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Epizootiology and Ecology of Western Equine Encephalomyelitis

Contributor

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Summary

Western equine encephalomyelitis (WEE) is an arthropod-borne virus infection that is confined to the Western Hemisphere and is caused by a WEE complex of four closely related viruses. WEE virus is associated more frequently with disease in animals and humans than are the remaining members of the WEE complex. WEE virus has been isolated from horses, wild birds, mammals, mosquitoes and humans in numerous states west of the Mississippi River. In Canada, the virus has been isolated from all western provinces. WEE virus dissemination rates are dependent upon the ecological dynamics of both mosquito and vertebrate host populations. These mosquito and vertebrate host populations, in turn, are modulated by several environmental factors including ambient temperature, landscape, and the type and volume of the water supply. Strong statistical relationships have been found among snow depth, water content of snow, river runoff, and WEE mosquito vector abundance on the San Joaquin Valley floor in California.

Knowledge of the bird species that are infected frequently with WEE virus in nature can be useful to delineate the time and place of endemic WEE virus persistence and transmission. Sixteen of 21 bird species that were studied in California were competent hosts for WEE virus. The purple finch, white-crowned sparrow, Brewer's blackbird, mourning dove, and house finch were the most competent of the species. These five species would seem to be important amplifying hosts for WEE virus, at least in California. Brewer's blackbirds, song sparrows, red-winged blackbirds, and American robins also could be important in transmission of WEE virus. Mallards, cattle egrets, and Bullock's orioles failed to produce a detectable viremia after having been exposed to several strains of WEE virus.

Extensive studies of the natural transmission of WEE virus in mosquito species were undertaken during the decades of 1940s to early 1970s. WEE virus was recovered from *Culex tarsalis* and *Aedes melanimon*, but not from others members of these two genera. WEE virus infection was found in 26 additional species of mosquito that were members of four additional genera. These species were from at least ten Western States in the United States and three Canadian provinces. *Aedes albifasciatus* (Macquart) was the first mosquito species that was incriminated as a vector of WEE in Argentina, South America. The summer transmission cycle of WEE virus in North America involves the primary enzootic and epizootic vector *Culex tarsalis* and passeriform birds. The *Culex*/passeriform components have been identified repetitively wherever the enzootic and epizootic transmission of WEE virus has occurred. The virus amplification cycle typically involves *Culex tarsalis,* with *Carpodacus mexicanus*, the house finch, and *Passer domesticus*, the nestling house sparrow, as amplifying hosts. Tangential transmission to horses and humans usually begins during summer, if epizootic transmission exceeds minimal thresholds. A summer transmission cycle involving jackrabbits and *Aedes melanimon* has been documented in parallel with the bird/*Culex tarsalis* cycle in the Sacramento Valley of California.

Epizootics and epidemics attributable to WEE virus occurred most frequently in North American agricultural ecosystems west of the Mississippi River drainage during the early 1900s. The number of incident cases of WEE in equines and humans had decreased dramatically by the late 1900s. The decrease in equine incident cases was attributed to a decrease in the size of the population-at-risk, a consequence of mechanization of agriculture, as well as an increase in immunity due to highly efficacious vaccination. However, epizootics of WEE in horses and other species were reported prior to and after WEE vaccines became widely available. An epizootic in horses in eastern North Dakota and northwestern Minnesota was reported during the summer of 1975. The epizootic was associated with extensive flooding of the Red River which created ecological conditions that enhanced the proliferation of *Culex tarsalis*. An epizootic in horses also was reported in Manitoba, Canada during the summer of 1975. Two epizootics of WEE in domesticated poultry flocks have been reported. One epizootic in turkeys was reported in 1993, and another epizootic in turkeys was reported in1957. WEE virus infection was diagnosed in emus in Texas during July 1992. Another epizootic of WEE virus in emus occurred in west-central Oklahoma during August and September, 1992. Each of these epizootics was reviewed in this report.

Geographically atypical WEE has been reported in the eastern US, specifically Florida. In 1978, WEE virus was recovered from *Culiseta melanura*, the primary vector of EEE virus, as well as *Coquillettidia perturbans* and three species of *Aedes*. However, WEE virus in Florida is thought to be weakly pathogenic for horses and humans. WEE virus was reported in a flock of emus in Palm Beach County, Florida in 1993. The WEE virus-positive emus had been imported from California, Louisiana, and Texas, thus raising concerns that an arbovirus can potentially be transported within these hosts from an endemic region to a non-endemic region where there are vectors that are suitable for establishing an active focus of virus.

Acronyms and Glossary

Acronyms

CDC	Centers for Disease Control and Prevention
EEE	eastern equine encephalomyelitis
EIA	enzyme immunoassay
EIP	extrinsic incubation period
HI	hemagglutination inhibition (test)
PRNT	plaque reduction neutralization test
RT-PCR	reverse transcription polymerase chain reaction
SN	serum neutralization (test)
VEE	Venezuelan equine encephalomyelitis
WEE	western equine encephalomyelitis

Glossary

Dead-end host—A species that has no involvement in virus transmission for one or more than one of the following reasons: (1) the species does not have a viremia of sufficient magnitude to infect vectors; (2) the species is not fed upon by vectors; (3) the species is disassociated from virus transmission, both in time and in space.

Diapause—A period of retardation, or suspension of development in some insects.

Ecology—The study of relationships among living organisms and their environment.

Epidemic—An outbreak of disease in humans. The occurrence in a region of cases of an illness, specific health-related behavior, or other health-related events clearly in excess of normal expectancy.

Epizootic—An outbreak of disease in an animal species.

Epizootiology—The study of outbreaks of diseases in an animal species.

Extrinsic incubation period—The period of time required for complete development of an arbovirus within a vector; the interval of time from acquisition of an arbovirus infection by a vector to transmission of the infection by the vector.

Passeriform—Resembling a sparrow in form or structure; *spec.* of, or relating to, the order Passeriformes, which comprises birds with feet adapted for perching, and includes all songbirds.

Riparian—Of, adjacent to, or living on, the bank of a river or, sometimes, of a lake, pond, etc.

Sylvan—An animal, especially a bird, living in or frequenting the woods.

Sympatric—Of, or pertaining to, closely related species of organisms occurring within the same geographic region.

Vernal amplification—Coming, appearing, happening, occurring, etc., in spring.

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Introduction

Western equine encephalomyelitis (WEE) is an arthropod-borne virus infection that is confined to the Western Hemisphere and is caused by a WEE complex of four closely related viruses (Reisen and Monath, 1989). WEE virus is associated more frequently with disease in animals and humans than are the remaining members of the WEE complex (Table 01). WEE virus has been isolated from horses, wild birds, mammals, mosquitoes and humans in California, Washington, North Dakota, South Dakota, Minnesota, Wyoming, Colorado, Nebraska, Montana, New Mexico, Utah, and Kansas. In Canada, the virus has been isolated from the provinces of Manitoba, Saskatchewan, Alberta, and British Columbia.

Table 01. Relationships among members of the western equine encephalomyelitis virus complex.

Virus Name	Primary Vector	Geographical Distribution	Disease	Host	
				Human	Equine
Western equine encephalitis	*Culex tarsalis* ; *Culex occasa*	Western North America Western South America Argentina	Encephalomyelitis	+	+
Y62-33	*Aedes species* *Culisetta melanura*	Former U.S.S.R.	Undetermined		
Highlands J	*Oeciacus vicarious*	Eastern North America	Encephalitis	+	+
Fort Morgan	*Culex univittatus* Other *Culex species* *Culiseta spp*. ; *Culex modestus*	Western North America	None		
Sindbis	*Culex modestus*	Africa, Europe, Asia, New Zealand, Former USSR	Fever, rash, arthritis	+	
Aura	*Aedes spp.*	South America	Undetermined		
From Reisen and Monath, 1989. Revised.					

Ecology of WEE Virus

WEE virus dissemination rates are dependent upon the ecological dynamics of both mosquito and vertebrate host populations, as is the case with all arboviruses. These mosquito and vertebrate host populations, in turn, are modulated by several environmental factors including ambient temperature, landscape, and the type and volume of the water supply.

Ambient Temperature

The period of time required for the development of arboviruses within a vector is the extrinsic incubation period (EIP). This period also is that period of time during which the vector becomes infected with an arbovirus and subsequently becomes infectious to susceptible hosts (Reisen et al., 1993). The EIP is a critical parameter in the epizootiology of arbovirus transmission, because it determines how long an infected vector must survive before it becomes capable of engaging in horizontal transmission of the virus. The combination of EIP, abundance, frequency of blood-meal consumption, and daily survivorship are used to delineate the size of the infectious vector population and the rate of virus transmission.

The ambient temperature alters the length of the EIP in mosquito/arbovirus systems (Hardy et al., 1988). An increase in the temperature will shorten the EIP by enhancing the rate of viral replication and mosquito metabolism. Differences in the EIP of arboviruses in response to temperature result in different rates of virus amplification and in different temporal and geographical patterns of virus distribution. When *Culex tarsalis* Coquillett female mosquitoes that had been infected with WEE virus were incubated at a higher temperature (i.e., 32°C), the infection rate actually decreased as a function of time-after-infection. The decrease in infection rate indicated that some females were able to modulate their viral titer and possibly clear their infections at

these higher temperatures.

The effect of constant ambient temperature on the duration of the EIP of WEE virus was investigated by Reisen (Reisen et al., 1993). High virus-producing strains of *Culex tarsalis* were infected *per os* by allowing them to feed on a mouse brain suspension of virus. The blood-fed mosquitoes were sorted into groups of 30 females and incubated at five different environmental temperatures, each of which was held constant. The different incubation temperatures were 10°, 15°, 20°, 25°, and 30°C. At the interval of 4 to 45 days after infection, 25 females per incubation temperature were evaluated for their ability to transmit WEE virus *per os*. The infection rate was calculated as the number of infected females divided by the total number tested. The dissemination rate was calculated as the number of females with WEE virus-positive legs divided by total number infected. The transmission rate was calculated as the number of females with WEE virus-positive salivary glands divided by the total number infected. The EIP was defined as the number of days from infection until virus was secreted by 50% of the infected females.

At least 92% of the females became infected with WEE virus within 10 days of exposure, regardless of incubation temperature to which they had been exposed. Dissemination of virus to the salivary glands progressed rapidly at 30°C and 25°C; 52% of these infected females were able to transmit virus 4 days after becoming infected, and 60% were able to transmit virus 7 days after becoming infected. At 20°C to 30°C, the transmission rate was maximal at 60% to 71%, within 10 days of infection, or less. At 15°C, the transmission rate was maximal at 68% by 30 days after infection. At 10°C, transmission was not detected until 24 days after infection, and was maximal at 14%. At 15°C to 30°C, the titer of WEE virus in the bodies of *Culex tarsalis* that secreted virus was greater than the titer of WEE virus in the bodies of *Culex tarsalis* that did not secrete virus. Virus titers varied among incubation temperatures, titers being highest at 25°C and lowest at 30°C. The number of days between the first and median points-of-transmission decreased as the incubation temperatures increased (Table 02). The temperature at which the rate of transmission of WEE virus was zero was 10.9°C.

Table 02. Duration of the extrinsic incubation period (EIP) in days when WEE virus was detected at each incubation temperature.

Temperature (°C)	First	Median	Rate
10	33	0.0	0.000
15	7	19.0	0.053
20	7	8.4	0.119
25	4	6.5	0.153
30	4	6.5	0.287

EIP, extrinsic incubation period, is the number of days from infection to first and median points of transmission. From Reisen et al., 1993. Revised.

Landscape, Temporal and Spatial Patterns of WEE Virus

Detailed comprehension of the landscape ecology of a disease is critical in developing effective surveillance and control strategies (Reisen et al., 1995A). Mosquito control agencies in California had monitored the abundance of *Culex spp.* populations, the WEE virus-infection rates in *Culex tarsalis*, and the virus infection rates in sentinel chickens, but the spatial dynamics to aid in the selection of surveillance sampling sites for WEE virus had not been investigated prior to 1990. Thus, the landscape ecology of WEE in the southern Coachella Valley, Riverside County, California was investigated to identify terrain features that are associated with the early-season detection of enzootic WEE virus activity.

