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Two *Rhabdoviridae:* Dillard's Draw virus, a putative new virus, and Merida virus from *Culex tarsalis* (*Diptera: Culicidae)* in New Mexico, USA

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Summary. – The U.S. Air Force conducts vector and arbovirus surveillance worldwide. We report on two *Rhabdoviridae* detected at Holloman Air Force Base in Otero County, New Mexico including the nearly complete 11-kb genome of Merida virus, which was not previously known from the USA, and a novel virus tentatively named Dillard's Draw virus. Merida virus was previously only known from Mexico. Dillard's Draw virus represents a novel arbovirus most closely related to an avian pathogen from Australia and shares some genetic similarity to Durham virus from the eastern United States.

Keywords: arbovirus; deep sequencing; novel virus; Culex; encephalitis; Rhabdoviridae

Introduction

Holloman Air Force Base in Otero County, New Mexico, sits in a high desert near White Sands Proving Grounds. Mosquitos are a seasonal problem, with pest and vector species developing in water pools from rain, snow melt, and manmade sources. The U.S. Air Force conducts vector and arbovirus surveillance at most bases throughout the United States and overseas. Since the introduction of West Nile virus and the subsequent spread across the country, there has been both mosquito and virus surveillance in the region on one or more military bases in New Mexico (Witt *et al.*, 2004). Mosquitoes are collected by public health or civil engineering staff for entomological surveillance, killed, sorted to remove nonmedically relevant insects, and shipped dead to the U.S. Air Force School of Aerospace Medicine Public Health Consult Service for identification and arbovirus screening (Reeves *et al.*, 2016).

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After identification, mosquitoes were pooled by species and tested for arboviruses. Most were tested with rapid screening assays (Stone *et al.*, 2005; Coleman *et al.* 2009). The Entomology Laboratory also sporadically tested arthropods for a wide range of vector-borne pathogens such as *Alphavirus*, *Orthobunyavirus*, *Phlebovirus*, *Rickettsia*, and *Coxiella* using a variety of assays (Wolf and Reeves, 2012; Reeves *et al.*, 2013, 2015; Tuten *et al.*, 2013; Taylor *et al.*, 2016). Identification of vector-borne pathogens in arthropods is important for protecting public health, which is especially true for the military, who often work outside and for whom locally acquired arboviruses have killed more U.S. Air Force associated personnel than foreign diseases such as malaria since the 1970s and cause significant morbidity in people (Anna *et al.*, 2012; Reeves and Bettano, 2014).

In 2012–2016, *Culex tarsalis* were collected in late summer through fall and initially screened using the RAMP West Nile virus test. We investigated the possibility that these positive pools had either novel virus in the same vector population. We attempted full genome sequencing of viral RNA from a West Nile virus-positive mosquito pools from 2015 to identify other probable arboviruses.

