NOTE

Infection of *Culicoides sonorensis* (Diptera: Ceratopogonidae) with Weldona Virus[1]

Will K. Reeves[2] and Myrna M. Miller

Arthropod-Borne Animal Diseases Research Laboratory-ARS-USDA, Laramie, Wyoming 82071 USA

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Blood-feeding species of Ceratopogonidae (Diptera) transmit arboviruses, filaria, and malarial parasites to domestic animals, humans, and wildlife. In the USA, *Culicoides* spp. transmit several arboviruses. Main Drain virus (Bunyaviridae: *Orthobunyavirus*) is transmitted by *Culicoides sonorensis* Wirth and Jones (Diptera: Ceratopogonidae) and was associated with encephalitis in horses and fetal malformations in sheep (Emmons et al. 1983, J. Am. Vet. Med. Assoc. 183:555 - 558; Edwards et al. 1997, Am. J. Trop. Med. Hyg. 56:171 - 176.). Cattle in the USA are infected with Lokern virus (Bunyaviridae: *Orthobunyavirus*), which is transmitted by biting midges, but this virus is not yet known to cause disease (Sahu et al. 2002, Am. J. Trop. Med. Hyg. 67:119 - 122.). *Culicoides sonorensis* transmits bluetongue and epizootic hemorrhagic disease viruses (Reoviridae: *Orbivirus*) to domestic and wild ruminants (Foster et al. 1963, Am. J. Vet. Res. 24:1195 - 1200; Foster et al. 1977, J. Wild. Dis. 13:9 - 16) and is an experimental vector of Akabane (Bunyaviridae: *Orthobunyavirus*) and African horse sickness viruses (Reoviridae: *Orbivirus*) (Jennings and Mellor 1989, Vet. Microbiol. 21:125 - 131; Boonnan et al. 1975, Arch. Virol. 47:343 - 349).

Weldona virus (Bunyaviridae: *Orthobunyavirus*), the only known Nearctic virus in the Tete serogroup, was isolated from pools of unidentified ceratopogonids from Colorado (Calisher et al. 1990, Am. J. Trop. Med. Hyg. 43:314 - 318.). Other isolates of Tete serogroup viruses were from birds and an Egyptian tick (Calisher et al. 1990, Am. J. Trop. Med. Hyg. 43:314 - 318; Converse et al. 1974, Arch. Ges. Virusforsch. 1 - 2:29 - 35). Ornithophilic biting midges such as *Culicoides crepuscularis* Malloch might be natural vectors of Weldona virus. *Culicoides crepuscularis* also feeds on mammals (Blanton and Wirth 1979, Arthropods of Florida and Neighboring Land Areas. vol. 10. Florida Dept. Agric. Consu. Serv., Gainesville). *Culicoides sonorensis* is the only species of Nearctic biting midge maintained in large continuously maintained colonies,

[1]Received 08 December 2008; accepted for publication 20 February 2009.

[2]Address inquiries. Present address: USAG-J, Unit 45013, Box 2582, APO, AP 96338 (email: wkreeves@gmail.com).

and it serves as a model laboratory organism. Were *C. sonorensis* susceptible to Weldona virus, it could be used in laboratory experiments, such as in vivo viral reassortments and controlled infections of livestock or poultry.

Working stocks of Weldona virus (isolate 76V-21935), from stock provided by the Centers for Disease Control and Prevention (Fort Collins, CO), were produced in African green monkey kidney cells (Vero) up to 4.8 $\log_{10}$ $TCID_{50}$. Total RNA was extracted from 1 ml of lysed Vero cells and culture media with an RNeasy Kit™ (Qiagen, Valencia, CA). We attempted to amplify RNA from the extract by RT-PCR using protocols described for the S, M, and L RNA segments of bunyaviruses (Bowen et al. 2001, Virol. 291:185 - 190; Kuno 1998, J. Virol. Meth. 72:27 - 41; Moreli et al. 2001, Trans. R. Soc. Trop. Med. Hyg. 95:108 - 113; Yandoko et al. 2007, J. Gen. Virol. 88:1761 - 1766). PCR products were separated by electrophoresis on 3% agarose gels and visualized under UV light with GelStar™ (Cambrex BioScience Rockland, Inc. Rockland, ME). Products of the appropriate size were purified with a QIAquick PCR Purification Kit™ (Qiagen, Valencia, CA). Sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit™ (Applied Biosystems, Foster City, CA) using PCR primers. Sequences were determined using an ABI 3730™ capillary sequencer (Applied Biosystems, Foster City, CA), aligned and assembled with Chromas Lite 2.01 (Technelysium, Tewantin, Australia) and ClustalW (Kyoto University Bioinformatics Center, Japan), and compared with sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, MD). We amplified a portion of the M segment using primers M14C (5′-CGGAATTCAGTAGTGTACTACC-3′) and M619R (5′-GACATATGITGATTGAAGCAAGCATG-3′) described by Bowen et al. (2001, Virol. 291:185 - 190), but all other RT-PCR failed. The sequences could be aligned with other orthobunyaviruses. Unfortunately, sequences of the M segment from other Tete group viruses were not available. Yandoko et al. (2007, J. Gen. Virol. 88:1761 - 1766) discussed their inability to amplify the S segment from African Tete serogroup viruses. They suggested that Tete serogroup viruses were divergent from other orthobunyaviruses. A 587 bp c-DNA sequence of the M segment envelope glycoprotein gene was accessioned in GenBank (EU409297).

