low threshold of infection and a less susceptible vector has a higher threshold (Chamberlain, 1968).

Following ingestion of an arbovirus, where the infecting dosage surpasses the infection threshold, virus will spread to and multiply in various cells and tissues of the mosquito. The salivary glands are among the last organs infected, but the level of infection reached in the salivary glands is higher than in other organs (McLintock, 1978). Once the infection barrier is passed and the necessary extrinsic incubation period for the virus in the mosquito is complete, virus is transmitted in the salivary gland secre-
tions when the mosquito feeds (Chamberlain and Sudia, 1961). However, it is increasingly evident that there are further barriers to successful transmission of arboviruses, particularly in the case of western equine encephalomyelitis (WEE) virus transmission by *Culex tarsalis* (Kramer et al., 1981).

It has long been recognized that *Cx. tarsalis* is a more competent vector of WEE virus than other mosquito species in the western United States. Hardy and Reeves (1973) first suggested that the vector competence (ability of a mosquito to become infected with a virus and subsequently to transmit the virus to a vertebrate host after an appropriate period of extrinsic incubation) of different populations of *Cx. tarsalis* might vary. Later research proved that *Cx. tarsalis* can vary greatly in its susceptibility to WEE virus (Hardy et al., 1976), and preliminary studies suggested that the ability of infected *Cx. tarsalis* to transmit WEE virus also might vary (Hardy and Reeves, 1974). Further studies (Hardy et al., 1978) have shown that the infection of *Cx. tarsalis* with WEE virus is genetically controlled, but reported results (Hardy et al., 1979) have not demonstrated conclusively genetic control of vector transmission ability.

Several recent reports (Gubler and Rosen, 1976; Tesh et al., 1976; Gubler et al., 1979) have substantiated the concept of variation in susceptibility of different geographical strains of a mosquito species to infection with a single
arbovirus. Other recent studies (Aitken et al., 1977; Grimstad et al., 1977) have shown variation in the ability of different geographical strains of a single mosquito species to transmit a particular arbovirus. Less convincing has been the evidence (Kramer and Scherer, 1976; Tesh et al., 1976) that variation occurs in the susceptibility of a particular laboratory strain of mosquito to infection with strains of a single arbovirus. However, in 1980, workers in Iowa (Green et al., 1980) documented the first evidence that field strains of a single mosquito species can vary in their susceptibility to infection with strains of an arbovirus (WEE). Variation in the transmission of three WEE viral strains by Aedes trivittatus was also noted (Green, 1979), but host susceptibility experiments were not conducted, thus preventing the conclusion that a single mosquito species can vary in its ability to transmit strains of an arbovirus. The results did, however, raise the interesting suggestion that a situation analogous to Venezuelan equine encephalitis (VEE) virus in Aedes spp. (Kramer and Scherer, 1976) might exist for WEE virus in Ae. trivittatus in Iowa.

Surveillance of arbovirus activity in Iowa over the last ten years has established that Cx. tarsalis is the primary vector of WEE virus, but numerous isolations also have been made from Ae. trivittatus (Rowley et al., 1973; Wong et al., 1973; Dorsey et al., 1978; Wong et al., 1978). During
surveillance activities in 1977, two isolates of WEE virus were obtained from mosquitoes collected in Ames, Iowa. One isolate came from a pool of Ae. trivittatus and the other from a pool of Cx. tarsalis. These isolates provided our laboratory the unique opportunity to investigate the vector competence of selected mosquito species from Iowa for two strains of WEE virus which were collected at the same location, on the same day, and which had identical low passage histories.

This paper reports the results of experiments undertaken to compare the oral and parenteral susceptibilities of Cx. tarsalis and Ae. trivittatus for two strains of WEE virus from Iowa and the Fleming strain from California. The transmission capabilities of these two species for the three strains of WEE virus, using a known susceptible line of chicks, are also compared.
MATERIALS AND METHODS

Viruses

Two strains of WEE virus from Iowa (Dorsey et al., 1978) and one strain of WEE virus from California (Buss and Howitt, 1941) were used to infect mosquitoes. The history and stock titer of these strains are described in Table 1. Stock viruses were prepared as 10% homogenates of infected suckling mouse brains in phosphate buffered saline, pH 7.5, containing 1% bovine albumin (BA-PBS) and were titrated in suckling mice. Aliquots of virus were used to prepare inoculating and feeding suspensions. Viral titers per 0.025 ml were estimated by the method of Reed and Muench (1938).

Mosquitoes

Recently colonized Cx. tarsalis and F₁ generation mosquitoes from field-collected adult Ae. trivittatus were used throughout the experiments. The adult Aedes were captured at the Iowa Conservation Commission Nursery in Ames, Iowa, and the egg rafts for the Cx. tarsalis colony were collected in western and central Iowa. Aedes trivittatus eggs from field-collected adults were handled according to the procedures developed by Christensen and Rowley (1978). Mosquitoes were reared uniformly by conventional laboratory techniques and were maintained at 26.5 ± 1°C and 80 ± 5% r.h.
Table 1. History and stock titer of three western equine encephalomyelitis (WEE) viral strains used to infect mosquitoes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source and date of collection(^a)</th>
<th>Passage history(^b)</th>
<th>Titer/0.025 ml ((\text{SMICLD}_{50})^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feeding trials</td>
</tr>
<tr>
<td>WEE-7738</td>
<td>Pool of Ae. trivittatus (8-12-77)</td>
<td>SM-p2</td>
<td>$10^{7.5}$</td>
</tr>
<tr>
<td>WEE-7746</td>
<td>Pool of Cx. tarsalis (8-12-77)</td>
<td>SM-p2</td>
<td>$10^{7.8}$</td>
</tr>
<tr>
<td>WEE Fleming</td>
<td>Human brain tissue (1940)</td>
<td>SM-p8</td>
<td>$10^{9.9}$</td>
</tr>
</tbody>
</table>

\(^a\) Specimens were collected at the Iowa Conservation Commission Nursery, Ames, Iowa, except the Fleming strain which came from Kern County, California, and was provided by the Center for Disease Control.

\(^b\) SM, suckling mice; p, passage level.

\(^c\) Suckling mouse intracerebral mean lethal dose.
under a 16-hr photoperiod. The larval diet consisted of tropical fish food (Tetramin®), and adult mosquitoes were maintained on a 0.3 M sucrose solution.

Infection of Mosquitoes

Mosquitoes (4-5 days old) used in oral susceptibility studies were taken off sucrose 24-36 hours before bloodfeeding. Blood-virus suspensions were prepared on the day of feeding by adding one part of stock virus to three parts of defibrinated dog blood. Female mosquitoes were allowed to feed on the virus-blood suspension through a natural lambskin membrane utilizing a feeder described by earlier workers (Rutledge et al., 1964). Temperature of the infective blood was maintained at 37°C by continuous circulation of heated water. Bloodfed mosquitoes were transferred to one-pint ice cream cartons, held for seven to 25 days at 26.5°C, and then frozen at -70°C for subsequent virus isolation attempts.

During parenteral susceptibility studies, female mosquitoes (4-5 days old) were inoculated through the neck membrane with 1.0 ± 0.1 μl quantities of viral suspensions by using a modification of the Rosen and Gubler technique (Rosen and Gubler, 1974) as described by Green et al. (1980). Inoculum suspensions were prepared by diluting the stock viruses with Dulbecco's PBS, pH 7.4, and Aedes physiological saline, pH 7.8. After inoculation, the mosquitoes were
transferred to one-pint ice cream cartons, held for five to 24 days at 26.5°C, and then frozen at -70°C for subsequent virus isolation attempts. In addition, some mosquitoes were used in transmission experiments 11-19 days after inoculation before being frozen.

Aliquots of each virus suspension were taken before and immediately after feeding or inoculation. The aliquots were frozen at -70°C for subsequent titration in Vero cell suspensions.

Chicks

To determine a susceptible chick line to be used in transmission experiments, dilutions of the three strains of WEE virus were inoculated subcutaneously into half-day-old chicks from ten different ISU Poultry Science chick lines provided by Dr. Arne Nordskog (Department of Animal Science, Iowa State University). Stock viruses were diluted in 1% BA-PBS, pH 7.5, before subcutaneous inoculation of chicks with 0.1 ml of virus suspension. Chick controls were inoculated subcutaneously with 0.1 ml of PBS, pH 7.4. Chicks were observed every four to eight hours for seven days, and dead chicks were frozen at -70°C for later viral assay.

Chick line PS-GHA (White Leghorn) was found to be the most susceptible and available (Andre et al., 1981) and
was therefore used in experiments designed to detect virus transmission capabilities of test mosquitoes.

Tests for Transmission of WEE Virus

Individual inoculated mosquitoes deprived of sucrose for 24-48 hours were transferred 11-19 days postinfection to 1 x 1 x 6 inch clear plexiglass cages covered on two sides with wire mesh. Half-day-old chicks (PS-GHA line) with wing bands were held against the screen until extensive probing or engorgement had occurred. Partially fed or fully engorged mosquitoes were then frozen at -70° C for later virus isolation attempts. Chicks that had been fed upon by mosquitoes were observed every four to 12 hours for seven days, and dead chicks were immediately frozen. At the end of seven days all remaining chicks (except controls) were sacrificed and frozen at -70° C. All experimental chicks were subsequently assayed for WEE virus.

Viral Tests

Each mosquito was triturated in 1.0 ml of 1% BA-PBS, pH 7.5, using a small amount of Alundum® as an abrasive agent, and then centrifuged at 2,000 rpm for ten min at 4° C. The supernatant fluid was frozen at -70° C in a small vial for later virus isolation attempts, at which time the fluid was unfrozen. Quantities of 0.025 ml of the mosquito
extract were then inoculated into four wells of microtiter plates, each well containing 0.075 ml of Vero cell tissue culture suspension. The Vero cell suspensions were then examined for cytopathic effect (CPE) on the second and third day following inoculation. On the fourth day, the material from the four wells was examined, combined, mixed, and a small quantity was placed into a well containing a monolayer of Vero cells. This well was then examined at 24, 48, and 72 hours after inoculation to confirm the presence or absence of CPE. In experiments using strains 7738 and 7746, suspensions that showed no CPE in Vero cells were further analyzed by intracerebral inoculation of suckling mice. Mice that became sick or that died were frozen, and brain tissue was later checked for evidence of WEE virus by the complement fixation (LBCF) test (U.S. Public Health Service, 1965). Suckling mice were not used in assaying any further experiments because it was determined that this expensive technique was detecting only a few additional positive mosquitoes (<5%).

Brains of moribund or dead chicks, used in the susceptibility tests and brains of all chicks fed upon by mosquitoes, were ground and inoculated into suckling mice. Mice that became sick or that died following inoculation were further tested in a second suckling-mouse passage. Virus isolated from this second passage was identified by
the LBCF test.

Dilutions of the feeding- and inoculum-suspension aliquots taken before and after each mosquito susceptibility test were inoculated into microtiter plate wells containing Vero cell tissue culture suspensions. Each dilution was inoculated into eight wells and examined for CPE on days two to five postinoculation. Viral titers (expressed as tissue culture infectious dose fifties or TCID$_{50}$'s) were estimated by the method of Reed and Muench (1938) and were found to have dropped less than $10^{0.25}$ TCID$_{50}$/0.025 ml during each experiment.
RESULTS

Both *Ae. trivittatus* and *Cx. tarsalis* were infected by each of the three strains of WEE virus after ingestion of virus-blood suspensions through a membrane (Table 2). The two mosquito species were somewhat less susceptible to strain 7738 than to strain 7746, and much less susceptible to the Fleming strain. In fact, the viral titer of the blood infected with the Fleming strain was two logs higher, but mosquito infection rates were 35-45% lower than they were with the other two strains. Infection rates of *Cx. tarsalis* were higher by 12-16% than those of *Ae. trivittatus* after exposure to the Fleming strain.

