IDENTIFICATION OF *AEDES CAMPESTRIS* FROM NEW MEXICO: WITH NOTES ON THE ISOLATION OF WESTERN EQUINE ENCEPHALITIS AND OTHER ARBOVIRUSES[1]

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ABSTRACT. An arbovirus survey was conducted during August 1985 at White Sands Missile Range in southcentral New Mexico following a suspected arboviral disease epizootic among feral horses. A total of 20,566 mosquitoes (18,505 females and 2,061 males) and 8,900 biting gnats were collected and assayed for virus. Female mosquitoes were principally *Aedes campestris* (54.8%), *Aedes dorsalis* (30.4%) and *Culex tarsalis* (13.2%). Arboviruses were not isolated from biting gnats, but mosquitoes yielded a total of 37 viral isolates, including western equine encephalitis (WEE) (18), California serogroup (15), Cache Valey (1), and Hart Park (1) viruses in addition to 2, as yet unidentified, rhabdoviruses. Isolates of WEE virus were from 9 pools of *Ae. campestris*, 6 of *Cx. tarsalis* and 3 of *Ae. dorsalis*. California serogroup viruses, including 2 subtypes, were obtained from 7 pools of females and 1 pool of males of *Ae. campestris* and from 4 pools of *Ae. dorsalis*. Cache Valley and Hart Park viruses were isolated from single pools of *Ae. dorsalis* and *Cx. tarsalis*, respectively, and the rhabdoviruses were obtained from *Ae. campestris* and *Psorophora signipennis*.

INTRODUCTION

In late July and early August 1985, heavy mortality occurred in feral horses at the White Sands Missile Range (White Sands), New Mexico. Twenty-nine (78%) of the 37 carcasses were found near salt marshes just south of Malpais Spring in Otero County (33°17'N, 106°18'W) and near Salt Creek approximately 7 km west in Sierra County (Fig. 1). Mortality was limited to a moderately cohesive herd composed of about 200 of the estimated 750 feral horses in the 3 herds at White Sands. Large numbers of mosquitoes were encountered by veterinarians soon after the first horses were found dead. As a result of these observations, and with extensive equine mortality of unclear etiology, an entomologic/virologic survey was undertaken to identify mosquitoes present in this locality and to determine if these mosquitoes were infected with a virus that might have caused the equine deaths.

METHODS AND MATERIALS

STUDY SITE. The survey was conducted in the Tularosa Basin, a flat valley in southcentral New Mexico bordered on the west by the San Andres Mountains and on the east by the

Sacramento Mountains. Elevation in the area of greatest mortality is approximately 380 m above sea level. The principal water sources are Malpais Spring, a freshwater spring (pH 7.4), and the highly alkaline Salt Creek (pH 8.0–8.6) which originates in the mountains north of the study area and terminates in alkali flats to the south. The area is characterized by desert grass/shrub vegetation. Salt cedar (*Tamarix* spp.) is the principal plant along the waterways, with alkali sacaton (*Sporobolus airoides*), mesquite (*Prosopis juliflora*), and chamiza (*Atriplex canescens*) between Salt Creek and Malpais Spring (Neher and Bailey 1976).

This area experiences a rainy season from July to September, usually providing about half the annual precipitation. Tularosa, 30 km southeast of Malpais Spring, the closest National Weather Service recording site, has an average annual precipitation of 26.2 cm (U.S. Department of Commerce 1984). The average daily maximum temperature during June, July and August ranges from 35.0 to 36.1°C. Because of the moderately high elevation, the average daily minimum temperature for these months is 14.4 to 17.8°C.

COLLECTION METHODS. On August 15–17, 1985, Army miniature solid-state light traps, supplemented with dry ice as a CO_2 source, were suspended at 1 to 2 m in salt cedar trees along Salt Creek and adjacent to Malpais Spring by 1930 hr. The following morning, collection containers from the traps were gathered by 0900 hr. Containers from each site were placed in coolers with dry ice for 5 min to immobilize the insects. The contents were then transferred to 50-ml plastic, screw-cap centrifuge tubes, sealed, labeled with site and date, and retained on dry ice to preserve any virus
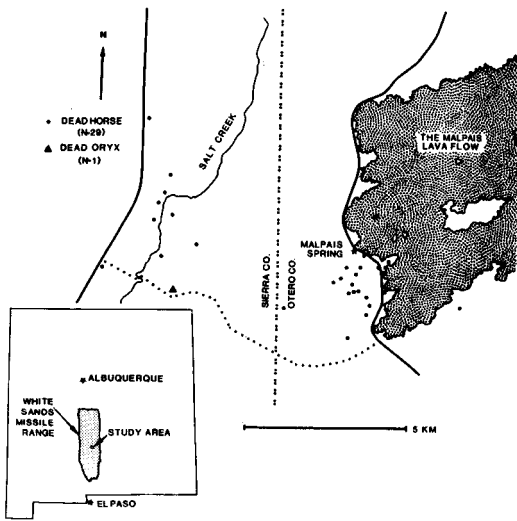
Fig. 1. Location of horse mortality in southcentral New Mexico, 1985.