The geographical location of the investigation was the southern half of the Coachella Valley, in the area from Palm Springs to the Salton Sea. The fish cultures and duck ponds in the area were concentrated below sea level near the Salton Sea, and they were located on poorly drained, alkaline soils. Large numbers of *Culex tarsalis* emerged usually after the duck ponds had become inundated during late summer. The agricultural production included row crops, dates, citrus, and grapes. The weather during winters was mild; the summers were hot and dry. Rainfall was infrequent during winters, but it increased slightly during summers due to monsoons that originated in the Gulf of Mexico.

Mosquitoes were collected in dry ice-baited CDC traps during March through November and tested for WEE virus using enzyme immunoassay. Virus transmission was monitored using sentinel flocks of white leghorn laying hens placed at 19 different

sites. The serological specimens from chickens were tested for WEE virus antibody with the indirect fluorescent antibody test. The 19 sites were assigned to one of nine different habitat categories, based on the dominant features of the terrain and vegetation of a given habitat. The nine habitat categories were: (1) duck ponds, (2) scrub/row crops (3) grapes/citrus/dates, (4) dates/grape/citrus, (5) saline marsh, (6) irrigated pasture, (7) desert, (8) sewage treatment plant, and (9) residential.

Culex tarsalis abundance varied significantly among habitat categories. Abundance was highest at the duck pond and saline marsh habitats, and it was lowest at the sewage treatment plants. The 19 sites were assigned further to one of three different groups, based on *Culex tarsalis* abundance, temporal patterns, and several other factors. *Culex tarsalis* abundance was highest at the group of sites that were located closest to the Salton Sea and its adjacent agricultural habitat; abundance was lowest in residential areas at distant proximity to the Salton Sea. The expansion of the Salton Sea during winter inundated marginal saline marshes and produced large vernal populations of *Culex tarsalis*. Contrarily, increased evaporation and decreased rainfall shrank the borders of the Salton Sea, dried the adjacent marsh breeding habitats, and decreased the *Culex tarsalis* population during summer. The *Culex* population remained low during fall and winter. *Culex tarsalis* abundance at the second group of sites was maximal during late summer and early fall, and was associated with intentionally flooding the duck ponds in preparation for the fall hunting season. Peak production of *Culex* occurred during the first month after flooding. *Culex tarsalis* at the third group of sites was intermittent, and was the result of focal water mismanagement within the agricultural communities.

Consistent patterns emerged from the spatial and temporal heterogeneity in vector and virus activity. WEE virus transmission was closely related to the dynamics of *Culex tarsalis* populations, and these populations, in turn, were related to creation of natural and human-made larval habitats during spring and late summer. Winter rain, vernal snowmelt, and reduced evaporation increased the depth of the Salton Sea during December through May. Inundation of adjacent, low-lying areas created a belt of saline marsh habitat which produced large populations of *Culex tarsalis*, even with salinity levels in excess of 9,000 parts per million. Increased *Culex tarsalis* abundance was followed by WEE virus amplification.

Landscape and WEE Virus Dissemination

Enzootic activity of WEE virus is detected during most summers in southern California by testing pools of the primary mosquito vector, *Culex tarsalis* Coquillett, for the virus, or by testing the sera of sentinel chickens for antibody (Reisen et al., 1995B). Consistent patterns of virus initiation, amplification, and dissemination in California provide insight into the refugia that were responsible for inter-seasonal persistence of the virus, but the mechanism that promoted inter-seasonal persistence of WEE virus remained obscure up through 1990. An investigation was undertaken to describe these patterns and to examine the patterns of initiation and spread of WEE virus, and to identify possible centers of enzootic WEE virus maintenance.

Virus activity was monitored at 15 different sites by testing pools of host-seeking, *Culex tarsalis* females for virus infection and by sequentially testing blood specimens from flocks of sentinel chickens to detect seroconversion due to WEE virus. The pattern of virus activity was evaluated based on the presence or absence of positive *Culex tarsalis* pools, or positive sentinel chicken sera at each sampling site during each month.

WEE virus was active in Coachella Valley during 1991 and 1992. *Culex tarsalis* mosquito pools were positive for the virus, and sentinel chicken sera were positive for virus antibody. Virus activity was detected for the first time at one of the sites on May 29, 1991, and it had become disseminated to numerous sites by August and September; most positive sites had reverted to a negative status by October. The maximum distance of dissemination of virus among the 15 sites was 16.3 km.

The pattern of WEE virus initiation, amplification, and dissemination was similar during 1991 and 1992. The earlier sites to have become infected were located adjacent to salt marsh habitat along the northern shore of the Salton Sea. The marsh was inundated by saline seepage from the Salton Sea during late winter and early spring, and it yielded increased vernal populations of *Culex tarsalis*. After amplification at the early sites, the virus underwent dissemination into an adjacent floodplain. The virus was never detected at those sites which were located more than 80 meters above sea level and situated in sandy, well-drained soils.

The association of arbovirus activity with specific foci had been demonstrated previously for other mosquito-borne encephalitis viruses, usually because of specific habitat requirements of the vector. Foci of eastern equine encephalomyelitis usually had been associated with bogs within

deciduous forest, the preferred habitat for *Culiseta melanura*, the primary enzootic vector. *Culex tarsalis* had been shown typically to seek hosts that were located along riparian vegetation; the distribution of human and equine cases had been associated with irrigated agricultural valleys along riparian habitats.

The repetitive appearance of early-season activity of WEE virus at one site indicated that the virus may be reintroduced consistently or that it may persist in the salt marsh habitat. Consistent reintroduction of virus from the south (i.e., Mexico) was thought to be an unlikely mechanism to explain this pattern because the virus appears earlier in Coachella Valley than Imperial Valley, the former of which is located farther north from Mexico. Also, most south-to-north bird migrations are completed by later spring, and completion of these northwardly migrations precedes the initial appearance of WEE virus activity. Thus, over-wintering along the margin of the Salton Sea may be the mechanism behind consistent vernal detection of WEE virus, but the exact reason for the consistency is unclear. The evidence to support an over-wintering mechanism through continuous, low-level horizontal transmission of WEE virus among *Culex tarsalis* and wild birds includes repeatable detection of infected *Culex tarsalis* during winter, repeatable detection of sentinel chicken seroconversions during winter, and early-season detection of virus near the latest positive site during the previous season.

The dissemination of WEE virus along the shore of the Salton Sea to the remainder of the Coachella Valley proceeded gradually. The rate was less than 1.3 kilometers per day. Dissemination was rarely beyond 18 kilometers to adjacent sites. The gradual spread of virus was attributed to infected host-seeking *Culex tarsalis*, because marked-and-released, host-seeking females were recaptured within 3 days, after undergoing dispersal from a site near the salt marsh habitat to several distant sites.

Weather

Weather affects the transmission of WEE virus by altering the quality and quantity of habitats for mosquito larvae, and these alterations lead to changes in abundance of adult females (Wegbreit and Reisen, 2000). Historically, the intensity of WEE activity in central California had been related to snowpack depth in the Sierra Nevada, vernal temperature, and runoff from the Kern River. These associations had been described in a qualitative manner during an abbreviated period of time, using mosquito population estimates that had been measured by resting counts, a sampling method that became obsolete eventually. Attempts to develop a quantitative approach to identify weather variables associated with changes in mosquito abundance were undertaken using data from years 1990-1998 (Wegbreit and Reisen, 2000). It was suggested that the identification and quantification of the weather factors that drive virus transmission would promote earlier interventions prior to virus amplification that exceeded the thresholds that are necessary for human infection to occur. Thus, the working hypothesis was investigated that winter snow-pack in the Sierra Nevada would be a useful predictor of: (1) mosquito abundance and (2) enzootic WEE virus activity during the following summer.

Mosquito abundance data were obtained from the Kern Mosquito and Vector Control District. Impaired runoff is a measure of actual river flow onto the San Joaquin Valley floor after removal of a portion of the river flow by impoundments such as irrigation canals. The snow depth and the water content of snow both represent a single measurement of the amount of water that has accumulated in snow. Data about precipitation, impaired runoff from the Kern River, snow depth and the water content of snow were obtained from the California Department of Water Resources.

Strong relationships were found among snow depth, water content of snow, river runoff, and mosquito abundance on the San Joaquin Valley floor. The relationship between impaired runoff of the Kern River and mosquito abundance was significant ($p < 0.001$); 56% of the variability among abundance of host-seeking mosquitoes was explained by impaired runoff. High runoff was associated with an abundance of *Culex tarsalis*, and low runoff was associated with a paucity of *Culex tarsalis*. The annual snow depth and the water content of snow were related significantly to total annual impaired runoff of the Kern River. The water content of the snow, when the snow was at its maximum level, explained 70% of the variability among the average number of *Culex tarsalis* per CDC trap-night during the subsequent summer (Figure 01).

WEE activity was not detected in Kern County during the 11-year period from 1984 to 1995. These 11 years include a period of six consecutive years of drought. During the summer of 1995, the runoff increased substantially beyond the runoff during preceding years and exceeded 150,000 acre-feet per month; the abundance of *Culex tarsalis* was highest during autumn 1995. During 1996, the number of sentinel chickens that underwent seroconversion, and the number of WEE virus isolates from mosquito pools increased. There were 34 seroconversions in sentinel chickens; three isolations of WEE virus were made from mosquito pools (Table 03). During 1996, 1997, and 1998, the snowpack and runoff increased, as did the

abundance of *Culex tarsalis* and WEE activity. During 1999, maximum water content of snow and runoff of the Kern River were 38% and 30% of the 1998 values, respectively. WEE virus was not detected during 1999. These quantifiable relationships among weather variables and mosquito abundance provide an early warning of enzootic activity and increased risk of human and equine infection.

Figure 01. Maximum water content of the snowpack in the Sierra Nevadas versus abundance of *Culex tarsalis* mosquitoes. GM = geometric mean. From Wegbreit and Reisen, 2000. Revised.

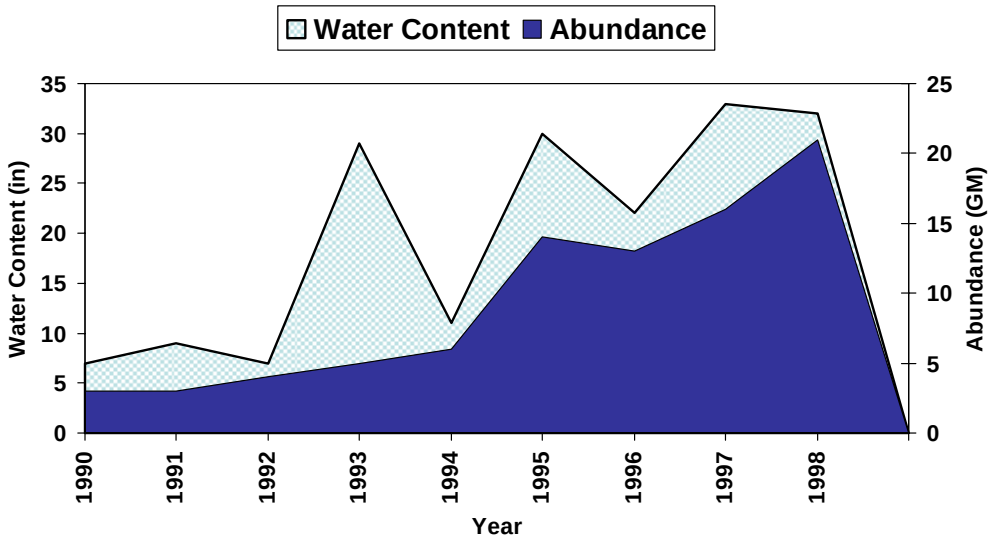


Table 03. Western equine encephalomyelitis virus activity in mosquitoes and sentinel chickens in Kern County, California, years 1990 to 1998.

		Year			
	Number	1990-1995†	1996	1997	1998
Mosquito pools	WEE virus isolates	0	3	0	42
	Tested	1667	168	598	770
Chicken flocks	Chickens Seroconverted	0	34	2	27
	Flocks Tested (#)	7 to 12	9	9	9

†Total number of mosquito pools and number of sentinel chicken flocks sampled annually, years 1990-1995**.** From Wegbreit and Reisen, 2000. Revised.