Over 75% of the *Ae. trivittatus* and *Cx. tarsalis* were susceptible to WEE virus inoculated intrathoracically (Table 3). Although the inoculum containing strain 7746 had the lowest titer, almost 100% of the *Aedes* and over 80% of the *Culex* were infected. The lowest infection rates for these two species were obtained in mosquitoes inoculated with strain 7738 despite the inoculum having a viral titer that was a log higher than that of the Fleming strain. *Aedes trivittatus* was more susceptible than *Cx. tarsalis* to parenteral infection of strain 7746 and the Fleming strain but less susceptible to strain 7738.

The PS-GHA chick line was highly susceptible to needle
Table 2. Infection of Ae. trivittatus and Cx. tarsalis after ingesting varying concentrations of three western equine encephalomyelitis (WEE) viral strains through a membrane

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mosquito species</th>
<th>Viral titer/0.025 ml in blood (SMICLD&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Days post-feeding assayed</th>
<th>Infection rate (&lt;# positive/# tested&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738</td>
<td>Ae. trivittatus</td>
<td>10&lt;sup&gt;6.9&lt;/sup&gt;</td>
<td>7-20</td>
<td>46% (5/11)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cx. tarsalis</td>
<td></td>
<td></td>
<td>62% (5/8)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WEE-7746</td>
<td>Ae. trivittatus</td>
<td>10&lt;sup&gt;7.2&lt;/sup&gt;</td>
<td>7-21</td>
<td>57% (29/51)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cx. tarsalis</td>
<td></td>
<td></td>
<td>69% (9/13)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WEE Fleming</td>
<td>Ae. trivittatus</td>
<td>10&lt;sup&gt;9.1&lt;/sup&gt;</td>
<td>7-25</td>
<td>12% (5/43)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Cx. tarsalis</td>
<td></td>
<td></td>
<td>23% (10/44)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Suckling mouse intracerebral mean lethal dose.

<sup>b</sup>Assayed in Vero cell cultures; negatives further assayed in suckling mice.

<sup>c</sup>Assayed in Vero cell cultures only.
Table 3. Infection of *Ae. trivittatus* and *Cx. tarsalis* by intrathoracic inoculation with varying concentrations of three western equine encephalomyelitis (WEE) viral strains

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mosquito species</th>
<th>Viral titer/0.025 ml in inoculum (SMICLD&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Days post-inoculation assayed</th>
<th>Infection rate (# positive/# tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738</td>
<td><em>Ae. trivittatus</em></td>
<td>10&lt;sup&gt;5.5&lt;/sup&gt;</td>
<td>5-20</td>
<td>52% (12/23)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><em>Cx. tarsalis</em></td>
<td></td>
<td></td>
<td>65% (17/26)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WEE-7746</td>
<td><em>Ae. trivittatus</em></td>
<td>10&lt;sup&gt;4.5&lt;/sup&gt;</td>
<td>5-20</td>
<td>97% (34/35)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><em>Cx. tarsalis</em></td>
<td></td>
<td></td>
<td>83% (29/35)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WEE Fleming</td>
<td><em>Ae. trivittatus</em></td>
<td>10&lt;sup&gt;5.2&lt;/sup&gt;</td>
<td>5-24</td>
<td>84% (42/50)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td><em>Cx. tarsalis</em></td>
<td></td>
<td></td>
<td>69% (36/52)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Suckling mouse intracerebral mean lethal dose.

<sup>b</sup>Assayed in Vero cell cultures; negatives further assayed in suckling mice.

<sup>c</sup>Assayed in Vero cell cultures only.
inoculation of the three strains of WEE virus (Table 4). All chicks died after receiving subcutaneous inoculations of virus at dilutions of $10^{-9}$ or less. An end-point was not reached with the Fleming strain and may be reflective of the higher stock virus titer. The speed with which each viral strain of WEE killed the chicks was quite different. Chicks succumbed to infection with strain 7738 very rapidly. In fact, this strain killed 50% of the chicks twice as fast as did strain 7746 and over three times as fast as did the Fleming strain. Suckling mice were less susceptible than the PS-GHA chick line to these three strains of WEE virus as evidenced by differences in LD$_{50}$ values of greater than 1.5 logs.

More than 50% of the infected *Ae. trivittatus* and *Cx. tarsalis* transmitted the three strains of WEE virus to susceptible chicks (Table 5). Strain 7738 was transmitted by all of the *Cx. tarsalis* and three-fourths of the *Ae. trivittatus*. Neither species transmitted strain 7746 as well as it did strain 7738, but the lowest percentage of transmission by both species of mosquitoes occurred in the experiment using the Fleming strain. With all three viral strains, *Cx. tarsalis* transmitted WEE virus more efficiently than *Ae. trivittatus* did.

Chick line PS-GHA was highly susceptible to mosquito inoculated WEE virus. The sensitivity of this host to
Table 4. Susceptibility of half-day-old chicks (ISU PS-GHA line) to three western equine encephalomyelitis (WEE) viral strains inoculated subcutaneously

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Stock virus titer/0.025 ml (SMICLD&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Virus dilution&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Control</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LT&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738 (trivittatus)</td>
<td>10&lt;sup&gt;8.8&lt;/sup&gt;</td>
<td>7/7&lt;sup&gt;e&lt;/sup&gt; 7/7 7/7 7/7 6/7 0/7 0/9</td>
<td>10&lt;sup&gt;4&lt;/sup&gt; 30 hrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEE-7746 (tarsalis)</td>
<td>10&lt;sup&gt;8.0&lt;/sup&gt;</td>
<td>7/7 7/7 7/7 7/7 2/7 1/7 0/9</td>
<td>10&lt;sup&gt;9.8&lt;/sup&gt; 65 hrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEE Fleming</td>
<td>10&lt;sup&gt;9.9&lt;/sup&gt;</td>
<td>6/6 7/7 7/7 7/7 7/7 7/7 0/9</td>
<td>&gt;10&lt;sup&gt;11&lt;/sup&gt; 102 hrs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Suckling mouse intracerebral mean lethal dose.

<sup>b</sup>Virus diluted in 1% bovine albumin in physiological buffered saline before subcutaneous inoculation of 0.1 ml.

<sup>c</sup>Baby chick subcutaneous mean lethal dose.

<sup>d</sup>Time at which 50% of chicks had died following subcutaneous inoculation of virus.

<sup>e</sup>Number of chicks that died/number of chicks inoculated.
Table 5. Transmission of three western equine encephalomyelitis (WEE) viral strains to chicks by *Ae. trivittatus* and *Cx. tarsalis* infected via intrathoracic inoculation

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mosquito species</th>
<th>Viral titer/0.025 ml in inoculum (SMICLD$_{50}$)$_a$</th>
<th>Fraction (%) mosquitoes infected</th>
<th>Day post-inoculation of feed</th>
<th>Fraction (%) infected transmitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738 (trivittatus)</td>
<td><em>Ae. trivittatus</em></td>
<td>10$^5.5$</td>
<td>8/12 (67)</td>
<td>18,19</td>
<td>6/8 (75)</td>
</tr>
<tr>
<td></td>
<td><em>Cx. tarsalis</em></td>
<td></td>
<td>3/3 (100)</td>
<td></td>
<td>3/3 (100)</td>
</tr>
<tr>
<td>WEE-7746 (tarsalis)</td>
<td><em>Ae. trivittatus</em></td>
<td>10$^4.5$</td>
<td>26/26 (100)</td>
<td>11,12,13</td>
<td>12/26 (46)</td>
</tr>
<tr>
<td></td>
<td><em>Cx. tarsalis</em></td>
<td></td>
<td>7/7 (100)</td>
<td></td>
<td>5/7 (71)</td>
</tr>
<tr>
<td>WEE Fleming</td>
<td><em>Ae. trivittatus</em></td>
<td>10$^5.2$</td>
<td>14/14 (100)</td>
<td>15,16</td>
<td>3/14 (21)</td>
</tr>
<tr>
<td></td>
<td><em>Cx. tarsalis</em></td>
<td>14/16$^b$ (88)</td>
<td></td>
<td></td>
<td>9/14 (64)</td>
</tr>
</tbody>
</table>

$^a$Suckling mouse intracerebral mean lethal dose.

$^b$One mosquito negative by Vero cell test.
mosquito transmission of WEE virus is evidenced by the fact that 14% of the successful transmissions were by mosquitoes that were negative in assays using Vero cells and suckling mice.
DISCUSSION

The variation in the susceptibility of different mosquito species to infection with a particular arbovirus has been proven frequently (Chamberlain and Sudia, 1961). Therefore, it is not surprising that our data show that Cx. tarsalis is more susceptible than Ae. trivittatus to infection with WEE virus in blood ingested through a membrane. What is surprising is that the Ae. trivittatus used in this study could be so readily infected. In the previous study on the susceptibility of this species to WEE virus (Green et al., 1980), Ae. trivittatus was relatively refractory to oral infection with WEE virus, even after imbibition of blood with a high viral titer.

Results from laboratory experiments (Gubler and Rosen, 1976; Tesh et al., 1976; Gubler et al., 1979) have confirmed that there is variation in the susceptibility of different geographical strains of a single mosquito species to infection with a single arbovirus strain. Our study did not examine this aspect specifically, but the different infection rates observed in Cx. tarsalis as compared to those cited in the literature (Hardy et al., 1978; Kramer et al., 1981) might be due to this phenomenon. In addition, previous susceptibility tests using Ae. trivittatus from eastern and central Iowa indicated that the eastern strain was more
susceptible than the same species from central Iowa (Green et al., 1980). However, the *Ae. trivittatus* that we tested were from the same location in central Iowa, but were more susceptible to infection with WEE virus than *Ae. trivittatus* from eastern Iowa was reported to be. Perhaps there was a change in susceptibility in the population that existed in central Iowa two years later.

Colonized *Ae. aegypti*, *Ae. albopictus*, and *Ae. taeniorhynchus* were shown to vary in their susceptibility to infection with strains of a single arbovirus (Kramer and Scherer, 1976; Tesh et al., 1976). The first report that field strains of a single mosquito species (*Ae. trivittatus*) can vary in their susceptibility to infection with strains of an arbovirus (Green et al., 1980) was strengthened by the fact that two of the three viral strains tested were isolated from mosquitoes at the same site (Ames, Iowa), on the same day. Using these two Iowa strains and a California strain rather than a New Jersey strain, we were able to confirm that *Ae. trivittatus* varies in its susceptibility to strains of WEE virus. We also found the same to be true for *Cx. tarsalis*. In addition, both species varied in their parenteral susceptibility to the three strains, supporting previous findings (Kramer and Scherer, 1976) concerning differences in the susceptibility of *Aedes* for epizootic and enzootic strains of VEE virus. These results also
indirectly support the hypothesis of Kramer et al. (1981), that there may be two dose-dependent barriers to the transmission of WEE virus.

