Studies on vector competence of Culex (Cx) tarsalis Coq from an epidemic/epizootic area in Iowa for an enzootic strain of western equine encephalomyelitis (WEE-7738) virus and vertical transmission of the WEE virus in Aedes (Ae) trivittatus (Coq)

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Iowa State University

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Studies on vector competence of *Culex (Cx.) tarsalis* Coq. from an epidemic/epizootic area in Iowa for an enzootic strain of western equine encephalomyelitis (WEE-7738) virus and vertical transmission of the WEE virus in *Aedes (Ae.) trivittatus* (Coq.)

by

Joon-Hak Lee

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Major: Entomology

Major Professor: Wayne A. Rowley

Iowa State University

Ames, Iowa

1998

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ABSTRACT

Experiments were designed to evaluate the vector competence of *Culex tarsalis* Coq. from an epidemic/epizootic area (Sioux City) in Iowa for a western equine encephalomyelitis virus (WEE-7738) and to elucidate maintenance mechanism of WEE in Iowa. WEE-7738 was isolated from *Aedes trivittatus* (Coq.) in 1977. Infection rate, dissemination rate, multiplication efficiency, and the transmission rate were examined for this virus in the SC strain of *Cx. tarsalis* and compared with previously published data. Susceptibility of the SC strain of *Cx. tarsalis* to WEE-7738 was similar to infection rates observed in studies conducted in California (Hardy et al. 1978; Reisen et al. 1996). However, the transmission of WEE-7738 by the SC strain of *Cx. tarsalis* was lower than transmission rates reported in other studies (Kramer et al. 1981; Reisen et al. 1993, 1996, 1997).

Virus multiplication was more rapid in the first week after feeding than it was in the second week. The average amount of virus (titer) present in infected *Cx. tarsalis* was proportional to virus concentration in a blood-meal (P<0.025).

Mosquitoes exposed to 4.7-5.0 log TCID₅₀/mosquito did not live as long as mosquitoes fed 2.7-3.0 log TCID₅₀/mosquito or controls and *Cx. tarsalis* exposed to the lower virus titer did not live significantly longer than the controls.

Infected *Cx. tarsalis* mosquitoes had 27.5% lower flight activity scores (P=0.0035) and 26.1% fewer spontaneous flights (P=0.024) than noninfected controls. Flight activity scores and initiation flights (flight numbers) decreased everyday. Virus infection did not affect on how long a mosquito flew in a 24-hr-period (the daily flying time) or the duration of individual flights. The circadian
rhythm (spontaneous flight activity patterns) of infected mosquitoes were identical to those of controls.

Vertical transmission (transovarial transmission) of WEE virus in *Ae. trivittatus* does not likely constitute maintenance mechanism of WEE virus in Iowa. The infection rate of *Ae. trivittatus* fed 7.0 log TCID$_{50}$/ml of WEE-7738 was only 8.47%. None of F1 adult progeny was infected after eggs were maintained at 4°C for 4 months to simulate winter conditions.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Interactions between arboviruses and their vectors are complex and are determined by intrinsic factors associated with the vector (Hardy et al. 1983). Intrinsic factors such as mesenteronal (midgut) infection, mesenteronal escape, salivary gland infection and salivary escape barriers (Hardy et al. 1983) are controlled by vector genes (Kramer et al. 1983; Tabachnick 1991) and are variable within a population.

The genomic nature of arboviruses ensures variability because the majority of arboviruses are RNA viruses in which the mutation rate is higher than in DNA viruses (Holland et al. 1982). The phenotypic or genotypic composition of an arbovirus population may be determined by interaction with its vertebrate host and its vector (Beaty et al. 1989). Variation in vector populations and mutation rates of a virus, along with involvement of vertebrate hosts, makes it difficult to estimate interaction between an arbovirus and its vector.

Western equine encephalomyelitis virus (WEE) belongs to the genus *Alphavirus* in the family Togaviridae (Karabatos 1985). A primary vector of WEE virus is *Culex (Cx.) tarsalis* Coq. (Reisen and Monath 1989). Variation in the susceptibility of *Cx. tarsalis* to WEE virus has been reported to be different over time (Hardy et al. 1990) and space (Hardy et al. 1976). Hardy et al. (1976) indicated that variation in susceptibility of a *Cx. tarsalis* population to WEE virus depends on the composition of subpopulations within a mosquito population. Also, differences in
virulence have been reported between epizootic and enzootic strains of WEE virus (Bianchi et al. 1993) and even in enzootic strains of WEE virus (Hardy et al. 1997).

Western equine encephalomyelitis in Iowa is essentially the same as it is in rest of the United States (Hess and Hayes 1967). Culex tarsalis is the primary vector of WEE virus (Reisen and Monath 1989). In Iowa Cx. tarsalis is more common in western sections than it is in central and eastern Iowa (Vandyk and Rowley 1995). Since a large vector population is required for epizootic and/or epidemic WEE virus to occur (Calisher 1994), it seems that an epizootic and/or epidemic in western Iowa is more likely than in central and eastern Iowa.

WEE-7738 strain is an enzootic strain occasionally isolated from Aedes trivittatus (Coq.) in Iowa. Susceptibility of Ae. trivittatus for this strain of WEE was lower than that of Cx. tarsalis although WEE-7738 was isolated from Ae. trivittatus. Field strains of Ae. trivittatus were relatively refractory to infection with WEE virus (Green et al. 1980). Aedes trivittatus probably is not a good epidemic/or epizootic vector for WEE virus but may function as an enzootic vector. An enzootic vector of Venezuelan equine encephalitis (VEE) virus, a member of the same genus as WEE virus, has a mesenteron escape barrier to epizootic strains of VEE virus but not to enzootic strains (Weaver et al. 1984).

It is of interest to investigate the interaction between an enzootic strain of WEE virus and the primary vector collected from a possible epidemic/epizootic area. Recent finding that reemergence of epidemic/epizootic VEE in Venezuela resulted when the virus evolved from an enzootic VEE strain (Rico-Hesse et al. 1995)
indicates that vector competence studies are needed especially in areas where the vector is common.

Longevity, feeding behavior, flight activity, and oviposition behavior of mosquitoes are important factors in vector competence and in arbovirus epidemiology (Bres 1988). The traditional view that arbovirus infection does not adversely affect the vector (Chamberlain and Sudia 1961; McLintock 1978; Hardy et al. 1983) has been challenged (Grimstad et al. 1980; Turell et al. 1985; Faran et al. 1987; Platt et al. 1997; McGaw et al. 1998). Several studies indicated that arbovirus infection reduced longevity (Turell et al. 1985; Faran et al. 1987; McGaw et al. 1998) and modified feeding behavior (Grimstad et al. 1980; Platt et al. 1997). Therefore, it is important to evaluate the impact of an arbovirus infection on the longevity and behavior of its vector.

An overwintering mechanism for WEE in Iowa was questioned by Green et al. (1980). WEE isolations from Ae. trivittatus during enzootic years and transovarial transmission of TVT virus in Ae. trivittatus suggest that WEE virus might overwinter in the eggs of Ae. trivittatus. Recent findings that natural vertical transmission of WEE occurs in Ae. dorsalis (Meigen) (Fulhorst et al. 1994) increase the probability that vertical transmission of WEE occurs in Ae. trivittatus.

The objectives of this study were: a) characterize an enzootic strain of WEE virus (WEE-7738) in Cx. tarsalis from the most probable epidemic/epizootic area in Iowa, b) investigate the impact of WEE-7738 on factors related to vector competence (longevity and flight activity), and c) examine whether vertical transmission of WEE in Ae. trivittatus is an overwintering mechanism of WEE-7738.
Dissertation Organization

This dissertation consists of five chapters. Three chapters will be submitted to a journal for publication. Chapter 1 is a general introduction composed of an introduction, thesis organization, literature review, and references cited in the literature review. Chapter 2 characterizes WEE-7738 in the primary vector, Cx. tarsalis. Parameters for characterization were infection rate, dissemination rate, transmission rate, and replication of the virus in the Sioux City strain of Cx. tarsalis. Data generated in these studies were compared with published data from other studies. Chapter 3 examines the effects of WEE-7738 infection on the longevity and spontaneous flight activity of the primary vector, Cx. tarsalis. Chapter 4 examines a possible overwintering mechanism for WEE-7738 in Iowa. Chapter 5 contains general conclusions. The primary author was responsible for the design and performance of all experiments included in this dissertation. All results and conclusions from these experiments were interpreted and written into manuscript form by the primary author.

Literature Review

**WEE epidemiology and history**

Western Equine Encephalomyelitis virus (WEE) has been recognized as a disease of horses and people since the 1930s (Reisen and Monath 1989). Historically, WEE was thought to be distributed throughout the Americas. However, recent studies revealed that WEE consists of a complex of six distinct viruses and that the distribution of WEE is considered to be limited to the area, west of the
Mississippi River. This is mostly because of the distribution of the primary vector, Cx. tarsalis Coq. (Reisen and Monath 1989). Epidemic or epizootic WEE has occurred most frequently in agroecosystems where its primary vector, Cx. tarsalis breeds (Hess and Hayes 1967). Although the incidence of WEE in humans and horses varies over time and space, California, Colorado, Kansas, Minnesota, Texas, and Utah have reported more than 50 cases in the past 30 years. The number of human and equine cases in the U. S. has declined during this period (Reisen and Monath 1989). Most human WEE cases occur during June, July, and August (McGowan et al. 1971) and frequently follow equine cases and infections in peridomestic, passeriform birds and/or domestic fowl. There is also a correlation between human cases and the isolation of WEE virus from mosquitoes. A marked increase in the abundance of female Cx. tarsalis has been associated with epidemics and/or epizootics of WEE (Hess and Hayes 1967).

**WEE virus**

There are three families of viruses responsible for encephalitis transmitted by mosquitoes (Calisher and Karabatos 1988). WEE virus belongs to the genus Alphavirus in the family Togaviridae (Karabatos 1985). The viruses in this family have a positive sense, single-stranded linear 42S-49S RNA genome, which is capped at the 5'-terminus and polyadenylated at the 3'-terminus, enclosed within an icosahedral nucleocapsid surrounded by a lipid bilayer containing two integral glycoproteins, E1 and E2 (Kaariainen and Soderlund 1978). Alphavirus structural proteins are translated as a polyprotein precursor in the order NH2-capsid-E3-E2-6K-E1-COOH from a subgenomic 26S mRNA identical to the 3'-terminal one third of
the 42S genomic mRNA (Strauss and Strauss 1986). The polyprotein precursor undergoes proteolytic cleavage to produce individual structural proteins. Within permissive cells, a polyprotein is initially translated from the 5'-two thirds of the 42S RNA genome that is processed post-translationally to produce nonstructural polypeptides nsP1, nsP2, nsP3, and nsP4 which are involved in viral transcription and replication (Strauss and Strauss 1986). A polymerase complex formed from nonstructural proteins uses the positive sense genomic RNA to synthesize a full-length minus-sense strand. This is used as a template from which both genome-sized and subgenomic strands are produced. The most abundant intracellular viral RNA is a subgenomic mRNA, or 26S RNA transcribed from an internal initiation site (RNA promoter) on the full-length minus-sense template. The core RNA promoter sequence contains 24 bases, which overlap the nonstructural coding region and subgenomic mRNA start site. The capsid protein complex with the 42S genomic RNA, which contains a selective packaging signal in the nsP1 gene, to form intracellular icosahedral nucleocapsids. Proteins E1 and E2 interact to produce a virion surface lattice composed of spikes of E1/E2 dimers. The 26S mRNA equivalents of eastern equine encephalitis (EEE) (Chang and Trent 1987) and WEE (Hahn et. al. 1988) have been sequenced. The entire nucleotide sequences of the RNA genomes of Sindbis (SIN) (Strauss et. al. 1984; Rice and Strauss 1981), Venezuelan equine encephalitis (VEE) (Kinney et al. 1989), Semliki Forest (SF) (Takkinen 1986; Garoff et al. 1980a,b) and Ross River (RR) (Strauss et al. 1988; Dalgamo et al. 1983), and O'Nyong-nyong viruses (Levinson et al. 1990) have been determined.
Phylogenetic studies using nucleotide sequencing and amino acid sequencing indicate that alphaviruses descended from a common ancestor by divergent evolution (Strauss and Strauss 1986). The ancestral virus diverged into two groups: one is a New World virus group (EEE and VEE) and the other includes Old World viruses (Sindbis, Middelburg, O'nyong-nyong, Ross River, and Semliki Forest). The position of WEE virus in the phylogenetic trees indicates that, both its capsid gene (Hahn et al. 1988) and its nonstructural genes were acquired from an EEE-like ancestor during recombination (Weaver et al. 1993). However, the envelope glycoproteins of WEE virus are most closely related to those of SIN virus (Hahn et al. 1988; Levinson et al. 1990).