Host Preferences

The identification of the species-of-origin of a mosquito's bloodmeal is an important tool that has been used to identify those vertebrates that may serve as hosts of arboviruses. *Culex tarsalis* in the Sacramento Valley had been shown to be a catholic feeder, taking 74% of its feedings from birds and 26% of its feedings from mammals. These early observations were extended during the 1960s, after specific diagnostic reagents became available for bloodmeal identification. *Culex tarsalis* was shown using these reagents to feed 75% of the time on birds and 25% of the time on mammals (Table 04). Because feeding patterns alone were not necessarily indicative of the ability of a species of bird to serve as a competent host of WEE virus, additional investigations of host competence were undertaken to identify competent avian hosts.

Table 04. Feeding patterns of *Culex tarsalis* and *Aedes melanimon* in Sacramento Valley, California, years 1969 to 1974.			
		Percent of Bloodmeals Taken From Host	
Group	**Host Animal**	*Culex tarsalis* †	*Aedes melanimon* ♦
Birds	Passerine	33	<1
	Dove	30	<1
	Pheasant and Quail	6	<1
	Others	5	<1
	Total Birds	**75**	**1**
Mammals	Rabbit	9	43
	Cattle	7	28
	Deer	4	14
	Sheep	<1	8
	Dog	<1	2
	Pig	<1	1
	Horse	<1	1
	Others	1	4
	Total Mammals	**25**	**99**
* Data are from the University of California Berkeley Arbovirus Research Program. †Percent of 4,797 bloodmeals. ♦Percent of 709 bloodmeals. From Hardy, 1987. Revised.			

Wild Birds as Hosts

Reisen et al. proposed that knowledge of the bird species that are infected frequently in nature could be used to delineate the time and place of endemic WEE virus persistence and transmission, and could be used to provide clues to the epidemiology of newly introduced and newly emerging arboviruses (Reisen et al., 2003). The accurate interpretation of prevalence of infection in wild birds and understanding the roles of different bird species in transmission requires knowledge of the responses of these species to viral infection, including susceptibility to infection, viremia, and immunity. Antibody-positive birds were presumed to have been susceptible to WEE virus and were presumed to have been infected at least once during their lifetime; however, the presence of antibody did not necessarily indicate that the infected bird species produced viremia at levels that would be sufficient to infect vector mosquitoes. For example, adult chickens had been shown to produce detectable viremia only rarely; thus, chickens had been categorized as appropriate sentinels for WEE virus, but they were not considered to be competent hosts for the virus. It was concluded from this that some species of wild birds may yield the same host response to WEE virus infection as chickens.

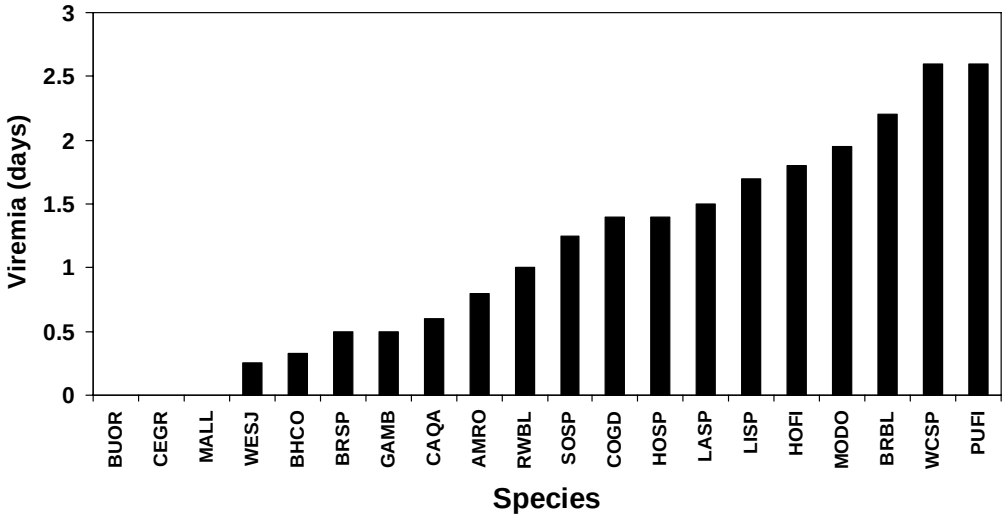
The failure to detect WEE virus antibody in wild birds of a given species suggested that the species was not susceptible to WEE virus and was not a competent host. That conclusion may have been invalid for several reasons: (1) the species may have been highly susceptible to infection, but it may have died rapidly, and would not have been accessible for sampling; (2) the species may have been refractory to infection; (3) the species may have been susceptible to infection, but responded with short-lived antibody that was undetectable; (4) the species was susceptible to infection, but was never exposed to virus-infected vectors because they were absent when the vectors were most active (e.g., the bird species was a winter resident only).

Experimental studies are required to distinguish birds that are negative for WEE virus antibody due to being refractory to infection, or birds that have rapidly decaying antibody, from those birds that are susceptible to infection, but have never been exposed to infectious vectors of WEE virus. Experimental investigations of the competence of various avian species for WEE virus were undertaken by Reisen et al. (2003). The birds were infected with sympatric WEE virus strains that had been isolated during recent enzootic and/or epidemic activity. All strains had produced viremia in experimentally infected house finches. Birds of each species were infected by subcutaneous inoculation of virus suspension containing 100 plaque-forming units of WEE virus. Blood specimens were collected on days 1 through 5, after they had been inoculated with WEE virus, to monitor the birds for viremia using plaque assay on Vero cells. Specimens were collected during weeks 1 through 6 to monitor antibody production using enzyme immunoassay (EIA) and plaque reduction neutralization assay (PRNT). Tissues collected during necropsy were screened for WEE virus RNA by using reverse transcription polymerase chain reaction (RT-PCR).

A total of 347 birds assigned to 27 different species and four orders were inoculated with strains of WEE. Approximately 63% of the birds developed viremia, and approximately 73% developed virus-specific antibody that was detectable using EIA and/or PRNT. Approximately 94% of the birds tolerated infection and survived more than two weeks post-inoculation. Most mortality that did occur was attributed to causes other than WEE virus (e.g., aggression). WEE viremia peaked on day 1 post-inoculation, began to decrease on day 2, and frequently was undetectable by days 3, 4, and 5. Most bird species developed viremia that exceeded the minimum threshold for infection of susceptible *Culex tarsalis*. All birds that became viremic also developed antibody, with the exception of 4 birds. Some birds developed measurable antibody, but viremia was not detectable (n=42; 12%).

Sixteen of 21 bird species were competent hosts for WEE virus. The purple finch, white-crowned sparrow, Brewer's blackbird, mourning dove, and house finch were the most competent of the 21 species (Figure 02). These five species would seem to be important amplifying hosts for WEE virus, at least in California. Only the mallards, cattle egrets, and Bullock's orioles failed to produce a detectable viremia after having been exposed to several strains of WEE virus. Song sparrows, red-winged blackbirds, and American robins also could be important in transmission of WEE virus because they were abundant, they were found in areas studied, and they were competent experimental hosts. During widespread epizootic WEE virus transmission, a large number of bird species could become infected and amplify virus.

Figure 02. Host competence index for species of California birds for WEE virus. Abbreviations are U.S. Geological Survey abbreviations. Refer to Appendix Table 01 for abbreviations. From Reisen et al., 2003. Revised.



Chronic infections in wild birds have been considered among the possible mechanisms responsible for the localized persistence and dispersal of WEE virus and other encephalitic viruses. Persistence and dispersal of virus over long distances was thought to be dependent upon the establishment of persistent or chronic infections that relapse later.

Mosquito Vectors

Culex tarsalis* and *Aedes melanimon

The definitive evidence of the involvement of *Culex tarsalis* in the natural transmission cycle of WEE virus was provided in 1941 (Hardy, 1987). The virus was isolated repeatedly from *Culex tarsalis*, but not from other hematophagous arthropods. Experimentally, *Culex tarsalis* became infected after it had been fed a suspension of WEE virus, and the virus was transmitted subsequently to chickens and

guinea pigs. Extensive studies of the natural transmission of WEE virus in mosquito species were undertaken during the decades of 1940s to early 1970s. The virus was recovered from *Culex tarsalis* and *Aedes melanimon*, but not from other members of these two genera (Table 05).

Table 05. Species of field-collected mosquitoes tested for WEE virus and number of virus isolations from the mosquitoes, Sacramento Valley, years 1969 to 1974.*

Species	Number Tested	Number of Pools	Number of Virus Isolations
Culex tarsalis	145,549	4,227	35
Culex peus	9,595	251	0
Culex pipiens	2,801	87	0
Culex erythrothorax	8,155	146	0
Aedes melanimon	85,397	996	33
Aedes nigromaculis	15,834	232	0
Aedes vexans	14,048	241	0
Culiseta incidens	950	100	0
Culiseta inornata	1,915	148	0
Anopholes freeborni	75,776	1,054	1
Miscellaneous species †	10,996	326	0

*Data from the University of California Berkeley Arbovirus Research Program. †Includes *Aedes increpitus, Aedes sierrensis, Aedes stricticus, Anopheles franciscanis, Anopheles punctipennis, Culex apicalis, Culex peus-thriambus, Culiseta particeps, and Mansonia perturbans*. From Hardy, 1987.

WEE virus activity decreased during much of 1970s, but returned to higher levels during the 1980s, allowing studies of mosquito vectors to be resumed. Both *Culex tarsalis* and *Aedes melanimon* were collected from riparian habitat, and temporally speaking, WEE virus was detected in *Culex tarsalis* about six weeks prior to detection in *Aedes melanimon* (Table 06). These studies confirmed that only two species of mosquitoes were involved in the summer transmission cycle of WEE virus in the Central Valley of California.

Table 06. WEE virus isolations from *Culex tarsalis* and *Aedes melanimon* by month of collection, Kern County, California, 1983.*

	Culex tarsalis		*Aedes melanimon*	
Month	**Number Tested**	**Number of Virus Isolations**	**Number Tested**	**Number of Virus Isolations**
May	4,396	0	2,140	0
June	4,931	2	978	0
July	2,143	11	1,732	0
August	4,300	23	1,633	8
September	5,300	13	1,220	5

*Includes only collections made at riparian and semi-riparian habitats where both mosquito species were present. From Hardy, 1987. Revised.

Aedes dorsalis in California

Although it had been well-established that *Culex tarsalis* was the primary mosquito vector of WEE virus in California, the vector *Aedes dorsalis* also had been shown to be capable of maintaining and amplifying WEE virus in a mammalian cycle (i.e., horizontal transmission) elsewhere in North America (Hardy, 1987). Subsequently, WEE virus was isolated from *Aedes dorsalis* that had been reared from their immature age (Fulhorst, 1994). The immature mosquitoes had been collected during two consecutive summers from a salt marsh habitat at Morro Bay estuary. These isolations from immature larvae were unique in that they were the first isolates of WEE virus from *Aedes dorsalis* in California, they were the first indication of trans-generational transmission of WEE virus by a mosquito, and it was a rare detection of WEE virus activity in coastal California. Transgenerational transmission of WEE virus by an *Aedes* species that engaged in oviposition of drought-resistant eggs could have

provided an effective mechanism for WEE virus persistence between episodes of horizontal transmission. This mechanism of transgenerational transmission had been documented for California encephalitis virus within *Aedes melanimon* populations in the Central Valley of California (Reisen et al., 1990). However, after the earlier successes, subsequent attempts to isolate WEE virus from *Aedes dorsalis* from Morro Bay estuary were largely unsuccessful. Thus, further investigations of the potential role of *Aedes dorsalis* in vertical and horizontal transmission of WEE virus were undertaken in the Sonoran Desert of southeastern California (Reisen et al., 1998).

The bases of these investigations were as follows: (1) WEE virus could be maintained between periods of enzootic activity within infected dormant eggs of *Aedes dorsalis;* (2) during wet years, as the Salton Sea would rise, these dormant eggs would hatch and produce an infected cohort of *Aedes dorsalis* that would amplify WEE virus horizontally within an *Aedes*/rabbit transmission cycle; finally, (3) the *Aedes*/rabbit transmission cycle would expand to the primary WEE virus transmission cycle, the *Culex tarsalis*/bird cycle. Immature mosquitoes were collected using a dipping procedure, they were transported to a laboratory, then reared to the adult stage. Flocks of sentinel chickens and rabbits were deployed at sites throughout the study area in the Coachella Valley. WEE virus was detected during 1995 only, based on seroconversion in the sentinel chickens that had been placed both within and outside the study area, the area where *Aedes dorsalis* resided. WEE virus was not detected during years 1994 and 1996 in the sentinel chickens, nor was it detected during either of the three years 1994–1996 in the sentinel rabbits.