More direct support of the above hypothesis can be derived from the results of our transmission experiments of WEE virus to chicks. Both Ae. trivittatus and Cx. tarsalis transmitted strain 7738 more efficiently than strain 7746, possibly because the inoculum titer of strain 7738 was a log higher. This higher dose may have overcome the "sali­vary gland infection" barrier that Kramer et al. (1981) discovered during experiments with the BFS 1703 strain of WEE virus. Although the inoculum titer of the Fleming strain was also higher than that of strain 7746, both species of mosquitoes were less efficient at transmitting the Fleming strain, especially Ae. trivittatus. The greater number of passages in suckling mice with the Fleming strain of WEE virus may account for this lower transmission efficiency by mosquitoes.

Overall, Cx. tarsalis was better at transmitting the three strains of WEE virus. In fact, eight Cx. tarsalis successfully transmitted the virus to chicks after having only probed, and all chicks succumbed to their infection. Although Ae. trivittatus seemingly did not support WEE virus as well as Cx. tarsalis, it is noteworthy that 44% of the Ae. trivittatus tested transmitted WEE virus to
chicks. In addition, five *Ae. trivittatus* transmitted WEE virus to chicks 12-14 days after ingestion of virus-infected blood through a membrane.

Observed results on the virulence of strains 7738 and 7746 in inoculated chicks suggest that these two strains may be somewhat analogous to the epizootic and enzootic strains of VEE virus discussed by Kramer and Scherer (1976). Further evidence for this analogy is provided by the finding that only one chick survived mosquito transmitted WEE strain 7738, whereas three chicks survived infections of WEE strain 7746. However, transmission studies of these two strains to equine hosts must be conducted to verify the epidemiological significance of these differences in virulence.

Field investigations in Iowa in the last few years determined that up to 20% of the bloodfed *Ae. trivittatus* collected contained blood from birds, even though this mosquito is usually mammalophilic (Ritchie and Rowley, 1981). Also, multiple isolations of WEE virus from this abundant, persistent, and fierce-biting mosquito have been made in the last ten years (Rowley et al., 1973; Wong et al., 1973; Dorsey et al., 1978; Wong et al., 1978). These findings coupled with our data substantiate the hypothesis (Green et al., 1980) that *Ae. trivittatus* is a competent vector and probably plays a role in the natural mosquito-bird cycle of WEE virus in Iowa.
Results of this study underline the importance of conducting vector competence studies in each endemic region for an arbovirus of public health concern. Such experiments should include not only different species of mosquitoes but also different strains of the same species. Lastly, the above information shows the need for testing different strains of the same virus from each area of interest to determine if enzootic and epizootic strains occur. If they do, then investigations as to which mosquito species are competent vectors of these viral strains should be made.
REFERENCES CITED


SECTION II. VECTOR COMPETENCE OF CULEX PIPiens,
CULEX RESTUANS, AND CULEX SALINARIUS
FOR THREE STRAINS OF WESTERN EQUINE
ENCEPHALOMYELITIS VIRUS
Vector Competence of Selected Species of Iowa Mosquitoes (Diptera: Culicidae) for Three Strains of Western Equine Encephalomyelitis Virus: II. Culex pipiens, Cx. restuans, and Cx. salinarius

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Wayne A. Rowley
Yau W. Wong

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INTRODUCTION

Considerable information pertaining to the epidemiology of mosquito-borne viruses in Iowa has been acquired during a continuing arbovirus surveillance program. In a 12-year period, thousands of pools of mosquitoes have been tested for the presence of arboviruses, and numerous virus isolations have been made (Rowley et al., 1973; Wong et al., 1973; Dorsey et al., 1978; Wong et al., 1978). Some of these viruses such as Cache Valley (CV), Flanders (FL), Turlock (Tur), and trivittatus (TWT) are of limited or unknown public health importance. In addition to these viruses, St. Louis encephalitis (SLE), western equine encephalomyelitis (WEE), and California (LaCrosse) encephalitis (LAC) viruses are routinely isolated from field collected mosquitoes in Iowa. During this time, both SLE and WEE antibodies have been frequently detected among thousands of avian sera tested. Also, hundreds of equine cases of WEE were diagnosed, and over 100 laboratory-confirmed human cases of LAC, SLE, and WEE were reported in the 12-year period from 1965 to 1977 (Dorsey et al., 1978).

Although LAC virus has been isolated only from pools of *Aedes triseriatus* in Iowa, SLE and WEE viruses have been detected in various species of mosquitoes (Rowley et al., 1973; Wong et al., 1973; Dorsey et al., 1978; Wong et al., 1978). *Culex tarsalis* is considered to be the primary
vector of SLE and WEE viruses in Iowa, but studies indicate that Ae. trivittatus also may play an important role in the natural cycle of WEE virus in Iowa (Green et al., 1980; Andre et al., 1981b; Ritchie and Rowley, 1981). In addition, isolations of SLE and WEE viruses have been made from pools of other Culex mosquitoes (Cx. pipiens, Cx. restuans, and Cx. salinarius) collected during the last ten years (Rowley et al., 1973; Dorsey et al., 1978; Wong et al., 1978).

Field studies by our laboratory staff indicate that large populations of Cx. pipiens, Cx. restuans, and Cx. salinarius may contribute to the amplification of SLE and WEE viruses in Iowa (Wong et al., 1978). These investigators suggest that unusually large populations of these mosquitoes in the spring and summer of 1974 and 1975 may have had some significance in the outbreak of encephalitis in Iowa during 1975. Also, research on the blood-feeding habits of these three species in Iowa has shown that they feed predominantly on birds but exhibit a midsummer increase in their feeding on mammals (Ritchie and Rowley, 1981). The authors stated that this feeding shift may increase the vector potential of these species. However, studies to compare the vector competency of these Culex species for Iowa strains of WEE virus have not been done.

This paper enumerates the results of vector competence
studies designed to determine the role *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* play in the transmission cycle of WEE virus in Iowa. Included are comparisons of the oral and parenteral susceptibilities of these mosquito species for two strains of WEE virus from Iowa and the Fleming strain from California. These three virus strains were shown to vary in their ability to infect and multiply in *Ae. trivittatus* and *Cx. tarsalis* (Green et al., 1980; Andre et al., 1981b). Also, *Ae. trivittatus* and *Cx. tarsalis* varied in their transmission capabilities with these three strains of WEE virus (Andre et al., 1981b). Therefore, we conducted experiments to compare the transmission of the three strains of WEE virus to susceptible chicks by *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*. 
MATERIALS AND METHODS

Viruses

Three strains of WEE virus (WEE-7738, WEE-7746, and Fleming) were used to infect mosquitoes. The history, preparation of stock viruses, and titer of these strains were elaborated upon previously (Andre et al., 1981b). Virus titers per 0.025 ml were estimated by the method of Reed and Muench (1938) and expressed as suckling mouse intracerebral mean lethal doses (SMICLD$_{50}$'s).

Mosquitoes

Adult Cx. pipiens, Cx. restuans, and Cx. salinarius were raised from field-collected egg rafts. These egg rafts were collected before each experiment from small artificial pools located at the Iowa Conservation Commission Nursery on the south edge of Ames in central Iowa. Mosquitoes were reared uniformly by conventional laboratory techniques as described earlier (Andre et al., 1981b).

Infection of Mosquitoes

Female Culex (4-15 days old) used in oral susceptibility tests were not allowed to feed on sucrose for 24-48 hrs before bloodfeeding. Virus-blood suspensions were prepared on the day of feeding by adding one part of stock virus to
two or three parts of defibrinated dog blood. Initially, mosquitoes were given the opportunity to feed on the suspension through a natural lambskin membrane, but only a few mosquitoes would attempt to feed. In order to increase feeding success, female Culex were offered small hanging-drops of the blood-virus suspension that had been warmed to 37°C. Bloodfed mosquitoes were transferred to one-pint ice cream cartons, held for eight to 38 days at 26.5°C, and then frozen at -70°C for subsequent virus isolation attempts.

Mosquitoes (4-15 days old) used in parenteral susceptibility studies were inoculated intrathoracically with virus suspensions as previously outlined (Andre et al., 1981b). Inoculated mosquitoes were held for five to 35 days at 26.5°C before being frozen for later virus isolation attempts. Some mosquitoes were used in transmission experiments nine to 18 days after inoculation.

Aliquots of each virus suspension were taken before and after feeding or inoculation. These were frozen at -70°C for subsequent titration in Vero cell suspensions.

Tests for Transmission of WEE Virus

A White Leghorn chick line (PS-GHA) was used in experiments designed to detect virus transmission capabilities of test mosquitoes. This line is highly susceptible to WEE
virus (Andre et al., 1981a) and was provided by Dr. Arne Nordskog (Department of Animal Science, Iowa State University).

Individual female mosquitoes that had been inoculated nine to 18 days earlier were transferred to 1 x 1 x 6 inch clear plexiglass cages covered on two sides with wire mesh. Mosquitoes were then deprived of sucrose for 24-48 hrs before being allowed to feed on 12-hour-old chicks. Once chicks had been fed upon by mosquitoes, they were observed every eight to 12 hrs for seven days. Dead chicks were frozen at -70°C, and at the end of seven days all remaining chicks (except controls) were sacrificed and frozen. Experimental chicks subsequently were assayed for WEE virus.

Viral Tests

Mosquitoes were assayed for virus in Vero cells using the procedures described in an earlier paper (Andre et al., 1981b). Mosquitoes that were negative in Vero cells were not tested further by intracerebral inoculation of suckling mice because less than 5% more positive mosquitoes were detected by this expensive method.

Brains of all chicks fed upon by mosquitoes were ground and inoculated into suckling mice. Mice showing signs of sickness or those that died were further tested by a second suckling-mouse passage. Virus isolated from the second
passage was identified by the complement fixation (LBCF) test (U.S. Public Health Service, 1965).

Feeding and inoculum suspensions were titered in Vero cell suspensions as outlined previously (Andre et al., 1981b). Virus titers in feeding and inoculum suspensions dropped insignificant amounts during the experiments.
RESULTS

*Culex restuans* was slightly susceptible to infection with the three strains of WEE virus following oral ingestion of infected blood, but *Cx. pipiens* and *Cx. salinarius* were refractory (Table 1). The Fleming strain infected the highest percentage of *Cx. restuans*, and strain 7738 infected the lowest percentage. Only four out of 34 *Cx. restuans* were susceptible to infection with strain 7746.

Over 60% of the *Cx. pipiens* and *Cx. restuans* were susceptible to WEE virus inoculated intrathoracically, however, *Cx. salinarius* was almost refractory (Table 2). Inoculated *Cx. pipiens* were uniformly susceptible to infection with strain 7746 and the Fleming strain, and slightly more susceptible to strain 7738. In contrast, *Cx. restuans* was least susceptible to strain 7738, yet, was uniformly susceptible to the other two strains. Even though more than 60 *Cx. salinarius* were inoculated, only one mosquito became infected with any of the three strains of WEE virus.