**Biology of the vector, Culex tarsalis**

*Culex tarsalis* is the primary vector of WEE and St. Louis encephalitis (SLE) in the western United States. Hammon et al. (1942) demonstrated that *Cx. tarsalis* transmits the viruses that cause these diseases.

Studies of feeding preferences of *Cx. tarsalis* on vertebrate hosts determined the maintenance mechanism of WEE and SLE viruses and indicated that although *Cx. tarsalis* preferred to feed on birds, the mosquito also readily feeds on a variety of other animals including mammals, reptiles, and amphibians (Anderson et al. 1957; Dow et al. 1957; Henderson and Senior 1961; Templis et al. 1965). Female mosquitoes become infected with these viruses by obtaining blood meals from viremic host animals (Horizontal transmission) although different populations show various susceptibilities to infection (Hardy et al. 1976, 1978). After ingestion of a bloodmeal, a secretion of peritrophic membrane precursors occurs at 8-12 hrs and
formation of a peritrophic membrane culminates with a fibrous, multilayered peritrophic membrane 20-24 hrs after bloodmeal ingestion (Houk et al. 1979). Viruses are maintained in adult *Cx. tarsalis* populations, which are active from spring to fall (Bellamy and Reeves 1963; Reisen et al. 1983b). Inseminated adult females diapause in natural sites such as animal burrows (Bennington et al. 1958a, 1958b; Mortenson 1953; Shemanchuck 1965), abandoned mines (Blackmore and Winn 1956), and rock piles (Rush et al. 1958). Man-made resting sites such as barns, sheds and cellars are also used as overwintering sites (Keener 1952; Bellamy and Reeves 1963). In addition, mosquitoes may use animal burrows as summer resting sites (Harwood and Halfhill 1960). In general, overwintering females are not infected with viruses because most are inseminated but have not blood-fed. However, in a few cases viruses have been isolated from overwintering populations of *Cx. tarsalis* suggesting that this is a possible mechanism by which the disease may be maintained between years (Blackmore and Winn 1956; Reeves et al. 1958).

Immature stages of *Cx. tarsalis* occur in a variety of freshwater habitats such as irrigation water, duck club ponds, log ponds and oil well swamps (Gjullin et al. 1965; Durso and Burguin 1988; Walton and Mulla 1991). Rice fields in California also provide extensive breeding sites for *Cx. tarsalis* (Collins and Washino 1980). The abundance of immature mosquitoes in rice fields varies from year to year. High larval density was correlated with a rotation of other crops planted immediately before the rice crop. This suggested that organic material in rice fields might affect oviposition and larval mortality of *Cx. tarsalis*. Mortality in the larval and pupal stages in temporary habitats is primarily attributed to fluctuations in water level and
pollution. However, predation is an important cause of mortality in relatively stable habitats with suitable water quality. Lack of food apparently causes comparatively low mortality. However, a low nutrient level lengthens the duration of the fourth stadium, delays pupation, and results in the emergence of small adults, many of which may be anautogenous (Reisen et al. 1989).

Male and female mosquitoes obtain sugars in nature from nectar and plant exudates. Sugars provide energy for flight and in the female, serve as precursors for the synthesis of fats (Van Handel 1984). Glucose and fructose are the predominant compounds in crop liquids (Schaeffer and Miura 1972). In addition, plant fluids may be an important water source in arid climates and are the only food ingested by males. Females typically leave resting sites at sunset (Gjullin et al. 1963). Mating occurs where males swarm. Mating occurs before host seeking, since most females collected at CO₂ baits are inseminated (Reisen et al. 1983a). Sexually mature male *Cx. tarsalis* commence swarming immediately after leaving diurnal shelters at dusk (Gjullin et al. 1963; Reisen et al. 1983a; Reisen et al. 1985).

Autogeny is important epidemiologically, since the initial bloodmeal is delayed, thereby reducing the number of potentially infective females (Reisen et al. 1983b). The expression of autogeny probably depends on both genetic and environmental factors (Clements 1992). Eberle and Reisen (1986) reported that autogeny in *Cx. tarsalis* appeared to be controlled by a dominant, autosomal gene(s) and the persistence of heterogeny in *Cx. tarsalis* during genetic selection for autogenous strains might result from male genetic factors. Cool temperature and short day length decrease the frequency of autogeny (Harwood 1966; Reisen et al.
Poor larval diet also reduces the proportion of autogenous mosquitoes in a population (Cardos 1959). In nature, the prevalence of autogeny varies spatially (Hardy and Reeves 1973) and temporally (Moore 1963; Spadoni et al. 1974; Reisen et al. 1983b). According to Reisen and Milby (1987), autogenous mosquitoes require one more day to complete immature development, but oviposit 1 to 2 days earlier than anautogenous mosquitoes. Autogenous females laid a significantly smaller number of eggs per raft during initial oviposition than anautogenous females (Reisen and Milby 1987).

**WEE virus interaction with the vector, Culex tarsalis**

Interactions between an arbovirus and its vector are complex and are determined by intrinsic factors associated with the vector (Hardy et al. 1983). Intrinsic factors involved in vector competence are mesenteronal (midgut) and salivary gland infection barriers, and mesenteronal and salivary gland escape barriers. These factors seem to be under the control of vector genes (Hardy et al. 1983; Kramer et al 1983; Tabachnick 1991). Most arboviruses are RNA viruses that have a higher mutational frequency than DNA viruses (Holland et al. 1982). Interaction of a virus with the vector and the vertebrate host may affect the genotypic and phenotypic composition of the arbovirus (Beaty et al. 1989). Variation in vector populations, higher mutation rates of the virus, and the involvement of vertebrate hosts, make it difficult to estimate interaction between an arbovirus and its vector. Susceptibility of *Cx. tarsalis* to WEE virus is under genetic control (Hardy et al. 1978). Houk et al. (1986) found that a mesenteronal infection barrier was responsible for interspecific variation in susceptibility. Higher WEE virus titer is
required to infect *Cx. pipiens pipiens* L. than the titer required to infect *Cx. tarsalis*. According to Houk et al. (1986), mesenteronal infection barrier to WEE virus in *Cx. pipiens pipiens* is associated with an inability of the virus to adsorb and/or penetrate mesenteronal epithelial cells when administered perorally. However WEE virus multiplied in midgut epithelial cells when inoculated intrathorathically into *Cx. pipiens pipiens*. There seems to be two-dose dependent barriers to transmission: the mesenteronal escape barrier and a salivary gland infection barrier (Kramer et al. 1981).

Susceptibility of *Cx. tarsalis* to WEE virus varies with the season of the year (Hardy et al. 1990) and with geographical differences (Hardy et al. 1976; Meyer 1989). Mosquitoes collected during May were 40 times more susceptible than those collected during August. These authors suggest that a wet winter and spring followed by cool temperature result in higher susceptibility of *Cx. tarsalis* to WEE virus than normal. Kramer et al. (1983) found that susceptibility of *Cx. tarsalis* to WEE virus was lower when the mosquitoes were maintained at 32°C after ingesting a viremic bloodmeal than at 18°C or 25°C. Meyer (1989) reported differences in susceptibility for WEE among *Cx. tarsalis* populations collected at different places in a contiguous geographical area. He also reported difference in susceptibility among cohorts of females collected as pupae from different breeding habitats sampled at the same location. Laboratory and field populations also had different susceptibilities to WEE virus (Hardy et al. 1976). This suggests that extrinsic environmental factors, as well as intrinsic factors, affect susceptibility of *Cx. tarsalis* to WEE virus.
There seems to be a difference in the virulence of epizootic and enzootic strains of WEE virus (Bianchi et al. 1993) and even within enzootic strains (Hardy et al. 1997). Virus strains associated with equine epizootics are neurovirulent and neuroinvasive, whereas enzootic viruses are neither neuroinvasive nor neurovirulent (Bianchi et al. 1993). However, all enzootic WEE virus strains isolated from California resulted in death when inoculated intracranially in adult mice (neurovirulence) but only some strains caused deaths when inoculated intraperitoneally in adult mice (neuroinvasiveness) (Hardy et al. 1997). Interestingly, WEE virus strains isolated from *Aedes* spp. did not infect neural tissues of adult mice (Bianchi et al. 1993; Hardy et al. 1997).

**WEE virus maintenance**

The summer transmission cycle of WEE virus in North America principally involves *Cx. tarsalis*, the primary vector and passeriform birds (Hess and Hayes 1967). However, mechanisms of spring amplification and of overwintering are not clearly understood. It has been postulated that spring amplification differs from one geographical region to another. In the U.S., virus isolations indicate that *Cx. tarsalis* and passeriform birds are involved in a spring-amplification cycle (Reeves et al. 1958; Cockburn et al. 1957; Hayes et al. 1967). However, in Canada, WEE virus has been recovered from mammals, snowshoe hares and ground squirrels, and early spring mosquitoes, *Culiseta inornata* (Williston) or several *Aedes* species, prior to diapause termination in *Cx. tarsalis*. These isolations also precede nesting activity by birds. Therefore, some mammals and early spring mosquitoes are
probably involved in early spring amplification of WEE virus (Burton et al. 1966; Leung et al. 1975; Mclintock et al. 1970).

In temperate regions of the world, mosquito-borne diseases are seasonal and adult mosquitoes are inactive in the winter. Three different plausible mechanisms have been postulated as overwintering mechanisms for WEE virus; survival of the virus through winter in a cold-resistant stage of infected primary or secondary vectors; survival of the virus in hibernating vertebrates; and extinction in autumn and reintroduction the following spring (Rosen 1987; Turell 1988; Reisen and Monath 1989). Of these mechanisms, the latter two have been extensively investigated. Overwintering of the virus in a mosquito may occur in one of two ways; transovarial transmission (TOT) or survival in overwintering or in long-lived vectors (Turell 1988).

Transovarial transmission of several mosquito-borne viruses has been demonstrated (LeDuc 1979; Tesh 1984; Rosen; 1987; Turell 1988). Watts et al. (1973) first showed transovarial transmission of La Crosse virus in *Aedes triseriatus* (Say). Many viruses in the Bunyaviridae and Flaviviridae are transmitted transovarially through *Aedes* spp. (Turell 1988). However, little is known about transovarial transmission in the Togaviridae. Virus isolation from field collected adult males and larvae, and adult mosquitoes reared from field collected larvae or eggs (or egg rafts) suggest TOT. In the laboratory, virus may be recovered from the progeny of artificially infected mosquitoes. In most studies of transovarial transmission (TOT), eggs are allowed to hatch shortly after embryogenesis. However, in order for transovarial transmission to serve as an overwintering mechanism, the virus should not only infect the eggs but also remain viable in the
eggs until the next period of vector activity. In addition, the virus must be transmitted to susceptible vertebrates by the bite of TOT infected adult female mosquitoes (Rosen 1987; Turell 1988).

A second mechanism for overwintering depends on the virus surviving the winter in vector mosquitoes and being transmitted to susceptible hosts in the spring. Many culicine mosquitoes overwinter as adult females. These overwintering mosquitoes could be a reservoir and reintroduce the virus in the next season of vector activity. In order to prove this mechanism for overwintering of the virus, the virus has to be recovered from overwintering vectors or overwintered vectors in early spring before vector activity begins (Rosen 1987; Turell 1988).

The family Bunyaviridae has the largest number of arboviruses (Matthews 1982) and the largest number that are transovarially transmitted. In the Bunyaviridae, transovarial transmission has been most thoroughly studied in the genus *Bunyavirus* where 11 viruses are presumed to be transovarially transmitted in their natural vectors (Tesh 1984). Seven of the California serogroup viruses in this genus, La Crosse, Keystone, California encephalitis, trivittatus, Jamestown Canyon, snowshoe hare and Yahyna have been recovered from naturally infected mosquito larvae (LeDuc 1979; Tesh 1984; Turell 1988). La Crosse (LAC) virus is the most important of these viruses causing human encephalitis in the United States, especially in the upper Midwest. Watts et al. (1973) demonstrated transovarial transmission of LAC virus in the laboratory. LAC virus isolations also have been made from field-collected larvae in widespread regions of upper Midwest, (Ohio, Berry et al. 1974; Minnesota, Balfour et al. 1975; and Wisconsin, Pantuwatana et al.
LAC virus also has been recovered from larvae collected in Wisconsin during early spring (Watts et al. 1974). Adult mosquitoes reared from field collected larvae transmitted LAC virus to suckling mice (Watts et al. 1974; Miller et al. 1977). However, it is still not certain whether transovarial transmission of LAC virus in *Ae. triseriatus* is the only overwintering mechanism. Recent studies indicate that LAC virus infection in diapausing eggs adversely affects survival of overwintering eggs (McGaw et al. 1998).