Although additional specimens of *Aedes dorsalis* were tested, WEE virus was not isolated. It was concluded that *Aedes dorsalis* was not essential for maintenance or amplification of WEE virus in southeastern California, because enzootic activity occurred in areas where and when *Aedes dorsalis* was collected rarely, if at all.

WEE Virus Vectors in Argentina

Traditionally, four criteria have been used to incriminate a mosquito species as a vector of WEE virus. These criteria are: (1) isolation of virus from wild-caught mosquito specimens, (2) demonstration of the ability of the mosquito species to become infected by feeding upon a viremic host, (3) demonstration of competence of the mosquito species to transmit WEE virus by bite, and (4) field evidence to confirm the association of the infected arthropod with the vertebrate population in which the infection is occurring. *Aedes albifasciatus* (Macquart) had received attention as a potential vector of WEE virus in Argentina. WEE virus had been isolated from *Aedes albifasciatus* (Macquart) during an epizootic in Santa Fe Province, Argentina in 1982–1983, and the mosquito had been shown to be susceptible to WEE virus infection via the oral route. The close association between the equine population and *Aedes albifasciatus* (Macquart) had been observed; the mosquito was an aggressive biter of equines as well as an avid feeder on humans. The success of *Aedes albifasciatus* (Macquart) at transmitting WEE virus by bite was evaluated by Aviles et al. (1992). The mosquitoes were infected by allowing them to feed on chicks that were 5 to 12 days old; WEE viremia had been induced in the chicks prior to the feedings. Transmission attempts were made after 9, 10, 15, and 16 days of incubation by allowing mosquitoes to re-feed on susceptible chicks that were 0.5 to six days old. Blood specimens were taken from the chicks 38 hours after being fed upon to test for WEE virus; these same chicks also were tested for WEE virus antibodies within 24 to 26 days of being fed upon. *Aedes albifasciatus* (Macquart) females collected in the vicinity of Cordoba, Argentina, were highly susceptible to WEE virus when allowed to feed on viremic chicks. Further transmission of WEE virus by bite from these females to chicks also was successful. Thus, *Aedes albifasciatus* (Macquart) was the first mosquito species that was incriminated as a vector of WEE in South America.

Other North American Mosquito Vectors

Mosquito species other than the two primary species, *Culex tarsalis* and *Aedes melanimon,* have been shown to be infected with WEE virus in North America. WEE virus infection was found in 26 additional species of mosquito that were members of four additional genera (Table 07). These species were from at least ten Western States in the United States and three Canadian provinces.

Summer Transmission Cycle of WEE Virus

The summer transmission cycle of WEE virus in North America involves the primary enzootic and epizootic vector *Culex tarsalis* and passeriform birds. The *Culex*/passeriform components have been identified repetitively wherever the enzootic and epizootic transmission of WEE virus has occurred. The transmission cycle consists of three temporal components: (1) vernal amplification, (2) summer maintenance, and (3) autumnal subsidence. The mechanism by which WEE virus persists between transmission seasons and is introduced into the amplification cycle is cryptic, although it has been studied intensely for years. At temperate latitudes,

Table 07. Mosquito species in North America that were infected naturally with WEE virus.			
		Geographical Location	
Genus	**Number of Species**	**Number of US States**	**Number of Canadian Provinces**
Aedes	13	8	2
Anopheles	03	2	1
Culex	06	4	1 to 3
Culiseta	01	1	2
Coquilletidia	01	None	2
Psorophora	03	5	None
From Reisen and Monath, 1989. Revised.			

WEE virus is recovered initially from *Culex tarsalis* and/or passerine birds. WEE virus isolations from nestling birds in Colorado and Texas frequently precede the isolation of virus from *Culex tarsalis.* Thus, it has been proposed that the virus was introduced into the *Culex tarsalis*/nestling bird amplification cycle from an unidentifiable sylvan source by species of mosquitoes other than *Culex tarsalis*. Others have postulated that the infection of peri-domestic passerines is the result of spillover of virus from a reservoir blackbird/*Culex tarsalis* cycle. However, virus has been detected in ground squirrels and snowshoe hares in Canada prior to termination of diapause by *Culex tarsalis,* and prior to the initiation of nesting activity by birds. This finding indicates that a mammalian transmission cycle involving early spring mosquitoes such as *Culiseta inornata* or several *Aedes* species may occur at northern latitudes.

The virus amplification cycle typically involves *Culex tarsalis,* with *Carpodacus mexicanus*, the house finch, and *Passer domesticus*, the nestling house sparrow, as amplifying hosts (Figure 03). Virus infection in peri-domestic passerines typically occurs prior to the involvement of peri-domestic columbiforms and domestic galliforms, both of which may contribute to virus amplification. WEE virus transmission among birds (both adults and fledglings) and *Culex tarsalis* continues during summer. Other ornithophilic mosquitoes such as *Culex erythrothorax, Culex pipiens, Culex quinquefasciatus*, and *Culex peux* also may become infected. If favorable ecological conditions persist, the virus may infect a wide variety of vertebrates, including other groups of birds, large and small feral and domestic mammals, and reptiles and amphibians, all of which are alternate or dead-end hosts. The infection of dead-end hosts occurs coincidentally with a seasonal shift in the host feeding patterns of the primary vector, *Culex tarsalis*. The shift in the host feeding pattern has been related to the fledging of most nestlings by midsummer and/or to the low tolerance of birds in comparison with mammals to increasing attacks by mosquitoes during summer. Infection in mammals has occurred concurrently with the recovery of virus from mammalophilic mosquitoes, including *Aedes*, *Culiseta*, and *Psorophora*.

A summer transmission cycle involving jackrabbits and *Aedes melanimon* has been documented in parallel with the bird/*Culex tarsalis* cycle in the Sacramento Valley of California (Figure 03). The *Aedes melanimon*/jackrabbit cycle may persist in desert environments where ecological conditions do not permit the establishment of large *Culex tarsalis* populations. An epizootic involving *Aedes dorsalis* and jackrabbits occurred in Utah in the absence of *Culex tarsalis*. More than 97 percent of the mosquito specimens collected was *Aedes dorsalis*. WEE virus was isolated from many of the *Aedes dorsalis* specimens, but it was not isolated from the *Culex tarsalis* specimens. Similarly, WEE viral amplification by *Aedes campestris* and *Aedes dorsalis* may have lead to an epizootic in New Mexico. The number of isolates from the large population of *Aedes campestris* exceeded the isolations from *Culex tarsalis.* Tangential transmission to horses and humans usually begins during summer, if epizootic transmission exceeds minimal thresholds.

The detection of WEE viral infection in vector mosquitoes and vertebrates subsides during autumn with the onset of colder ambient temperatures and fewer daylight hours. *Culex tarsalis* populations undergo bifurcation into: (1) a non-overwintering host-seeking component and (2) an overwintering/diapausing component. The non-overwintering host-seeking and potentially infectious component gradually decreases in abundance during fall, leading to a coincidental decrease in the numbers of new vertebrate infections. Few human infections have been contracted after and even during October.

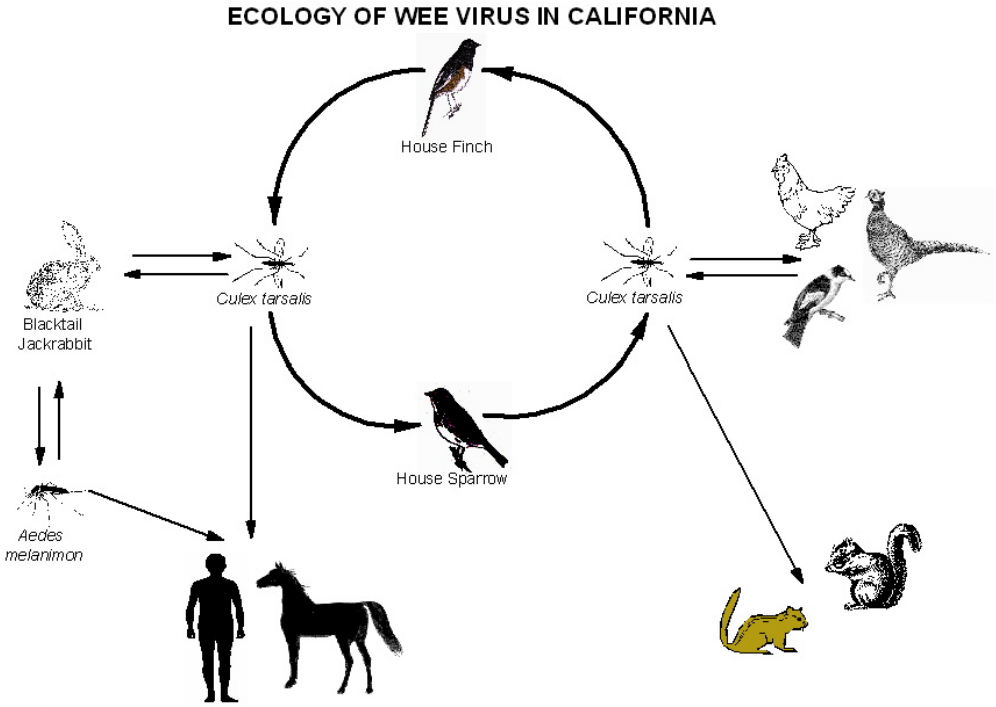


Figure 03. Summer transmission cycle of WEE virus in Central Valley of California. From Hardy, 1987. Revised.

Epizootiology of WEE Virus

Epizootics and epidemics attributable to WEE virus have occurred most frequently in North American agricultural ecosystems west of the Mississippi River drainage and in association with the distribution and abundance of the primary vector, *Culex tarsalis*. Epizootics were reported in Kern County California, 1952; Hale County, Texas,1965; and Manitoba Province, Canada, 1981. The number of equine and/or human cases was correlated directly with abundance of *Culex tarsalis* (Figure 04) (Figure 05). The relationship between *Culex tarsalis* abundance and western encephalomyelitis cases in humans was similar to that of horses (Appendix Figures 01, 02, 03, 04). As few as 450 and as many as 3,258 clinically diagnosed cases of WEE in equines occurred annually in the U.S. during years 1955 to 1969; laboratory diagnosed cases were reported thereafter, and the number of laboratory diagnosed cases was 20 to 650 (Figure 06). The annual distribution of human cases was similar to that of horses; an average of 34 confirmed cases in humans occurred annually in the U.S. during the same years 1955 to 1984 (Appendix Figure 05). The number of incident cases of WEE in equines and humans had decreased dramatically by the late 1900s. The decrease in equine incident cases has been attributed to a decrease in the size of the population-at-risk, a consequence of mechanization of agriculture, as well as an increase in immunity due to highly efficacious vaccination (Appendix Figure 06). According to a national survey of U.S. horse operations, 46% to 63% of the operations were vaccinating all or some resident horses against encephalitis in 1998 (Figure 07).

Geographically speaking, approximately 53% to 73% of the operations in the four geographical regions were vaccinating all or some horses against encephalitis (Figure 08). However, several epizootics of WEE in horses and other species were reported prior to and after WEE vaccines became widely available.

WEE Epizootic in Horses, Northern Red River Valley, 1975

During the early summer of 1975, eastern North Dakota and northwestern Minnesota were flooded by the Red River. The extensive areas of standing water provided conditions that were favorable for proliferation of large numbers of *Culex tarsalis* mosquitoes, the principal vector of WEE.

Figure 04. Temporal relationship between *Culex tarsalis* abundance and cases of WEE in horses, Kern County, California, 1952. From Reisen and Monath, 1989. Revised.