Substantial differences in the susceptibility of *Cx. pipiens* and *Cx. restuans* to WEE virus, with respect to mosquito age and season, occurred during parenteral susceptibility experiments (Table 3). Infection rates of both species were 30 to 50% lower in experiments with older adults (9-15 days old) that were raised from egg rafts collected
Table 1. Infection of Cx. pipiens, Cx. restuans, and Cx. salinarius after ingesting varying concentrations of three western equine encephalomyelitis (WEE) viral strains through a membrane or from a hanging drop of blood

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mosquito species</th>
<th>Viral titer/0.025 ml in blood (SMICLD\textsuperscript{50})\textsuperscript{a}</th>
<th>Days post-feeding assayed</th>
<th>Infection rate (# positive/# tested)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738 (trivittatus)</td>
<td>Cx. pipiens</td>
<td></td>
<td>10\textsuperscript{7.4}</td>
<td>8-21</td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEE-7746 (tarsalis)</td>
<td>Cx. pipiens</td>
<td></td>
<td>10\textsuperscript{7.2}</td>
<td>14-27</td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEE Fleming</td>
<td>Cx. pipiens</td>
<td></td>
<td>10\textsuperscript{8.0}</td>
<td>15-38</td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Suckling mouse intracerebral mean lethal dose.

\textsuperscript{b}Assayed in Vero cell cultures.

\textsuperscript{c}Died on day two postfeeding.
Table 2. Infection of Cx. pipiens, Cx. restuans, and Cx. salinarius by intrathoracic inoculation with varying concentrations of three western equine encephalomyelitis (WEE) viral strains

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mosquito species</th>
<th>Viral titer/0.025 ml in inoculum (SMICLD&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Days post-inoculation assayed</th>
<th>Infection rate (# positive/# tested)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738 (trivittatus)</td>
<td>Cx. pipiens</td>
<td>10&lt;sup&gt;4.7&lt;/sup&gt;</td>
<td>5-36</td>
<td>63% (51/81)</td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td></td>
<td></td>
<td>57% (48/84)</td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td></td>
<td></td>
<td>0% (0/4)</td>
</tr>
<tr>
<td>WEE-7746 (tarsalis)</td>
<td>Cx. pipiens</td>
<td>10&lt;sup&gt;4.5&lt;/sup&gt;</td>
<td>5-27</td>
<td>58% (25/43)</td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td></td>
<td></td>
<td>71% (30/42)</td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td></td>
<td></td>
<td>0% (0/26)</td>
</tr>
<tr>
<td>WEE Fleming</td>
<td>Cx. pipiens</td>
<td>10&lt;sup&gt;5.7&lt;/sup&gt;</td>
<td>5-34</td>
<td>57% (27/47)</td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td></td>
<td></td>
<td>69% (24/35)</td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td></td>
<td></td>
<td>3% (1/33)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Suckling mouse intracerebral mean lethal dose.

<sup>b</sup>Assayed in Vero cell cultures.
Table 3. Differences in the susceptibility of \textit{Cx. p\text{ipiens}} and \textit{Cx. restuans} to two Iowa strains of western equine encephalomyelitis (WEE) virus inoculated intrathoracically, with respect to mosquito age and season

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>Virus strain</th>
<th>Date egg rafts collected in 1980$^a$</th>
<th>Age of adults when inoculated</th>
<th>Infection rate ($#$ positive/$#$ tested)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Cx. p\text{ipiens}}</td>
<td>WEE-7738 (trivittatus)</td>
<td>June 24</td>
<td>4 days</td>
<td>82% (31/38)</td>
</tr>
<tr>
<td></td>
<td>WEE-7746 (tarsalis)</td>
<td>July 10</td>
<td>5 days</td>
<td>89% (16/18)</td>
</tr>
<tr>
<td></td>
<td>WEE-7738 (trivittatus)</td>
<td>June 24</td>
<td>5 days</td>
<td>82% (32/39)</td>
</tr>
<tr>
<td>\textit{Cx. restuans}</td>
<td>WEE-7746 (tarsalis)</td>
<td>July 10</td>
<td>5 days</td>
<td>86% (18/21)</td>
</tr>
<tr>
<td></td>
<td>Sep 1</td>
<td>14 days</td>
<td>36% (20/43)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sep 19</td>
<td>9 days</td>
<td>36% (9/25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sep 1</td>
<td>15 days</td>
<td>36% (16/45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sep 13</td>
<td>15 days</td>
<td>57% (12/21)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Collected from small artificial pools at the Iowa Conservation Commission Nursery, Ames, Iowa.

$^b$Assayed in Vero cell cultures.
in September. High infection rates resulted when young adult females (4-5 days old), raised from egg rafts collected in late June and early July, were inoculated. The differences in parenteral susceptibility of the two species to WEE virus strains 7738 and 7746, with respect to adult age and collection date, were similar.

A majority of the field-collected Culex used in this study did not feed on chicks in the laboratory. Various modifications to the feeding method were tried but to no avail. Although only low numbers of infected Culex mosquitoes successfully fed on chicks, a high percentage of those that did, transmitted WEE virus nine to 18 days after parenteral infection (Table 4). Culex pipiens transmitted virus strain 7738 to susceptible chicks more efficiently than it did virus strain 7746. Culex restuans and Cx. salinarius transmitted virus strain 7738 to all susceptible chicks fed upon.
Table 4. Transmission of two Iowa strains of western equine encephalomyelitis (WEE) virus to chicks by Cx. pipiens, Cx. restuans, and Cx. salinarius infected via intrathoracic inoculation

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Mosquito species</th>
<th>Viral titer/0.025 ml (SMICLD&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fraction (%) mosquitoes infected&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day post-inoculation of feed</th>
<th>Fraction (%) infected transmitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738 (trivittatus)</td>
<td>Cx. pipiens</td>
<td>10&lt;sup&gt;4.7&lt;/sup&gt;</td>
<td>4/6&lt;sup&gt;c&lt;/sup&gt; (67)</td>
<td>3/4 (75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td>10&lt;sup&gt;4.7&lt;/sup&gt;</td>
<td>2/3 (67)</td>
<td>2/2 (100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td>10&lt;sup&gt;4.7&lt;/sup&gt;</td>
<td>1/3&lt;sup&gt;c&lt;/sup&gt; (33)</td>
<td>1/1 (100)</td>
<td></td>
</tr>
<tr>
<td>WEE-7746 (tarsalis)</td>
<td>Cx. pipiens</td>
<td>10&lt;sup&gt;4.5&lt;/sup&gt;</td>
<td>5/6 (83)</td>
<td>2/5 (40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. restuans</td>
<td>10&lt;sup&gt;4.5&lt;/sup&gt;</td>
<td>R.F.&lt;sup&gt;d&lt;/sup&gt;</td>
<td>N.D.&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cx. salinarius</td>
<td>10&lt;sup&gt;4.5&lt;/sup&gt;</td>
<td>0/3 (0)</td>
<td>N.D.&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Suckling mouse intracerebral mean lethal dose.

<sup>b</sup>Assayed in Vero cell cultures.

<sup>c</sup>One mosquito negative by Vero cell culture test.

<sup>d</sup>Refused to feed.

<sup>e</sup>Not determined.
DISCUSSION

The differences observed in the susceptibilities of Cx. restuans and Cx. salinarius to infection with WEE virus following oral ingestion of virus-infected blood are similar to those already reported (Chamberlain et al., 1954). These authors stated that 18% of the Cx. restuans tested were susceptible to infection with WEE virus, but Cx. salinarius was not susceptible. It should be noted, however, that the eastern strain of WEE virus used in their experiments is now classified as Highlands J (HJ) virus (Trent and Grant, 1980). Chamberlain et al. (1954) did not test the susceptibility of Cx. pipiens, but in our experiments Cx. pipiens was refractory to oral infection with two Iowa strains and a California strain of WEE virus. Although natural infections of WEE virus have been detected in field-collected Cx. pipiens (Ferguson, 1954), our laboratory results indicate that this species would have to feed on a host whose blood contained a WEE virus titer of greater than $10^8$ SMICLD$_{50}$/0.025 ml to become infected.

In contrast to the results of the oral susceptibility study, we found Cx. pipiens and Cx. restuans to be almost uniformly susceptible to parenteral inoculation of WEE virus. Hayes (1979), however, reported that following intrathoracic inoculation of virus, Cx. restuans was much more susceptible than Cx. pipiens to infection with WEE
virus. Also, he calculated that Cx. salinarius was 200 times less susceptible than Cx. restuans to WEE virus, and six times less susceptible than Cx. pipiens. We determined that Cx. salinarius was almost completely refractory to infection with WEE virus inoculated parenterally. The differences between our results and those reported by Hayes may be because he used an eastern strain of WEE virus (= HJ virus). Also, he used colonized mosquitoes from the eastern United States in his experiments. However, his results led him to propose that intrinsic barriers other than the midgut act to modulate virus replication in mosquitoes. Kramer et al. (1981) also discussed this possibility in reference to WEE virus infection in Cx. tarsalis. Our data from this study and the previous one (Andre et al., 1981b) seem to substantiate further this hypothesis.

The two Iowa strains of WEE virus overcame the "salivary gland infection" barrier (Kramer et al., 1981) in the field-collected Culex used in our study. However, the single specimen of Cx. salinarius that transmitted WEE virus to a chick apparently contained only a small amount of virus, yet this barrier was still circumvented. Of the few Cx. restuans that fed on susceptible chicks, all those infected transmitted WEE virus. In contrast, only 56% of the Cx. pipiens successfully transmitted WEE virus to susceptible chicks. The apparent difference in the ability of this species to
transmit the two Iowa strains of WEE virus may be significant. Our results indicate that Culex mosquitoes in Iowa, once infected, could transmit strains of WEE virus to susceptible hosts. This may be important epidemiologically when large populations of these species are present.

Our study comparing the susceptibility of Cx. pipiens and Cx. restuans to parenteral infection of WEE virus indicates adult age or season as possible factors influencing mosquito susceptibility. We found that female mosquitoes (9-15 days old), which were raised from egg rafts collected in September, were much less susceptible than young females (4-5 days old) that were raised during midsummer. Unfortunately, our study was not designed to determine which factor was most important.

Baqar et al. (1980) showed that adult age slightly affected the susceptibility of Cx. tritaeniorhynchus to infection with West Nile (WN) virus. But in studies using the same mosquito species, Takahashi (1976) determined that the pattern of transmission of Japanese encephalitis (JE) virus was not influenced by mosquito age. We had one group of late season Cx. pipiens that developed an infection rate that was 56% lower than that in the early season group; yet, the late season group was only four days older when inoculated. This may indicate that age is the less important factor.
The effect of season on mosquito susceptibility to virus infection also has been reported in the literature. Seasonal changes in the susceptibility of *Cx. tarsalis* to infection with WEE virus have been documented (Hardy et al., 1979). However, these authors reported that late spring and early summer populations of *Cx. tarsalis* were less susceptible to infection than were subsequent populations. In contrast, the *Culex* mosquitoes we collected in late summer were less susceptible. This difference may be because the major seasonal changes in Iowa do not correlate temporally with those in California. Eldridge et al. (1972) have discussed the implications of the physiological changes that occur in *Cx. restuans* and *Cx. salinarius* under simulated fall conditions. They suggested that mosquito species whose females have undergone prehibernation diapause are more likely to harbor arboviruses. However, our results suggest that older, late season *Culex* mosquitoes would be less supportive of virus growth, and therefore, less likely to be an overwintering mechanism for the virus. More importantly, basic physiological changes may occur in these mosquitoes that reduce their vector competence.