Crane et al. (1977) isolated California encephalitis (CE) virus from two pools of *Ae. dorsalis* (Meigen) adults reared from field-collected larvae in Utah and Turell et al. (1982a) showed that some populations of *Ae. dorsalis* transmitted CE virus transovarially to more than 90% of their progeny. These studies strongly suggested that transovarial transmission of CE is an overwintering mechanism. There are reports that transovarial transmission of CE virus and a CE-like virus occurred in *Ae. melanimon* Dyar (Turell et al. 1982a; Turell et al. 1982b) and in *Ae. squamiger* (Coq.) (Eldridge et al. 1991). Therefore, at least some viruses in the Bunyaviridae overwinter by transovarial transmission.

Flaviviruses are the most important encephalitis causing mosquito-borne viruses. Unlike mosquito-borne viruses in the Bunyaviridae, overwintering mechanisms of the Flaviviridae are unknown. Two viruses, St. Louis encephalitis (SLE) virus and Japanese encephalitis (JE) virus, in the Flaviviridae are responsible for encephalitis in temperate climates. SLE virus and JE virus may possibly overwinter in infected *Culex* spp. Mifune (1965) found that *Cx. tritaeniorhynchus* Giles orally infected in the fall transmitted JE virus to pigs after hibernation in Japan.
Bellamy et al. (1968) experimentally infected *Cx. tarsalis* and *Cx. quinquefasciatus* Say with SLE virus and transmitted the virus to chickens the following spring. Some experimental data suggest that JE and SLE viruses survive the winter in vector mosquitoes and that these viruses might be transmitted to susceptible hosts the following spring. However, virus isolation from winter or early spring field populations of vector mosquitoes is not common (Rosen 1987). JE virus was isolated from overwintering *Cx. pipiens pallens* Coq. mosquitoes in Korea (Lee 1971) and SLE virus from *Cx. tarsalis* in California during March (Reeves et al. 1958) and from *Cx. p. pipiens* in Maryland and Pennsylvania during January and February (Bailey et al. 1978). The rareness of flavivirus isolations from overwintering mosquito populations in nature may be associated with the possibility of inappropriate methods used for virus isolation in diapausing vector mosquitoes. Another factor may be the rare number of infected mosquitoes in overwintering populations (Rosen 1987). It is generally believed that at least a majority of overwintering populations of *Culex* species are nulliparous or non-blood fed (Rosen 1987; Turell 1988). However, blood-fed *Cx. p. pipiens* that have been preconditioned for diapause in the laboratory survive natural winter conditions without maturing ovarian follicles like wild-caught hibernating *Cx. p. pipiens* mosquitoes (Eldridge and Bailey 1979; Bailey et al. 1982). This suggests that mosquitoes fed a viremic bloodmeal may overwinter and serve as a vector of a virus (Eldridge and Bailey 1979; Bailey et al. 1982).

Transovarial transmission of JE virus in *Ae. albopictus* (Scuse) and *Ae. togoi* (Theobald) (Rosen et al. 1978) and in *Cx. tritaeniorhynchus* (Rosen et al. 1980) has
been demonstrated in the laboratory. SLE virus has been vertically transmitted in *Ae. albopictus* and *Ae. epaticus* Dyar & Knab (Hardy et al. 1980), *Ae. atropalpus* (Coq.) (Pelz and Freier 1990), *Cx. pipiens* complex (Francy et al. 1981), and *Cx. tarsalis* (Hardy et al. 1984) in the laboratory. Field evidence of vertical transmission of SLE virus or JE virus in vectors does not exist. Transovarial transmission of yellow fever virus and dengue virus were demonstrated both in the laboratory and in the field (Aitken et al. 1979; Beaty et al. 1980; Hull et al. 1984; De Souza and Freier 1991; Jousset 1981; Khin and Than 1983). However, since vectors of yellow fever virus and dengue virus are distributed in tropical and subtropical regions of the world, overwintering is not required to maintain these viruses in nature. In general, vertical transmission of flaviviruses as an overwintering mechanism has not been demonstrated.

There are two alphaviruses in the family Togaviridae that cause encephalitis in temperate regions; western equine encephalomyelitis (WEE) virus and eastern equine encephalitis (EEE) virus. The same overwintering mechanisms hypothesized for flaviviruses have been proposed for alphaviruses. WEE virus has been isolated from naturally infected adult *Cx. tarsalis* in California in every month of the year except December (Reeves et al. 1958). *Cx. tarsalis* infected with WEE virus in the fall survive the winter and transmit the virus to susceptible hosts the following spring (Bellamy et al. 1967). These data indicate that WEE virus could survive the winter in overwintering vectors at least in California where winters tend to be mild. However, there are questions as to whether *Cx. tarsalis* enters hibernation
after blood feeding and whether the proportion of mosquitoes hibernating after a blood meal is sufficient to maintain the virus through the winter.

There was no evidence that alphaviruses are transovarially transmitted by mosquitoes until Fulhorst et al. (1994) isolated WEE virus from adult *Ae. dorsalis* collected in the field as larvae. WEE virus isolation from naturally infected *Ae. dorsalis* adults reared from field collected larvae suggested that vertical transmission of WEE virus could be a overwintering mechanism or at least a mechanism to maintain WEE virus.

**Effects of WEE virus infection on the vector**

The traditional view of arboviral interaction with vectors has been that arbovirus infections do not adversely affect invertebrate hosts (Chamberlain and Sudia 1961; McLintock 1978; Hardy et al. 1983). However, several arboviruses seem to effect vector mosquitoes deleteriously or modify their behavior(s) (Grimstad et al. 1980; Turell et al. 1985; Faran et al. 1987; Turell 1992; Platt et al. 1997; McGaw et al. 1998). Longevity, feeding behavior, flight activity, and oviposition behavior are considered to be important factors in vector competence and in arbovirus epidemiology (Bres 1988). All of these have been shown to be altered when a mosquito is infected with a virus.

**Longevity and mortality**

Turell et al. (1985) reported that a presumptive epizootic vector, *Cx. pipiens* larvae inoculated with Rift Valley fever (RVF) virus did not survive. Whereas, more than 50% of the *Cx. pipiens* larvae inoculated with La Crosse virus successfully emerged as adults. RVF virus infection also decreases adult *Cx. pipiens* survival
(Faran et al. 1987). *Cx. pipiens* mosquitoes that transmitted the RVF virus had a higher mortality rate than those not transmitting and mosquitoes with disseminated infections had lower survival rates than those with nondisseminated infections (Faran et al. 1987). The more severe RVF infection in *Cx. pipiens* results in the lower survival. The number of mosquitoes with severe infection (transmission and disseminated infection) correlate with the amount of virus ingested (Hardy et al. 1983). Both studies indicate that reduced survival rates of mosquitoes infected with RVF virus might be associated with a short evolutionary relationship between the mosquito and the virus. However, there is evidence that a virus infection adversely affects survival of a natural vector. Larvae of *Ae. mcintoshi* Huang inoculated with RVF virus failed to emerge (Turell 1992). Eggs of *Ae. triseriatus* infected by transovarial transmission with La Crosse virus had higher mortality rate than uninfected eggs (McGaw et al. 1998).

**Flight ability**

Several studies indicate that infection with parasites adversely affects the flight ability of infected mosquitoes. Filarial worm infection alters mosquito flight activity (Townson 1970; Paige and Craig 1975; Hockmeyer et al. 1975; Berry et al. 1986, 1987, 1988). Development of *Brugia pahangi* (Buckley & Edeson) microfilariae damaged indirect flight muscle (Beckett 1990) and reduced flight ability and/or activity of *Ae. aegypti* L. The spontaneous laboratory flight ability of *Ae. aegypti* infected with filarial worms was much lower than it was in uninfected mosquitoes (Townson 1970; Paige and Craig 1975; Hockmeyer et al. 1975; Berry et al. 1986). Dog heartworm, *Dirofilaria immitis* (Leidy), infection in malpighian tubules
of *Ae. aegypti*, reduced spontaneous flight activity (Berry et al. 1987). However, flight activity of *D. immitis*’ natural vector, *Ae. trivittatus*, increased (Berry et al. 1988). Berry et al. (1988) suggested that infection of *D. immitis* possibly irritated the mosquito causing it to be more active. Malaria parasites (*Plasmodium* spp.) reduced the distances flown and total flight times of *Anopheles (Ano.) stephensi* Liston in a flight mill test (Schiefer et al. 1977). The spontaneous flight activity of *Ano. stephensi* infected with *Plasmodium yoelii* measured by an acoustic actograph system was also reduced (Rowland and Boersma, 1988).

Flight activity or flight ability of mosquitoes infected with parasites and viruses seems to be inversely correlated with severity of infection. Possible reasons are that the parasite(s) in the mosquito compete for nutrients with the host, or damages and impairs host vitality and nutrient utilization (Schiefer et al. 1977; Rowland and Boersma 1988).

In contrast, the effect of an arbovirus infection on flight activity of mosquitoes was first reported by Berry et al. (1987). *Ae. trivittatus* infected with trivittatus (TVT) virus has a similar flight activity pattern as control mosquitoes. The lack of change in spontaneous flight activity of *Ae. trivittatus* infected with TVT virus suggested that *Ae. trivittatus* was an effective natural vector of TVT with perhaps a long evolutionary relationship (Berry et al. 1987).

**Feeding behavior**

Transmission of a pathogen by a mosquito depends on probing time and the duration of blood feeding (Molyneux and Jefferies 1986). Some researchers have demonstrated that *Ae. aegypti* infected with *Plasmodium gallinaceum* Brumpt probe
longer (Rossignol et al. 1984) and have an increased biting rate (Rossignol et al. 1986). Apyrase, a salivary gland enzyme, is a principal factor in determining probing time and biting rate (Ribeiro et al. 1984; Ribeiro et al. 1985). The presence of Plasmodium gallinaceum in salivary glands decreases activity of apyrase (Rossignol et al. 1984) which resulted in longer probing times.

Grimstad et al. (1980) showed that La Crosse virus infection in Ae. triseriatus modified feeding behavior of its natural vector; infected females had a tendency to probe more and engorge less than uninfected females. Recently, Platt et al. (1997) reported the same results with dengue virus-infected Ae. aegypti. However, Putnam and Scott (1995) did not find evidence that dengue-2 virus infection modified feeding behavior of its natural vector, Ae. aegypti.

Oviposition

Decreased fecundity in arbovirus infected mosquitoes has been demonstrated in Cx. pipiens infected with RVF virus (Turell 1985) and possibly in Ae. albopictus infected with either San Angelo (SA) or Kunjin (KUN) viruses (Tesh et al. 1980).

References


CHAPTER 2. CHARACTERIZATION OF A STRAIN OF WESTERN EQUINE ENCEPHALOMYELITIS (WEE-7738) VIRUS IN CULEX TARSALIS COQ. (DIPTERA: CULICIDAE) FROM AN EPIDEMIC AND/OR EPIZOOTIC AREA OF IOWA, USA

A paper to be submitted to the journal of Medical Entomology

Joon-Hak Lee, Wayne A. Rowley, and Kenneth B. Platt

Abstract

Experiments were designed to evaluate the vector competence of Culex tarsalis Coq. from an epidemic/epizootic area (Sioux City) in Iowa for western equine encephalomyelitis virus (WEE-7738). WEE-7738 was isolated from Aedes trivittatus (Coq.) in 1977. 1977 was an enzootic year following two years, 1975 and 1976 when an epidemic of WEE occurred in the upper Midwest. Four parameters were used to evaluate and compare the vector competence of a Sioux City (SC) Iowa strain of Cx. tarsalis for WEE-7738 with previously published data. An infection rate, dissemination rate, multiplication efficiency, and transmission rate were determined for this virus in the SC strain of Cx. tarsalis. Susceptibility of the SC strain of Cx. tarsalis to WEE-7738 was similar to infection rates observed in studies conducted in California (Hardy et al. 1978; Reisen et al. 1996). However, the transmission of WEE-7738 by the SC strain of Cx. tarsalis was lower than transmission rates reported in other studies (Kramer et al. 1981; Reisen et al. 1993, 1996, 1997). The transmission rate (4.2%) for WEE-7738 in Iowa mosquitoes was lower than it (ca. 10%) was in the Coachella Valley strain of Cx. tarsalis (Reisen et al. 1996). Both a

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mesenteronal escape barrier and a salivary gland barrier for the WEE-7738 in SC strain of *Cx. tarsalis* seem to exist. Virus multiplication occurred more rapidly in the first week after feeding than it did in the second week. The average amount of virus (titer) present in infected *Cx. tarsalis* was proportional to the virus concentration in the blood-meal \((P<0.025)\). For example, 14 days after an infectious blood meal, individual mosquitoes fed 1.8 to 2.1 logs of WEE-7738 virus had 5.25 logs of virus in them, while mosquitoes fed 4.8-5.1 logs of virus had 6.7 logs of virus.