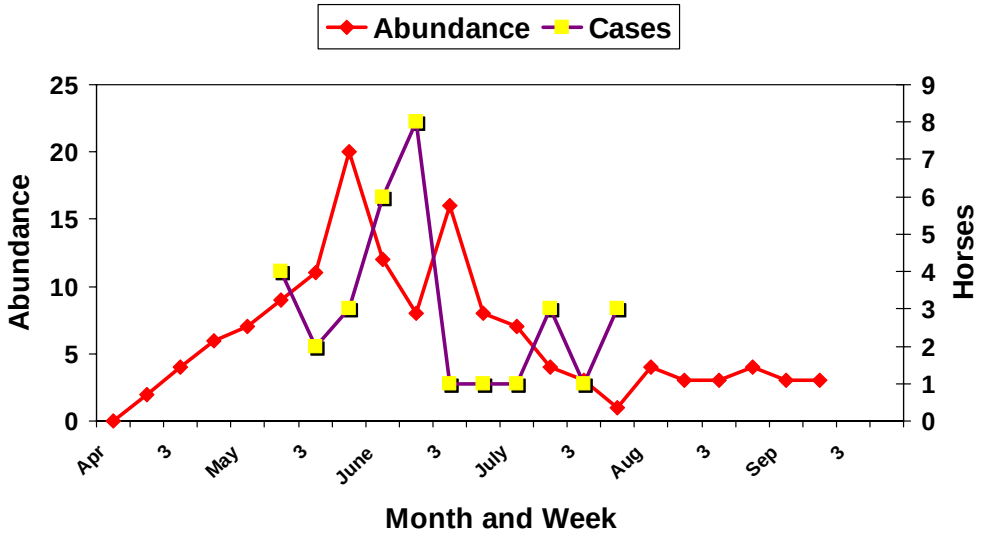


Figure 05. Temporal relationship between *Culex tarsalis* abundance and cases of WEE in horses, Manitoba, Canada, 1981. From Reisen and Monath, 1989. Revised.

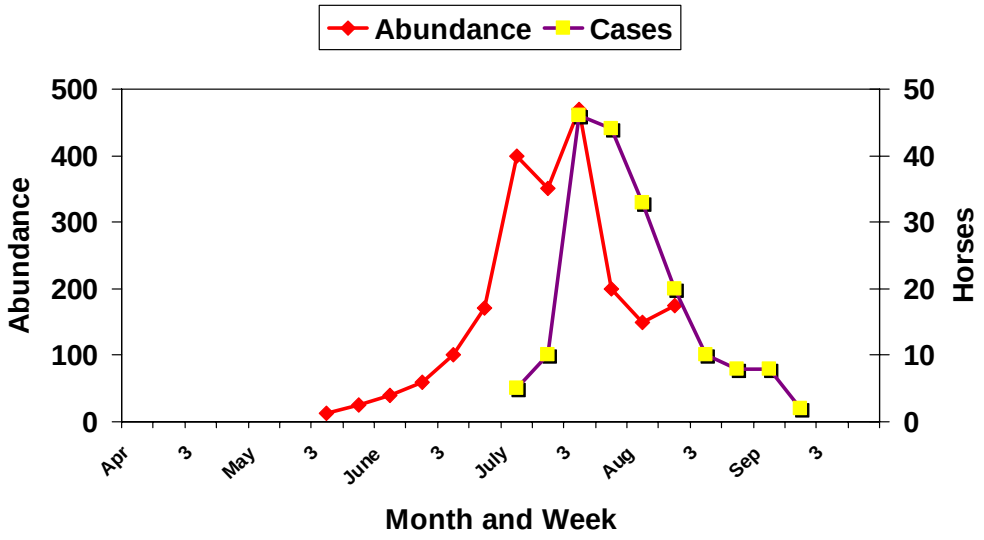


Figure 06. Annual distribution of incident cases of WEE virus infection in horses in the United States during years 1955 to 1976. Cross-hatched area represents laboratory-diagnosed cases only. From Reisen and Monath, 1989. Revised.

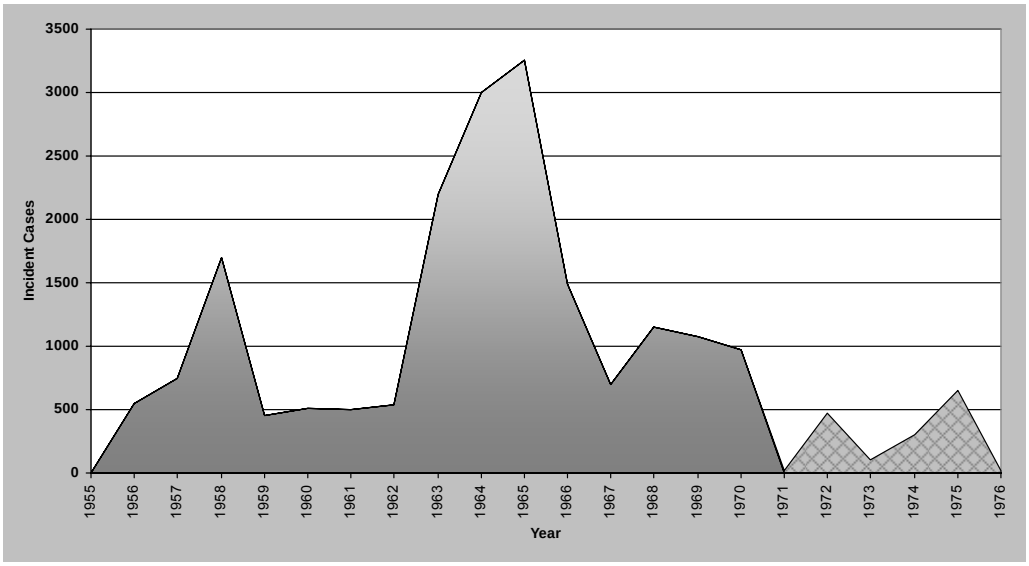


Figure 07. Percent of United States operations vaccinating all or some resident horses against encephalitis by age class/type category. From USDA:APHIS:Veterinary Services, 1999. Revised.

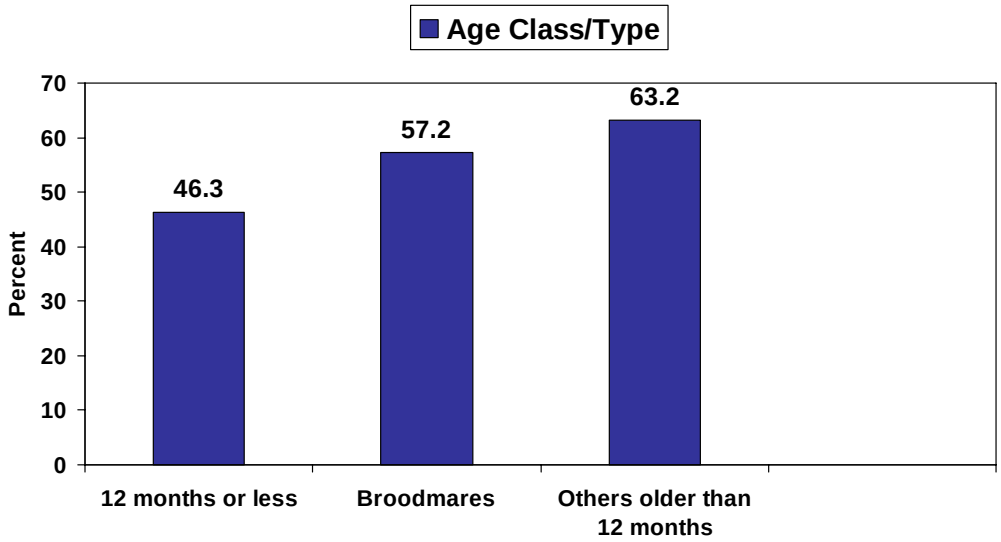
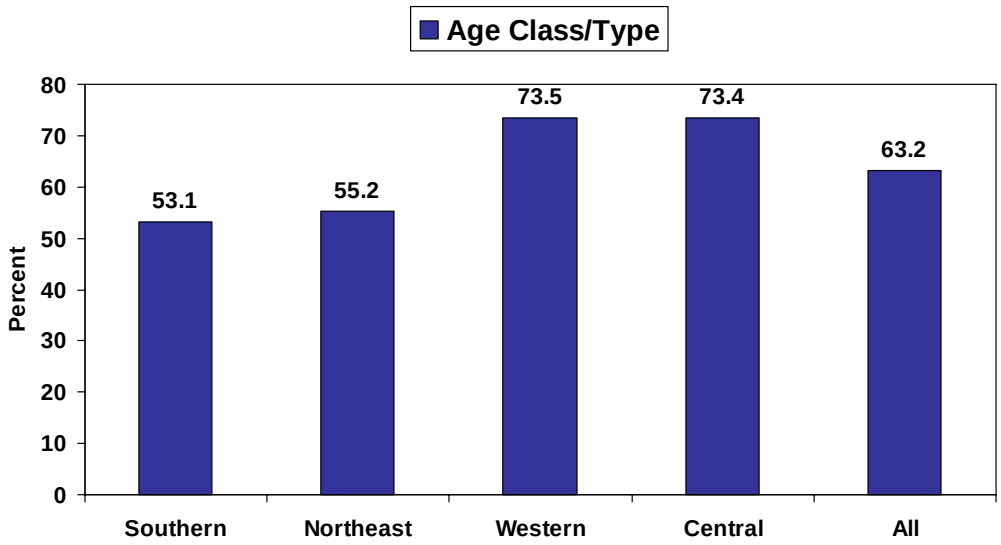


Figure 08. Percent of United States operations by geographical region vaccinating resident horses against encephalitis. "Operations" refers to those operations that had horses (other than broodmares) more than 12 months of age. From USDA:APHIS:Veterinary Services, 1999.

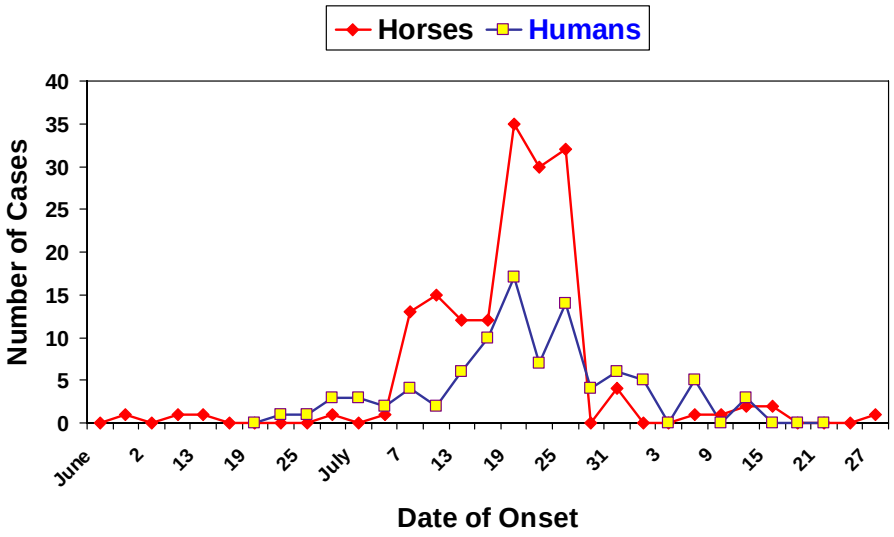


WEE virus was isolated during July from mosquitoes collected within the flood zone. Because clinical signs of WEE become apparent in horses prior to humans, surveillance of the disease in horses was initiated. Surveillance of central nervous system disease in the human population within the flood zone was amplified by the State departments of health and the Centers for Disease Control and Prevention.

Veterinarians from 61 of 65 equine practices were contacted by telephone by members of the surveillance team. Twenty-four of the 61 practices were from a 28-county area in eastern North Dakota. The remaining 37 practices were from a 34-county area of western Minnesota. Each practice was contacted via telephone an average of four times during the four-week period July 25 to August 19, 1975 to inquire about diagnosis of WEE in horses in 1975. During the first interview, information was solicited from each veterinarian about: (1) the approximate number of horses in the practice, (2) the number of horses vaccinated annually, (3) the number of horses vaccinated during the current year, (4) the number of WEE cases expected each year, and (5) the temporal distribution of the WEE cases. A case of WEE was defined as a horse that was diagnosed with WEE by an equine veterinarian who participated in the surveillance effort. During the subsequent interviews, data about the recent cases were collected. Laboratory supplies were distributed to facilitate collection of blood specimens for serological evaluation. On August 22, 1975, an initial request was made to all veterinarians to record cases of WEE for approximately five weeks, through September 30, 1975. Additional requests to these veterinarians were made, if necessary. The owners of 90 clinically normal horses in Richland County, North Dakota were interviewed two weeks after the last equine case of WEE had been reported from the county to determine if their horses had been vaccinated. Blood specimens were collected from the horses in Richland County that had not been vaccinated during the preceding two years. The laboratory specimens were examined for antibodies against WEE, EEE, and VEE using hemagglutination inhibition (HI) and serum neutralization (SN) tests. The temporal distribution of equine cases and human cases was compared.

