The significance of western equine encephalomyelitis viral infections in *Aedes trivittatus* (Diptera: Culicidae) in Iowa

by

Dennis Warren Green

A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Departments: Entomology
Veterinary Microbiology and Preventive Medicine
Co-majors: Medical Entomology
Immunobiology

Iowa State University
Ames, Iowa

1979

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GENERAL INTRODUCTION

Western equine encephalomyelitis (WEE) has been a serious public health concern in western North America for more than 30 years. WEE virus was first isolated from the brain tissue of two horses in California that exhibited signs and symptoms of inflammation and injury to the meninges, brain, and spinal cord (Meyer et al. 1931). The relationship of WEE virus to human disease was confirmed later by Howitt (1938) who isolated virus from the brain of a child who died of encephalitis in California.

It was observed that WEE epidemics occurred during the warmer periods of the year and were limited to areas with large mosquito populations. Experimental transmission work supported the concept that an arthropod vector could be involved in the spread of WEE virus. Kelser (1933) was able to achieve transmission of the virus by Aedes aegypti; more importantly, he also demonstrated that A. aegypti could transmit the virus to a horse, resulting in an encephalitic disease. A number of studies were done to evaluate the vector competence of other Aedes species (Ferguson 1954) as a result of Kelser's observations. The first field isolation of WEE virus from mosquitoes, however, was made from Culex tarsalis in Washington (Hammon et al. 1941), and subsequent studies on the feeding habits and transmission capabilities of this mosquito established it as the most likely epidemic vector of WEE virus (Reeves and Hammon 1962). On the basis of viral recovery

from wild birds and the viremic response of wild birds to experimental infection, these animals were incriminated as the most important enzootic vertebrate hosts (Hammon et al. 1951; Sooter et al. 1951).

Since 1960, most scientific interest in WEE has focused on defining the dynamics of the basic cycle, especially on determining the overwintering mechanism of the virus in temperate climates. No local winter reservoir in arthropod vectors of any stage or in vertebrate hosts has been demonstrated, and evidence is lacking for reintroduction of virus by migrating birds.

Occasionally, WEE viral isolates are obtained from other arthropods collected concurrently with C. tarsalis, or sometimes earlier in the season. The significance of such findings and their relationship to WEE viral activity is unknown. Aedes melanimon has recently been incriminated as a vector of WEE virus in California in a jackrabbit-mosquito cycle (Hardy and Bruen 1974). Even though epidemics and epizootics of WEE are confined to the range of C. tarsalis, it is possible that other mammalophilic species may serve to amplify WEE viral activity in a region if conditions are suitable.

Following the multiple isolations of WEE virus from Aedes trivittatus in 1971 and 1977, studies were initiated to more carefully define the WEE virus cycle in Iowa. This thesis enumerates the results of vector competence experiments designed to determine the role A. trivittatus might play in that cycle.

Explanation of Thesis Format

The thesis is composed of two sections. Section I is concerned with the susceptibility of A. trivittatus to infection with different WEE viral strains. This section has been accepted for publication in the January 1980 issue of the American Journal of Tropical Medicine and Hygiene and is essentially the same copy that will appear in that article. Section II is concerned with the transmission of WEE viral strains that infected A. trivittatus per os. This section will also be submitted in a modified form to the American Journal of Tropical Medicine and Hygiene as Part Two in a continuing series of articles dealing with the significance of WEE viral infections in A. trivittatus in Iowa.

The senior author is responsible for the design of experiments in both sections and for the performance of the entomological aspects of each experiment. Virus stock was prepared and titered by the State Hygienic Laboratory at the University of Iowa, Iowa City, Iowa. Potentially infectious specimens generated in our laboratory at Iowa State University were processed by the Hygienic Laboratory as well. Results were assimilated, interpreted, and written into manuscript form by the senior author. Ideas and recommendations expressed in both sections of this thesis are those of the senior author.

SECTION I. VARIATION IN SUSCEPTIBILITY OF *AEDES TRIVITTATUS* TO EXPERIMENTAL INFECTION WITH THREE STRAINS OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS

The Significance of Western Equine Encephalomyelitis Viral Infections in *Aedes trivittatus* (Diptera: Culicidae) in Iowa. I. Variation in Susceptibility of *Aedes trivittatus* to Experimental Infection with Three Strains of Western Equine Encephalomyelitis Virus

Dennis W. Green, B.S., B.A.

Wayne A. Rowley, Ph.D.

Yau W. Wong, M.A.

James P. Brinker, B.S.

Donald C. Dorsey, M.A.

William J. Hausler, Jr., Ph.D.

From the Department of Entomology (Green, Rowley), Iowa State University, Ames, Iowa 50011; and the University Hygienic Laboratory (Wong, Brinker, Dorsey, Hausler), University of Iowa, Iowa City, Iowa 52240

INTRODUCTION

The epidemiology of western equine encephalomyelitis (WEE) in Iowa is basically the same as in other western States and Canada (Hess and Hayes 1967). The disease exists endemically in numerous foci throughout the state with a low incidence rate among humans (Rowley et al. 1979). Epizootics in horses are a more common occurrence. From 1963 to 1977, an annual average of 375 clinical cases of equine viral encephalomyelitis was reported, with a low of 41 cases during the 1976 drought and a high of 1287 cases in 1964 (Iowa State Dept. of Health 1963-1977). The peak period of WEE virus activity generally occurs from mid-July to mid-September following a sharp, sustained rise in Culex tarsalis populations, and transmission seldom continues past October. Virus isolations are made primarily from this mosquito. The mechanism for endemic persistence of WEE virus in Iowa through harsh winter weather remains unresolved.