**Introduction**

The epidemiology of WEE in Iowa is essentially the same as it is in other areas of western North America (Reisen and Monath 1989). Throughout this region, *Culex tarsalis* Coq. is the primary vector of WEE virus (Reisen and Monath 1989). In Iowa, *Cx. tarsalis* is more common in western areas of the state than it is in central and eastern Iowa (Rowley unpublished data). Large vector populations usually have been associated with epizootics and/or epidemics of WEE (Reisen and Monath 1989; Calisher 1994). For this reason, it is more likely that an epizootic and/or epidemic of WEE would occur in western Iowa than central or eastern Iowa. No data exist on the vector competence of *Cx. tarsalis* for WEE in this area.

WEE-7738 is a strain of WEE isolated from *Aedes (Ae.) trivittatus* (Coq.) in Iowa during the enzootic year, 1977 which followed two years (1975 and 1976) when there was considerable WEE activity in this area (Dorsey et al. 1978). WEE virus isolations have been made occasionally from *Ae. trivittatus* in Iowa during enzootic years (Dorsey et al. 1978). However, *Ae. trivittatus* may not be an efficient epidemic/or epizootic vector for WEE virus because of its low susceptibility to WEE virus (Green et al. 1980).
The objectives of this study were: a) to evaluate the vector competence of *Cx. tarsalis* from an epidemic/epizootic area in Iowa for an enzootic strain of WEE virus; and b) to characterize the 7738 strain of WEE virus in an Iowa strain of *Cx. tarsalis*.

Materials and Methods

Mosquitoes

A strain of *Culex tarsalis* mosquitoes was colonized from adult female mosquitoes collected in Sioux City, Iowa in July, 1995. The colony, Sioux City strain (SC strain or SC) has been maintained in the Department of Entomology at Iowa State University. For all experiments, approximately 500-600 first instars were placed in distilled water in a 40 x 24 x 6 cm enamel pan and fed Tetramin® ad lib. Five rabbit pellets were added to each pan. Pupae were manually separated with a wire scoop. Pupae were placed in a 46 X 56 X 46 cm cage for emergence and mating. Adult mosquitoes had continuous access to a 0.3 M sucrose solution soaked in cotton pads. To minimize inbreeding effects in the colony, field collected, female *Cx. tarsalis* were introduced into the rearing cage every summer.

Preparation of WEE-7738 for oral infection

The WEE-7738 virus was originally isolated in suckling mice from a pool of *Ae. trivittatus* collected in Ames, Iowa in 1977 (Dorsey et al. 1978). WEE-7738 strain was subsequently passaged twice in suckling mouse brain. Ten percent homogenates of infected suckling mouse brain in phosphate buffered saline, pH 7.5, containing 1% bovine albumin (BA-PBS) was stored at -80°C. The stored virus was multiplied once in Vero African green monkey cells. This constituted the stock virus for these studies. Titer of the stock virus was determined by microtitration.
(Hierholzer and Killington 1996) and calculated at 8.0-8.3 log TCID$_{50}$/ml (Reed and Munch 1938).

**Virus infection of mosquitoes**

Mosquitoes were infected with WEE using glass membrane-feeders (Rutledge et al. 1964). Membranes were commercially available condoms made of sheep intestine (Naturallamb®) (Novak et al. 1991).

Blood used for feeding was obtained from sheep that did not have neutralizing antibody to WEE virus. The blood was mechanically defibrinated with glass beads, sweetened with sucrose to 2.5% (w/v) and maintained at 37±0.5°C in feeders.

At 3-4 days of age, female mosquitoes were transferred to 0.51 l ice cream cartons and starved for 24 hrs prior to feeding. Mosquitoes had access to the blood-virus mixture for 60-90 min. Fully engorged mosquitoes were separated from partially or unengorged mosquitoes. Twenty-five fully engorged mosquitoes were maintained in a 0.51 l ice cream carton at 25±1°C and 75±10% R. H. After feeding, mosquitoes had unlimited access to 0.3 M sucrose. Control mosquitoes engorged on virus free blood. All stages of mosquitoes were kept at 25±1°C and 75±10% relative humidity, with a 16:8 hr light/dark with a 90 min. crepuscular period before lights on and lights off respectively.

All experiments with infected mosquitoes were performed in a biosafety level 3 laboratory at the Animal-holding facility, college of veterinary medicine, Iowa state university.

**Virus assay**

To determine the virus titer of the virus-blood mixture, two fully engorged mosquitoes were removed after feeding and placed individually at -80°C for virus titration. These mosquitoes were ground in 1 ml of grinding medium (DMEM
containing 10% fetal bovine serum and 230 μg/ml gentamycin) and centrifuged at 10,000 rpm for 90 sec. Serial dilutions of the supernatant were made with Vero cell maintenance medium containing Dulbecco’s Modified Eagle’s medium (DMEM, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178) with 2% Fetal Bovine Serum (Sigma Chem. Co.). One hundred microliters of each viral dilution was inoculated in each well of a 96-well plate of 24-hr old Vero cells. Four wells were used for each dilution. Cell plates were incubated in a highly humidified chamber with 5% CO₂ at 37°C and checked for CPE after 2-3 days. Virus titers (log TCID₅₀/mosquito) were determined using the Reed and Muench method (1938) and hereafter, referred as logs.

To determine infection, dissemination, and transmission, virus isolations from saliva, the head, or the body of infected mosquitoes were made in 96 well plates containing 24-hr-old monolayers of Vero cells. Control mosquitoes were processed exactly as were infected mosquitoes. Cell monolayers were observed for cytopathic effect (CPE) with a phase contrast microscope (LH 50A, Olympus, Japan) 2-3 days after inoculation. Virus infection was confirmed by immunofluorescence assay (IFA). Cell plates were fixed in a solution (75% acetone and 25% methanol) for 10 min and air-dried. Fifty microliters of WEE antibody (mouse hyperimmune ascitic fluids) diluted to 1:400 with PBS were placed in each well of 96-well plates and incubated at 37°C for 45 min. After incubation, the plates were washed three times with PBS and 50 ul of FA conjugate (KPL lab. Inc., 2 Cessna Court, Gaithersburg, MD 20879) diluted with PBS (1:40) was placed in each well of the plates. After 45 min incubation at 37°C, fluorescence was observed under the FA microscope (Leitz Co., Germany). CPE and fluorescence of infected cells were compared with the controls.
WEE-7738 infection and dissemination

Adult female *Cx. tarsalis* mosquitoes 3-4 days after emergence were permitted to engorge on four 10-fold dilutions of infectious blood. After an extrinsic incubation period of 3, 5, 7, 10, and 14 days, 10 mosquitoes fed on each dilution were cold-anesthetized and the wings and legs were removed. The head and the rest of body were severed and put into 1.5 ml centrifuge tubes separately and kept in -80°C for future analysis. The body and a head were triturated with 0.5 ml of grinding medium (DMEM containing 10% fetal bovine serum and 230 ug/ml gentamycin) respectively and centrifuged in a microcentrifuge at 10,000 rpm for 90 sec. The supematant was used for virus assay.

The infection rates and dissemination rates were analyzed by General Linear Models Procedure and Linear Regression method (SAS Institute Inc. 1988).

WEE-7738 multiplication in *Cx. tarsalis*

Three to 4-day-old *Cx. tarsalis* mosquitoes were permitted to engorge on four 10-fold dilutions of infectious blood. Ten mosquitoes fed on each dilution were chill-anesthetized at 3, 5, 7, 10, and 14 days PF and stored at -80°C for microtitration. Mosquitoes were individually triturated in 1ml of grinding medium (DMEM containing 10% fetal bovine serum and 230 ug/ml gentamycin) and clarified by centrifugation at 10,000 rpm for 90 sec. Microtitration of the supematant allowed us to calculate titers following the Reed and Muench (1938) method. Data were analyzed by General Linear Models Procedures (SAS Institute Inc. 1988).

In vitro transmission of WEE virus

Mosquitoes 3-4 days old were fed on blood containing 4.7-5.0 log TCID₅₀/mosquito. Seventy-one mosquitoes (8-10 days postfeeding) were starved for 24 hrs before being permitted to feed. Blood fed mosquitoes were anaesthetized by chilling with ice and their wings and legs were removed on day 8, 9, and 10.
postfeeding. Each mosquito was permitted to salivate into a capillary tube containing 3.5 ul of feeding solution consisting of fetal bovine serum (FBS) mixed with 10% sucrose solution at a ratio of 1:1 for 20 min. The saliva was ejected in 0.3 ml of 20% FBS in PBS with 230 ug/ml gentamycin (personal communication with L. D. Kramer). After salivating, each head was severed from the rest of the body and used to determine dissemination and infection rates.

Results

**WEE-7738 in Cx. tarsalis**

WEE-7738 virus readily infected the Sioux City (SC) strain of Cx. tarsalis. Fifty percent of mosquitoes were infected at day 3 PF when fed more than 3.8 logs of virus (Table 1). However, less than 10% of mosquitoes that ingested less than 3.1 logs of virus were infected at day 3 PF. There was a correlation between infection rate in SC Cx. tarsalis and virus titer, and the extrinsic incubation time (days PF) (P<0.001). At days 10-14 PF, 90-100% of mosquitoes fed 4.8-5.1 logs of virus were infected. At the same time (PF), only 40-60% of the mosquitoes fed two lower virus titers were infected (Table 1).

**Dissemination of WEE-7738 into the head of SC Cx. tarsalis**

None of the mosquitoes had disseminated infections in their heads on day 3 PF regardless of the virus titer in the infectious blood meal (Table 2). However by day 5 PF, the virus was present in the heads of 10% of the mosquitoes fed 4.8-5.1 logs. By day 7 PF, WEE-7738 had disseminated into 10-30% of the heads of mosquitoes fed more than 1.8 logs of virus.
Table 1. Relationship between the concentration of virus (WEE-7738) in infectious blood meals and the resultant infection (%)\(^1\) of mosquitoes (Sioux City strain of *Culex tarsalis*).

<table>
<thead>
<tr>
<th>Amount of virus in blood meal (log TCID(_{50}) /mosq.)</th>
<th>Infection rate (%)</th>
<th>Days Post-Feeding</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>3</td>
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<tr>
<td>4.8-5.1</td>
<td></td>
<td>50 (5/10)</td>
</tr>
<tr>
<td>3.8-4.1</td>
<td></td>
<td>50 (5/10)</td>
</tr>
<tr>
<td>2.8-3.1</td>
<td></td>
<td>10 (1/10)</td>
</tr>
<tr>
<td>1.8-2.1</td>
<td></td>
<td>10 (1/10)</td>
</tr>
</tbody>
</table>

\(^1\)Infection rate (%): number of mosquito infected/number of mosquito tested x 100.
Table 2. Relationship between percent of mosquitoes with disseminated infections (dissemination rate)\(^1\) and amount of WEE-7738 virus in the blood meal fed the Sioux City, Iowa strain of *Culex tarsalis*.

<table>
<thead>
<tr>
<th>Virus conc. in blood meal (log TCID(_{50}) /mosq.)</th>
<th>Dissemination rate (%)</th>
<th>Days Post-Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3</td>
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<tr>
<td>4.8-5.1</td>
<td></td>
<td>0 (0/10)</td>
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<td>3.8-4.1</td>
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<tr>
<td>2.8-3.1</td>
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<td>0 (0/10)</td>
</tr>
<tr>
<td>1.8-2.1</td>
<td></td>
<td>0 (0/10)</td>
</tr>
</tbody>
</table>

\(^1\)Dissemination rate (%) equals the number of mosquito heads infected/the number of mosquito tested (10) x 100.
The disseminated infection rate was dependent on virus concentrations ($p<0.0036$) and the extrinsic incubation time ($P<0.0001$). At day 14 PF, more than 50% of mosquitoes fed on the two higher virus concentrations had disseminated infections, whereas only 20% of mosquitoes that ingested the lowest virus concentrations (1.8-2.1 logs) had disseminated infections (Table 2).