Because the above findings could contribute to the overall understanding of mosquito-virus relationships, we are planning future experiments in Iowa to determine the effects of adult age and of season on mosquito susceptibil-
ity to infection with WEE virus. Experiments also should be carried out in other locations using different mosquito species and arboviruses. In addition, earlier findings (Green et al., 1980; Andre et al., 1981b) indicate that different virus strains should be tested in such studies.
REFERENCES CITED


SECTION III. THE SUSCEPTIBILITY OF DIFFERENT CHICK LINES TO THREE STRAINS OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS
The Susceptibility of White Leghorn Chicks of Different Genotypes to Three Strains of Western Equine Encephalomyelitis Virus

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Gene A. Erickson
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INTRODUCTION

During arbovirus surveillance activities in Iowa in the summer of 1977, two isolates of western equine encephalomyelitis (WEE) virus, Family Togaviridae, were obtained from mosquitoes collected in the city of Ames. One isolate came from a pool of *Aedes trivittatus* and the other from a pool of *Culex tarsalis*. These isolates provided our laboratory the unique opportunity to investigate the vector competence of selected mosquito species for two strains of WEE virus which were collected not only at the same location but at the same time. However, a necessary part of a vector competence study is the determination of the ability of a mosquito species to transmit the virus to a host. To insure that variations in transmission results are due to mosquito-virus interactions, the susceptibility of the laboratory host (e.g., baby chicken) for each virus strain must be determined ahead of time.

Tests to determine the susceptibility of juvenile chickens to infection with WEE virus were first conducted to elucidate the role played by domestic chickens in the epidemiology of WEE (Hammon and Reeves, 1946). Studies to find a suitable laboratory host for WEE virus were later carried out (Chamberlain et al., 1954), and these studies showed that chicks were more sensitive than mice in quantifying newly isolated strains of WEE virus and in detecting mosquito
transmission of WEE virus. However, the primary strain (L2-34a) of virus utilized in the experiments has been reclassified as a Highlands J (HJ) virus strain (Trent and Grant, 1980). In addition, Chamberlain et al. (1954) stated that any breed of chick is a suitable host for WEE virus, but they did not verify this statement by testing chicks other than New Hampshire Reds. Consequently, the susceptibilities of specific White Leghorn chick populations to infection with WEE virus have not been determined.

During genetic studies on the blood-group systems in two White Leghorn populations (lines S1 and S2), several important findings have been made. The B locus blood group was found to influence adult viability and egg production (Nordskog et al., 1973). Later work demonstrated that an immune response gene may be associated with this major histocompatibility locus (Pevzner et al., 1975). Also, B^1.B^1 chicks proved to be low responders to Salmonella pullorum bacterin, human serum albumin, and GAT^10 (L-glutamic acid^60 L-alanine^30 L-tyrosine^10)n. In addition, the results indicated that inbred lines (genotypes) GH and 19 differed in their response to GAT^10. Various immune responses to GAT^10 also were noted among haplotypes of B^19.B^19 birds (Pevzner et al., 1978). Recent studies have shown that the locus controlling the fate of tumors induced by Rous sarcoma virus (RSV), Family Retroviridae, was closely linked to the locus controlling the
immune response to GAT (Gebriel et al., 1979). However, nothing has been reported on the susceptibility of these chick lines to viruses in the genus Alphavirus, Family Togaviridae.

We selected three different lines (genotypes GHA, 19, and S1) of chicks for inclusion in this study for two reasons. First, we wanted to determine if genotypes with proven resistance to Marek's disease virus and RSV also would have reduced susceptibility to infection with an Alphavirus like WEE virus. Second, we hoped to find an established line of chicks that would be uniformly susceptible to infection with three different strains of WEE virus. If we knew the response of the chicks to needle-inoculated WEE virus, then we could directly compare the ability of various mosquito species to transmit the different strains of the virus to known susceptible hosts.
MATERIALS AND METHODS

Viruses

One strain of WEE virus from California (Buss and Howitt, 1941) and two strains of WEE virus from Iowa (Dorsey et al., 1978) were used to infect chicks. The history and stock titer of these strains are described in Table 1. Stock viruses were prepared as 10% homogenates of infected suckling mouse brains in phosphate buffered saline, pH 7.5, containing 1% bovine albumin (BA-PBS) and were titrated in suckling mice. Virus titers per 0.025 ml were estimated by the method of Reed and Muench (1938).

Chick Lines

The three lines (genotypes) of White Leghorn chicks used in this study were obtained from the Poultry Science Center at Iowa State University, Ames, Iowa. Genetic backgrounds of inbred lines GHA and 19 have been listed previously (Nordskog et al., 1977). Seven haplotypes (B'B'L, B'B'H, B'B',
B'B'L, B'B'H, B'L'B'H, and B'B'L) of the third line (Sl) were used, and their backgrounds have been described earlier (Gebriel et al., 1979).
Table 1. History and stock titer of three western equine encephalomyelitis (WEE) virus strains used to infect chicks

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source and date of collection&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Passage history&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Date produced</th>
<th>Titer/0.025 ml (SMICLD&lt;sub&gt;50&lt;/sub&gt;)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE-7738</td>
<td>Pool of <em>Ae. trivittatus</em> mosquitoes (8-12-77)</td>
<td>SM-p2</td>
<td>2-5-80</td>
<td>10&lt;sup&gt;8&lt;/sup&gt;.8</td>
</tr>
<tr>
<td>WEE-7746</td>
<td>Pool of <em>Cx. tarsalis</em> mosquitoes (8-12-77)</td>
<td>Sm-p2</td>
<td>8-31-78</td>
<td>10&lt;sup&gt;8&lt;/sup&gt;.0</td>
</tr>
<tr>
<td>WEE Fleming</td>
<td>Human brain tissue (1940)</td>
<td>SM-p8</td>
<td>1-7-80</td>
<td>10&lt;sup&gt;9&lt;/sup&gt;.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Specimens were collected at the Iowa Conservation Commission Nursery, Ames, Iowa, except the Fleming strain which came from Kern County, California, and was provided by the Center for Disease Control.

<sup>b</sup>SM, suckling mice; p, passage level.

<sup>c</sup>Suckling mouse intracerebral mean lethal dose.
Infection of Chicks

Twelve-hour-old chicks were wing banded, and the numbers were tabulated according to genotype or haplotype, before inoculation trials were begun. Chicks from each genotype or haplotype were then randomly assigned to treatment or control groups. Stock viruses were diluted in 1% BA-PBS, pH 7.5, before subcutaneous inoculation of chicks with 0.1 ml of virus suspension. Chick controls were inoculated subcutaneously with 0.1 ml of PBS, pH 7.4. After inoculation, chicks from each treatment or control group were placed into separate compartments of standard hatchery cardboard boxes (designed to ship 100 chicks). To avoid crowding, no more than eight chicks were placed into each of the four compartments of the box. Separate boxes were used for each strain of virus inoculated, for each chick line, and for control chicks. Hardware cloth was used to cover each box to allow the entrance of light.

Chicks were kept in an animal-rearing room at 30°C, and were provided standard chick ration and water. Every three to eight hrs for seven days, chicks were observed for signs of illness, and dead chicks were frozen at -70°C. At the end of seven days, all remaining chicks were sacrificed and frozen. Experimental chicks, from each group which had been inoculated with the highest dilution of virus causing 100% mortality, were subsequently assayed for WEE virus.
Control chicks that were moribund or dead also were assayed for virus. Each chick line was tested twice against each of the three strains of WEE virus.

**Viral Assays**

Brains of moribund or dead chicks were triturated in 2.0 ml of 1% BA-PBS, pH 7.5, and centrifuged at 2,000 rpm for 10 min at 4°C. One-day-old suckling mice were inoculated with 0.025 ml of the supernatant and were monitored 14 days for signs of illness. Mice that became sick or that died following inoculation were further tested in a second suckling-mouse passage. Virus isolated from this second passage was identified by the complement fixation (LBCF) test (U.S. Public Health Service, 1965).
RESULTS

Over 900 chicks were inoculated with WEE virus. Most chicks died after receiving subcutaneous inoculations of virus at dilutions of $10^{-9}$ or less. All dead experimental chicks assayed for WEE virus were positive. Only 4% (three out of 73) of the control chicks died, and none of these contained WEE virus.

The three chick lines were highly susceptible to needle inoculation of the three strains of WEE virus (Table 2). Genotype 19 was extremely susceptible to infection with WEE virus, and consequently, LD$_{50}$ endpoints were not reached in trials using either strain 7738 or the Fleming strain. Genotype GHA had higher LD$_{50}$ values than the mean values calculated for genotype SI. However, haplotype $B^{1}H-B^{19}L$ was slightly more susceptible than genotype GHA and haplotypes $B^{19}-B^{19}L$ and $B^{19}-B^{19}H$ were to infection with virus strain 7738. Also, haplotype $B^{1}H-B^{19}L$ was a log more susceptible than haplotype $B^{1}-B^{1}H$ was to infection with virus strain 7738. In fact, haplotype $B^{1}-B^{1}H$ had the lowest LD$_{50}$ values for all three strains of WEE virus. Chicks were more susceptible than suckling mice to the two Iowa strains of WEE virus as evidenced by differences in LD$_{50}$ values of greater than 1.5 logs (Tables 1 and 2). Genotypes 19 and GHA were more susceptible than suckling mice to infection with the Fleming
Table 2. Susceptibility of 12-hour-old chicks to three western equine encephalomyelitis (WEE) virus strains inoculated subcutaneously

<table>
<thead>
<tr>
<th>White Leghorn chick genotype</th>
<th>WEE-7738&lt;sup&gt;a&lt;/sup&gt;</th>
<th>WEE-7746</th>
<th>WEE Fleming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>No. tested</td>
</tr>
<tr>
<td>19</td>
<td>28 &gt; 11</td>
<td>10.3</td>
<td>26 &gt; 11</td>
</tr>
<tr>
<td>GHA</td>
<td>70</td>
<td>10.4</td>
<td>40</td>
</tr>
<tr>
<td>S1 (Haplotype)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;H-B&lt;sup&gt;19&lt;/sup&gt;L</td>
<td>22</td>
<td>10.7</td>
<td>21</td>
</tr>
<tr>
<td>B&lt;sup&gt;19&lt;/sup&gt;-B&lt;sup&gt;19&lt;/sup&gt;L</td>
<td>32</td>
<td>10.4</td>
<td>32</td>
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<tr>
<td>B&lt;sup&gt;19&lt;/sup&gt;-B&lt;sup&gt;19&lt;/sup&gt;H</td>
<td>26</td>
<td>10.4</td>
<td>26</td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;L-B&lt;sup&gt;19&lt;/sup&gt;H</td>
<td>27</td>
<td>10.2</td>
<td>27</td>
</tr>
<tr>
<td>B&lt;sup&gt;2&lt;/sup&gt;-B&lt;sup&gt;2&lt;/sup&gt;</td>
<td>31</td>
<td>9.9</td>
<td>29</td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;-B&lt;sup&gt;1&lt;/sup&gt;L</td>
<td>40</td>
<td>9.9</td>
<td>40</td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;-B&lt;sup&gt;1&lt;/sup&gt;H</td>
<td>32</td>
<td>9.7</td>
<td>32</td>
</tr>
<tr>
<td>Mean of S1</td>
<td></td>
<td>10.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Virus strain.

<sup>b</sup>Expressed as inverse log of dilution killing half of animals inoculated with 0.1 ml.

<sup>c</sup>Based on serological blood type and high or low response to GAT10.
virus strain, but the mean LD_{50} value of genotype S1 was similar to that obtained in mice.