Aedes trivittatus is a floodwater mosquito that is widely distributed in North America. It has been reported from Nova Scotia, Ontario, and Manitoba in Canada; from 40 of the continental United States as far south as Georgia and Texas and west to Idaho, Utah, and Arizona; and from Mexico as far south as Oaxaca (Carpenter and LaCasse 1955; Arnell 1976). Aedes trivittatus can be the most abundant species in many areas of Iowa and is found throughout the state (Rowley et al. 1973). The mosquito is a strong flier, is long-lived, is multivoltine, and takes several bloodmeals during its lifetime (Rowley, unpublished

data). It is predominantly a crepuscular, mammalophilic feeder but occasionally feeds on amphibians, reptiles, and birds (Pinger and Rowley 1975). It is a fierce, persistent biter and can be an extremely annoying pest.

In 1971, WEE virus was isolated from A. trivittatus collected in central and western Iowa (Rowley et al. 1973). WEE virus also was recovered from this species in central and eastern Iowa in 1977. Statewide recovery of WEE virus from A. trivittatus is of interest for several reasons. First, isolations were made during enzootic years, thus excluding the possibility that A. trivittatus only become infected during periods of extensive zoonotic involvement. Second, the bionomics and abundance of this mosquito would make it a good amplifying force in the natural cycle of WEE virus even if only a small proportion of the population becomes infected. Third, conclusive evidence has established that transovarial transmission in A. trivittatus is an overwintering mechanism for another arbovirus in Iowa, trivittatus virus (Bunyaviridae) (Christensen et al. 1978). Recent reports indicate that vertical transmission may be a common phenomena in Aedes species associated with arboviruses requiring an overwintering mechanism to persist endemically (Watts and Eldridge 1975; Rosen et al. 1978).

Laboratory studies were initiated to determine the significance of WEE viral infections in A. trivittatus in Iowa. This report is concerned with several parameters of WEE viral infection in this species. Limited data concerning infection in C. tarsalis are included for comparative purposes.

MATERIALS AND METHODS

Viruses

Three strains of WEE virus were used to infect mosquitoes. The history, titer, and plaque morphology of these strains are described in Table 1. Stock viruses were prepared as 10% homogenates of infected suckling mouse brains in phosphate buffered saline, pH 7.5, containing 1% bovine albumin (BA-PBS) and were titrated in suckling mice. Aliquots of virus from these 3 pools were used to prepare feeding and inoculating suspensions. Viral titers of all suspensions were expressed as suckling mouse intracerebral mean lethal dose ($SMICLD_{50}$) per 0.025 ml and were estimated by the method of Reed and Muench (1938).

Mosquitoes

F_1 generation mosquitoes from field-collected adult A. trivittatus and from field-collected C. tarsalis egg rafts were used throughout the experiments. Two geographical strains of A. trivittatus and one geographical strain of C. tarsalis were employed. The Ames strains of A. trivittatus and C. tarsalis were collected at the Iowa Conservation Commission Nursery on the south edge of Ames in central Iowa. This site has been described elsewhere (Pinger and Rowley 1975). The Weise Slough strain of A. trivittatus was collected at the Weise Slough Game Management Area in Muscatine County in eastern Iowa, approximately one mile south of U.S. highway 6 along the Cedar River. Field-collected adults were titrated in suckling mice to remove the possibility of transovarial

Table 1. History, titer, and plaque characteristics of three WEE viral strains used to infect mosquitoes.

Strain	Source and date of collection[a]	Passage history[b]	Titer/0.025 ml ($SMICLD_{50}$)[c]	Plaque size (mm)[d]
7738	Pool of A. trivittatus (8-12-77)	SM-p2	$10^{7.5}$	4-6
7746	Pool of C. tarsalis (8-12-77)	SM-p2	$10^{8.0}$	4-6
NJ-275	Pool of A. vexans (9-14 and 10-3-61)	C-p1, SM-p6	$10^{8.5}$	1-2

[a] Specimens were collected at the Iowa Conservation Commission Nursery, Ames, Iowa except strain NJ-275 which was collected in Mays Landing, New Jersey.

[b] SM, suckling mice; C, chick; p, passage level.

[c] Suckling mouse intracerebral mean lethal dose.

[d] Diameter measured 5 days after infection of Vero cell cultures.

transmission of virus, particularly trivittatus (TVT) virus, to F_1 progeny.

Mosquitoes were reared uniformly by conventional laboratory techniques (Christensen and Rowley 1978) and were maintained at 26.5 ± 1° C and 80 ± 5% r.h. under a 16-hr photoperiod. The larval diet consisted of tropical fish food (Tetramin^R); adult mosquitoes were maintained on 0.3 M sucrose solution before and after infection.

