Culicoides mohave (Diptera: Ceratopogonidae): New Occurrence Records and Potential Role in Transmission of Hemorrhagic Disease

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ABSTRACT Bitting midges of the genus Culicoides are important in the transmission of viral diseases affecting wild and domestic ungulates, including bluetongue (BLU) and epizootic hemorrhagic disease (EHD). The primary known vector for these viruses is C. sonorensis Wirth & Jones, however, it has been speculated that other species of Culicoides may also be involved. One potential candidate is C. mohave, a poorly studied species found in inland desert areas of the southwestern United States. In 2000 and 2001, we collected C. mohave and C. sonorensis at six sites in a previously unsurveyed area in the Sonoran Desert of southwestern Arizona and used PCR to detect nucleic acids associated with BLU and EHD viruses. C. mohave was abundant at two low-elevation sites on the study area, but uncommon or absent elsewhere. C. sonorensis commonly occurred along with C. mohave at one site, but was much less abundant. All C. mohave pools were negative for BLU viral RNA, however, 35% yielded positive results for EHD. All C. sonorensis were negative for both BLU and EHD. Our results suggest that C. mohave is a potential vector of EHD virus in this area, however additional studies are needed to determine its ability to transmit EHD.

KEY WORDS Culicoides mohave, Culicoides sonorensis, vectors, bluetongue, epizootic hemorrhagic disease

Culicoides mohave Wirth is an hematophagous ceratopogonid found in inland desert areas of the southwestern United States and northern Baja California, Mexico. Previous occurrence records in the United States are primarily from southern California (Wirth and DeMoraes 1979, Brenner et al. 1984, Mullens and Dada 1992) but include two collections in southeastern Arizona (Wirth and DeMoraes 1979). Many areas within the potential range of C. mohave, including southwestern Arizona, have not been surveyed previously. Another North American species of Culicoides, C. sonorensis Wirth & Jones is considered the primary vector for two viruses affecting wild and domestic ungulates, bluetongue (BLU; Tabachnik 1996) and epizootic hemorrhagic disease (EHD; Nettles et al. 1991). In the southeastern United States, species other than C. sonorensis have been suggested as potential vectors of BLU and EHD viruses (Smith and Stallknecht 1996); however, this question has not been previously addressed in the desert Southwest. The objectives of our study were to obtain information on the distribution and abundance of C. mohave in a previously unsurveyed area and to conduct a preliminary assessment of its potential role as a vector for BLU and EHD viruses.

Materials and Methods

Study Area. We trapped Culicoides midges at six sites in southwestern Arizona. Trap sites were located in Yuma and La Paz counties, on the U.S. Army Yuma Proving Ground, Kofa National Wildlife Refuge, and adjacent Bureau of Land Management lands. Four sites (KV, K23, 541, and 967) were located along ephemeral washes in relatively ßat terrain. Two sites (K4, K10) were located in rocky canyons adjacent to desert mountain ranges. The dominant plant community at all sites is Sonoran Desertscrub, Lower Colorado River Valley Subdivision (Turner and Brown 1982). Elevations of trap sites ranged from 305 to 839 m. The area is extremely arid, with maximum daily summer air temperatures frequently exceeding 40°C and average annual precipitation of 9.0 cm (nearest weather station, located at U.S. Army Yuma Proving Ground).

Insect Collection and Identification. Trapping was conducted once per month in September 2000 and April through October 2001. At each site, we set two CDC Miniature Light Traps (John W. Hock Co., Gainesville, FL) spaced 250 m apart. Initial trapping sessions in September 2000 yielded large numbers of nontarget insects, thus, in subsequent efforts, we modiﬁed traps by removing the light bulb (Brenner et al. 

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1984) and placing a 3-mm mesh hardware cloth screen over the trap inlet. Traps were suspended 1 m above ground level, baited with \( \approx 1.5 \) kg dry ice, and operated from sunset to sunrise. Insects were trapped directly into 75% ethanol.

Trap samples were sorted in absolute ethanol in the lab, with at least one change of ethanol during sorting. Identification was based on descriptions in Wirth (1952) and Wirth and DeMoraes (1979). Voucher specimens were cleared in 10% KOH (except wings, which were mounted uncleared) and mounted on microscope slides in Canada balsam. These slides were deposited as voucher specimens at the University of Arizona, Tucson.

**Virus Detection.** We used reverse transcriptase-polymerase chain reaction (RT-PCR) procedures (Wilson 1994, Shad et al. 1997) to screen female *C. mohave* and *C. sonorensis* for nucleic acids associated with EHD and BLU viruses. Midges of each species were pooled by trap and capture occasion before PCR analysis. Pool sizes for *C. mohave* and *C. sonorensis* ranged from 1 to 173 and 1–54 individuals, respectively. RNA was extracted using the RNA Easy kit (Qiagen, Valencia, CA).