The speed with which each virus strain of WEE killed the chicks was quite different. Chicks succumbed to infection with virus strain 7738 very rapidly (Table 3). This strain of virus killed 50% of the chicks (genotype S1), inoculated with a virus dilution of 10^{-9}, twice as fast as did strain 7746 and over three times as fast as did the Fleming strain. The LT_{50} values obtained following inoculation of chicks with virus strain 7738 were similar for the three genotypes. However, LT_{50} values obtained after inoculation of virus strain 7746 varied significantly (42 to 116 hrs). Less variation was noted in experiments using the Fleming strain.

Observations on the duration of illness before chicks died indicated that the three virus strains may have multiplied in different body organs. The majority of the chicks infected with Fleming virus strain showed distinct CNS symptoms (paralysis, convulsions, tremor). In some chicks, symptoms persisted for over two days before death (Table 3). However, chicks receiving an inoculation of virus strain 7738 showed signs of illness for only a few hours before death occurred. The duration of illness in chicks inoculated with virus strain 7746 ranged from six to 37 hrs. Genotype 19,
Table 3. Response of 12-hour-old chicks to three western equine encephalomyelitis (WEE) virus strains inoculated subcutaneously, with respect to duration of signs and time of death

<table>
<thead>
<tr>
<th>White Leghorn chick genotype</th>
<th>WEE-7738a Duration</th>
<th>LT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>WEE-7746 Duration</th>
<th>LT&lt;sub&gt;50&lt;/sub&gt;</th>
<th>WEE Fleming Duration</th>
<th>LT&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>19&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>31&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6</td>
<td>55</td>
<td>12</td>
<td>114</td>
</tr>
<tr>
<td>GHA</td>
<td>5</td>
<td>40</td>
<td>12</td>
<td>67</td>
<td>25</td>
<td>102</td>
</tr>
<tr>
<td>S1 (haplotype)&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sup&gt;19&lt;/sup&gt;-B&lt;sup&gt;19&lt;/sup&gt;H</td>
<td>2</td>
<td>30</td>
<td>14</td>
<td>42</td>
<td>62</td>
<td>116</td>
</tr>
<tr>
<td>B&lt;sup&gt;19&lt;/sup&gt;-B&lt;sup&gt;19&lt;/sup&gt;L</td>
<td>7</td>
<td>41</td>
<td>12</td>
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<tr>
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<td>6</td>
<td>41</td>
<td>28</td>
<td>66</td>
<td>27</td>
<td>116</td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;-B&lt;sup&gt;1&lt;/sup&gt;H</td>
<td>1</td>
<td>41</td>
<td>21</td>
<td>97</td>
<td>33</td>
<td>126</td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;H-B&lt;sup&gt;19&lt;/sup&gt;L</td>
<td>3</td>
<td>41</td>
<td>22</td>
<td>104</td>
<td>36</td>
<td>126</td>
</tr>
<tr>
<td>B&lt;sup&gt;1&lt;/sup&gt;-B&lt;sup&gt;1&lt;/sup&gt;L</td>
<td>7</td>
<td>42</td>
<td>29</td>
<td>114</td>
<td>36</td>
<td>126</td>
</tr>
<tr>
<td>B&lt;sup&gt;2&lt;/sup&gt;-B&lt;sup&gt;2&lt;/sup&gt;</td>
<td>6</td>
<td>30</td>
<td>37</td>
<td>116</td>
<td>45</td>
<td>126</td>
</tr>
<tr>
<td>Mean of S1</td>
<td>4</td>
<td>38</td>
<td>23</td>
<td>86</td>
<td>40</td>
<td>125</td>
</tr>
</tbody>
</table>

<sup>a</sup>Virus strain.

<sup>b</sup>Mean number of hrs before death that chicks exhibited signs of illness; at 10<sup>-9</sup> dilution.

<sup>c</sup>Mean number of hrs when 50% of the chicks had died; at 10<sup>-9</sup> dilution.

<sup>d</sup>Results from 10<sup>-10</sup> dilution.

<sup>e</sup>In hrs.

<sup>f</sup>Based on serological blood type and high or low response to GAT<sub>10</sub>.
when infected with virus strain 7738 or 7746, rarely showed signs of illness for more than a few hours before death.
DISCUSSION

Previous studies have determined that young chicks are susceptible to infection with WEE virus and are more sensitive than suckling mice to certain WEE virus strains (Chamberlain et al., 1954; Sponseller et al., 1966). However, some of the virus strains tested are now considered to be strains of HJ virus (Trent and Grant, 1980). In our studies, two WEE virus strains from Iowa and a WEE virus strain from California were used. We found three different chick lines (genotypes) to be highly susceptible to infection with WEE virus. Also, chicks were more sensitive than mice to the two Iowa virus strains, but most haplotypes of genotype Sl were less sensitive than mice to the Fleming strain. The lower susceptibility of chicks to infection with the Fleming strain has been noted previously (Chamberlain et al., 1954), and explained by the fact that the strain has become largely neurotropic in mice. Therefore, it was surprising that chicks of genotypes 19 or GHA were much more susceptible than suckling mice to infection with the Fleming strain. Seemingly, these two genotypes were just extremely sensitive to infection with WEE virus.

Experimental evidence has substantiated that WEE virus, as well as other arboviruses with neurotropic properties, is altered by intracerebral passage in laboratory animals (Johnson, 1963). Low-passage WEE virus strains have produced a variety of disease syndromes in ponies (Sponseller et al.,
1966), but the strains came from several geographical locations. Also, geographic variation among other arbovirus strains in the viremic responses of avian and mammalian hosts has been documented (Bowen et al., 1980; Monath et al., 1980). Therefore, the difference we noted in virulence between WEE virus strain 7738 and strain 7746 in infected chicks was unexpected because the strains were collected at the same location, on the same day, and had identical low passage histories. The responses of chicks infected with these two strains of WEE virus are somewhat analogous to the responses of horses infected with epizootic and enzootic strains of Venezuelan equine encephalitis (VEE) virus (Kramer and Scherer, 1976).

Earlier studies on the three chick lines used in our experiments determined that some genotypes were resistant to Marek's disease virus and RSV tumor induction (Nordskog et al., 1977; Gebriel et al., 1979). However, the $B^1B^1$ genotype was highly susceptible to Marek's disease virus. In our study, haplotype $B^1B^1H$ had the lowest susceptibility to infection with WEE virus, and genotype 19 had the highest susceptibility. Although there were variations in the LD$_{50}$'s, no profound differences were detected among the three genotypes. The chicks were probably too young to be immunologically competent. However, other studies have shown that newly hatched chicks may be protected against infection with WEE
virus by transovarian passage of maternal antibodies (Reeves et al., 1954; Holden et al., 1973).

Although chicks of genotype GHA were not the most susceptible to infection with WEE virus, they were still very sensitive to infection with all three strains of virus. In addition, these chicks were most often available in numbers large enough to satisfy the requirements of vector competence studies. Therefore, only GHA chicks were used in subsequent experiments with the three WEE virus strains, and this chick line proved to be highly sensitive in detecting successful mosquito transmission of WEE virus (Andre et al., 1981a, 1981b).
REFERENCES CITED


SECTION IV. SURVEILLANCE OF ARBOVIRUS ACTIVITY
IN IOWA, 1978-1980
Surveillance of Arbovirus Activity in Iowa, 1978-1980

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Wayne A. Rowley
Yau W. Wong
Don C. Dorsey

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INTRODUCTION

The arthropod-borne encephalitides are an important cause of equine and human morbidity in the United States of America (USA). Between 1975 and 1978, 6970 human cases of arboviral encephalitis were reported, and 1000-4000 cases of equine encephalitis occurred annually (Monath, 1979). Unfortunately, these numbers probably represent just a fraction of the true incidence of these diseases. Western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), and California encephalitis (CE) accounted for nearly all of the reported infections, but cases of eastern equine encephalitis (EEE) and Venezuelan equine encephalomyelitis (VEE) also were noted (Shope, 1980). All of these viruses are mosquito-borne, but the epidemiology of the diseases they cause may vary from state to state.

Considerable information pertaining to the epidemiology of mosquito-borne viruses in Iowa has been acquired during a continuing arbovirus surveillance program. Since the start of the program in 1966, thousands of pools of mosquitoes have been tested for the presence of arboviruses, and hundreds of virus isolations have been made (Wong et al., 1970, 1971; Rowley et al., 1973; Wong et al., 1973; Dorsey et al., 1978; Wong et al., 1978; Rowley et al., 1979). The viruses isolated are identified as follows: Cache Valley (CV), Flanders (FLA), LaCrosse (LAC), SLE, trivittatus (TVT), Turlock (Tur), and
WEE. Both SLE and WEE antibodies frequently are detected among avian sera tested, and hundreds of equine cases of WEE have been diagnosed. Many laboratory-confirmed human cases of LAC, SLE, and WEE also have been reported (Dorsey et al., 1978).

Although SLE and WEE viruses have been detected in various species of mosquitoes collected in Iowa, *Culex tarsalis* is the primary vector (Rowley et al., 1973; Wong et al., 1973; Dorsey et al., 1978; Wong et al., 1978). In addition, *Culex* spp. (*Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius*) may contribute to the amplification of SLE and WEE viruses (Wong et al., 1978). More recent findings, however, indicate that *Aedes trivittatus* also may play an important role in the natural cycle of WEE virus (Green et al., 1980; Andre et al., 1981c; Ritchie and Rowley, 1981). The other arbovirus of public health significance in Iowa, LAC virus, has been isolated only from pools of *Ae. triseriatus*.

Field studies in the last few years determined that up to 20% of the bloodfed *Ae. trivittatus* collected in Iowa contained avian blood, even though this mosquito is usually mammalophilic. Research on the blood-feeding habits of *Culex* spp. indicates that they feed predominantly on birds in Iowa but increase their feeding on mammals in midsummer. Also, some *Ae. trivittatus* and *Culex* spp. take multiple bloodmeals (Ritchie and Rowley, 1981). These findings strengthen the
hypothesis that *Ae. trivittatus* and *Culex* spp. are important in the natural maintenance of arthropod-borne viruses in Iowa.

In August of 1977, two isolates of WEE virus were obtained from mosquitoes collected in Ames, Iowa. One isolate came from a pool of *Cx. tarsalis* and the other from a pool of *Ae. trivittatus*. These isolates provided our laboratory the opportunity to conduct vector competence studies using two strains of virus which were collected from the same place on the same day. Laboratory experiments during the past three years tested these strains of WEE virus in *Ae. trivittatus*, *Cx. tarsalis*, *Cx. pipiens*, *Cx. restuans*, and *Cx. salinarius* (Green et al., 1980; Andre et al., 1981b, 1981c). We also studied the relationships of these five mosquito species with certain viruses during arbovirus field studies in Iowa. This paper enumerates the results of arbovirus surveillance activities from 1978 through 1980, with an emphasis on the isolation of viruses from *Ae. trivittatus*, *Culex* spp., and *Cx. tarsalis* and detection of concomitant arbovirus activity in birds.
MATERIALS AND METHODS

Collection of Mosquitoes

Adult mosquitoes were collected for virus isolation studies with battery-operated, CO₂-baited CDC light traps. These traps were operated periodically from June through August during the three-year study. Traps were set in or near eight Iowa cities: Ames, Council Bluffs, Davenport, Des Moines, Dubuque, Iowa City, Sioux City, and Waterloo. Collection and processing of mosquitoes for subsequent virus isolation attempts were accomplished in accordance with standard techniques (Chamberlain and Sudia, 1967).