Infection of Mosquitoes

Female mosquitoes for oral susceptibility studies were taken off sucrose 18-24 hrs before bloodfeeding. Blood-virus suspensions were prepared on the day of feeding by diluting viral strains to the desired concentration by using 1% BA-PBS, pH 7.5, and then mixing these suspensions 1:3 with defibrinated dog blood. Mosquitoes were exposed to WEE viral strains by using a natural lambskin membrane feeder (Rutledge et al. 1964). Temperature of the infectious bloodmeal was maintained at 37° C by continuous circulation of heated water. Aliquots of each suspension were taken before and immediately after feeding and were frozen at -70° C for subsequent titration. Bloodfed mosquitoes were separated under light CO_2 anesthesia, held for 5-26 days, and then frozen at -70° C for subsequent virus isolation attempts.

Female mosquitoes also were inoculated through the neck membrane with 0.95 ± µl quantities of viral suspensions by using a modification of the Rosen and Gubler technique (Rosen and Gubler 1974).

Suspensions were prepared by diluting viral strains to the desired concentration by using Dulbecco's PBS, pH 7.4, and then mixing this material 1:1 with Aedes physiological saline, pH 7.8 (Hayes 1953). Needles were made by drawing borosilicate glass capillary tubing to a point (end diameter 1 μ or less) by using a Narishige^{R} PN-3 Micromanipulator. The tip was broken at a selected point with jeweler's forceps to allow for fluid uptake. The untapered portion of the tubing was marked off at 1-mm intervals so that one could calculate the approximate amount injected. The needle was then attached to a tuberculin syringe with epoxy resin, which in turn was attached via rubber tubing to a 50-cc syringe for better control of the inoculum administered. Once the glass tubing was filled with inoculum by immersing the tip in the suspension and withdrawing the plunger of the 50-cc syringe, the tuberculin syringe was mounted in a movable holder with 3-dimensional control under a dissecting microscope. Mosquitoes under nitrogen anesthesia were held in place by fine suction to the end of a 16-gauge needle whose end was specially formed to receive the dorsum of the insect. After inoculation, the mosquitoes were transferred to a 1-pint ice cream carton, held for 8-9 days, and then frozen at -70° C for subsequent virus isolation attempts. Aliquots of all inoculums were taken before and immediately after inoculation and were frozen at -70° C for subsequent titration.

Viral Assay

Most mosquitoes were assayed for WEE virus by using methods described by Sudia and Chamberlain (1967). Each pool was triturated in 1.0 ml of 1% BA-PBS, pH 7.5, using a small amount of Alundum^{R} as an abrasive agent, and then centrifuged at 2000 rpm for 10 minutes at 4° C. One- to 2-day-old suckling mice were inoculated with 0.025 ml of the supernatant and were observed for 14 days for signs of illness. Brains of moribund or dead mice were passed an additional time in suckling mice. Virus isolated from this second passage was identified by the complement fixation (LBCF) test (U. S. Communicable Disease Center, Atlanta 1965).

Two inoculation treatment groups were assayed for virus in Vero cell cultures. Mosquito pools were triturated as just described. After centrifugation, a 1:10 dilution of the supernatant was prepared in Medium 199 containing 5% fetal calf serum and 0.14% sodium bicarbonate. Quantities of 0.025 ml were inoculated into duplicate wells of microtiter plates containing approximately 5000 Vero cells per well, bringing the total volume per well to 0.1 ml. Results were read 5 days after inoculation and were recorded only as positive (plaques present or cell layer absent) or negative (plaques absent, cell layer intact).

RESULTS

A. trivittatus varied little in their susceptibility to infection with 3 WEE viral strains via intrathoracic inoculation (Table 2). The 50% infective dose seemed to be approximately $10^{2.1}$ $SMICLD_{50}$. More than 10^{3} $SMICLD_{50}$ probably would be required to infect all A. trivittatus inoculated, regardless of the strain of virus inoculated. In contrast, C. tarsalis were more susceptible to infection with WEE-7738 virus via intrathoracic inoculation than were A. trivittatus. A dose of $10^{1.6}$ $SMICLD_{50}$ units was sufficient to infect all C. tarsalis inoculated. This agreed closely with the 100% infective dose reported by others (Hardy et al. 1976) for field and laboratory strains of this species.

There was a significant difference between WEE viral strains in the minimum viral concentration required to infect A. trivittatus (Ames) by membrane feeding (Table 3). Infection thresholds were approximately $10^{2.5}$ and $10^{5.8}$ $SMICLD_{50}$ for the 7738 and 7746 strains, respectively. Aedes trivittatus (Ames) were not infected by exposure to as much as $10^{7.2}$ $SMICLD_{50}$ of the NJ-275 strain of WEE virus. This represented a 2000-fold difference in susceptibility by membrane feeding between the 7738 and 7746 WEE viral strains, and at least a 12,000-fold difference between the 7738 and NJ-275 strains. Aedes trivittatus (Weise Slough) and C. tarsalis (Ames) seemed equally if not more susceptible to infection with WEE-7738 virus.