**Multiplication of WEE-7738 in SC strain of *Cx. tarsalis***

Mosquitoes fed between 3.8 and 5.1 logs of virus had 5.4 logs (TCID$_{50}$/mosquito) of virus on day 3 postfeeding. While WEE virus between 3.67 and 3.92 logs was present in *Cx. tarsalis* that fed on the lower virus titers (Table 3). The average amount of virus in infected *Cx. tarsalis* was proportional to the virus concentration in the blood meal and was also a function of the extrinsic incubation time ($P<0.025$). Table 3 shows that at day 14 PF the amount of WEE-7738 virus in individual mosquitoes ranged from 5.3 to 6.7 logs (TCID$_{50}$/mosq.). There were similar amount of virus in infected mosquitoes on day 14 PF that fed on 1.8-2.1 logs of virus or 4.8-5.1 logs of virus. However, the number of infected mosquitoes varied considerably. Ninety percent (9/10) became infected when fed 4.8-5.1 logs of virus while only twenty percent (2/10) of those that fed on 1.8-2.1 logs became infected.

**Transmission rate***

Table 4 shows the infection rate at different days PF, the dissemination rate (percent of mosquitoes with virus in their heads) and the transmission rate for WEE-7738. All (100%) of the mosquitoes were infected on day 9 PF. Only two of the 23 (8.7%) mosquitoes actually passed virus into the feeding tubes.
Table 3. Relationship between the amount of WEE-7738 virus in a blood meal and the amount of virus in individual *Cx. tarsalis*

<table>
<thead>
<tr>
<th>Amount of virus in blood meal (log TCID$_{50}$/mosq.)</th>
<th>Concentration of Virus (log TCID$_{50}$/mosq.)</th>
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<tr>
<td></td>
<td>Days Post-Feeding</td>
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<td>3</td>
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<tr>
<td>4.8-5.1</td>
<td>5.41 (8)$^2$</td>
</tr>
<tr>
<td>3.8-4.1</td>
<td>5.48 (6)</td>
</tr>
<tr>
<td>2.8-3.1</td>
<td>3.92 (2)</td>
</tr>
<tr>
<td>1.8-2.1</td>
<td>3.67 (1)</td>
</tr>
</tbody>
</table>

$^1$Mean Square Error (MSE) = 0.84

$^2$The number of mosquitoes infected out of 10.
The mean transmission rate (4.2%) was lower than that of day 9 PF because none of the 24 mosquitoes transmitted virus on day 10 PF. There was not a correlation between percent infection, the dissemination rate and transmission.

Table 4. Transmission rate (%) for WEE-7738 by a Sioux City, IA strain of Cx. tarsalis, fed on 4.7-5.0 log TCID$_{50}$/mosquito

<table>
<thead>
<tr>
<th>Days Post-Feeding</th>
<th>No. of mosquitoes</th>
<th>Infection rate (%)</th>
<th>Dissemination rate (%)</th>
<th>Transmission rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>24</td>
<td>19/24 (79.2)</td>
<td>2/24 (8.3)</td>
<td>1/24 (4.2)</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>23/23 (100)</td>
<td>7/23 (30.4)</td>
<td>2/23 (8.7)</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>22/24 (91.7)</td>
<td>7/24 (29.2)</td>
<td>0/24 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>66/71 (93.0)</td>
<td>16/71 (22.5)</td>
<td>3/71 (4.2)</td>
</tr>
</tbody>
</table>

Discussion

Variation in the susceptibility of vector mosquitoes to arboviruses is well known (Beaty et al. 1989; Reisen and Monath 1989). Variables include the vector population as well as geographic or genetic strains of a particular virus (Hardy et al. 1983; Beaty et al. 1989). The susceptibility of Cx. tarsalis to WEE virus varies over time (Hardy et al. 1990) and space (Hardy et al. 1976). Hardy et al. (1976) indicated that variation in susceptibility of a population to WEE virus depends on the composition of subpopulations of mosquitoes within a mosquito population. Weaver et al. (1984) found that an enzootic vector mosquito for Venezuelan equine encephalitis (VEE) virus, Culex taeniopus Dyar and Knab, was more susceptible to
enzootic strains than epizootic strains of VEE virus. He also found that replication of an epizootic strain of VEE was confined in the mesenteron of *Cx. taeniopus* (Weaver et al. 1984). Rico-Hesse et al. (1995) discovered that reemergence of VEE epidemic/epizootic in Venezuela resulted when the virus evolved from an enzootic strain. Based on findings of Weaver et al. (1984) and Rico-Hesse et al. (1995), it is a good idea to investigate vector competence for strains of an arbovirus in a particular area, where vectors are common.

Reisen et al. (1996) found that a 50% oral infectious dose of WEE virus (OID50) for *Cx. tarsalis* collected in the Coachella Valley of California was less than 2.0 log PFU/mosquito at day 10 PF. Hardy et al. (1978) reported OID50's for three geographically different *Cx. tarsalis* strains. These OID50's were 2.8 for the Knight Landing strain, 4.4 for the Chico strain, and 5.4 for log PFU/0.006 ml for the Fort Collins strain at 10-12 days PF. The OID50 of our Sioux City (SC) strain at day 10 PF was 2.93 log TCID50/0.006 ml. Therefore, the susceptibility of SC strain of *Cx. tarsalis* for WEE-7738 was similar to that of other strains of WEE virus and other strains of *Cx. tarsalis*.

Hardy (1987) found that WEE virus titer in the blood of ring necked pheasant was 7-9 log PFU/0.1 ml (5.78-7.78 log PFU/0.006 ml) 24-48 hrs after being inoculated subcutaneously with 3 log PFU. Hardy (1987) also found that the OID50 from *Cx. tarsalis* fed on viremic chickens was approximately 100 fold lower than it was from *Cx. tarsalis* fed on virus-soaked pledgets. In this study 90 to 100 percent of the SC strain of *Cx. tarsalis* became infected 10 to 14 days after feeding on ca. 5.0 log TCID50 of WEE-7738/mosquito.

As indicated in Table 2, the dissemination rate for WEE-7738 in the SC strain of *Cx. tarsalis* was 20-35.5% lower than the infection rate during 3-14 days PF. Difference between infection rates and dissemination rates of WEE virus in *Cx.*
*tarsalis* suggests that there is a mesenteronal escape barrier (Kramer et al. 1981, 1983; Reisen et al. 1996, 1997).

In the SC strain of *Cx. tarsalis*, infection rates and dissemination rates were correlated with virus concentration in the infectious blood meal. Also Thomas (1961) and Kramer et al. (1981) found that both the infection and dissemination rates increased as the extrinsic incubation period increased.

Table 3 shows that the amount of WEE-7738 virus present in a whole body of individual *Cx. tarsalis* mosquitoes markedly increased in the first week after blood feeding and then, slowed. Thomas (1963) reported similar results with WEE virus. The highest amount of WEE-7738 virus in a whole mosquito fed 4.8-5.1 logs was 7.5 logs at day 14 PF. The second highest virus amount was 7.23 logs at day 3 PF. Hardy (1987) suggested that WEE virus multiplication is higher in transmitting *Cx. tarsalis* than in nontransmitting *Cx. tarsalis*. Nontransmitting *Cx. tarsalis* have genetically controlled dissemination and transmission barriers (Kramer et al. 1981; Hardy et al. 1983). In our studies, WEE virus multiplication varied within infected mosquitoes even when fed the same virus concentration.

WEE-7738 transmission by the SC strain of *Cx tarsalis* was low when compared with WEE transmission studies with *Cx. tarsalis* in California (Kramer et al. 1981; Reisen et al. 1993, 1996, 1997). Kramer et al. (1981) reported that 64% of their mosquitoes transmitted the virus 13-16 days after ingesting 4.1 log PFU of the Bakersfield strain (BFS)-1703 of WEE virus. Reisen et al. (1993) found that ca. 60% of the mosquitoes fed 5.3 log PFU of WEE virus transmitted virus 7-8 days after blood feeding. The transmission rate of the Coachella Valley strain of *Cx. tarsalis* was ca. 10% on day 10 PF (Reisen et al. 1996). On day 14 PF, 12-50% of the Bakersfield strain of *Cx. tarsalis* transmitted WEE virus when exposed to 4.3 log PFU (Reisen et al. 1997). However, only 4.2% of the Sioux City strain of *Cx. tarsalis*
transmitted WEE-7738 virus. The lower transmission rate of WEE-7738 by the SC strain of *Cx. tarsalis* may be a result of a short evolutionary relationship between WEE-7738 and the SC strain of *Cx. tarsalis*. Also, the SC strain of *Cx. tarsalis* may have a salivary gland infection barrier or salivary gland escape barrier to WEE-7738 as discussed by Hardy et al. (1983).

**Acknowledgement**

The author would like to thank Dr. Momodou Niang at Dept. of MIPM, ISU for his kind help with bleeding sheep. We also thank Jun Zhu at Dept. of Statistics, ISU for assisting data analysis.

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dissemination of Venezuelan encephalitis viruses in the middle American
CHAPTER 3. LONGEVITY AND SPONTANEOUS FLIGHT ACTIVITY OF *Culex tarsalis* Coq. (Diptera: Culicidae) Infected with an Iowa Strain of Western Equine Encephalomyelitis Virus

A paper to be submitted to the journal of Medical Entomology

Joon-Hak Lee¹, Wayne A. Rowley¹, and Kenneth B. Platt²

Abstract

The effect of an Iowa strain of western equine encephalomyelitis virus (WEE-7738) was examined on the longevity and spontaneous flight activity of an Iowa strain (Sioux City) of *Culex (Cx.) tarsalis* Coq.

Mosquitoes exposed to 4.7-5.0 log TCID₅₀/mosquito did not live as long as mosquitoes fed 2.7-3.0 log TCID₅₀/mosquito or controls. Survival of *Cx. tarsalis* exposed to the lower virus titer was not significantly different than that of the controls. Only 1% of mosquitoes fed the higher virus titer survived to day 18 PE. However, 13% those fed the lower virus titer and 19.5% of the controls were still alive on day 18 PE.

Infected *Cx. tarsalis* mosquitoes had 27.5% lower flight activity scores and 26.1% fewer spontaneous flights than noninfected controls. After day 8 post-infection, infected *Cx. tarsalis* had 37.1% lower activity scores and 40.0% fewer spontaneous flights than noninfected controls. Virus infection did not affect how long a mosquito flew in a 24-hr-period (the daily flying time) or the duration of individual flights.

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The circadian rhythm (spontaneous flight activity patterns) of infected mosquitoes were identical to those of controls. Both infected and noninfected mosquitoes began spontaneous flight activity at 2000-2100 hrs (at light off or at the beginning of a 90 min. twilight period) and were active throughout the dark phase of the 24 hr cycle. Although mosquitoes were active throughout the night, there was a burst or peak of activity at 2200-2300 hrs when the complete dark cycle began.

**Introduction**

Traditionally, arboviral infection has been considered to be harmless to invertebrate vector mosquitoes (Chamberlain and Sudia 1961; McLintock 1978; Hardy et al. 1983). However, since Mimes et al. (1966) reported that Semliki Forest virus infection resulted in the degeneration of the salivary glands in *Aedes aegypti* L. this view has been challenged. Several studies indicated that viruses adversely affect vectors or modify their behavior. Rift Valley fever virus caused higher mortality in a putative epidemic vector, *Cx. pipiens* L. (Turell et al. 1985; Faran et al. 1987) and recently, overwintering *Ae. triseriatus* (Say) eggs infected with La Crosse virus (LAC) by transovarial transmission had a higher mortality rate than uninfected eggs (McGaw et al. 1998). In addition to mortality, arboviruses affect the feeding behavior of vector mosquitoes. La Crosse virus infection in *Ae. triseriatus* and dengue virus infection in *Ae. aegypti* L. lengthen the probing and feeding time of their vectors (Grimstad et al. 1980; Platt et al. 1997). However, the spontaneous flight activity of *Ae. trivittatus* (Coq.) was not affected by trivittatus virus (Berry et al. 1987b). Other parasites reportedly also affect flight activity of vector mosquitoes (Townson 1970; Paige and Craig 1975; Hockmeyer et al. 1975; Berry et al. 1986; Berry et al. 1987a; Berry et al. 1988; Beckett 1990).
WEE virus exists endemically throughout Iowa where isolations have been made not only from *Cx. tarsalis* Coq. but also from *Ae. trivittatus*. However, WEE is thought to be more important in western Iowa than it is in central and eastern Iowa because its primary vector, *Cx. tarsalis* is considerably more abundant in western Iowa (Vandyk and Rowley 1995).

This paper examines the effect of a western equine encephalomyelitis virus strain (WEE-7738) on adult survival (longevity) and the spontaneous flight activity of a Sioux City (western Iowa) strain of *Cx. tarsalis*.