There were 267 cases of WEE reported by 61 veterinarians during the telephone surveillance. The 22 veterinarians who completed and returned surveillance logs reported 14 additional cases. Thus, the total number of WEE cases reported from June through September, 1975 was 281 (Figure 09). The normal number of equine cases during June to September was estimated to be 120. The incident cases in horses were at their peak during the three-day period of July 28–31, 1975, when 36 cases were reported.

Figure 09. Epizootic and epidemic curves of 281 cases of western encephalomyelitis in horses and 39 cases in humans, Northern Red River Valley, June to September, 1975. From Potter et al., 1977. Revised.



The seroprevalence of WEE in the 281 horses with clinically diagnosed WEE was 23 percent (65 of 281 horses), based on presence of HI and SN antibody. The specimens were not positive for EEE, or for VEE. The case fatality, based on naturally occurring deaths or euthanasia, was at least 6.4% (18 of 281 horses); complete information on all horse deaths was not available. More than one case per premises was rare. Of the 281 clinically diagnosed cases of WEE, 90.3% (n=254) had not been vaccinated in 1975, 16 had been vaccinated, and the vaccination status of 11 was undeterminable. The number of days between vaccination and onset of clinical signs in the 16 cases that had been vaccinated was calculated to determine if there was inadequate time for development of immunity in vaccinated horses. The vaccine had been administered within three days of onset in eight cases, five days of onset in one case, and eight days of onset in one case; the date of vaccination was unknown for the remaining six cases. The short duration between vaccination and onset of clinical signs suggest that the horses may already have undergone natural exposure to WEE virus prior to vaccination, not that immunization was a failure.

Of the 90 clinically normal horses in Richland County, the vaccination status of 95% (n=86) was determined, and 73% (n=63) of these horses had been vaccinated during 1974 or 1975, or during both years. Serological samples from 17 of the 23 unvaccinated horses were positive for HI and SN antibodies against WEE; all 17 were negative for antibodies against EEE and VEE. The estimates of the total number of horses vaccinated annually by 43 of the 61 veterinarians was 8,000, but 56% more horses (n=12,500) had been vaccinated by August 1975.

Incident cases were first diagnosed during late June and early July in Richland County, North Dakota and in Swift County, Minnesota. During mid-July, cases were reported in counties that border South Dakota; the epizootic spread northward then to counties along the Canadian border. After the initial appearance of cases in the adjacent counties of Swift, Stevens, and Big Stone in Minnesota, the epizootic spread to counties that border the Red River, as well as the eastern counties (Figure 10).

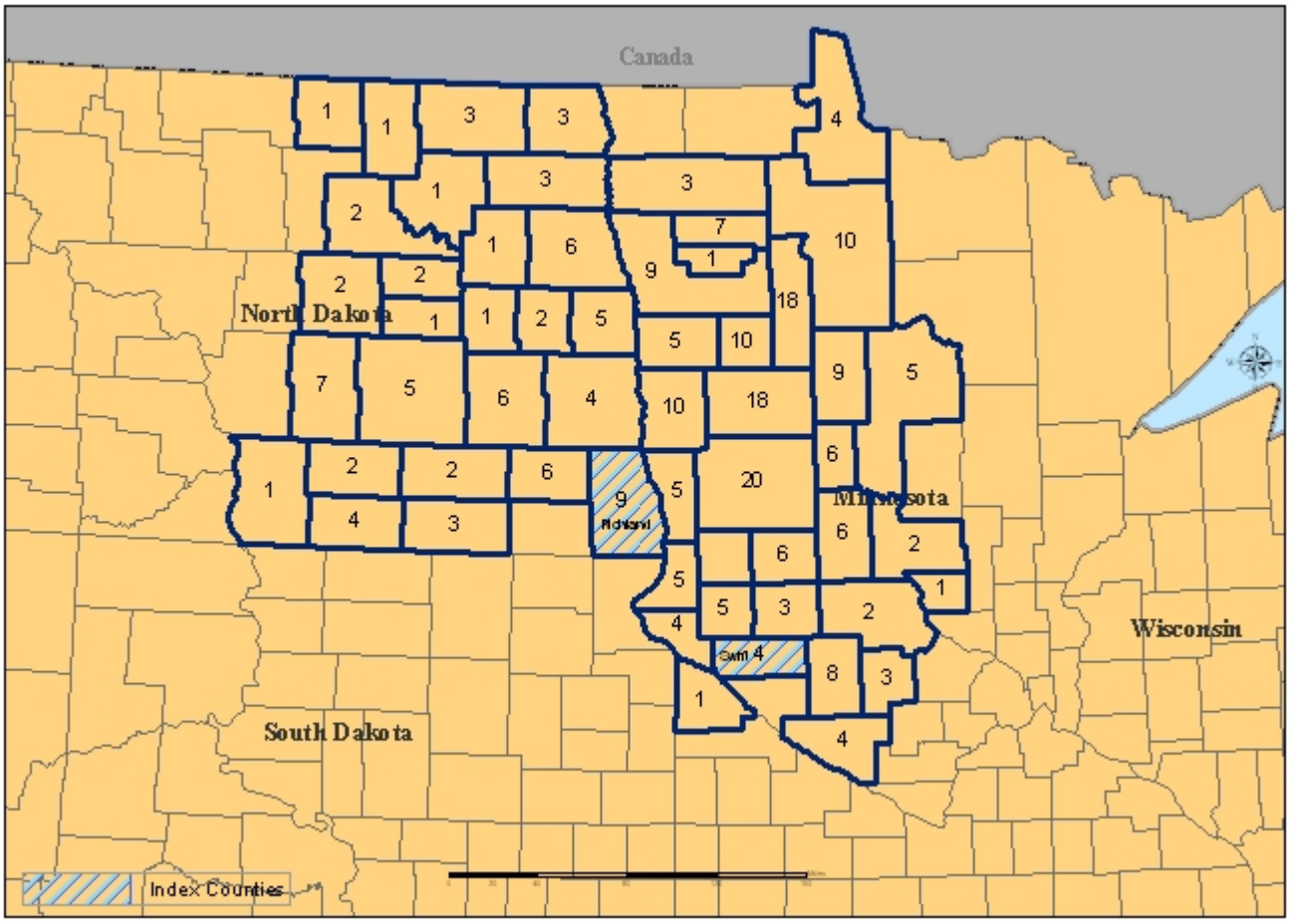


Figure 10. Geographical distribution by county of 281 cases of western equine encephalomyelitis in horses, Northern Red River Valley, June to September, 1975. From Potter et al., 1977. Revised

Human incident cases of encephalitis and aseptic meningitis attributed to arboviruses were first reported during mid-July, and they were at their peak during early August. A total of 52 cases were confirmed: 39 cases of WEE, and 13 cases of St. Louis encephalitis (Figure 09).

WEE virus infection in horses proved to be a good sentinel for the disease in humans. The first equine incident case occurred five weeks prior to the first suspected human incident case in the Red River Valley. The most rapid increase in equine incident cases occurred three weeks prior to a similar increase in human incident cases.

WEE Epizootic in Horses in Manitoba, Canada 1975

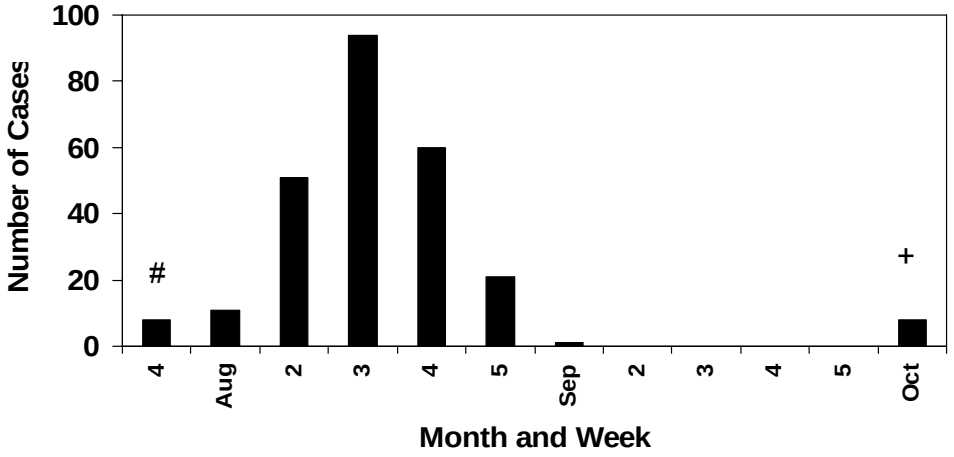
WEE was diagnosed in western Canada repeatedly during the 1930s, and it culminated in a major epizootic during 1938 to 1939. Thereafter, numerous horses were vaccinated annually and the incidence of the disease plummeted. After introduction of the vaccine, the number of clinically diagnosed cases in horses seldom exceeded 50 per year. However, the introduction of the vaccine coincided with the progressive mechanization of agriculture and concomitant decrease in the horse population. The horse population in Manitoba in 1922 was 370,800. By 1954, the population had decreased to 185,400, and the decrease continued to 30,000 horses in the early 1970s.

Both epidemic and epizootic WEE occurred in Manitoba during 1975. As the possibility of an epizootic became more apparent, veterinarians in clinical practice were placed on alert and were requested to report suspected cases of WEE to the Manitoba Veterinary Services Branch. The veterinarians also were requested to collect acute and convalescent serological specimens, as well as brain tissue from suspected cases. The sera were tested for antibodies against WEE virus with the direct complement fixation test. Isolation of WEE virus was determined from inoculation of infant mice and cytopathogenic effects in tissue culture. Where possible, laboratory specimens also were evaluated

for rabies virus and equine infectious anemia as other possible diagnoses.

A total of 261 clinically suspect cases of WEE were reported from the veterinarians to the Manitoba Veterinary Services Branch. The index case was reported on June 5, 1975, and eight additional cases had been reported by July 26. During each subsequent weekly period, 11, 51, 94, 60, 21 and 1 cases were reported (Figure 11).

Figure 11. Temporal distribution of 254 of 261 clinically suspect equine cases of WEE, Manitoba,1975 epizootic. "#" represents 8 cases diagnosed during June 5 to July 26. "+" represents 8 cases diagnosed during September 7 to October 9. From Lillie et al., 1976. Revised.



The date-of-onset for seven cases was not reported. Cases were reported from all areas of the Province. The valleys of the Red River and Assiniboine River and their tributaries, and the Brandon-Virden region were three areas where the infection was most severe. Incident cases were reported from 81 (67%) of 120 geo-political units, including rural municipalities, local government districts, unorganized territory, etc. (Figure 12). Biological specimens from 233 (89%) of the 261 clinical cases were received for laboratory confirmation. These cases were assigned to one of six diagnostic groups. Serological specimens had been received for horses assigned to four groups; in addition to serological specimens, tissues for virus isolation and histopathological exam had been received for horses assigned to a fifth group. Regarding serological diagnoses, 63 cases were interpreted as "confirmed positive", 76 cases were interpreted as "presumptive positive", 79 cases were interpreted as "presumptive negative", and 3 cases were interpreted as "confirmed negative" (Table 08). Regarding virus isolation and histopathological examinations of horses, two cases were confirmed positive based on virus isolation, four were presumed positive based on histopathological examination, and one case was positive for rabies virus. The remaining five cases were not interpretable.

WEE Epizootics in Turkeys

Despite the widespread distribution of WEE virus infection in wild birds, infections in avian species other than emus rarely cause disease. Two epizootics of WEE in domesticated poultry flocks have been reported. One epizootic in turkeys was reported in 1993, and another epizootic in turkeys was reported in1957.

A decrease in egg production associated with WEE virus infection was reported in four different turkey breeder flocks located on two separate ranches in the central valley of California in 1993 and 1994 (Cooper and Medina, 1999). This was the first report of a decrease in egg production in breeder turkeys associated with WEE virus infection.