Table 2. Infection of A. trivittatus and C. tarsalis by intrathoracic inoculation with varying concentrations of three WEE viral strains.

Strain	Mosquito species (strain)	Log_{10} virus dosage ($SMICLD_{50}$ units)[a]	Days post-inoculation	Infection rate
7738	A. trivittatus (Ames)	2.6	9	75% (39/52)[b]
	C. tarsalis (Ames)	1.6	8	100% (19/19)[c]
7746	A. trivittatus (Ames)	2.1	8	50% (15/30)[c]
	A. trivittatus (Ames)	3.1	8	93% (37/40)[b]
NJ-275	A. trivittatus (Ames)	2.6	8	73% (22/30)[c]

[a] Suckling mouse intracerebral mean lethal dose.

[b] Assayed in Vero cell cultures.

[c] Assayed in suckling mice, intracerebral inoculation.

Table 3. Infection of A. trivittatus and C. tarsalis after ingesting varying concentrations of three WEE viral strains through a membrane.

Strain	Mosquito species (strain)	Log_{10} virus dosage[a]	Days post-exposure	Number mosquitoes tested	Number pools tested	Number pools positive	Minimum percent infected
7738	A. trivittatus (Ames)	2.5	16	26	13	1	3.8
		5.3-5.5	12	10	5	1	10.0
			16	10	5	2	20.0
		6.0-6.5	12	15	5	2	13.3
			16	8	4	2	25.0
		6.8-7.0	14	56	11	10	17.9
	A. trivittatus (Weise Slough)	5.3	12	10	5	1	10.0
			16	10	5	1	10.0
		6.3-6.6	12	10	5	3	30.0
			16	8	5	3	37.5
	C. tarsalis (Ames)	7.0	14	45	9	9	20.0

[a] $SMICLD_{50}$/0.025 ml.

Table 3 (cont.).

Strain	Mosquito species (strain)	Log_{10} virus dosage[a]	Days post-exposure	Number mosquitoes tested	Number pools tested	Number pools positive	Minimum percent infected
7746	A. trivittatus (Ames)	5.3	12	10	5	0	0.0
			16	10	5	0	0.0
		6.3	12	20	7	3	15.0
			16	20	7	4	20.0
NJ-275	A. trivittatus (Ames)	5.5	5-11	28	28	0	0.0
			12-26	43	43	0	0.0
		6.6-7.2	14	18	18	0	0.0

F_1 generation A. trivittatus were quite heterogeneous in regard to viral susceptibility. About 20% of the Ames strain was infected after imbibition of $10^{5.3}$ $SMICLD_{50}$ of WEE-7738 virus, and no less than 25% was infected when the concentration of this strain was $10^{6.8-7.0}$ $SMICLD_{50}$, a 50-fold increase. Using a finite population model for estimating infection rates in pooled mosquitoes (Bhattacharyya et al. 1979), an infection rate of 37.8% was estimated for A. trivittatus exposed to the latter concentration of WEE-7738 virus. A similar pattern occurred in A. trivittatus exposed to WEE-7746 virus. All field strains of A. trivittatus were relatively refractory to infection with WEE virus.

Single isolates of WEE and TVT virus were recovered from 120 A. trivittatus collected at Weise Slough. Virus was not isolated from 91 adult progeny reared from the eggs laid by pooled mosquitoes yielding WEE virus.

DISCUSSION

Variation in the susceptibility of different mosquito species to infection with a particular arbovirus is an expected phenomena in arbovirus research (Mangiafico 1971; Watts et al. 1973). The concept of variation in susceptibility of different geographical strains of an arthropod to infection with a single arbovirus also has been substantiated by several recent reports (Gubler and Rosen 1976; Hardy et al. 1976; Tesh et al. 1976; Grimstad et al. 1977; Hardy et al. 1978; Jones and Foster 1978). Evidence has been less convincing that variation occurs in the susceptibility of a particular strain of mosquito to infection with strains of a single arbovirus (Kramer and Scherer 1976; Tesh et al. 1976). Varied responses were demonstrated in laboratory colonies of A. aegypti, Aedes taeniorhychus, and Aedes albopictus. Such responses may or may not reflect the true biological capabilities of these species as vectors of a given arbovirus strain. This report represents the first evidence that field strains of a single mosquito species can vary in their susceptibility to infection with strains of an arbovirus. Moreover, these data suggest that interactions that occur in vector-virus-host cycle(s) at a particular focus of WEE virus may be more complex than previously believed, since both strains infecting A. trivittatus were mosquito isolates and the site, date of collection, and passage history of these isolates were identical.