that the insects might contain until returned to the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick, Frederick, Maryland. Mosquito larvae were collected with a standard dipper from a 3 m × 3 m isolated ground pool and from numerous water-filled hoofprints near Malpais Spring. The larvae were allowed to emerge to adults for species identification and viral assay.

LABORATORY METHODS. Specimens from the light trap collections and laboratory-reared mosquitoes were placed on a chill table (4°C), and bloodsucking Diptera were separated from other arthropods. Mosquitoes were identified to species under a stereomicroscope with the aid of a key produced by Darsie and Ward (1981) and placed in pools of up to 50 according to sex, site of collection, date and presence or absence of visible blood. Ceratopogonidae (*Culicoides* spp.) were placed in pools of 100 without regard to species or bloodfed status. Procedures for initial viral assay were described previously (Clark et al. 1985).

Arthropod suspensions also were inoculated intracranially into each of a group of eight 2- to 4-day-old suckling mice and observed for 14 days for evidence of morbidity or mortality. Suspensions of brains from sick or dead mice were tested by complement fixation (CF) by the $LBCF_{50}$ method modified for microtiter (Casey 1965); crude alkaline-extracted, infected suckling mouse brain was clarified and used as antigen (Calisher and Maness 1975). For the viral identification tests, we used hyperimmune mouse ascitic fluids (Tikasingh et al. 1966)

prepared against the following viruses: Jamestown Canyon (California serogroup), Flanders (Hart Park serogroup), Turlock (Turlock serogroup), St. Louis encephalitis (arbovirus serogroup B = flaviviruses), western equine encephalitis (WEE) (arbovirus serogroup A = alphaviruses), and a grouping fluid for Bunyamwera serogroup viruses. Representative California (CAL) group viruses were subtyped by a serum dilution-plaque reduction neutralization test (Lindsey et al. 1976). Viral stocks were prepared from supernatant fluids from virus-infected Vero cells. Titers of the stock viral preparations were determined by plaque assays in Vero cells and subsequent neutralization tests performed by using infection-immune hamster sera prepared with Jamestown Canyon, California encephalitis (CE), and San Angelo (SA) viruses (Karabatsos and Mathews 1980).

RESULTS

A total of 20,566 mosquitoes were collected, pooled, and assayed for virus (Table 1). The principal species collected were *Aedes campestris* Dyar and Knab, *Aedes dorsalis* (Meigen) and *Culex tarsalis* Coquillett. At Malpais Spring, *Ae. campestris* and *Cx. tarsalis* were found in equal numbers, whereas over 5 times as many *Ae. campestris* as *Cx. tarsalis* were collected near Salt Creek.

Males represented 10.0% of the total mosquito collection (Table 1). At Malpais Spring, 24.0% of the *Cx. tarsalis* were males, compared with 3.4% at Salt Creek. *Aedes* males, predominantly *Ae. campestris*, comprised 23.4% of all *Aedes* collected at Malpais Spring and 10.4% of the *Aedes* mosquitoes from Salt Creek. While sorting the mosquitoes, *Ae. campestris* pairs were frequently found *in copula*.

All laboratory-reared mosquitoes (25) collected from hoofprints at Malpais Spring were *Cx. tarsalis*. Adults reared from immature mosquitoes collected from the larger ground pool at this location were all *Ae. campestris* (17 females and 5 males). Numerous *Ae. campestris* males were also present in the low vegetation in and around this pool. When disturbed, they would fly to a height of approximately 30 cm before settling back to the vegetation.