Materials and Methods

Mosquitoes were submitted weekly during the summer months from Holloman AFB from 2011–2016. Mosquitoes were primarily trapped using Mosquito Magnets® (Woodstream Corp., Litiz, PA) in a gulch named Dillard's Draw on Holloman Air Force Base, Otero County, New Mexico. As a surveillance program, traps were run for 1 week and insects were sorted and removed. A pool of 50 *Cx. tarsalis* from a collection on 22 July 2015 was positive for West Nile virus. RNA was extracted from 100 µl of clarified mosquito pool supernatant using spin columns [ZR viral RNA kit (Zymo Research, Irvine, CA)]. Viral RNA was transformed into complementary DNA libraries with TruSeq Sample Prep (Illumina, San Diego, CA), sequenced using the Illumina MiSeq System (Roy J. Carver Biotechnology Center/WM Keck Center, University of Illinois at Urbana-Champaign), and sequences assembled *de novo* using the Ray Assembler program (Ray v2.3.1, free software maintained by Sebastien Boisvert). Contiguous sequences were compared to reference virus sequences in GenBank with NCBI BLAST. Using all contigs over 300 nucleotides in length, over 50 matched other viral sequences, ranging from portions of *Rhabdoviridae*, insect bunyaviruses, Flavivirus including West Nile virus, to an Iflavirus. We compared novel *Rhabdoviridae* virus sequence with the complete genetic sequences of *Rhabdoviridae* in GenBank, including American bat vesiculovirus TFFN 2013 isolate liver2008, Arboretum virus isolate Lo 121, Berrimah virus strain DPP 63, Bovine ephemeral fever virus isolate Bovine/China/Henan1/2012, Chandipura virus isolate CIN 0451, Coastal Plains virus strain DPP53, *Culex tritaeniorhynchus* rhabdovirus strain TY, Curionopolis virus isolate BE AR 440009, Dolphin rhabdovirus, Drosophila melanogaster sigma virus isolate AP30, Drosophila obscura sigma virus 10A, Eggplant mottled dwarf virus isolate Agapanthus, Fikirini bat rhabdovirus isolate KEN352, Grass carp rhabdovirus V76, Hart Park virus strain AR7C, Hybrid snakehead virus isolate C1207, Kolente virus isolate DakAr K7292, Lettuce necrotic yellows virus isolate 318, Long Island tick rhabdovirus strain LS1, Malpais Spring virus strain 85 488NM, Merida virus isolate MERD Mex07, Moussa virus isolate C23, Ngaingan virus MRM14556, Niakha virus DakArD 88909, Oak Vale virus strain CSIRO 1342, Pike fry rhabdovirus isolate F4, Puerto Almendras virus isolate LO 39, Rabies virus strain PV 2061, *Scophthalmus maximus* rhabdovirus, *Siniperca chuatsi* rhabdovirus from China, Snakehead rhabdovirus, *Spodoptera frugiperda* rhabdovirus isolate Sf, Sunguru virus isolate Ug#41, Tench rhabdovirus S64, Tupaia rhabdovirus, Vesicular stomatitis Indiana virus variant popA, and Xiburema virus isolate XIBV/BE AR 362159 viruses, and a phylogenic tree was constructed using MEGA-5 as described by Tamura *et al.* (2011).

The full sequence of Dillard's Draw virus was designated isolate DDrV-2015 and the annotated sequence was submitted to GenBank (MG251664). A voucher of *Culex tarsalis* from the Holloman Air Force Base was deposited in the Ohio Museum of Biological Diversity.

Results

Two complete or nearly complete rhabdovirus genomes of were found. Fourteen contigs combined covered 97.8% of the total genome (11,536/11,799 nt) of the recently identified Merida virus, with 98% identity. In addition, a single contig of 11,142 nt contained the complete genome of a second, novel rhabdovirus we tentatively named Dillard's Draw virus. We named Dillard's Draw virus for the location where mosquitoes were trapped. The assembled genome was ~11 kb. BLAST results partially matched numerous rhabdovirus genomes or genes. The full genome was most similar (70% identical) to the complete genomes of Oak-Vale virus strains CSIRO 1342 and K13965 (GenBank# JF705876, JF705877) isolated from *Culex edwardsi* in Australia. Oak-Vale virus was fully described by Quan *et al.* (2011). Individual genes and segments were 65–100% matches to over 100 other genes