Preliminary findings have not determined if genetic factors are responsible for the variation in susceptibility of A. trivittatus to WEE viral strains. Miles et al. (1973) reported that substantial

amounts of Whataroa virus, a virus antigenically related to WEE virus and maintained in a similar zoonotic cycle, could be recovered from hemolymph of Aedes australis during the first 48 hrs after infection. Hemoglobin has been observed in the hemocoel of A. trivittatus in other studies shortly after a bloodmeal, indicating that this mosquito may become infected via a "leaky gut" as well. This phenomena could vary with the age of the mosquito, the quantity of blood ingested, and/or other factors affecting the integrity of the midgut wall. Intercellular passage of virus, however, would not account for the differences in susceptibility noted in this study because similar infection profiles were not obtained with all 3 strains. Variation seemed to be related to a "gut barrier" as shown in other studies (McLean 1955; Gubler and Rosen 1976; Hardy et al. 1976) since variation in susceptibility was shown only when virus was imbibed and not when virus was inoculated into the hemocoel. In light of several reports suggesting or confirming a genetic basis for infection of some arthropods with arboviruses (Jones and Foster 1974; Gubler and Rosen 1976; Tesh et al. 1976; Hardy et al. 1978), it is probable that the susceptibility of A. trivittatus for WEE viral strains also is an inherited trait.

Non-genetic factors (MacDonald 1967), such as age, nutrition, and method of exposure, did not seem to be responsible for the variability in A. trivittatus susceptibility to WEE virus. Mosquitoes were similar in age, ranging from 3 to 13 days old in the oral susceptibility trials and from 7 to 18 days old in the inoculation trials. The use of younger and older mosquitoes was the same in trials in which variation in

susceptibility was observed. Mosquitoes were reared on identical artificial diets from the time they emerged and were not provided a bloodmeal prior to or subsequent to virus exposure. Sucrose was not included in the blood-virus suspensions during membrane feeding, and therefore it is unlikely that the bloodmeal was transferred into the ventral diverticulum instead of passing directly into the midgut.

It is not clear why A. trivittatus were refractive to infection with the NJ-275 strain of WEE virus and yet were susceptible to infection with WEE viral strains 7738 and 7746. After 6 serial intracerebral passages in suckling mice, it is probable that the more neurotropic subpopulation of virions was selected over the more viscerotropic subpopulation presumably present in the original isolate; this may have affected the ability of virus to attach to and/or replicate in midgut tissue. Several authors also have reported that WEE viral strains isolated in the western United States and Canada are antigenically distinct from strains isolated in the East (Karabatsos et al, 1963; Henderson 1964). Henderson, however, found that serial passage of WEE virus through infant mice resulted in an alteration of antigenic properties and that in most cases eastern strains of WEE virus converted to the antigenic phase of western strains by the sixth intracerebral passage. This would indicate that regional differences may not have played a role in the refractiveness of A. trivittatus for WEE-NJ275 virus. Further studies are needed to clarify this matter. A serological comparison of these strains is in progress and will be published separately.

The importance of using more than one strain of virus in vector competence studies, especially more than one strain from a given region and from a given source, cannot be overemphasized. WEE viral strains originating from different sources have been shown to produce different clinical symptoms, viremic responses, and mortality patterns in infected mammals (Sponseller et al. 1966; Hardy et al. 1974) and birds (J. L. Hardy, School of Public Health, U.C. Berkeley, personal commun.); and our data show that vector susceptibility may vary with the strain of WEE virus employed even when strains are taken simultaneously from the same location. Other aspects of vector competence of A. trivittatus for WEE virus may also vary with the strain of virus employed and are currently being investigated.

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SECTION II. VARIATION IN TRANSMISSION OF TWO STRAINS OF WESTERN EQUINE ENCEPHALOMYELITIS VIRUS BY *AEDES TRIVITTATUS*

The Significance of Western Equine Encephalomyelitis Viral Infections in *Aedes trivittatus* (Diptera: Culicidae) in Iowa. II. Variation in Transmission of Two Strains of Western Equine Encephalomyelitis Virus by *Aedes trivittatus*

Dennis W. Green, B.S., B.A.

Wayne A. Rowley, Ph.D.

Yau W. Wong, M.A.

James P. Brinker, B.S.

Donald C. Dorsey, M.A.

William J. Hausler, Jr., Ph.D.

From the Department of Entomology (Green, Rowley), Iowa State University, Ames, Iowa 50011; and the University Hygienic Laboratory (Wong, Brinker, Dorsey, Hausler), University of Iowa, Iowa City, Iowa 52240

INTRODUCTION

The successful completion of an arboviral cycle depends upon a number of factors, including the distribution of virus to the salivary glands via the hemolymph following viral multiplication in the midgut of the vector, viral multiplication in the salivary glands, and finally the injection of virus into a susceptible vertebrate host. In mosquitoes, infection rates may be quite high and yet transmission attempts may be unsuccessful (Chamberlain et al. 1954; Jupp et al. 1966; Saliba et al. 1973; Watts et al. 1975). Studies designed to evaluate the vector competence of a particular species for a given arbovirus must therefore have a transmission component.

Variation in the transmission of several arboviruses by different mosquito species has been documented in a number of comparative studies (Kissling and Chamberlain 1967; Mangiafico 1971; Watts et al. 1973). Variation between species probably is never absolute. Large differences in transmission rates by strains of the same mosquito species have been noted by Chamberlain (1968), who reported differences in the extrinsic incubation period of eastern encephalitis virus in strains of Aedes aegypti; by Grimstad et al. (1977), who found that geographic strains of Aedes triseriatus outside the known endemic area of LaCrosse virus were more competent transmitters of that virus than were endemic geographic strains; and by Beaty and Aitken (1979), who reported that African strains of A. aegypti were more competent transmitters of yellow fever (YF) virus than was a strain of A. aegypti from Asia, where YF is not known

to occur. Studies demonstrating variation in the transmission of different arboviral strains by a single laboratory strain of mosquito are equally important (Saliba et al. 1973; Kramer and Scherer 1976).