The rainfall data recorded at Tularosa for June 1 through August 18 are presented in Fig. 2. Only 0.2 cm of rain was recorded in May, which was below normal. However, the 1.7 cm and 4.8 cm of rainfall in June and July, respectively, were normal for Tularosa. The most significant climatic events recorded during the 2 weeks, prior to the initial observation of dead horses were the 2.3- and 1.2-cm

Table 1. Mosquitoes collected for viral assay at White Sands Missile Range, New Mexico, August 16–18, 1985.

Species	Collection area		
	Malpais Spring	Salt Creek	Total
Ae. campestris	650 (36.8)*	9,495 (56.7)	10,145 (54.8)
Ae. dorsalis	374 (21.2)	5,243 (31.3)	5,617 (30.4)
Ae. sollicitans	8 (0.5)	32 (0.2)	40 (0.2)
Ae. vexans	32 (1.8)	58 (0.3)	90 (0.5)
Aedes spp. (males)	249	1,539	1,788
An. pseudopunctipennis	15 (0.8)	34 (0.2)	49 (0.3)
Cx. erythrothorax	34 (1.9)	4 (<0.1)	38 (0.2)
Cx. tarsalis	649 (36.8)	1,800 (10.8)	2,449 (13.2)
Cx. tarsalis (males)	209	64	273
Cs. inornata	1 (0.1)	0	1 (<0.1)
Ps. confinnis	2 (0.1)	71 (0.4)	73 (0.4)
Ps. signipennis	0	3 (<0.1)	3 (<0.1)
Total	2,223	18,343	20,566
No. females	1,765	16,740	18,505

* No. collected (% of females at this site).

rainfalls of July 15 and 27, respectively. The temperature for the 10 days following the first precipitation in July ranged from 13.3 to 36.1° ($\bar{x}$ = 24.6°C).

Thirty-six virus isolations were obtained from 18,505 female mosquitoes collected at White Sands during 3 nights of light-trapping (Table 2). Following initial virus isoltion, all isolates were reisolated in Vero cells and suckling mice. Eighteen isolates were identified as WEE virus. Nine of these were from *Ae.*

campestris; however, of the 3 principal species, *Cx. tarsalis* had the highest minimum field infection rate (MFIR).

Fourteen CAL serogroup virus isolates were obtained from pools of female mosquitoes, 4 from *Ae. dorsalis* and 11 from *Ae. campestris* (Table 2). Another CAL virus isolate, not shown in Table 2, was from a pool of 50 male *Aedes* spp. This pool of males as well as one of female *Ae. campestris* were identified as CE virus, while one pool of *Ae. dorsalis* yielded a
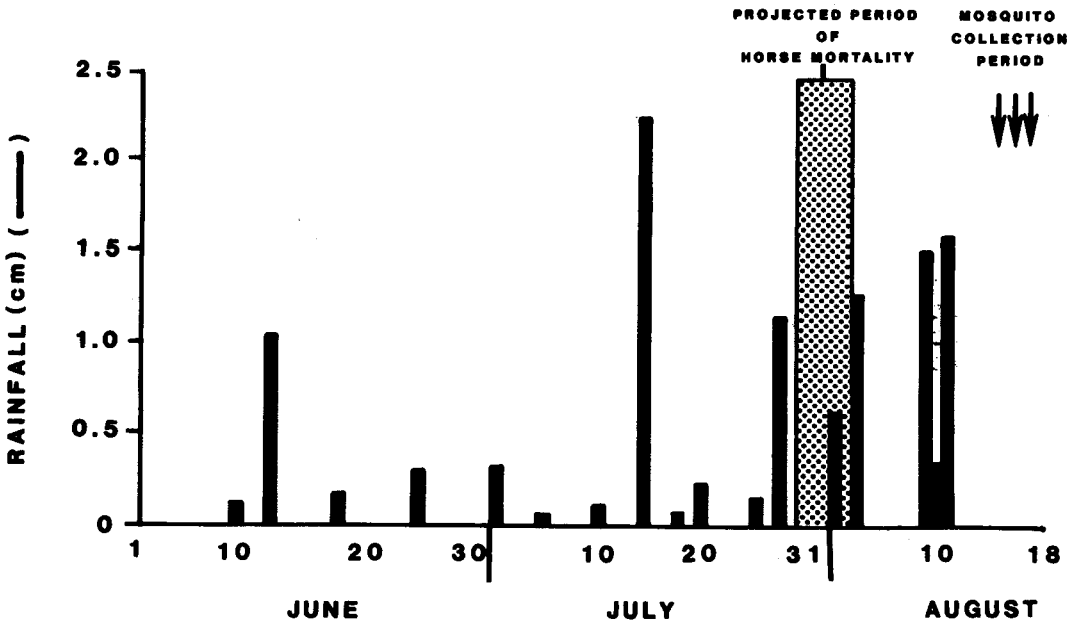

Fig. 2. Relationship of rainfall and horse mortality at White Sands Missile Range, New Mexico, June–August, 1985.