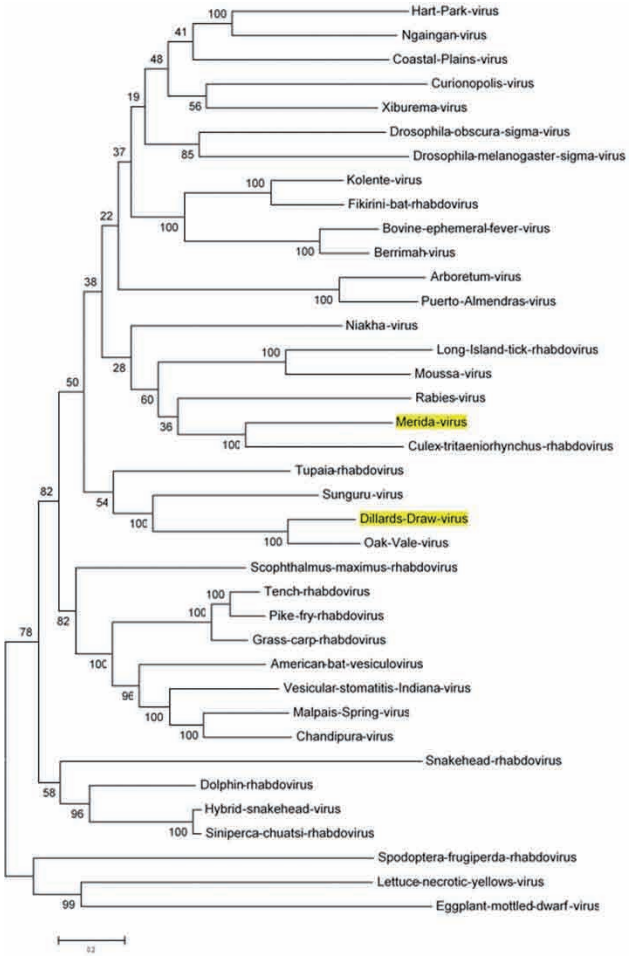

Fig. 1

Phylogenetic reconstruction of select *Rhabdoviridae* with Dillard's Draw virus using complete genome sequences

of rhabdovirus isolates in GenBank. The phylogenetic tree (Fig. 1) reconstructed using representatives of the majority of known Rhabdovirus groups place Dillard's Draw virus with Oak-Vale virus and Sunguru virus in the Sandjimba group or Almpiwar group.

In addition, we sequenced ~11 kb genome of Merida virus in 14 overlapping contigs. The BLAST result was a near match (98%) for the full genome of Merida virus (GenBank# KU194360). Merida virus is an unclassified *Rhabdoviridae* recently described from *Culex* spp. in Mexico (Charles *et al.*, 2016).

Discussion

We sequenced almost the entire genome of Merida virus and it was essentially a complete match to the recently described virus from Mexico. Merida virus has not been cultured and is not known to cause disease in humans or vertebrates and might be an obligate insect virus (Charles *et al.*, 2016). Merida virus was the most recently described rhabdovirus from *Culex* spp. in North America (Charles *et al.*, 2016) and was not similar to Dillard's Draw virus. Our sequencing-based detection represents a new host association for the Merida virus and the first detection in the United States.

Based on genetic similarity, Dillard's Draw virus is almost certainly in the Rhabdoviridae and, along with Oak-Vale virus, in the Sandjima virus or Almpiwar group. Dillard's Draw virus is most similar to some Australian viruses, but there is no indication that we detected a foreign virus. It was not similar to Hart Park virus, which represented a group of rhabdoviruses isolated from *Cx. tarsalis* in North America. Deep sequencing of possible insect vectors allows the detection of unknown viruses without culture including those from dead mosquitoes (Reeves *et al.*, 2016) as do PCR screening assays (Wellehan *et al.*, 2012). Dillard's Draw virus shares a very close molecular similarity to known potentially fatal pathogens of birds. Based on continued detection from mosquitoes, they are probably transmitted by arthropods, but there is at least some data to refute mosquito-borne transmission of Sunguru virus due to limited persistence in one mosquito species (Allison *et al.*, 2011; Quan *et al.*, 2011; Ledermann *et al.*, 2014). The strong sequencing results from our sample imply large viral copy numbers in the mosquito pool. In North America the closest match is Durham virus, which was isolated from a moribund American coot (*Fulica americana*) in North Carolina (Allison *et al.*, 2011). Durham virus is possibly a fatal pathogen in some birds. Viruses in the Sandjima virus or Almpiwar group are relatively poorly studied for their relevance to human or animal health. Dillard's Draw virus might remain a largely innocuous arbovirus.

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