Collection of Birds

From 1978 through 1980, periodic collections of wild birds were made during the summer months. Birds were trapped with nylon mist nets and in baited cages placed in Ames, Council Bluffs, Davenport, and Des Moines. Captured birds were identified to species, and their approximate age (adult or juvenile) and other pertinent information were recorded. Avian blood was collected from the jugular vein using a technique described earlier (McClure and Cedeno, 1955). Approximately 0.2 ml of blood was added to 0.8 ml of field diluent (1% bovine albumin in phosphate-buffered saline, pH 7.6). Diluted bloods were centrifuged at 1000 rpm for 10
min, and the sera were pipetted into new tubes. Sera were stored at 4-8° C until they were processed for serology.

Sentinel Flocks

Two sentinel chicken flocks were set out in rural areas near Council Bluffs and Sioux City during the summers of 1979 and 1980. White Leghorn chicks (line PS-GHA), a line proven to be highly susceptible to infection with WEE virus (Andre et al., 1981a), were used at both locations. Six to 12 young chickens with wing bands were placed in sheds similar in construction to the sentinel shed described by Rainey et al. (1962). Chickens were bled from the wing vein before being put outdoors and every seven to ten days thereafter. Approximately 1 ml of blood was collected and allowed to clot. Each sample was centrifuged at 1000 rpm for 10 min, and the serum was pipetted into another tube. The serum was refrigerated, and the clot was frozen at -70° C for subsequent viral assay.

Viral Tests

Mosquitoes were processed in pools of 10 to 200. The procedure outlined in an earlier paper (Wong et al., 1971) was followed for arbovirus isolation using suckling mice. The minimal field infection rate (MFIR) for each mosquito
species was calculated by dividing the total number of specimens of a given species collected during 1978-1980 by the number of virus isolations from that species. This rate was determined individually for each arbovirus isolated.

Human cases were verified as a routine procedure in the Virology Division of the University Hygienic Laboratory. Sera from suspected cases of central nervous system disease were examined for complement-fixing (CF), hemagglutination-inhibition (HI), and/or neutralizing (N) antibodies against WEE, SLE, and LAC antigens (U.S. Public Health Service, 1965; Lennette and Schmidt, 1969). Sucrose acetone extracted infected mouse brain antigens were used in both the HI and CF tests. The microtiter system was employed for the N, HI, and CF tests. Baby hamster kidney (BHK-21) cells were used for the N test.

Bird sera were examined for HI antibodies against WEE and SLE antigens (Clarke and Casals, 1958). Sera were treated with protamine sulfate to remove acetone-insoluble inhibitors and then acetone extracted before testing (Holden et al., 1966). Blood clots from chickens that seroconverted were inoculated into suckling mice for arbovirus isolation.
RESULTS

One hundred and nine mosquito-borne human encephalitis cases were confirmed serologically by the University of Iowa Hygienic Laboratory from 1970 through 1980 (Table 1). An unusually high number of human cases of California (LAC) encephalitis were confirmed in 1978, and considerable concern was associated with a fatal case of this disease in a three-year-old girl in De Soto, Wisconsin (less than one-half mile from the Iowa state line). Fewer cases of LAC occurred in 1979 and 1980. In 1980, two of the eight cases were in Dubuque, a large city in northeastern Iowa. Very few human WEE or SLE cases have been confirmed since the epidemic year of 1975, however, one nonfatal case of SLE was seen in Council Bluffs in 1980.

A total of 2,298 bird bloods representing 38 species was collected during the summers of 1978, 1979, and 1980. Antibodies (HI) against WEE virus were detected in 41 of the bird bloods, but only 10 birds were positive for SLE antibodies. Table 2 lists by location and month of collection (1978-1980) the results of HI testing of birds for WEE antibodies. Nine out of the 41 birds positive for WEE antibodies were juveniles (eight house sparrows and one American robin). Five species of adult birds (American robin, Baltimore oriole, cardinal, catbird, and house sparrow) had WEE antibodies at a
Table 1. Mosquito-borne encephalitis in Iowa, 1970-1980

<table>
<thead>
<tr>
<th>Year</th>
<th>LaCrosse (California)</th>
<th>Western equine</th>
<th>St. Louis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>14</td>
<td>1</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>1971</td>
<td>3</td>
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<td>5</td>
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<td>12</td>
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<td>18</td>
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<td>-</td>
<td>18</td>
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<tr>
<td>1979</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>6</td>
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<tr>
<td>1980</td>
<td>8</td>
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<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>7</td>
<td>25</td>
<td>109</td>
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Table 2. Birds with hemagglutination-inhibition (HI) antibodies (1:20 or greater) to western equine encephalomyelitis WEE virus, by location and month of collection, Iowa, 1978-1980

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Number (%) with titers &gt; 20/no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>May</td>
</tr>
<tr>
<td>1978</td>
<td>Ames</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Davenport</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Des Moines</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>Ames</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>Council Bluffs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Davenport</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Des Moines</td>
<td>1/110 (1)</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Totals</td>
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</table>
serum dilution of 1:20 or greater. The blood of one sentinel chicken exhibited a seroconversion (WEE HI titer ≥ 80) between the 22nd and the 28th of August 1979 in Council Bluffs. The number of birds tested for the presence of HI antibodies against SLE virus is listed in Table 3 by location and month of collection (1978-1980). Almost all the birds with SLE antibodies (HI titer ≥ 20) were house sparrows. Antibodies were also detected in one juvenile American robin and in one adult catbird in Council Bluffs during July of 1980. Most birds with HI antibodies against WEE virus or SLE virus were captured in Des Moines, a major population area. More birds with HI antibodies against these two viruses were bled in 1978 than in 1979 or in 1980.

During the summers of 1978 through 1980, 120,896 mosquitoes were collected in CO₂-baited CDC light traps, identified to species, and divided into 2,109 pools for subsequent arbovirus isolation. The arboviruses isolated from mosquitoes collected in or near eight Iowa cities are recorded by year in Table 4. Flanders and TVT viruses were isolated most frequently, but WEE and LAC viruses also were isolated. Six different viruses were isolated from 119 positive pools in 1979. The lowest percentage of virus isolations from mosquito pools occurred in 1980. Most arbovirus isolations were from mosquitoes collected in Ames, Council Bluffs, and Davenport. None of the mosquito pools from Iowa City was positive for
### Table 3. Birds with hemagglutination-inhibition (HI) antibodies (1:20 or greater) to St. Louis encephalitis (SLE) virus, by location and month of collection, Iowa, 1978-1980

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
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<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
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<td>1978</td>
<td>Ames</td>
<td>--</td>
<td>--</td>
<td>0/47</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Davenport</td>
<td>--</td>
<td>0/89</td>
<td>0/93</td>
<td>0/69</td>
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</tr>
<tr>
<td></td>
<td>Des Moines</td>
<td>--</td>
<td>0/93</td>
<td>1/102 (1)</td>
<td>1/63 (2)</td>
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<tr>
<td></td>
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<td>--</td>
<td>0/182</td>
<td>1/242 (0.4)</td>
<td>1/132 (0.8)</td>
<td>3/92 (3)</td>
</tr>
<tr>
<td>1979</td>
<td>Ames</td>
<td>0/9</td>
<td>0/30</td>
<td>0/25</td>
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<td>--</td>
</tr>
<tr>
<td></td>
<td>Council Bluffs</td>
<td>--</td>
<td>--</td>
<td>0/43</td>
<td>0/25</td>
<td>--</td>
</tr>
<tr>
<td></td>
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<td>--</td>
<td>0/121</td>
<td>0/43</td>
<td>0/98</td>
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</tr>
<tr>
<td></td>
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<td>1/128 (1)</td>
<td>0/137</td>
<td>0/123</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td>0/119</td>
<td>1/279 (0.4)</td>
<td>0/248</td>
<td>0/246</td>
<td>--</td>
</tr>
<tr>
<td>1980</td>
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<td>--</td>
<td>--</td>
<td>2/41 (5)</td>
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<td>--</td>
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<tr>
<td></td>
<td>Davenport</td>
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<td>0/103</td>
<td>0/115</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Des Moines</td>
<td>--</td>
<td>1/229 (0.4)</td>
<td>1/86 (1)</td>
<td>0/43</td>
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</tr>
<tr>
<td></td>
<td>Totals</td>
<td>--</td>
<td>1/370 (0.3)</td>
<td>3/230 (1)</td>
<td>0/158</td>
<td>--</td>
</tr>
<tr>
<td>Year or city <em>b</em></td>
<td>No. tested</td>
<td>No. arbovirus isolates ^a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
<td>---------------------------</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specimens</td>
<td>Pools (% pos.)</td>
<td>Total</td>
<td>WEE</td>
<td>LAC</td>
<td>CV</td>
</tr>
<tr>
<td>1978</td>
<td>36,593</td>
<td>906 (10)</td>
<td>92</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1979</td>
<td>61,462</td>
<td>880 (14)</td>
<td>119</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1980</td>
<td>22,841</td>
<td>323 (6)</td>
<td>21</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>120,896</td>
<td>2,109 (11)</td>
<td>232</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
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<p>| | | | | | | | | | |</p>
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<thead>
<tr>
<th></th>
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<th></th>
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<tbody>
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<td>Ames</td>
<td>27,401</td>
<td>455 (10)</td>
<td>47</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>C.B.</td>
<td>41,274</td>
<td>749 (9)</td>
<td>70</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>28</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>Dav.</td>
<td>21,266</td>
<td>367 (19)</td>
<td>69</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>20</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>D.M.</td>
<td>2,323</td>
<td>66 (4)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dub.</td>
<td>4,845</td>
<td>90 (11)</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>I.C.</td>
<td>3,556</td>
<td>71 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S.C.</td>
<td>6,104</td>
<td>147 (11)</td>
<td>16</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Wat.</td>
<td>14,127</td>
<td>164 (10)</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>120,896</td>
<td>2,109 (11)</td>
<td>232</td>
<td>8</td>
<td>1</td>
<td>3</td>
<td>79</td>
<td>1</td>
<td>140</td>
</tr>
</tbody>
</table>

^a WEE - western equine encephalomyelitis; LAC - LaCrosse; CV - Cache Valley; FLA - Flanders; Tur - Turlock; TVT - trivittatus.

^b C.B. - Council Bluffs; Dav. - Davenport; D.M. - Des Moines; Dub. - Dubuque; I.C. - Iowa City; S.C. - Sioux City; Wat. - Waterloo.
arbovirus. Trivittatus and FLA viruses were isolated consistently from mosquitoes collected in most regions of the state. Three isolations of WEE virus were from mosquitoes collected in north central Iowa, and five isolations were from mosquitoes collected in western Iowa. LaCrosse virus was isolated from a pool of 34 *Ae. triseriatus* collected on July 19, 1979, in Dubuque.