The two ranches were located in low-lying areas close to the San Joaquin River, in areas that are usually heavily infested with mosquitoes during summer and fall. The number of birds per flock was

Figure 12. Distribution of 261 clinically suspect cases of WEE among 120 geo-political units during the 1975 epizootic, Manitoba, Canada. From Lillie et al., 1976. Revised.

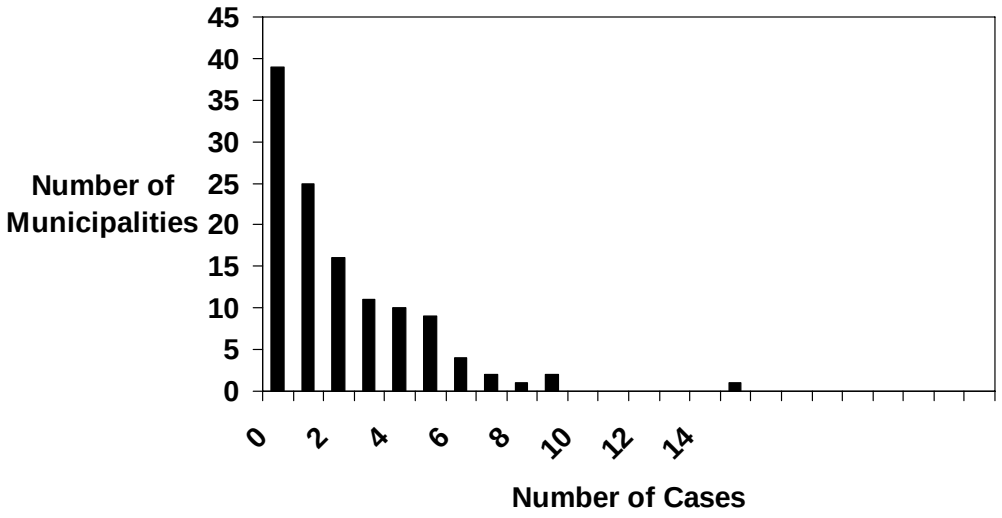


Table 08. Laboratory confirmations of 233 equine cases of western equine encephalomyelitis in Manitoba, Canada based only on serological test results, 1975 epizootic.

		Laboratory Results			
Group	**Number of Cases**	**Acute Serum**	**Convalescent Serum**	**Tissues**	**Interpretation of Result**
1†	3	Negative	Negative		Confirmed Negative
2*	79	Negative	Not submitted	Not submitted	Presumptive Negative
3♦	76	Positive	Not submitted	Not submitted	Presumptive Positive
4♀	63	Positive	Positive	Not submitted	Confirmed Positive

†No detectable complement fixation titer in acute or convalescent specimen. *Complement fixation titer from acute specimen was < 1:8. ♦Complement fixation titer from acute specimen was ≥ 1:8. ♀Complement fixation titers of acute specimen and convalescent specimen were different by four-fold value, or the titer was persistently increased. From Lillie et al., 1976. Revised.

5,413 to 13,127. The age at onset was 46 weeks to 56 weeks. The four episodes occurred during three different months; the first two episodes were in August, 1993, the third episode was in November, 1993, and the fourth episode was in September, 1994. The week-of-onset in the production cycle was from the 14th to the 23rd week. The duration of the episodes was from four weeks to six weeks (Table 09).

Clinical signs were unapparent for the most part, and the abnormalities associated with the infection were alterations in productivity. The percentage decreases in egg production was from 8.76% to 10.12%. The percentage increases in deformed egg was from 1.04% to 7.6%. Morbidity and mortality rates were nil. Turkeys from flock B underwent postmortem examination; there were no lesions, but WEE virus was isolated from a pool of mixed tissues. Turkeys from flock A, flock C, and flock D also underwent postmortem examination, but there were no lesions, nor were viruses isolated.

Serological samples were collected at three different phases of the production cycle: (1) during “normal” (i.e., pre-acute) production, (2) during the early (i.e., acute) phase of production losses, and (3) during the late (i.e., convalescent) phase of production losses. Flock A was examined during the acute phase, flock B during the acute and convalescent

Table 09. Four episodes of decrease in egg production in breeder turkeys associated with western equine encephalitis virus infection on two ranches in California, years 1993 and 1994.				
	Flock Identification			
Factor	**Flock A**	**Flock B**	**Flock C**	**Flock D**
Year	1993	1993	1993	1994
Ranch identification	Ranch 1	Ranch 2	Ranch 2	Ranch 2
Bird type	Breeder hen	Breeder hen	Breeder hen	Breeder hen
Birds per flock (#)	11,622	13,127	6,341	5,413
Age of onset (weeks)	56	46	48	53
Month of onset	August	August	November	September
Initiating week # *	23rd	14th	15th	20th
Terminating week #*	27th	20th	18th	24th
Duration of loss (wks)	5	6	4	5
Egg production loss (%)	9.71	9.57	8.76	10.12
Deformed eggs (%)	6.7	1.04	7.6	1.56
Mortality (%)	0	0	0	0
Morbidity (%)	0	0	0	0
Postmortem exam	Yes	Yes	No	Yes
Virus isolation	Negative	WEE	Not reported	Negative
Pre-acute phase serology	No	No	Yes (20)	Yes (10)
Acute phase serology	Yes (50)†	Yes (20)†	Yes (20)†	Yes (10)†
Convalescent serology	No	Yes (10)†	Yes (10)†	Yes (10)†
(20), numbers in parentheses represent sample size. * "week #" equals week number into a production cycle. † Indicates laboratory specimens were serologically positive for WEE virus based on results of serum neutralization test. From Cooper and Medina, 1999. Revised.				

phase, and flocks C and D during all three phases. Serological results were negative during the pre-acute phase, but were positive during each acute phase and each convalescent phase.

An epizootic of WEE virus in meat-type turkeys was reported in Nebraska in 1957 (Woodring, 1957). The disease was associated with high mortality. The diagnosis was based primarily on serological evidence, and was not confirmed by isolation of WEE virus.

WEE Epizootics in Emus

WEE virus infection was diagnosed in emus in Texas during a three-week period in July 1992 (Ayers et al., 1994). The percent morbidity in the eight affected flocks varied from 15% to 50%. The percent mortality among the 193 emus in the flocks was 8.8%. The age of affected emus was three months to three years. The onset of clinical signs was rapid, and all signs were consistent with severe neurological disease, given that the interval between the onset of signs and death was as brief as 24 hours in some emus. However, some emus recovered within seven days, even without therapeutic intervention. WEE virus was isolated from pooled tissue homogenates in the chicken egg and Vero cell cultures. The isolates were identified by one laboratory as WEE virus by use of hyper-immune equine serum produced against WEE virus and EEE virus, and these findings were confirmed by another diagnostic laboratory.

Serum samples were tested for antibodies to WEE virus using HI tests for the detection of equine encephalitis viruses. The titers in 77% of 13 emus from the eight affected flocks were 20 to 1,280. Sera from 281 additional ratites in Texas were tested during an eight-month period in 1992. The seroprevalence was 37%, with titers that were 40 to 1,280. Sera from 64 ratites in states adjacent to Texas also were tested during the same period. The seroprevalence was 25%. Thus, the seroprevalence was 35% for the 345 samples that were tested.

Another epizootic of WEE virus in emus occurred in west-central Oklahoma during August and September, 1992 (Randolph et al., 1994). The six affected premises were located within a 25-mile radius of Cordell, Oklahoma. Clinical signs were apparent in nine emus, and these signs were consistent with severe neurological disease. The percent morbidity was 10% to 50%, and a single 4-year old emu died. The age of affected emus was 34 to 72 months. The diagnosis of WEE virus was

confirmed by isolation of the virus from the brain of the dead emu. Serological specimens from the remaining eight emus were tested for antibodies to WEE, EEE, and VEE using hemagglutination inhibition. The samples from two emus from one premises were positive for WEE virus at titer of 320, and samples from one bird from each of two other premises was positive at titer of 640. The outbreak occurred in a geographical region where WEE virus and EEE virus infections in equine are common, and the mosquito vector *Culex tarsalis* is abundant.

WEE virus was reported in emus in a single flock that was located in the central San Joaquin valley of California during September, 1997 (Cooper et al, 1999). An embryo-lethal virus was isolated from the brain, and the virus was identified as WEE virus. According to surveillance data from the California Department of Health, WEE virus transmission was highly active during 1997. The initial seroconversions in sentinel chickens were recorded in late June and early July, and reached their peak in late July and August. WEE virus was diagnosed simultaneously in at least one horse in the same county in which the affected emu flock was located.

Geographically Atypical WEE

The enzootic and epizootic nature of WEE in the Western United States and Canada was clearly established during the middle of the 20th century, but the infection also has been documented in the Eastern United States, specifically Florida. The earliest evidence of WEE virus in Florida was reported in 1958, when antibodies were recovered from wild birds. The virus was isolated in 1960 from blue jays, mosquitoes, and sentinel mice. A comprehensive analysis of the geographical and temporal distribution of WEE virus in Florida was completed using data that had been collected during years 1955 to 1974, and was reported by the Florida Department of Health and Rehabilitative Services (Hoff et al., 1978). There were 463 laboratory-confirmed cases of equine encephalomyelitis in Florida, and 436 (94.2%) were attributed to EEE virus, 25 (5.4%) were attributed to WEE virus, and 2 (0.49%) were attributed to VEE virus. Nearly 1,000 additional horses that were suspected of having encephalomyelitis underwent serological examination, and 24 (2.4%) were positive for WEE virus. The equine cases of WEE occurred during March through December, but 44% of the cases were reported during July and September.

Twenty-nine isolates of WEE virus were recovered from mosquitoes. *Culiseta melanura*, the primary vector of EEE virus, was the mosquito species from which WEE virus was recovered most frequently. The remaining isolates were recovered from *Coquillettidia perturbans* and three species of *Aedes*. Eleven isolates were recovered from host species other than mosquitoes and the horse; these species were the blue jay, catbird, warbler, chukar, sentinel mice, and sentinel chickens. Serological specimens from 2.9% of 3,871 backyard chickens were positive for WEE virus, but none of 1,080 sentinel chickens were positive.

WEE virus was detected via virus isolation or serologically in 37 of the 67 counties in Florida. Thus, it was concluded that the virus is enzootic throughout Florida, with the exception of the lower Florida peninsula. However, WEE virus in Florida is thought to be weakly pathogenic for horses and humans.

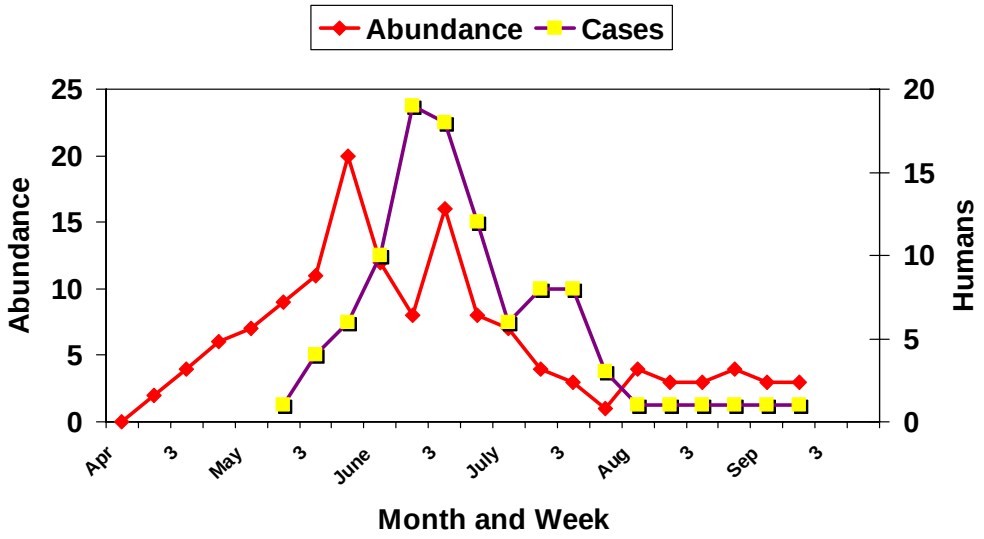
WEE virus was reported in a flock of emus in Palm Beach County, Florida in 1993 (Day and Stark, 1998). The ranch was located within a slash pine and saw palmetto habitat in the western part of the county. Approximately 150 juvenile emus had been imported from locations in California, Texas, Louisiana, Pennsylvania, and Florida. Investigation of disease in the flocks began during November, 1993, when the initial morbidity was observed. Serological specimens were collected, and the emus were assigned to one of three age groups: (1) hatching year emus were 1 to 120 days old, (2) juvenile emus were 121 to 365 days old, and (3) adult emus were 366 days old, or older. The sera were tested for WEE virus antibody using a neutralization test in which the challenge virus was WEE Fleming strain VR-1251, American Type Culture Collection, Rockville, MD. The VR-1251 strain did not cross-react with Florida EEE virus, nor Highlands J strains of WEE virus. Sera from 59 emus were tested for antibodies to three arboviruses during the 15-month period from November 1993 through January 1995. Twenty-six of the emus originated in California, 13 in Florida, 11 in Louisiana, 8 in Texas, and one in Pennsylvania. Thirty-four were juvenile, 20 were adult, and five were of hatching year age. Fourteen of the 59 emus were tested for antibodies to WEE virus, and eight of those 14 were positive for naturally-acquired infection, versus maternally-derived or vaccine-derived antibodies. The WEE virus-positive emus had been imported from California, Louisiana, and Texas. Emus are sold and transported throughout North America; thus, an arbovirus can potentially be transported within these hosts from an endemic region to a non-endemic region where there are vectors that are suitable for establishing an active focus of virus.