**Materials and Methods**

**Mosquitoes**

A strain of *Culex tarsalis* mosquitoes was colonized from adult female mosquitoes collected in Sioux City (SC), Iowa in July, 1995. The mosquito colony (SC strain) was maintained in the Department of Entomology at Iowa State University. Routine laboratory rearing was accomplished by placing 500-600 first instars in distilled water in a 40 x 24 x 6 cm enamel pan where were afforded access to Tetramin® ad lib. Five rabbit pellets were also added to each tray. Pupae were separated manually and placed in a 46 X 56 X 46 cm cage for emergence and mating. Adult mosquitoes were afforded continuous access to a 0.3 M sucrose solution soaked in cotton pads. Fifty female mosquitoes 3-4 days old were placed in 0.51-liter ice cream cartons. Mosquitoes were starved for ca. 24 hrs prior to being fed a virus-blood mixture. All stages of mosquitoes were maintained at 25±1°C and 75±10% RH, with a 16:8 hr light/dark cycle. Rearing conditions also included a 90 min crepuscular period before complete lights on and lights off respectively. To minimize inbreeding effects in the colony, field collected *Cx. tarsalis* were introduced into the rearing cage each summer.
Preparation of WEE-7738 for oral infection

WEE-7738 isolated from *Ae. trivittatus* collected in Ames, IA in 1977 had been passaged twice in suckling mouse brain and was passaged once in a Vero African green monkey cell culture to produce the stock virus used in this study. The stock virus had a titer of 8.0-8.3 log TCID$_{50}$/ml (Reed and Munch 1938) and was used for the entire study.

Virus infection of mosquitoes

Glass membrane-feeders (Rutledge et al. 1964) with commercially available condom membranes made of sheep intestine (Naturallamb®) (Novak et al. 1991) were used to feed mosquitoes. Blood was obtained from adult sheep in which no neutralizing antibodies to WEE were present. Blood was mechanically defibrinated, sweetened to 2.5% (w/v) with sucrose and maintained at 37±0.5°C in feeders. Mosquitoes were starved for 24 hrs before being afforded access to the blood-virus mixture. After feeding, fully engorged mosquitoes were separated from partially or unengorged mosquitoes. At most, 25 fully engorged mosquitoes were maintained in 0.51-liter cups at 25±1°C in a relative humidity of 75±10%. After blood feeding all mosquitoes had constant access to 0.3 M sucrose. Control mosquitoes, allowed to engorge on blood without virus, were prepared in the same manner. The virus titration technique used in these studies was modified from Reisen et al. (1993). Two fully engorged mosquitoes were removed from each group and triturated to determine virus titer. Each mosquito was ground in 1 ml of grinding medium (DMEM containing 10% fetal bovine serum and 230 ug/ml gentamycin) and centrifuged at 10,000 rpm for 90 sec. A serial dilution of supernatant was made with Vero cell maintenance medium containing Dulbecco's Modified Eagle's medium (DMEM, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178) with 2% Fetal Bovine Serum (Sigma Chem. Co.). One hundred ul of each viral dilution was inoculated in
each well of a 96-well plate of 24-hr old Vero cells. Four wells were used for each dilution. Cell plates were incubated in a highly humidified chamber with 5% CO₂ at 37°C and checked for CPE (Cytopathic effects) at 2-3 days.

All experiments with live infected mosquitoes were performed in a BL-3 (Biosafety Level 3) laboratory, animal-holding facility, at the college of Veterinary Medicine at Iowa State University.

**Longevity**

Two different concentrations of WEE-7738 (4.7-5.0 log TCID₅₀/mosquito and 2.7-3.0 log TCID₅₀/mosquito) were used to feed mosquitoes. Fifty *Cx. tarsalis* were fed on blood containing each virus titer. Mosquitoes were aspirated into a 0.51-liter ice cream carton immediately after blood feeding and provided access to a 0.3 M of sucrose solution in cotton pads. Twenty-five mosquitoes were housed in a 0.51-liter ice cream carton at 25±1°C. Mortality was observed daily and recorded until 21 days post-feeding. Each experiment was repeated four times. Data were combined and survival rates were compared by LIFETEST (SAS Institute 1985).

**Spontaneous flight activity**

On day 5 post-exposure (PE) to WEE virus, 30 mosquitoes (12 control and 18 treatment) were assigned randomly to individual chambers in an acoustic flight activity system (Rowley et al. 1987). Mosquitoes were allowed to acclimate for 12 hrs. The light regime and temperature were identical to that of our mosquito rearing system. Spontaneous flight activity was monitored through day 11 PE. Variables measured included the activity score, the number of individual flights, and the flying time for each individual mosquito in each 30 min period (Rowley et al. 1987). Flight values for the 4 replicates were combined, yielding hourly and daily values of each variable for each mosquito. On day 11 PE, mosquitoes were removed from the chambers and placed individually in microcentrifuge tubes. All treatment
mosquitoes were tested for infection by RT-PCR. Variables tested were the least square (LS) mean of daily activity scores (the number of minutes in 24 hrs during which a mosquito made at least one flight of any duration), daily numbers of flights, daily flying time, and the duration of each flight. Least Square means of each variables/each day were obtained and analyzed by MIXED procedure in analysis of repeated measure data (Littell et al. 1996). The overall average of LS means of each variables/day was compared by General Linear Models Procedures (SAS Institute Inc. 1988). Circadian activity patterns of spontaneous flight activity were also determined and compared for each group. Circadian activity patterns represent an average of all of the mosquitoes’ spontaneous activity during each hour of a 24-hr-period (Rowley et al. 1987). The activity of all mosquitoes (infected or control) was averaged for each hour of each 24-hr-period. The data in Fig. 2 represent averages of all mosquito activity.

Virus assay

Individual mosquitoes were assayed for infection with WEE virus using RT-PCR. A total RNA extraction procedure was modified from that of Chomczynski and Sacchi (1987). Two oligonucleotide primers were selected from published information (Vodkin et al. 1994), which are the 223-bp region that encompasses the 3’ region of the capsid and the 5’ region of the contiguous envelope gene. The primer sequences are as follows; WEEV549 for CAGTCCAGTATGAGAATGGG, WEEV773 rev GCGCTGTAACTAGTGACCAC.

Ten microliters of RT reaction and 50 ul of PCR were run with GeneAmp® (RNA PCR kit) from Perkin-Elmer according to the manufacturer’s instructions (Perkin Elmer, Applied Biosystems Division, 850 Lincoln Centre Drive, Foster City, CA 94404). PCR products were visualized by electrophoresis in 1.2% agarose-ethidium bromide gel in TAE buffer. A 10 ul aliquot of the fluid from each PCR tube
was mixed with 2 ul of a standard gel loading solution (Invitrogen Co., 3985 B Sorrento Valley Blvd., San Diego, CA 92121) and 10ul of this mixture was loaded into a 4-mm x 1-mm slot in an agarose gel under TAE buffer (0.04 M Tris-acetate, 0.001 M EDTA). DNA size standard (100 bp DNA Ladder, Invitrogen), negative control and positive control were included in each gel.

Results

Survival rate

Mosquitoes exposed to blood containing 5 logs (high titer) of WEE-7738 virus did not live as long as control mosquitoes (P<0.0001). Mortality was similar to that of the controls during the first 8 days (Fig. 1). However, after day 9 PE, mortality increased more rapidly than it did in the control mosquitoes. Seventy-five percent (LT75) of the infected mosquitoes were dead by day 14 PE. In the control group an LT75 did not occur until day 16 PE. By day 18 PE, 99% of the infected mosquitoes were dead, while 19.5% of the control mosquitoes were still alive.

The survival of Cx. tarsalis fed blood containing with 3 logs (low titer) of WEE-7738 virus was similar to that of the control group (Fig. 1). The LT75 occurred at day 15 PE in the infected mosquitoes and at day 16 PE in the controls. Thirteen percent of the mosquitoes fed the lower virus titer survived until day 18 PE.

Spontaneous flight activity

Flight activity scores represent the number of minutes during a 24-hr-period when a mosquito made at least one flight of any duration. Mean flight activity scores of infected mosquitoes (89.3) were 27.5% lower than those of the controls (123.2) (p<0.0035) during the experiment (Table 1). Flight activity scores of infected mosquitoes (113.6) were identical to those of the controls (113.8) until day 6 PI. Flight activity scores of infected mosquitoes decreased daily after day 6 PI (Table 1).
Fig. 1. Survival of *Cx. tarsalis* maintained at 25±1°C after exposure to blood infected with WEE-7738 titers of 4.7-5.0 log TCID<sub>50</sub>/mosquito (high titer) and 2.7-3.0 log TCID<sub>50</sub>/mosquito (low titer).
Table 1. Least Square (LS) Mean daily flight activity scores and number of flights for *Culex tarsalis* infected with WEE-7738 virus titer of 4.7-5.0 log TCID$_{50}$/mosquito at 25±1°C

<table>
<thead>
<tr>
<th>Day PI$^3$</th>
<th>LS Mean Activity Score/24 hrs$^1$</th>
<th>LS Mean No. of Flights/24 hrs$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
</tr>
<tr>
<td>6</td>
<td>113.6</td>
<td>113.8</td>
</tr>
<tr>
<td>7</td>
<td>125.9</td>
<td>107.7</td>
</tr>
<tr>
<td>8</td>
<td>137.9</td>
<td>88.6</td>
</tr>
<tr>
<td>9</td>
<td>131.4</td>
<td>88.9</td>
</tr>
<tr>
<td>10</td>
<td>113.3</td>
<td>73.0</td>
</tr>
<tr>
<td>11</td>
<td>116.8</td>
<td>63.8</td>
</tr>
<tr>
<td>Average</td>
<td>123.2</td>
<td>89.3</td>
</tr>
</tbody>
</table>

1. Activity scores represent the number of minutes in a 24-hr-period that a mosquito made at least one flight of any duration.

2. Number of flight initiated in 24 hrs

3. PI: day post-infection
After day 8 PI, infected mosquitoes (78.6) had significantly lower flight activity scores (37.1% lower) than the controls (124.9) (p<0.0016). The number of flights/day taken by infected mosquitoes (201.8) was also significantly lower (26.1%) than those initiated by the controls (273.1) during days 6-11 PI (p<0.24) (Table 1). The number of flights/day of infected mosquitoes also decreased daily after day 6 (Table 1). After day 8 PI infected mosquitoes (168.2) spontaneously flew significantly fewer times (40.0%) than those of the controls (271.2) (p<0.0055). There was not a significant difference between infected mosquitoes and the controls in mean daily flying time (p=0.11) nor was there a difference in the mean duration of individual flights (p=0.88) (Table 2).

The circadian flight activity patterns of infected mosquitoes were identical to those of the control mosquitoes (Fig. 2). The mean spontaneous flight activity (activity scores, flight number, and flying time) in Fig. 2 represents an average of spontaneous flight activity of all mosquitoes (infected or control) for each hour of a 24-hr-period. Flight activity markedly increased during the period of 2030-2200 hrs (the crepuscular period). Peak activity occurred during the hour between 2200-2300 hrs when the first complete scotophase began (Fig. 2). *Culex tarsalis* mosquitoes were active throughout the entire night. Flight activity ceased at full daylight, 0700 hr (Fig. 2).
Table 2. Least square (LS) mean daily flying time\(^1\) and duration of individual flights for *Culex tarsalis* infected with WEE-7738 virus titer of 4.7-5.0 log TCID\(_{50}\)/mosquito at 25±1°C

<table>
<thead>
<tr>
<th>Day Pr(^1)</th>
<th>Flying Time (Sec./24 hrs)</th>
<th>Flight duration (Sec./Flight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
</tr>
<tr>
<td>6</td>
<td>1004.2</td>
<td>1160.2</td>
</tr>
<tr>
<td>7</td>
<td>1122.5</td>
<td>910.6</td>
</tr>
<tr>
<td>8</td>
<td>1206.1</td>
<td>848.4</td>
</tr>
<tr>
<td>9</td>
<td>991.1</td>
<td>718.6</td>
</tr>
<tr>
<td>10</td>
<td>890.7</td>
<td>620.3</td>
</tr>
<tr>
<td>11</td>
<td>774.2</td>
<td>398.6</td>
</tr>
<tr>
<td>Average</td>
<td>998.1</td>
<td>777.1</td>
</tr>
</tbody>
</table>

\(^1\) Daily flying time represents a total length of time that a mosquito has flown for 24 hrs.