We identified the presence of viral nucleic acids of the correctly sized nest amplicon (EHD, 135bp; BLU, 218bp) using agarose gel electrophoresis. For each specimen, we included a negative (nontemplate) PCR control in an adjacent lane to control for false positive results or specimen carry-over. We included a positive control consisting of viral RNA in each set of PCR's (1–20 specimens).

**Results and Discussion**

We trapped *C. mohave* at four of the six sites (967, K4, 541, KV). Initial captures at one site (967) using light plus CO\(_2\) yielded similar numbers of both sexes (226 males, 240 females). Subsequent captures with CO\(_2\) only were almost exclusively host-seeking females; out of 3,138 individuals, only a single male *C. mohave* was collected. Numbers of individuals varied greatly among sites and among trapping occasions, ranging from 1 to 1,501 individuals (Fig. 1). *C. mohave* occurred most frequently and in greatest numbers at two lower elevation sites in the western portion of the study area (967, K4) and in low numbers (<5 individuals per sample, total of four occasions) at two other sites (KV, 541). Abundance at the 967 and K4 sites followed similar seasonal patterns and was highest during late summer (September and October). The 967 site consistently yielded the largest catches of *C. mohave*. *C. mohave* was absent at the two higher elevation sites (K10, K23). Female *C. sonorensis* were also present in trap samples on all but two occasions at the 967 site, though in considerably lower numbers (1–54 individuals). *C. sonorensis* did not occur at any other trap sites.

We found *C. mohave* in habitats similar to those reported in previous studies (Wirth and DeMoraes 1979, Mullens and Dada 1992). The relative lack of *C. mohave* at higher elevations agrees with data from southern California (Mullens and Dada 1992). However, a notable difference on our study area was the apparent lack of suitable developmental sites in close proximity to capture sites. Immature *C. mohave* are generally associated with saturated sand/soil substrates in saline aquatic habitats (Foulk 1966, Wirth and DeMoraes 1979, Brenner et al. 1984, Mullens and Dada 1992). The 967 and K4 trap sites that yielded abundant *C. mohave* were 2.7 and 19.6 km, respectively, from the closest presumed developmental sites (two small water treatment brine ponds). Brenner et al. (1984) reported that *C. mohave* were captured up to 4.6 km from larval habitat, but numbers decreased precipitously at distances >2.5 km. Our results suggest that *C. mohave* has greater dispersal ability or may use breeding habitats other than those previously described.

Fig. 1. Captures of *Culicoides mohave* at four sites in the Sonoran Desert, southwestern Arizona, 2000–2001.
We analyzed a total of 55 and 22 pools of C. mohave and C. sonorensis, respectively, for nucleic acids associated with BLU and EHD viruses. For C. mohave, 19 pools (35%) were positive for EHD and all were negative for BLU. The bulk of EHD-positive C. mohave (79%) were from the 967 site and occurred in all sampling periods except April and October 2001. EHD-positive C. mohave were also found at the K4 site (September 2000, June 2001, and July 2001) and at the KV site (April 2001). All pools of C. sonorensis had negative PCR results for both EHD and BLU viruses.

Currently, C. sonorensis is the primary suspect vector for EHD virus in the United States; however, Nettles et al. (1991) noted that other species of Culicoides might be involved in transmission of EHD. Our results strongly suggest that C. mohave may fill this niche in southwestern Arizona, though the PCR-based approach did not indicate presence of live virus or determine whether or not C. mohave is actually capable of transmitting EHD virus. Thus, further work is needed to isolate EHD virus and confirm virus replication in C. mohave.

The local abundance of C. mohave and relatively high proportion of EHD-positive pools present opportunities for viral transmission among wild ungulates in our study area. EHD virus is a common disease of white-tailed deer (Odocoileus virginianus Zimmerman) in the southeastern United States (Nettles and Stallknecht 1992) and has recently been reported in Arizona white-tailed and mule deer (Odocoileus hemionus Rafinesque) (Noon et al. 2002). Lower elevation areas where we trapped EHD-positive C. mohave support large numbers of mule deer. Preliminary results of agar gel immunodiffusion tests using serum from fall 2002 hunter-killed deer yielded multiple positives for antibodies to EHD virus (D. Stallknecht, University of Georgia, personal communication). Forthcoming surveys in our study area will provide a more complete picture of EHD antibody prevalence and serotype distribution in potentially affected wild ungulates.

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