Table 2. Viral isolations from female mosquitoes collected at White Sands Missile Range, New Mexico, August 16–18, 1985.

| Species | Virus* | | | | | |
---	CV	CAL gp.	HP	WEE	Rhabdo-viridae	Total
Ae. campestris	0	10 (1.0)**	0	9 (0.9)	1	20
Ae. dorsalis	1 (0.2)	4 (0.7)	0	3 (0.5)	0	8
Cx. tarsalis	0	0	1 (0.4)	6 (2.4)	0	7
Ps. signipennis	0	0	0	0	1	1
Total	1	14	1	18	2	36

* CV = Cache Valley, CAL gp = California serogroup, HP = Hart Park, WEE = western equine encephalitis

** No. virus isolates (Minimum field infection rate/1,000).

subtype of SA virus. Single strains of Cache Valley (CV) and Hart Park viruses were isolated from *Ae. dorsalis* and *Cx. tarsalis*, respectively.

Two additional viral isolates have not been specifically identified. However, electron microscopic examination revealed morphologic characteristics similar to viruses in the family Rhabdoviridae. Preliminary serologic results indicate that these two are identical to each other, yet different from the 6 rhabdoviruses known from this region of the USA.

Virus was not obtained from the 22 adult *Ae. campestris* reared from immatures. Likewise, virus was not detected in *Culicoides* (89 pools) collected on August 16 near the lava flow in the Malpais Spring area.

The product of the WEE virus MFIR and the percentage of the 3 principal species in the collection yielded a "population infection index" (Table 3). In this analysis, the overall index value in both areas for *Ae. campestris* was 3.2 times as great as *Ae. dorsalis* and 1.5 as large as *Cx. tarsalis*.

DISCUSSION

The presence of substantial numbers of *Cx. tarsalis* and *Ae. dorsalis* in our collections was not unexpected, based on the reported distribution of these 2 species (Darsie and Ward 1981). *Aedes campestris* has been reported from northern New Mexico (Sudia et al. 1967) and from southwestern Texas (McGregor and Eads 1943), but not from southcentral New Mexico (Darsie and Ward 1981). Initially regarded as a univoltine species of late spring and early summer (Rees 1943), *Ae. campestris* was suggested by Chapman (1966) to be multivoltine but with only one generation per year because of climatologic and ecologic factors. Apparently, midsummer rainfall and elevated ambient temperatures caused the emergence of this species prior to the occurrence of horse deaths at White Sands. The portrayal of the habitat, mating and anthropophilic behavior of this species and concentration of *Ae. campestris* at an emergence site were similar to those reported by Knab (Carpenter and Lacasse 1955).

Culex tarsalis is regarded as the principal vector of WEE virus in the western USA (reviewed by Hess and Hayes 1967). The MFIR that we observed for this species was similar to the 2.3/1,000 found in 1983 in Kern County, California, by Reisen (1984). However, our findings are the first reported isolations of WEE virus from *Ae. campestris* in this country.

Table 3. Western equine encephalitis virus isolations from and population infection index of three mosquito species collected at White Sands Missle Range, New Mexico, August 16–18, 1985.

| Species | Collection area | | |
---	Malpais Spring	Salt Creek	Both areas
Ae. campestris	0/650	9/9,495 (0.9)* 51.0**	9/10,145 (0.9) 49.3
Ae. dorsalis	0/374	3/5,243 (0.6) 18.8	3/ 5,617 (0.5) 15.2
Cx. tarsalis	1/649 (1.5) 55.2	5/11,800 (2.8) 30.2	6/ 2,449 (2.4) 31.7

* No. viral isolates/no. mosquitoes assayed (minimum field infection rate/1,000).

** Population infection index = (minimum field infection rate/1,000) × species' percentage of female collection.

In the mid-1960s, McLintock et al. (1970) reported 3 WEE virus isolates, and J. O. Iversen (personal communication, 1985) obtained a single WEE virus isolate in 1976 from *Ae. campestris* collected in Saskatchewan, Canada.