\(^2\) PI: day post-infection
Fig. 3. Circadian rhythm (spontaneous flight activity) in a 24 hr period of *Cx. tarsalis* mosquitoes infected with WEE-7738 virus.
Discussion

Survival

Exposure to WEE-7738 virus affected the longevity of adult female Cx. tarsalis Coq. Mosquitoes exposed to a high titer (4.7-5.0 log TCID<sub>50</sub>/mosquito) of WEE-7738 did not live as long as those fed a lower titer (2.7-3.0 log TCID<sub>50</sub>/mosquito) or the controls. In other studies in our laboratory, infection rates and the dissemination of WEE-7738 in Cx. tarsalis was dependent on virus titer (Lee et al. unpublished data). Also Faran et al. (1987) found that Cx. pipiens with disseminated infections of Rift Valley fever virus had a higher mortality than those with nondisseminated infection. Rift Valley fever virus transmitting Cx. pipiens did not survive as long as nontransmitting Cx. pipiens (Faran et al. 1987).

Thomas (1963) found Cx. tarsalis infected with WEE virus still alive 67 days after infection. While Johnson and Rowley (1972) used 84-day-old non-blood fed Cx. tarsalis in a flight-mill study. We found that 9.5% of the SC strain of Cx. tarsalis survived for 21 days after feeding, whereas ca. 70% of a Californian strain of Cx. tarsalis (Bakersfield strain) lived for 21 days under similar conditions (Lee unpublished data). The longest any SC strain Cx. tarsalis lived was 27 days postfeeding. Differences in the longevity of Cx. tarsalis in this and other studies might, in part, be related to slight differences in rearing and holding. Also, the strain of Cx. tarsalis and the physiological conditions of the mosquitoes studied may affect adult longevity.

Romoser et al. (1992) suggested that virus infection in some organs and tissues of mosquitoes may be deleterious to the mosquito and may disrupt proper
functioning of infected organs and tissue. This could, in turn, affect longevity. Eastern equine encephalitis (EEE) virus infection and EEE induced cytopathology in the posterior midgut epithelial cells were detected in Cs. melanura (Coq.) (Weaver et al. 1988). Similar results with WEE infected Cx. tarsalis were also reported by Weaver et al. (1992). Pathology associated with infection of WEE virus in the posterior midgut epithelial cells of Cx. tarsalis and the tendency of alphaviruses to infect fat body (Scott et al. 1984; Weaver 1986; Bowers et al. 1995) suggest that WEE virus may adversely affect energy metabolism and ultimately reduce the survival of infected mosquitoes.

**Spontaneous flight activity**

Some parameters of spontaneous flight activity (number of flights initiated per day and flight activity scores) were significantly lower in WEE virus infected mosquitoes. However, there was not a difference in the length (duration) of individual flights taken by infected and noninfected mosquitoes. These data suggest that WEE virus infected mosquitoes do not initiate flight as readily as noninfected controls. The overall flight activity, as expressed by the activity score, was markedly lower (37.1%) than it was in the controls after day 8 PI. The daily decrease in both flight activity scores and flight numbers seems to be associated with severity of infection. Lee et al. found that number of SC strain of Cx. tarsalis with disseminated infections increased as days postinfection increased (unpublished data).

Biogenic amines such as octopamine are important in mediating flight behavior in insects. Several studies with moths, locusts, and cockroaches (Claassen and Kammer 1986; Orchard et al. 1993; Weisel-Eichler and Libersat
indicated that octopamine plays an essential role in flight behavior. Weisel-Eichler and Libersat (1996) found that octopamine lowered the threshold for the initiation of flight in *Periplaneta americana* (L.). Novak (personal communication) found that an amine-depleting compound (Reserpine) markedly decreased spontaneous flight activity in the mosquito, *Ae. triseriatus* (Say). Studies by Scott et al. 1984, Weaver 1986, and Bowers et al. 1995 indicate that some of the alphaviruses infect neural tissue in mosquito vectors. Thomas (1963) found WEE virus located (concentrated) in the head of *Cx. tarsalis*. A concentration of WEE virus in the neural tissues of *Cx. tarsalis* might alter the ability of this mosquito to produce octopamine that in turn could modify its flight behavior.

Berry et al. (1987b) found that the spontaneous flight activity of *Ae. trivittatus* (Coq.) infected with trivittatus (TVT) virus was almost identical to that of noninfected controls. A suggested reason was a probable lack of TVT virus in the flight muscle of *Ae. trivittatus*. The fact that TVT virus infection did not alter the flight ability of *Ae. trivittatus* suggests a long evolutionary relationship between the virus and *Ae. trivittatus* (Berry et al. 1987b). The relationship between WEE-7738 virus and *Cx. tarsalis* may not be as long as the relationship is between TVT virus and its primary vector, and as a consequence, WEE virus has more of an effect on *Cx. tarsalis*.

**Circadian rhythm of spontaneous flight activity**

The circadian flight activity patterns of WEE infected *Cx. tarsalis* were identical to those of control mosquitoes (Fig. 3). The abrupt increase in the flight activity of *Cx. tarsalis* during the crepuscular period (2030-2200 hrs) probably correspond to flights, in part, for mating (Reisen et al. 1983) and sugar-feeding
(Reisen et al. 1986). Peak flight activity during the hour (2200-2300 hrs) when the first complete scotophase began corresponds to host-seeking activity (Nelson and Spadoni 1972, Meyer et al. 1984). Thereafter, flight of *Cx. tarsalis* for host-seeking activity decreased, whereas that for sugar-feeding activity increased (Reisen et al. 1986).

Little effect of parasitic or virus infection on circadian rhythms (spontaneous flight activity) of mosquitoes has been reported (Berry et al. 1986; Berry et al. 1987a, b; Berry et al. 1988; Rowland and Boersma 1988). *Aedes aegypti* L. infected with *Brugia pahangi* (Buckley and Edeson) (Berry et al. 1986) and dog heart worm, *Dirofilaria immitis* (Leidy) (Bery et al. 1987a) had the same circadian flight activity patterns as uninfected mosquitoes. *Dirofilaria immitis* infection in its natural vector, *Ae. trivittatus* did not affect circadian rhythms of spontaneous flight activity (Berry et al. 1988). Rowland and Boersma (1988) found that there was no effect of *Plasmodium yoelii* infection on circadian activity patterns of spontaneous flight activity of *Anopheles stephensi* Liston. Trivittatus virus infection did not affect the circadian flight activity patterns of *Ae. trivittatus* (Berry et al. 1987b).

Circadian rhythm as a function of flight activity in mosquitoes is controlled endogenously (Jones et al. 1972; Jones 1974; Chiba et al. 1993) and entrained by environmental cycles such as light (Jones et al. 1967; Jones et al. 1972; Jones 1976; Jones 1982) and temperature (Chiba et al. 1993). The circadian pacemaker controlled by light entrainment is located in the brain of mosquito (Chiba and Tomioka 1987). WEE virus infection in *Cx. tarsalis* may not affect this pacemaker.
(control mechanism) of circadian flight activity rhythms because it (WEE virus) may not infect cells in the brain of this particular mosquito.

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References


CHAPTER 4. THE INABILITY OF Aedes trivittatus (Coq.) (Diptera: Culicidae) to Transovarially Transmit Western Equine Encephalomyelitis Virus

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Abstract

An attempt was made to transmit western equine encephalomyelitis (WEE) virus vertically in Aedes (Ae.) trivittatus (Coq.) and elucidate an overwintering mechanism for this virus in Iowa. Aedes trivittatus and a WEE virus strain (WEE-7738) isolated from Ae. trivittatus in Ames, IA in 1977 were used in these experiments.

We were unable to show that vertical transmission (transovarial transmission) of WEE virus occurs in Ae. trivittatus. The infection rate of Ae. trivittatus fed 7.0 log TCID₅₀/ml of WEE-7738 was only 8.47%. None of F1 adult progeny was infected after eggs were maintained at 4°C for 4 months to simulate winter conditions.

Introduction

Western equine encephalomyelitis (WEE) virus is endemic in Iowa and its epidemiology is basically the same as it is in the western United States and Canada (Reisen and Monath 1989). Culex tarsalis Coq. is the primary vector but a mechanism for endemic persistence is unknown.

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Several bunyaviruses and flaviviruses are transmitted vertically (Turell 1988). Proof of transovarial transmission (TOT) does not necessarily mean it (TOT) is an overwintering mechanism for a virus (Rosen 1987; Turell 1988). Watts et al. (1974) proved that transovarial transmission of La Crosse virus in *Aedes triseriatus* (Say) is an overwintering mechanism. Other bunyaviruses (California encephalitis, San Angelo, trivittatus, Tahyna, and snowshoe hare) overwinter transovarially (LeDuc 1979; Turell 1988).

Little information is available on transovarial transmission of alphaviruses (Togaviridae) through mosquito systems. In particular, information on vertical transmission of WEE virus in mosquitoes was not available until Fulhorst et al. (1994) demonstrated vertical transmission of WEE in *Aedes dorsalis* (Meigen).

In Iowa, Green et al. (1980) suggested that transovarial transmission in *Aedes (Ae.) trivittatus* is a possible mechanism for WEE virus maintenance during the winter in Iowa. Because *Ae. trivittatus* is locally abundant in Iowa (Vandyk and Rowley 1995) and WEE virus has been occasionally isolated from pools of this mosquito during enzootic years (Rowley et al. 1973; Dorsey et al. 1978).

In addition to the abundance of *Ae. trivittatus* and isolations of WEE virus from this mosquito in Iowa, studies demonstrating the vertical transmission of WEE virus in *Ae. dorsalis* (Fulhorst et al. 1994) indicates that vertical transmission of WEE virus in *Ae. trivittatus* might be a possible.

The objective of this study was to determine whether vertical transmission of WEE virus occurs in *Ae. trivittatus* and if it does, whether it is a mechanism for virus maintenance during the winter in Iowa.
Materials and Methods

Virus and virus assay

Virus

The WEE-7738 strain of virus was used in this study. WEE-7738 strain was isolated from a pool of 50 *Ae. trivittatus* collected in Ames, IA in 1977. This virus has undergone two intracranial passages in suckling mice and has been stored at -80°C. The virus was amplified once in Vero African green monkey cell cultures prior to use.

Microtitration

The titer of the virus-blood mixture was determined by microtitration (Hierholzer and Killington 1996) in a prepared 96 well plate of 24-hr old Vero African green monkey cells. The virus concentration in the virus-blood mixture was determined both immediately before and after mosquitoes were blood-fed.

RT-PCR

Presence of WEE-7738 virus in adult *Aedes trivittatus* and mouse brain was determined by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). The RNA extraction procedure was modified from Chomczynski and Sacchi (1987). Samples were ground in 300 ul of 4 M denaturation solution (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7; 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) in a microcentrifuge tube using a plastic pestle (Kontes, Vineland, NJ). And sequentially in the ground samples, equal volumes of phenolchloroform-isoamyl alcohol mixture and 1/10 volume of 2 M sodium acetate pH 4.0 were mixed. A final suspension was vigorously vortexed and cooled on ice for 15 min. Samples were centrifuged at 4°C and 10,000 rpm for 10 min. After centrifugation, the aqueous phase was transferred to a new tube, mixed with 0.7 volume of the aqueous phase of chilled isopropanol, and then placed at -20°C for at least 2 hrs to precipitate RNA.
Sedimentation at 10,000 rpm for 15 min at 4°C was performed and the resulting pellet was resuspended with 1 ml of 70% ethanol. Centrifugation at the same conditions was performed and the resulting pellet was allowed to dry. Dry pellets were resuspended with 10 ul of DEPC-treated water and kept in -20°C for future RT-PCR reaction.

Two oligonucleotide primers were selected from published information (Vodkin et al. 1994). The product is a 223-bp region that spans the 3' region of the capsid and the 5' region of the contiguous envelope gene. The primer sequences are as follows; WEEV549 for CAGTCCAGTATGAGAATGGG, WEEV773 rev GCGCTGTAACTAGTGACCAC. Ten microliters of RT reaction and 50 ul of PCR were run with GeneAmp® (RNA PCR kit) from Perkin-Elmer according to the manufacturer's instructions (Perkin Elmer, Applied Biosystems Division, 850 Lincoln Centre Drive, Foster City, CA 94404). The RT reaction was primed with 2.5 uM random hexamers: the thermal profile consisted of 10 min at room temperature, 15 min at 42°C, 5 min at 99°C and 5 min at 5°C (Vodkin et al. 1994).

The PCR was primed with 20 uM of the primer pair. The thermal profile consisted of 39 cycles of 1 min at 94°C, 1 min at 56°C and 1 min at 72°C. A final cycle with a 5 min extension at 72°C was added to ensure that full-sized product was synthesized.