WEE virus had been isolated from birds and mosquitoes in Florida during the five years preceding 1964, but it had never been associated

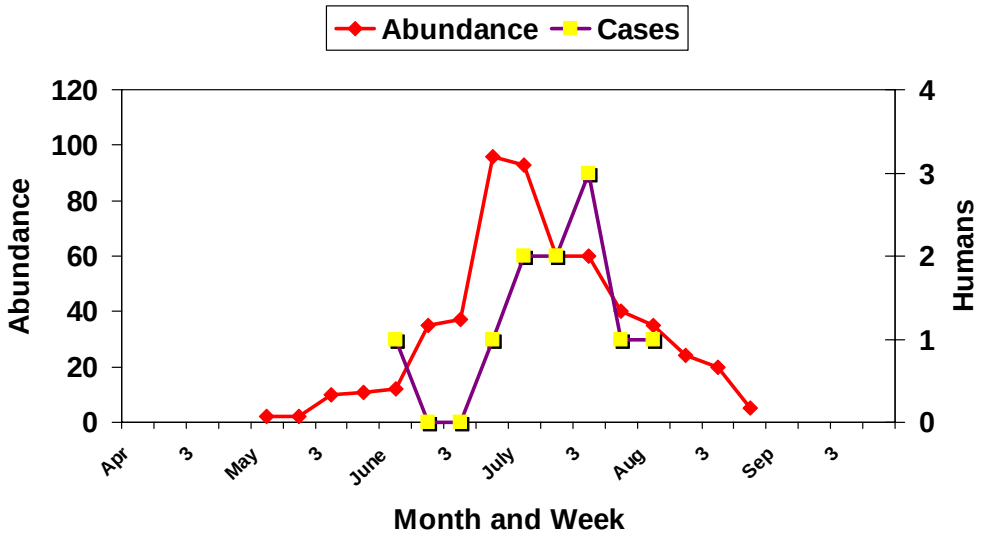
with an equine death. The virus was diagnosed for the first time in a horse in Florida in 1964. The three-year-old male horse was born in Hillsborough County, Florida and had been reared there. The clinical signs appeared initially on May 09. Within two days, the horse became blind, was ataxic, and walked in circles. The hemagglutination inhibition (HI) for antibodies against EEE virus was negative, but the HI test for WEE virus was positive at titer 1:40. The horse was euthanatized three days after the onset of clinical signs. A series of intra-cerebral inoculations of tissue from the brain of the horse into chicks and mice lead to a definitive diagnosis of WEE virus.

APPENDIX

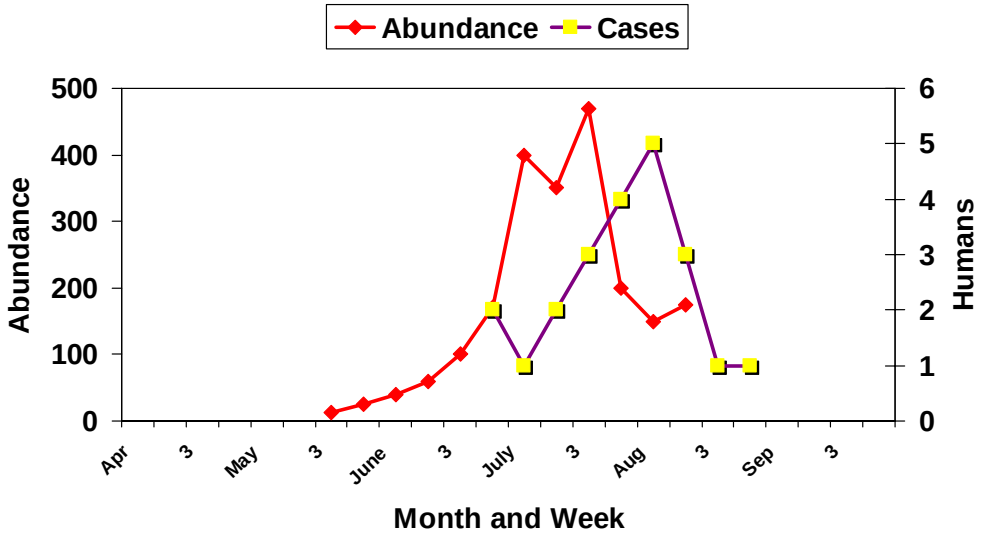
Appendix Figure 01. Relationship between *Culex tarsalis* abundance and cases of WEE in humans, Kern County, California, 1952. From Reisen and Monath, 1989. Revised.



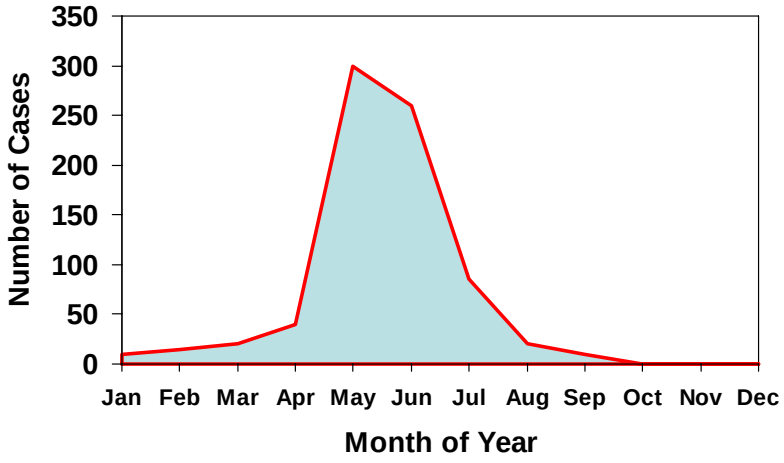
Appendix Figure 02. Relationship between *Culex tarsalis* abundance and cases of WEE in humans, Hale County, Texas, 1965. From Reisen and Monath, 1989. Revised.



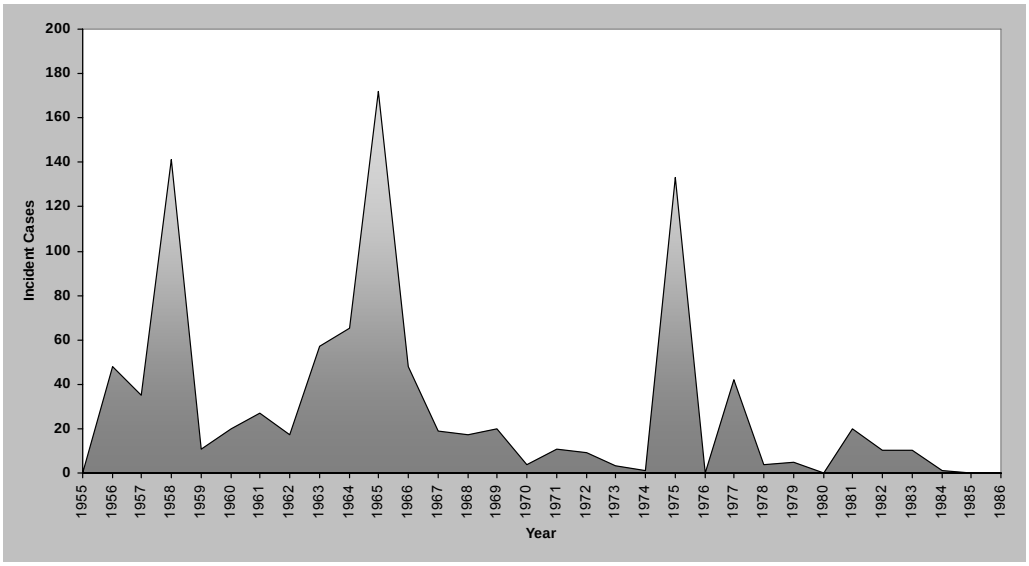
Appendix Figure 03. Relationship between *Culex tarsalis* abundance and cases of WEE in humans, Manitoba, Canada, 1981. From Reisen and Monath, 1989. Revised.



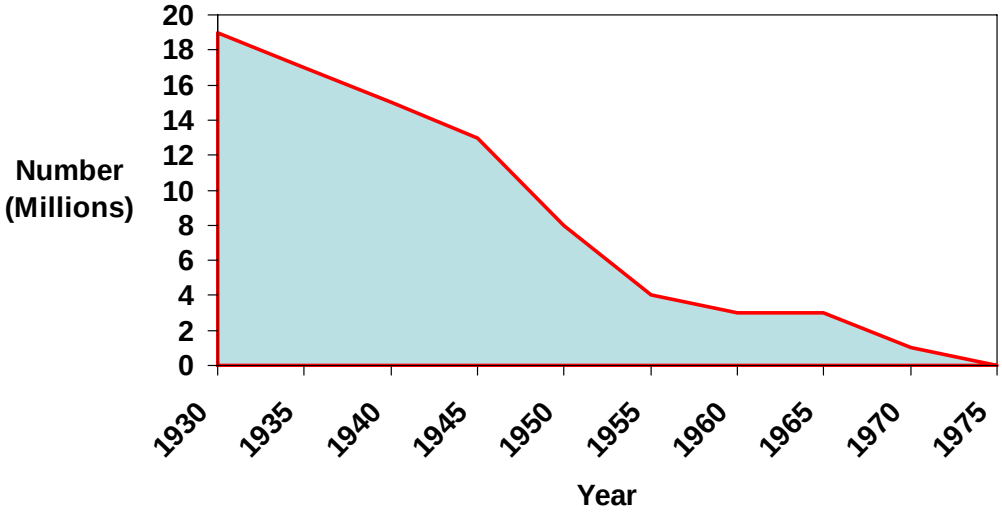
Appendix Figure 04. Seasonal distribution of WEE in humans in California during years 1945 to 1985. From Reisen and Monath, 1989. Revised.



Appendix Figure 05. Annual distribution of incident cases of WEE virus infection in humans in the United States during years 1956 to 1984. From Reisen and Monath, 1989. Revised.



Appendix Figure 06. Number of horses and mules on United States farms, 1930 to 1974. From Reisen and Monath, 1989. Revised.



Appendix Table 01. Abbreviations and common names of species of California birds that were tested for host competence for western equine encephalomyelitis virus.	
Species Abbreviations	**Species Common Name**
BUOR	Bullock's oriole
CEGR	Cattle egret
MALL	Mallard
WESJ	Western scrub-jay
BHCO	Brown-headed cowbird
BRSP	Brewer's sparrow
GAMB	Gambel's quail
CAQA	California quail
AMRO	American robin
RWBL	Red-winged blackbird
SOSP	Song sparrow
COGD	Common ground-dove
HOSP	House sparrow
LASP	Lark sparrow
LISP	Lincoln's sparrow
HOFI	House finch
MODO	Mourning dove
BRBL	Brewer's blackbird
WCSP	White-crowned sparrow
PUFI	Purple finch
Source: U.S. Geological Survey	

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