Colonies of *C. sonorensis* were continuously maintained as described by Hunt (1994, ARS-121, pp. 1 - 68. National Technical Information Service, Springfield, VA). Aliquots of 4.8 $\log_{10}$ $TCID_{50}$ virus in cell culture media (Medium 199 with Earle's salts in 10% fetal bovine serum) were mixed 1:1 with sheep blood and fed to females of *C. sonorensis* (Ausman colony) in 2 separate trials. We infected females of *C. sonorensis* with similar doses of other arboviruses. Adult midges were held in a cage without a sugar meal for 3 d prior to feeding. Females of *C. sonorensis* fully digest their blood meal and oviposit in 3 - 4 d post feeding (dpf) (Hunt 1994). After virus feeding, midges were maintained on a sugar water diet for 12 d at 24°C.

To ensure that the blood meals were fully digested and that the virus had time in infect the midges, we tested midges 8 - 10 dpf. The extrinsic incubation period for Weldona virus is unknown but Akabane virus can be transmitted in 7 - 10 dpf (Mellor et al. 2000, Ann. Rev. Entomol. 45:307 - 340). A total of 70 flies were tested for Weldona virus at 8 (37 flies), 9 (12 flies), 10 (21 flies), and 12 (46 flies) dpf by virus isolation. Female flies were sorted on a chill table, decapitated, and the heads and bodies were macerated separately in gnat antibiotic media (GAM) with gold-plated tungsten beads (Spirit River, Roseburg, OR) using a Tissue Lyser™ (Qiagen, Valencia, CA) as previously described by Kato and Mayer (2007, J. Virol. Meth. 140:140 - 147). To avoid contamination, a separate sterile scalpel was used for each decapitation, and all tools

used to move heads or bodies were sterilized with 4% bleach and then 70% ethanol between each transfer. Duplicate inoculums of 100 µL of midge homogenate and GAM were added to 100 µL of Vero cells (2.5 x 10^5 cells/ml). GAM consists of 400 U/ml penicillin, 400 µg/ml streptomycin, 200 µg/ml gentamicin, 25 µg/ml ciprofloxacin, and 5 µg/ml amphotericin B in Medium 199 with Earle's salts and 10% fetal bovine serum. These cells were incubated at 37°C with 5 - 6% CO_2 for 10 d. Supernatant was removed from all wells on day 10, the cells were stained with crystal violet to show plaques, and examined for cytopathic effect (CPE). RNA was extracted from the supernatant of wells with CPE and tested by RT-PCR as previously described. DNA sequencing was used to verify the presence of Weldona virus. The sequences were identical to those from virus stocks.

Weldona virus was isolated in duplicate from 1/37 and 1/21 of the 8 and 10-dpf midges. Virus was isolated from the bodies of the midges but not the heads. This implies that the virus did not disseminate from the midgut or the titer in the head was not high enough to cause CPE. Higher viral titers or a longer extrinsic incubation period might have allowed viral dissemination. Based on the results *C. sonorensis,* is unlikely to be a good laboratory model for transmission studies with Weldona virus. Unfortunately, there are no large-scale colonies of ornithophilic species of *Culicoides.* A voucher specimen of *C. sonorensis* (Ausman colony) was deposited in the University of Wyoming Insect Museum (Laramie).

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