From the 16 species of mosquitoes tested, arbovirus isolates most frequently were obtained from *Ae. trivittatus*, *Culex* spp., and *Cx. tarsalis*. Minimal field infection rates of six arboviruses in seven species of mosquitoes are listed in Table 5. The MFIRs ranged from 1:95 to 1:39,295. Of four mosquito species which yielded FLA virus isolates, *Ae. trivittatus* had the lowest MFIR; yet, this species had the highest MFIR, 1:392, with TVT virus. Most of the isolations of WEE virus were from pools of *Cx. tarsalis*, but one isolation was from a pool of *Culex* spp. *Anopheles quadrimaculatus* and *An. punctipennis* had high MFIRs with CV virus, 1:95 and 1:1,766, respectively.
Table 5. Minimal field infection rates (MFIR) of arboviruses in mosquitoes, Iowa, 1978-1980

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mosquito species</th>
<th>No. tested</th>
<th>(% pools pos.)</th>
<th>No. isolates</th>
<th>MFIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>WEE</td>
<td>Cx. tarsalis</td>
<td>7,796</td>
<td>329 (2)</td>
<td>7</td>
<td>1:1,114</td>
</tr>
<tr>
<td></td>
<td>Cx. spp.</td>
<td>39,295</td>
<td>628 (0.2)</td>
<td>1</td>
<td>1:39,295</td>
</tr>
<tr>
<td>LAC</td>
<td>Ae. triseriatus</td>
<td>2,841</td>
<td>110 (0.9)</td>
<td>1</td>
<td>1:2,841</td>
</tr>
<tr>
<td>CV</td>
<td>An. quadrimaculatus</td>
<td>95</td>
<td>6 (17)</td>
<td>1</td>
<td>1:95</td>
</tr>
<tr>
<td></td>
<td>An. punctipennis</td>
<td>3,532</td>
<td>113 (2)</td>
<td>2</td>
<td>1:1,766</td>
</tr>
<tr>
<td>FLA</td>
<td>Cs. inornata</td>
<td>161</td>
<td>12 (8)</td>
<td>1</td>
<td>1:161</td>
</tr>
<tr>
<td></td>
<td>Cx. tarsalis</td>
<td>7,796</td>
<td>329 (8)</td>
<td>27</td>
<td>1:289</td>
</tr>
<tr>
<td></td>
<td>Cx. spp.</td>
<td>39,295</td>
<td>628 (8)</td>
<td>49</td>
<td>1:802</td>
</tr>
<tr>
<td></td>
<td>Ae. trivittatus</td>
<td>54,029</td>
<td>721 (0.3)</td>
<td>2</td>
<td>1:27,014</td>
</tr>
<tr>
<td>Tur</td>
<td>Cx. tarsalis</td>
<td>7,796</td>
<td>329 (0.3)</td>
<td>1</td>
<td>1:7,796</td>
</tr>
<tr>
<td>TVT</td>
<td>Ae. trivittatus</td>
<td>54,029</td>
<td>721 (19)</td>
<td>138</td>
<td>1:392</td>
</tr>
<tr>
<td></td>
<td>An. punctipennis</td>
<td>3,532</td>
<td>113 (0.9)</td>
<td>1</td>
<td>1:3,532</td>
</tr>
<tr>
<td></td>
<td>Cx. spp.</td>
<td>39,295</td>
<td>628 (0.2)</td>
<td>1</td>
<td>1:39,295</td>
</tr>
</tbody>
</table>

a WEE - western equine encephalomyelitis; LAC - LaCrosse; CV - Cache Valley; FLA - Flanders; Tur - Turlock; TVT - trivittatus.

b Cx. spp. - Cx. pipiens, Cx. restuans, Cx. salinarius.
DISCUSSION

In 1978, the incidence of CE in the USA was unusually high, with 109 cases reported (Monath, 1979). Two of these cases resulted in death, and LAC virus was isolated from the brain tissue of a little girl who lived less than one-half mile from the state line of Iowa. Eighteen cases were confirmed serologically in Iowa, but fortunately, no deaths resulted. During 1979, only six laboratory-confirmed cases were reported, however, two of these occurred in Dubuque, a large city where LAC virus also had been isolated from a pool of 34 Ae. triseriatus. In addition, two of eight cases in 1980 occurred in Dubuque.

The most intense and geographically extensive epidemic of SLE on record occurred in the USA in 1975, with 1815 cases being reported (Monath, 1979). In Iowa, 19 cases were confirmed by serological techniques. During the Chicago and Memphis outbreaks, SLE virus was isolated from pools of Cx. restuans and Cx. salinarius. Monath (1979) suggested that Cx. salinarius may play an important role in enzootic transmission of SLE virus, and Cx. restuans may be important in overwintering and early spring amplification of the virus. Field studies by our laboratory staff (Wong et al., 1978) also indicate that Culex spp. (Cx. pipiens, Cx. restuans, and Cx. salinarius) may contribute to the amplification of SLE virus in Iowa. Two
reasons are given: unusually large populations of these mosquitoes were present during the epidemic of 1975, and field infection ratios were very high (1:19 to 1:158) in 1975. Although isolations of SLE virus have been made from pools of Cx. tarsalis collected in central and western Iowa (Rowley et al., 1973, 1979), Cx. pipiens may be more important epidemiologically in central Iowa because this species transmits highly virulent and viremogenic strains of SLE virus (Bowen et al., 1980; Monath et al., 1980). Since 1975, only a few human cases of SLE have been confirmed serologically in Iowa. One case was reported in Council Bluffs in 1980. Interestingly, two of 41 birds collected in this city in July had HI antibodies to SLE virus, thereby indicating virus activity prior to the occurrence of illness in the human population.

The rate of WEE virus transmission, and consequently the risk of human and equine infection, can be quantified by measuring vector population density, incidence of nestling bird viremias, and by serological conversions in sentinel chickens (Monath, 1979). Through the use of these techniques, Reeves and Milby (1979) predicted the major resurgence of WEE virus in the Central Valley of California during 1978. Also in 1978, an extensive outbreak of WEE in horses occurred in north eastern Utah (Romney et al., 1980). Although no human cases of WEE were diagnosed in Iowa in 1978, many of the
wild bird bloods tested had HI antibodies (titer > 20).
In 1979, WEE virus activity in Iowa increased during the late summer. The sentinel chicken flock located in Council Bluffs seroconverted in late August, and eight laboratory-confirmed cases of WEE in horses were reported in September. Also, seven isolations of WEE virus were made from Cx. tarsalis. In addition, this virus was isolated from a pool of Culex spp. in 1980. Although we cannot be certain which of the three Culex species was present in the positive pool, laboratory experiments have shown that Cx. restuans is susceptible and Cx. pipiens and Cx. salinarius are refractory to oral infection with WEE virus in Iowa (Andre et al., 1981b).

From 1978 through 1980, numerous isolations of viruses of limited or unknown public health importance were made from various species of mosquitoes in Iowa. Trivittatus virus was the most frequently encountered virus, with 140 isolations. The MFIRs calculated for TVT virus in mosquito species yielding isolates ranged from 1:392 to 1:39,295, however, most of the isolations were from pools of Ae. trivittatus, a mammalophilic species. In contrast, FLA virus rarely was found in this species, but FLA virus frequently was encountered in ornithophilic species such as Culiseta inornata, Cx. tarsalis, and Culex spp. Similarly, FLA virus was isolated only from ornithophilic mosquito species collected during a ten-year period in Connecticut (Main et al., 1979).
A long-term arbovirus surveillance program in Iowa has provided valuable information concerning the epidemic and endemic occurrence of several arboviruses of public health interest. Although there have been fluctuations in the surveillance program because of budget restrictions, an "early warning" system for virus activity has remained available.
REFERENCES CITED


SUMMARY AND DISCUSSION

Experiments were conducted to compare the oral and parenteral susceptibilities of Culex tarsalis and Aedes trivittatus for two strains of western equine encephalomyelitis (WEE) virus from Iowa and the Fleming strain from California. The transmission capabilities of these species for the three WEE virus strains also were compared. The results indicate that Cx. tarsalis is more susceptible than Ae. trivittatus to infection with WEE virus in blood ingested through a membrane. However, Ae. trivittatus was more susceptible than previously reported. The parenteral susceptibility to the three virus strains differed in both species. This supports earlier findings of differences in the susceptibility of mosquitoes for epizootic and enzootic strains of Venezuelan equine encephalitis (VEE) virus. Our data on the virulence of the two Iowa strains of WEE virus in chicks also support these findings. Culex tarsalis transmitted the three strains of WEE virus more effectively than Ae. trivittatus, but almost half of the Ae. trivittatus tested transmitted WEE virus to chicks. Seemingly, Ae. trivittatus can be a competent vector and probably plays an important role in the natural mosquito-bird cycle of WEE virus in Iowa.

Vector competence studies determined the role(s) of Culex spp. (Cx. pipiens, Cx. restuans, and Cx. salinarius)
in the transmission cycle of WEE virus in Iowa. Comparisons were made of the oral and parenteral susceptibilities of these three mosquito species for the three strains of WEE virus. The transmission of WEE virus to susceptible chicks by these Culex species was determined also. Culex pipiens and Cx. salinarius were refractory to oral infection with all three strains of WEE virus, however, Cx. restuans was susceptible. In contrast, Cx. pipiens and Cx. restuans were almost uniformly susceptible to parenteral inoculation of WEE virus. But, Cx. salinarius was almost completely refractory to infection with WEE virus inoculated parenterally. These results indicate that intrinsic barriers other than the midgut act to modulate virus replication in mosquitoes. Older, later season, Culex mosquitoes were less supportive of virus growth, suggesting that basic physiological changes may have occurred in these mosquitoes that reduced their susceptibility to these viruses. The three Culex species successfully transmitted WEE virus to chicks. These data suggest that when large populations of these species are present, they may be important epidemiologically in Iowa.

Three different lines (genotypes GHA, 19, and S1) of White Leghorn chicks were tested for their susceptibility to needle-inoculated WEE virus. Chicks were susceptible to infection with WEE virus. In fact, they were more sensitive to the two Iowa strains than mice. Five haplotypes
(B^{1H}-B^{19L}, B^{1L}-B^{19H}, B^{2}-B^{2}, B^{1}-B^{1L}, and B^{1}-B^{1H}) of genotype S1 were less sensitive than mice to the Fleming strain. The lower susceptibility of chicks to infection with the Fleming strain has been noted previously; therefore, it was surprising that genotypes 19 and GHA were more susceptible to this strain of the virus. Haplotype B^{1}-B^{1H} had the lowest susceptibility to infection with WEE virus. Variations in LD_{50}'s occurred, however, profound differences were not detected among the three genotypes. The chicks probably were immunologically incompetent because of age. In other experiments with the three WEE virus strains, chick line GHA proved to be the most sensitive to infection with mosquito-transmitted WEE virus.

Considerable information pertaining to the epidemiology of mosquito-borne viruses in Iowa has been acquired during a continuing arbovirus surveillance program. Virus isolation results indicate that Cx. tarsalis is the primary vector of St. Louis encephalitis (SLE) and WEE viruses in Iowa, but that Culex spp. may contribute to the amplification of SLE virus. Also, Ae. trivittatus may be important in the natural maintenance of WEE virus in Iowa. From 1978 through 1980, numerous isolations of viruses such as Flanders (FLA) and trivittatus (TVT) were made from various species of mosquitoes in Iowa. Many of the wild bird bloods tested had hemagglutination-inhibition (HI) antibodies (titer $\geq$ 20) to
WEE virus, and a few had antibodies to SLE in Council Bluffs, where a human case occurred in 1980. Eighteen human cases of California (LaCrosse) encephalitis occurred in Iowa in 1978, and a little girl who lived near the Iowa state line died from the effects of this virus. LaCrosse virus was isolated from a pool of *Ae. triseriatus* collected in Dubuque, where two human cases occurred in 1979 and again in 1980.
LITERATURE CITED


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