OCCURRENCE OF *CULEX ERYTHROTHORAX* IN SOUTHEASTERN COLORADO AND REPORT OF VIRUS ISOLATIONS FROM THIS AND OTHER MOSQUITO SPECIES

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ABSTRACT. Significant numbers of *Culex erythrothorax* were collected during arbovirus surveillance in 1987 and 1988 at Las Animas, Bent County, on the high plains of southeastern Colorado. This finding extends the range of this species to the eastern side of the Rocky Mountains. The isolation of western equine encephalitis virus in 1988 from both *Cx. tarsalis* and *Cx. erythrothorax* suggests that the latter also may be involved in the endemic cycle of this virus in the area. St. Louis encephalitis, Hart Park, Turlock, and a Bunyamwera group virus were also isolated from the mosquitoes collected.

INTRODUCTION

The Colorado Department of Health and the Division of Vector-Borne Viral Diseases (DVBVD) of the Centers for Disease Control (CDC) collaborated in 1987 to assess an apparent increase in arboviral activity along the Front Range of the Rocky Mountains. The studies included mosquito collections by one of the authors (T.D.) in a high plains area of southeastern Colorado, approximately 120 km east of the Rocky Mountains. The collection contained a significant number of a *Culex* (*Culex*) species which appeared distinct from all species known to occur in the area and could not be definitively identified since no males were recovered. These specimens remained unidentified despite discussions with and the examination of voucher specimens by colleagues at the Walter Reed Biosystematics Unit (WRBU), Smithsonian Institution, Washington, DC.

Additional collections were made at the site by the authors on August 2–3, 1988. Again, although more than 2,000 females of the unknown *Culex* sp. were caught, no males were found in collections. Immatures collected from an adjacent small seepage area densely vegetated with sedge (*Carex* sp.) yielded a single female of this unidentified species and numerous *Cx. tarsalis* Coq. adults. Further collections by the authors on August 16–17 yielded more than 6,000 females of the unknown species and, fortunately, 6 males.This paper reports the identification of this species as *Cx. erythrothorax* Dyar and an extension of its range. We also present data on arbovirus isolations from the mosquitoes collected during these studies in southeastern Colorado.

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MATERIALS AND METHODS

The high plain of southeastern Colorado in which the collections were made (Fig. 1) is an intensively irrigated agricultural region. Alfalfa, corn and grains are the major crops; pastures and small cattle feedlots are also common. The collection site was located outside the town of Las Animas along the Purgatory River drainage near its confluence with the Arkansas River. Marshes abound along both river basins with cattail *(Typha* spp.) and tule *(Scirpus* spp.) the predominant vegetation.

All adult mosquito collections were made with CDC miniature light traps supplemented with dry ice. The specimens were put in vials and transported on dry ice to the DVBVD in Fort Collins, CO. In the laboratory, the mosquitoes were identified and pooled on refrigerated chill tables to prevent loss of virus.

Pooled mosquitoes were triturated in 1.6 ml of diluent composed of 1% bovine albumin in pH 7.6 Tris-buffered saline containing antibiotics. Suspensions were clarified by high-speed centrifugation, and supernatant fluids were stored at −65°C for later testing; 0.2 ml of each mosquito suspension was inoculated onto monolayer cultures of a continuous line of African green monkey kidney (Vero) cultures grown at 37°C in 6-well cell culture plates. After adsorption of inocula for 1 h, cultures were overlaid with nutrient agar (Hayes et al. 1976). Cultures were observed daily for plaques, and cells were harvested for passage when plaques appeared. Recovered virus strains were identified by indirect immunoflourescence tests using mouse immune ascitic fluids.

Approximately 60 live females and 1 male of the unknown *Culex* were aspirated from the collection bags of August 17 and returned to the DVBVD for colonization attempts. Adults were initially offered a bloodmeal on the arm of the senior author (10 fed to repletion) and thereafter were allowed to feed on restrained baby chicks. Immatures were reared from egg rafts by standard insectary procedures; pupae were reared individually to adults in darkness. Emerged adults

were frozen at −65°C, lyophilized and pinned as reference specimens. Egg rafts were also supplied to colleagues at the Walter Reed Biosystematics Unit, Smithsonian Institution for their own rearing of associated specimens. The terminal abdominal segments were snipped from field-collected males for clearing and dissection of the genitalia by previously described methods (Jakob et al. 1979).

RESULTS

The genitalia of the *Culex* sp. males from the 1988 collections and the reared males were compatible with that depicted for *Cx. erythrothorax* (Carpenter and LaCasse 1955) and with specimens recovered from collections along the lower Colorado River after water was released from lakes along the California-Arizona border because of excessive spring runoff in 1983 (CDC, unpublished data 1984). Adult males reared at the WRBU were also determined to be *Cx. erythrothorax* (E. L. Peyton, personal communication 1988).

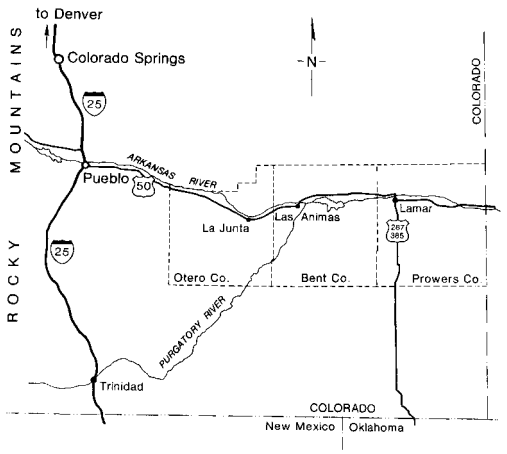

Fig. 1. Area of southeastern Colorado where *Culex erythrothorax* were collected.