Vector competence studies were initiated to determine the significance of western equine encephalomyelitis (WEE) viral infections in Aedes trivittatus in Iowa following statewide recovery of virus from this species. Variation in the susceptibility of this species to infection with local WEE viral strains (Green et al. 1980) suggested that other aspects of vector competence also might vary with the strains of virus employed. This report is concerned with several parameters of WEE viral transmission by A. trivittatus.

MATERIALS AND METHODS

Viruses

Two strains of WEE virus (WEE-7738 and WEE-7746) were used to infect mosquitoes. The history, titer, and plaque morphology of these strains have been enumerated previously (Green et al. 1980). Stock viruses were prepared as 10% homogenates of infected suckling mouse brains in phosphate buffered saline, pH 7.5, containing 1% bovine albumin (BA-PBS), and were titrated in suckling mice. Aliquots of virus from these 2 pools were used to prepare inoculating suspensions. Titers of all viral suspensions were expressed as suckling mouse intracerebral mean lethal dose ($SMICLD_{50}$) per 0.025 ml and were estimated by the method of Reed and Muench (1938).

Mosquitoes

F_1 generation mosquitoes from field-collected adult A. trivittatus were used throughout this experiment. Mosquitoes were collected at the Iowa Conservation Commission Nursery on the south edge of Ames in central Iowa. This site has been described elsewhere (Pinger and Rowley 1975). Mosquitoes were reared uniformly by conventional laboratory techniques (Christensen and Rowley 1978) and were maintained at 26.5 $\pm$ 1° C and 80 $\pm$ 5% r.h. under a 16-hr photoperiod. The larval diet consisted of tropical fish food (Tetramin^R); adult mosquitoes were maintained on 0.3 M sucrose solution before and after infection.

Infection of Mosquitoes

As A. trivittatus are relatively refractory to infection with WEE virus (Green et al. 1980), infection by intrathoracic inoculation was considered the method of choice to evaluate the transmission capabilities of this species. Female mosquitoes were inoculated through the neck membrane with 0.95 ± 0.2 µl quantities of viral suspensions by using a modification of the Rosen and Gubler technique (Rosen and Gubler 1974) as previously outlined (Green et al. 1980). After inoculation, mosquitoes were transferred to 1-pint ice cream cartons and held for transmission trials.

Tests for Transmission of WEE Virus

Two lines of White Leghorn chicks were donated by Dr. Arne Nordskog (Poultry Science Center, Iowa State University) and by Mr. Jack Merikal (Pioneer Hybrid International, Dallas Center, Iowa). The Poultry Science (PS) line, derived from the Pioneer Hybrid (PH) line in 1974, was used to evaluate the transmission of WEE-7738 virus. The PH line was used to evaluate the transmission of WEE-7746 virus.

Individual mosquitoes deprived of sucrose for 24-36 hrs were transferred 8-9 days postinfection to 6 x 1 x 1 inch clear plexiglass cages covered on two sides with wire mesh. Chicks (1-day-old cockerels) were held against the screen until extensive probing or engorgement had occurred. Mosquitoes were frozen at -70° C for subsequent viral titration. Chicks were banded and observed every 12 hrs for 10 days for

signs of illness. Moribund or dead chicks were frozen at -70° C for subsequent viral assay.

The transmission rate was defined as the percentage of infected mosquitoes that transmitted WEE virus to chicks resulting in observable illness. Only mosquitoes that visibly engorged blood were included in these calculations.

Viral Tests

Individual mosquitoes were assayed for virus in Vero cell cultures. Mosquitoes were triturated in 1.0 ml of 1% BA-PBS, pH 7.5. After centrifugation at 2000 rpm for 10 min at 4° C, 10-fold dilutions of the supernatant were prepared in Medium 199 containing 5% fetal calf serum and 0.14% sodium bicarbonate. Quantitites of 0.025 ml of each dilution were inoculated into duplicate wells of microtiter plates containing approximately 5000 Vero cells per well, bringing the total volume per well to 0.1 ml. Cytopathology in duplicate wells after 4-5 days was considered evidence of WEE virus at that dilution.

Brains of moribund or dead chicks were triturated in 2.0 ml of 1% BA-PBS, pH 7.5, and centrifuged at 2000 rpm for 10 min at 4° C. One- to 2-day-old suckling mice were inoculated with 0.025 ml of the supernatant and were monitored 14 days for signs of illness. Virus isolated from this passage was identified by the complement fixation (LBCF) test (U. S. Communicable Disease Center, Atlanta 1965).