PCR products were visualized by electrophoresis in 1.2% agarose-ethidium bromide gel in TAE buffer. A 10 ul aliquot of the fluid from each PCR tube was mixed with 2 ul of a standard gel loading solution (Invitrogen Co., 3985 B Sorrento Valley Blvd., San Diego, CA 92121) and 10ul of this mixture was loaded into a 4-mm x 1-mm slot in an agarose gel under TAE buffer (0.04 M Tris-acetate, 0.001 M
EDTA). DNA size standard (100 bp DNA Ladder, Invitrogen), negative control and positive control were included in each gel.

**Mosquito and virus infection**

*Aedes trivittatus* mosquitoes were host-seeking adult females collected in Sioux City, Iowa with CO₂-baited CDC light traps on July 25, and Aug. 17, 1996. One hundred female *Ae. trivittatus* were placed into 0.51-l ice cream cartons and allowed to feed on 7.0 log TCID₅₀/ml WEE virus in defibrinated sheep blood (Rutledge et al. 1964) through a natural lamb skin membrane (Naturallamb®) (Novak et al. 1991). The virus-blood mixture was maintained at 37°C.

**Transovarial transmission**

Fully engorged mosquitoes were separated from partially and unengorged specimens. Mosquitoes engorged on uninfected blood were used as controls. Each blood-fed mosquito was placed in an oviposition cage (Horsefall 1973). Cages were placed on a wet cheesecloth substrate in white enamel pans (25 x 42 x 7 cm) covered with glass plates (Christensen et al. 1978) in a BL3 laboratory maintained at 25±1°C and 75±10% relative humidity (RH). Cotton pads saturated with 0.3 M sucrose were placed on the oviposition cages. After oviposition, eggs were collected with a Pasteur pipette, counted, and transferred to moist filter paper in petri dishes. Mosquitoes were fed on uninfected adult mice after each oviposition in order to obtain as many egg batches as possible from each mosquito. Mosquitoes were maintained in oviposition cages for 24 days.

Dead mosquitoes were removed from the cages daily and processed for WEE virus. After 24 days, all live mosquitoes were chill-anesthetized and processed for WEE virus.

Eggs were embryonated on moist filter paper at 25±1°C for 14-21 days and then stored at 4°C (simulated winter conditions) for 4 months. Approximately 3 wks
before hatching, eggs were warmed at 25±1°C and 75±10% RH. Egg batches from infected *Ae. trivittatus* were immersed in deoxygenated water for 2 hrs, and larvae were removed and placed in deionized water with Tetramin® as a food source. Unhatched eggs were immersed in a nutrient broth-deionized water solution (about 1:4,000 W/V) for 10-12 hrs. Larvae were removed and maintained as described above. Larvae were reared to the adult stage.

**Transmission capability of F1 progeny**

To determine oral transmission capability of F1 progeny, adult female mosquitoes 5 to 10 days old were starved for 36 hrs and allowed to feed on 2-3-day-old suckling mice. A suckling mouse was placed in a 0.51-l of ice cream carton with a female mosquito. Mosquitoes were allowed access to the suckling mice for 3hrs. After feeding, mosquitoes were cold anesthetized and kept individually at -80°C for virus assay. Suckling mice were observed for 14 days. Suckling mice with CNS symptoms were anesthetized and processed for virus assay. When the experiment was terminated, the remaining suckling mice were sacrificed. Mouse brains were harvested and assayed for virus.

**Results**

**Infection rate of parent mosquitoes**

Of 873 host-seeking *Aedes trivittatus* mosquitoes collected in Sioux City, Iowa, 1996, 74 mosquitoes (8.48%) engorged with WEE-7738 virus-blood mixture. Only 6 of the 74 mosquitoes were infected (8.1%) (Table 1). Virus was detected in mosquitoes with a postinfection range of 16-24 days.
Table 1. Infection rate of *Aedes trivittatus* mosquitoes fed on virus-blood mixture with a virus titer of 7.0 log TCID$_{50}$/ml

<table>
<thead>
<tr>
<th>Days of Post Infection</th>
<th>No. of tested</th>
<th>No. of Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>16-20</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>21-24</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>6</td>
</tr>
</tbody>
</table>

Infection and transmission rate of F1 progeny mosquitoes

One hundred twenty-nine eggs were recovered from the 6 infected mosquitoes. All adult mosquitoes (49) of F1 progeny were tested for the presence of WEE virus. None of the F1 progeny and none of the mice fed on by infected mosquitoes were infected.

Discussion

Infection rate of parental mosquitoes

Only 8.48% of field collected *Ae. trivittatus* mosquitoes fed to repletion through a membrane. According to Christensen et al. (1978), 67 out of 92 mated *Ae. trivittatus* mosquitoes using the same system blood fed. Novak et al. (1991) reported that more than 56% of laboratory reared *Ae. triseriatus* and *Ae. aegypti* mosquitoes blood fed within 20 min.

Six of the 74 engorged mosquitoes were infected with WEE-7738. An infection rate was 8.1% (Table 1). Green et al. (1980) obtained infection rates of 3.8-37.5% with an Ames (central Iowa) strain of *Ae. trivittatus* with the same virus strain and feeding system. Green et al. (1980) also reported that the Ames strain of
Ae. trivittatus was more susceptible to WEE-7738 than the Weise Slough (eastern Iowa strain) of this species.

**Vertical transmission rate and transmission rate of F1 progeny**

None of the F1 mosquitoes was infected with WEE virus. None of the F1 females exposed to suckling mice took a detectable amount of blood. Ae. trivittatus does not mate in a cage (Christensen and Rowley 1978) and unmated Ae. aegypti mosquitoes more actively seek hosts than mated individuals (Klowden and Fernandez 1996). No unmated Ae. trivittatus fed to repletion on suckling mice. This may be associated with a feeding preference of Ae. trivittatus for medium to large mammals rather than small rodents, especially suckling mice (Pinger and Rowley 1975).

Transovarial transmission is an overwintering mechanism in some bunyaviruses (LeDuc 1979; Turell 1988). Trivittatus virus in Ae. trivittatus was transovarially transmitted virus to 11.6% of the F1 progeny which underwent simulated winter conditions (4°C for 4 months) (Christensen et al. 1978). Transovarial transmission of alphaviruses (Togaviridae) is not an overwintering mechanism (Rosen 1987; Turell 1988) although vertical transmission of some alphaviruses has been reported. Low levels of vertical transmission of Ross River virus in Ae. vigilax (Skuse) (Kay 1982) has been demonstrated in the laboratory. Two EEE virus isolations were made in field-collected Culiseta melanura larvae and in field-collected male mosquitoes (Chamberlain and Sudia 1961). However, EEE virus isolation from male mosquitoes could result from contamination of pools with parts of infected female mosquitoes. Fulhorst et al. (1994) reported that three WEE virus isolations were made from 666 pools of 29,841 adult Ae. dorsalis in California during August. In spite of considerable efforts to confirm vertical transmission of
WEE virus in *Ae. dorsalis*, no WEE virus isolations have been made since then (Reisen, W. K. personal communication).

We were unable to demonstrate vertical transmission of WEE virus (Alphavirus, Togaviridae) in *Ae. trivittatus*. It may not be possible for *Ae. trivittatus* to transovarially transmit WEE-7738. If vertical transmission of alphaviruses occurs in mosquitoes, it probably does not constitute an overwintering mechanism.

**Acknowledgement**

We are grateful to Ms. Jodi Pierce for her help in sorting *Aedes trivittatus* mosquitoes from the other field collected mosquitoes. We also thank Dr. Momodou Niang for his help in bleeding sheep.

**References**


CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

The primary objective of this research was to characterize an enzootic strain of WEE virus (WEE-7738) in Cx. tarsalis from an epidemic/epizootic area in Iowa. A second objective was to evaluate the impact of WEE-7738 on factors related to vector competence (longevity and flight activity), and to examine whether vertical transmission of WEE in Ae. trivittatus is an overwintering mechanism for this virus. Information on the vector competence for an enzootic strain of WEE virus from an epidemic/epizootic area could be valuable in assessing risk of a WEE epidemic/epizootic in Iowa. Second, the fact that WEE virus infection decreases the survival of adult Cx. tarsalis and alters spontaneous flight activity challenges conventional views that arbovirus infections do not have an adverse effect on their mosquito vectors (Chamberlain and Sudia 1961; McLintock 1978; Hardy et al. 1983). Third, the inability of Ae. trivittatus to transovarially transmit WEE virus suggests that transovarial transmission in Aedes mosquitoes is not an overwintering mechanism for this virus in Iowa.

The first aspect of this study evaluated vector competence of Sioux City (SC) strain of a Cx. tarsalis (susceptibility, transmissibility, and multiplication efficiency) for WEE-7738 and compared them with previously published information. Susceptibility of the SC strain of Cx. tarsalis was within a range of susceptibility in other studies (Hardy et al. 1978; Reisen et al. 1996). Transmissibility by the SC strain of Cx. tarsalis was lower than that of other studies (Kramer et al. 1981; Reisen...
et al. 1993, 1996, 1997). It is unknown why transmission of WEE-7738 by Cx. tarsalis from Sioux City, IA was lower than WEE transmission in other studies.

A second part of this study evaluated the effect of WEE-7738 infection on survival and flight activity of the SC strain of Cx. tarsalis. Survival and flight activity of mosquitoes are epidemiologically important factors in the assessment of vector-borne disease (Smith 1987; Bres 1988). Both the survival and spontaneous flight activity of the SC strain of Cx. tarsalis decreased when they were infected with WEE-7738. Little information is available that indicates whether or not an arbovirus infection adversely affects survival of a vector mosquito (Turell et al. 1985; Faran et al. 1987; McGaw et al. 1998). In this study WEE-7738, an alphavirus lowered the survival of its vector, Cx. tarsalis. It is not known exactly how arbovirus infection decreases the survival or longevity of a vector mosquito.

There are no data that virus infection affects the flight activity of vector mosquitoes. Biogenic amines such as octopamine are important in mediating flight behavior in insects. Several studies with moths, locusts, and cockroaches (Claassen and Kammer 1986; Orchard et al. 1993; Weisel-Eichler and Libersat 1996), indicated that octopamine plays an essential role in flight behavior. Weisel-Eichler and Libersat (1996) found that octopamine lowered the threshold for the initiation of flight in Periplaneta americana (L.). Novak (personal communication) found that an amine-depleting compound (Reserpine) markedly decreased spontaneous flight activity in the mosquito, Ae. triseriatus (Say).

In experiment three, I examined transovarial transmission of WEE-7738 in Ae. trivittatus to ascertain if it could be a maintenance mechanism in Iowa during
winter. Vertical transmission of trivittatus virus in *Ae. trivittatus* is an overwintering mechanism (Christensen et al. 1978). WEE virus is occasionally isolated in *Ae. trivittatus* during enzootic years (Dorsey et al. 1978). *Aedes dorsalis* in California vertically transmit WEE virus to its progeny (Fulhorst et al. 1994). We were unable to demonstrate that transovarial transmission of WEE virus occurs in *Ae. trivittatus*.

**Recommendations for Future Research**

For risk assessment of WEE epidemics or epizootics in the area where vector and human or equine populations overlap in Iowa, studies on the susceptibility and transmission ability of *Cx. tarsalis* for other strains are needed. Transmission of SC strain of *Cx. tarsalis* for WEE-7738 was lower than in some other studies. Several factors could have contributed to this lower vector potential.

*Culex tarsalis* exposed to WEE-7738 virus did not live as long as uninfected controls. Research on distribution of WEE virus in *Cx. tarsalis* is needed to elucidate exactly how WEE infection affects survival of *Cx. tarsalis*. It would be interesting to compare mortality rates of *Cx. tarsalis* infected with a strain of WEE isolated from *Cx. tarsalis* the same species with that of the mosquito infected with a strain of WEE from a different species of mosquito such as WEE-7738. WEE-7738 virus infection seemed to hamper the initiation of flight in *Cx. tarsalis*. Octopamine was reported to lower threshold of flight initiation in *Periplaneta americana* (Weisel-Eichler and Libersat 1996).

Alphaviruses in general are not transmitted transovarially by mosquitoes. However, Fluhorst et al. (1994) demonstrated vertical transmission of WEE virus by
Ae. dorsalis in California. In spite of considerable efforts to confirm vertical transmission of WEE virus in Ae. dorsalis, no WEE virus isolations have been made since then (Reisen, W. K. personal communication). We were unable to show that transovarial transmission of WEE virus in Ae. trivittatus is an overwintering maintenance mechanism in Iowa. Extensive efforts are needed to confirm vertical transmission of WEE virus in Ae. trivittatus.

References


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