Virus isolation studies with the 1987 collections (Table 1) yielded virus strains only from *Cx. tarsalis*, the known enzootic and major epidemic vector of western equine encephalitis (WEE) and St. Louis encephalitis (SLE). In addition to WEE; SLE, Hart Park and Turlock viruses were isolated from this species. Although not all *Cx. erythrothorax* were pooled for virus studies, it was the most abundantly collected species. No virus was isolated from it in 1987.

The 1988 collections (Table 1) yielded 30 arbovirus strains with 4 WEE isolations from both *Cx. tarsalis* and *Cx. erythrothorax* and a single WEE strain from *Aedes vexans*. In addition, 20 Hart Park virus strains were obtained from *Cx. tarsalis* and *Cx. erythrothorax*. A single Bunyamwera group virus was obtained from *Anopheles quadrimaculatus* Say. *Culex erythrothorax* was again the most prevalent mosquito in the collections.

DISCUSSION

Prior to these studies, *Cx. erythrothorax* was found in Colorado only in the Grand Junction, Mesa County area on the western edge of the state (Harmston and Lawson 1967). Thus, the finding of this species on the plains of southeastern Colorado represents a significant extension of its range, particularly since a great natural barrier, the Rocky Mountains, lies between these areas. Compared to the discovery of relatively low numbers of this species in Texas in 1953–1954 (Menzies et al. 1955) and in New

Table 1. Virus isolations from mosquitoes collected near Las Animas, Bent County, CO, in 1987 and 1988.

Species[a]	No. mosq. (pools)	Virus isolations WEE[b]	SLE	TUR	HP	BUN
August 5, 1987						
Aedes vexans	273 (6)					
Anopheles quadrimaculatus	133 (3)					
Culex erythrothorax	1,690 (34)					
Cx. tarsalis	613 (21)	2 (3.3)[c]	1 (1.6)	1 (1.6)	1 (1.6)	
August 2–3 and 17–18, 1988						
Aedes vexans	3,008 (44)	1 (0.3)				
Anopheles quadrimaculatus	969 (17)					1 (1.0)
Culex erythrothorax	8,669 (126)	4 (0.5)			6 (0.7)	
Cx. tarsalis	1,917 (32)	4 (2.1)			14 (7.3)	

[a] *Ae. dorsalis*, *Ae. melanimon*, *Ae. trivittatus*, and unidentifiable *Aedes*, *Coquillettidia perturbans*, *Culex restuans* and *Culiseta inornata* were also present in the collections.

[b] WEE = western equine encephalitis; SLE = St. Louis encephalitis; TUR = Turlock virus; HP = Hart Park virus; BUN = Bunyamwera group virus.

[c] Minimum infection rate per 1,000 mosquitoes (number of positive pools per total number of mosquitoes).

Mexico from September 1963–September 1964 (Sublette and Sublette 1970), large numbers of *Cx. erythrothorax* were taken in 1987 in Las Animas, CO, even though trapping was done only on a single night (August 5) with 6 traps. In 1988, collections on 4 nights (24 trap nights) yielded more than 8,000 *Cx. erythrothorax*, even though no measurable rain had fallen in the area for 3 months. Thus, the species appears to be well established in the area.

In California, *Cx. erythrothorax* occurs mainly in the coastal and foothills areas: larvae are found in the tule margins of lakes and ponds (Bohart and Washino 1978), and the riverine ecology of this species in the Imperial Valley has been described (Walters and Smith 1980). In Utah this species is most frequently found in large permanent swamps containing considerable vegetation (Nielsen and Rees 1961).

Feeding preferences for *Cx. erythrothorax* are variable, depending on the geographic area of study. In Kern County, CA, over 85% of the feedings identified were on domestic mammals with the remainder on birds, whereas nearly all feeding were on birds in Salt Lake County, UT (Tempelis 1970). Approximately equal feeding occurred on mammals and birds near the Salton Sea in California (Gunstream et al. 1971). The species appears to be an opportunistic feeder, using whatever hosts are most abundant in the area.

Western equine enecphalitis, SLE, and Turlock viruses have all been isolated from *Cx. erythrothorax* in California; and SLE virus was recovered from specimens collected in Yuma, AZ (Emmons et al. 1974). In our study of collections made in southeastern Colorado in 1988, 4 WEE virus strains were recovered from both *Cx. erythrothorax* and *Cx. tarsalis*, although the minimum infection rate (MIR) in the latter species (2.1) was 4 times that of *Cx. erythrothorax* (0.5). The possible role of *Cx. erythrothorax* in the ecology of WEE virus in Colorado is unknown, although laboratory studies with southern California populations of this species have shown it to be an inefficient vector (Meyer et al. 1988). Six strains of Hart Park virus were also recovered from *Cx. erythrothorax*, although the MIR (0.7) was 10-fold less than that for *Cx. tarsalis* (7.3).

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