The infrequent isolation of WEE virus from *Ae. campestris* may reflect the relatively small numbers that have been assayed for virus (i.e., 5,726—Iversen et al. 1979; 4,087—McLintock et al. 1966; 2,338—McLintock et al. 1970; 974—Burton et al. 1973; less than 1,000—Shemanchuk and Morgante 1968; 18—Crane et al. 1977; "few"—Sudia et al. 1967; Hammon et al. 1941). No *Ae. campestris* were found by Hayes et al. (1976) among 44,444 mosquitoes collected during 1972 in 8 New Mexico counties, not including Otero and Sierra counties.

Although infection rates are not synonymous with transmission rates (Reeves et al. 1961), our "population infection index" was similar to one calculated for St. Louis encephalitis virus and birds by Lord et al. (1974). This index clearly suggested that *Ae. campestris* was potentially the most important WEE virus vector based on biomass and field infection rate. Although we collected more *Ae. campestris* near Salt Creek than near Malpais Spring, the 10-mile [16 km] flight range for this species (Rees 1943) easily encompasses the latter area, where the greatest number of dead horses was observed. The relatively high percentage of males in light trap collections, the "fresh" condition of the specimens, and the frequency of pairs found *in copula*, implied that there was a recent emergence of *Ae. campestris* at this locality.

The possibility of transovarial transmission of WEE virus in this remote location should be explored. The MFIR in females without visible blood and the large percentage of males in the collection, but relatively small number of males assayed, reinforce the need for this study.

The isolation of CE virus from a pool of *Ae. campestris* males was suggestive of transovarial transmission of this virus. Previously, Crane et al. (1977) reported transovarial transmission of CE virus in *Ae. dorsalis* at Blue Lake, Utah; they isolated CE virus from 2 pools of *Ae. dorsalis*, one of males and one of females, reared from field-collected larvae.

Although rarely isolated, SA virus has been obtained from *Ps. signipennis* collected at Las Cruces, New Mexico (Tesh 1980). The only prior report of an SA virus isolation from the genus *Aedes* was from a single mixed pool of *Aedes atlanticus-infirmatus* (Bartnett et al. 1967). The role of these 2 *Aedes* species in the maintenance of these CAL serogroup viruses in this area requires further definition.

The postmortem conditions of the horses at White Sands precluded collection of usable diagnostic samples required to ascertain the etiology of the sudden die-off. While WEE virus was the only recognized equine pathogen that we isolated, it was not possible to unequivocally link the isolation of this virus from mosquitoes to the deaths of the horses. Transmission of WEE virus in this locality by *Aedes* mosquitoes, involving the principal species that we collected, is a tenable hypothesis and should be pursued by further field, as well as laboratory studies. These results warrant laboratory studies to define the ability of this species to 1) become infected with WEE virus after feeding on sources with viremias equivalent to those developed by naturally infected vertebrates and 2) to successfully transmit this virus to other susceptible vertebrate hosts.

The most abundant mammal observed during ground and aerial surveillance at White Sands was the black-tailed jackrabbit (*Lepus californicus*). A natural transmission cycle involving this hare, WEE virus, and *Aedes melanimon* Dyar has been described from northern California (Hardy and Bruen 1974). Recently, Reisen (1984) reported an MFIR of 1.9/1,000 for *Ae. melanimon* in Kern County, a rate twice that we found in *Ae. campestris*. *Aedes melanimon* is reported only from northwestern New Mexico (Darsie and Ward 1981) and was not found in our collections. However, in Hale County, Texas, Bowers et al. (1969) obtained 5 WEE virus isolates from *L. californicus* in 1965 and found that 48 (86%) of 56 had hemagglutination-inhibiting antibody to WEE virus.

The ecological situation described here is an example of a unique focus that could provide the basis for important field studies on the ecology of WEE virus in southwestern United States. In this small, well-defined area with minimal human activity, we found WEE virus, a recognized epizootic (*Cx. tarsalis*) and potential maintenance (*Ae. campestris*) vector of WEE virus, and a possible vertebrate host (*L. californicus*). Because of restricted access to this area and the presence of an endangered species, the White Sands pupfish (*Cyprinodon tularosa*), the current land-use policy and regulations against use of insecticides strongly preclude significant man-made changes in this desert habitat in the near future.

ACKNOWLEDGMENTS

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