RESULTS

Aedes trivittatus were able to transmit both strains of WEE virus used in this experiment. Transmission rates for the two strains were markedly different, even though both strains were mosquito isolates and the site, date of collection and passage history of the isolates were identical (Table 4). Over 50% (16/31) of A. trivittatus infected with WEE-7738 virus transmitted virus to chicks, whereas only 3% (1/37) of A. trivittatus infected with WEE-7746 virus transmitted virus to chicks. This difference was not caused by differences in the multiplication of each strain in A. trivittatus. No significant difference in titer was noted between groups of mosquitoes inoculated with WEE-7738 and WEE-7746 viruses ($t^{66} = 0.665$, $p > 0.5$).

F_1 generation A. trivittatus were quite heterogeneous in their response to both strains of WEE virus following intrathoracic inoculation (Table 4). Individual mosquitoes ranged in titer from undetectable levels to $10^{6.1}$ $TCID_{50}$ (tissue culture mean infective dose) at 8-9 days postinoculation. There was no significant difference in titer between mosquitoes transmitting and those not transmitting virus. The mean viral titer of mosquitoes transmitting WEE-7738 virus was $10^{5.5}$ $TCID_{50}$, although titers ranged from $10^{2.1}$ to $10^{6.1}$ $TCID_{50}$. The single mosquito that transmitted strain 7746 had a titer of $10^{6.1}$ $TCID_{50}$, equal to the highest viral concentration attained in either inoculation treatment group.

Transmission of virus to chicks was indicated in most cases by the

Table 4. Transmission of two WEE viral strains by A. trivittatus 8-9 days after infection by intrathoracic inoculation.

Virus strain	Log_{10} virus inoculated ($SMICLD_{50}$)[a]	Fraction (%) infected	Fraction (%) infected mosquitoes transmitting	Mean (Range) viral titer (mosq.)[b] ($TCID_{50}$)[c]	
				Transmitters	Non-transmitters
7738	2.6	31/39 (79%)	16/31 (52%)	5.5 (2.1-6.1)	5.0 (2.1-5.6)
7746	3.1	37/40 (93%)	1/37 (3%)	6.1	5.1 (2.1-6.1)

[a] Suckling mouse intracerebral mean lethal dose.

[b] Assayed in Vero cell cultures.

[c] Tissue culture mean infective dose.

occurrence of illness compatible with central nervous system disease. A difference in the pattern of illness was observed, however, between the two experimental groups. Chicks becoming ill or dying following the bite of A. trivittatus infected with WEE-7738 virus did so within 3 days; virus was isolated from the brain of 89% (16/18) of all chicks assayed. Chicks becoming ill or dying following the bite of A. trivittatus infected with WEE-7746 virus did so 2-8 days postexposure; virus was recovered from the brain of only 1 of 9 chicks assayed, and that chick died 4 days postexposure.

DISCUSSION

The cause of the significant difference in transmission of WEE viral strains by A. trivittatus is not clear. Several possibilities exist. First, in spite of a common genetic background, breeding patterns of chick lines since 1974 may have selected for a more susceptible or a more refractory population. Chicks of the PS line have been used in histocompatibility studies and were identical at the B locus for blood group antigens; their response may have been more uniform to viral challenge than the PH line used in commercial breeding operations. Second, WEE-7746 virus may be less virulent for White Leghorn chicks. Chicks exposed to mosquitoes infected with the 7746 strain died later in general than those exposed to mosquitoes infected with the 7738 strain; and viral isolation from brain tissue was 78% less successful with the 7746 strain, suggesting that mortality may have been associated with viral multiplication in tissue other than the central nervous system. It is unfortunate that viral assay of other tissues was not done and that blood samples were not taken from surviving chicks of both groups to determine if inapparent infections had occurred. Third, A. trivittatus may respond differently to infection with strain 7738 and 7746, i.e. WEE-7746 virus may not multiply as well as WEE-7738 in salivary gland tissue. Since significant differences in titer were not noted between the two groups of mosquitoes or between mosquitoes transmitting and those not transmitting virus, this hypothesis would imply that a "salivary gland barrier" exists in A. trivittatus (1) that discrimi-

nates between strains of WEE virus and (2) that does not exhibit a viral threshold for infection as demonstrated for the "midgut barrier" (Green et al. 1980).

If it can be established that chick lines PS and PH are equally susceptible to WEE viral challenge, these data suggest that a situation analogous to Venezuelan equine encephalitis (VEE) virus might exist for WEE virus in A. trivittatus. Findings of Kramer and Scherer (1976) showed that laboratory colonies of A. taeniorhynchus and A. aegypti responded differently to epizootic and enzootic strains of VEE virus, another alphavirus. Epizootic strains multiplied to slightly higher titers and were transmitted much more frequently by these epidemic vectors. It was thought that vector competence of these mosquitoes could serve as a marker to distinguish epizootic from enzootic VEE viral strains, in addition to HI subtyping (Young and Johnson 1969) and virulence for equines (Walton et al. 1973). Although the possibility of epizootic and enzootic strains of WEE virus existing in nature has not been investigated, this idea should not be ruled out because parallels to VEE virus do exist. WEE viral strains isolated in the western United States have been shown to produce different responses in mammals (Hardy et al. 1974), birds (J. L. Hardy, School of Public Health, U. C. Berkeley, personal commun.), and mosquitoes (Green et al. 1980); and immunologic characterization of these strains has shown that western strains form predominantly two antigenic groups (Henderson 1964). It would be interesting to determine to which antigenic group WEE viral strains 7738 and 7746 belong. A serological study of these strains

will be published separately.

It is not known if the ability to transmit arboviruses is genetically controlled in mosquitoes. In three leafhopper-plant virus systems, the genetic mechanisms for transmission have been characterized (Storey 1932-33; Black 1943; Nagaraj and Black 1962). Aedes trivittatus may be quite heterogeneous in their ability to transmit WEE virus, and transmission may not depend on the viral concentration in the mosquito. This may be a reflection of the large genetic variability in field populations from which A. trivittatus were collected. Further studies need to determine if there is a genetic basis for the variation in transmission observed in this study, particularly with regard to salivary gland infection.

While comparative vector competence studies using colonized mosquitoes may generate a great deal of interest in the epidemiology of arthropod-borne disease, the response of these mosquito strains to a given arbovirus may not resemble the variable response of natural populations. If it can be established that chick lines PS and PH were equally susceptible to WEE viral strains 7738 and 7746, this report represents the first evidence that field strains of a single mosquito species can vary in their transmission of strains of an arbovirus. The fact that both WEE viral strains were taken simultaneously from the same location is noteworthy.

Fitness for transmission of selected viruses was suggested by Aitken et al. (1977) as a marker for mosquito population differences.

A more revealing marker of population differences might well be fitness for transmission of strains of a single virus. Field populations should be evaluated for each virus-vector system and periodically monitored for changes that may take place in vector competence.

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SUMMARY AND CONCLUSIONS

F_1 progeny obtained from field-collected Aedes trivittatus were evaluated for susceptibility to infection with western equine encephalomyelitis (WEE) virus by intrathoracic inoculation and by oral imbibition of virus-blood suspensions through a membrane. Mosquitoes were uniformly susceptible to infection by intrathoracic inoculation of three strains of WEE virus, but minimum infective doses varied as much as 2,000- to 12,000-fold between strains by membrane feeding. Dose-response data obtained by membrane feeding also indicated that field strains of A. trivittatus were quite heterogeneous in their susceptibility to WEE virus since some individual mosquitoes could be infected by ingestion of low virus concentrations while others could not be infected by a 20,000-fold increase in virus concentration. Moreover, A. trivittatus showed a greater affinity for a WEE viral strain isolated from this species than for a WEE viral strain isolated from Culex tarsalis, even though the site, date of collection, and passage history of these isolates were identical. Field strains of A. trivittatus were relatively refractory to oral infection with WEE virus.

F_1 progeny obtained from field-collected A. trivittatus also were evaluated for their ability to transmit WEE virus after infection by intrathoracic inoculation. Mosquitoes were able to transmit two strains of WEE virus, but transmission rates for the two strains were markedly different. Over 50% of A. trivittatus transmitted a WEE viral strain isolated from this species in contrast to a 3% transmission rate for

a WEE viral strain isolated from C. tarsalis. Aedes trivittatus were quite heterogeneous in their response to both strains of WEE virus; individual mosquitoes ranged in titer from undetectable levels to $10^{6.1}$ $TCID_{50}$ 8-9 days postinoculation. No significant differences in titer were noted between groups of mosquitoes inoculated with different WEE viral strains or between mosquitoes transmitting and those not transmitting WEE virus. Dose-response data suggested that a "salivary gland barrier" may exist for WEE virus in A. trivittatus.

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ACKNOWLEDGMENTS

During my years at Iowa State, invaluable assistance and support came from numerous sources:

Foremost is Dr. Wayne A. Rowley, who introduced me to science as a profession. Though sometimes an elusive spirit, his support, encouragement, and persistence in the face of overwhelming odds only become more admirable as time passes on.

I would like to express my gratitude to Mr. Yau Wong of the State Hygienic Laboratory at the University of Iowa, who not once failed to support a research effort requiring his facilities and his time. The careful attention of Mr. Jim Brinker and Mr. Don Dorsey in this laboratory is also acknowledged.

The efforts of my committee members, Drs. Patricia Gough and George Beran, are duly appreciated. Dr. Gough is thanked for her early guidance at my point of entry into graduate education. Dr. Beran must be commended for his boundless, exhaustive enthusiasm.

My graduate associations have been colorful and fulfilling. I thank Mr. Ron Keith, Mr. Gregg "Jose" Hunt, and Mr. Les "Bush" Shipp for their comraderie and for their interest in medical entomology. My thanks, too, to Jeff and Clare Flosi, whose open house and open icebox provided a welcome haven for reflection, away from departmental stress.

I am indebted to my parents and best friends, Bud and Kay, for

their patience, understanding, and concern. I am unworthy of such devotion.

Best wishes are extended to Sharon Fletcher, Nancy Jo Stansberry, and Monique Darlix for their good humor, good food, good times, and special love.

Finally, I am blessed with the memories of my former "family" on Stanton Street-- Margaret Grove, Beth DeBruin, and Keith Stansberry. As out of a novel, they took me in, warmed my heart, filled my sails, and set me dreaming again.