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West Nile Virus Isolated from a Virginia Opossum (*Didelphis virginiana*) in Northwestern Missouri, USA, 2012

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Abstract

We describe the isolation of West Nile virus (WNV; *Flaviviridae*, *Flavivirus*) from blood of a Virginia opossum (*Didelphis virginiana*) collected in northwestern Missouri, USA in August 2012. Sequencing determined that the virus was related to lineage 1a WNV02 strains. We discuss the role of wildlife in WNV disease epidemiology.

West Nile virus (WNV; *Flaviviridae*, *Flavivirus*) was introduced into the US in 1999 and has since become the leading cause of domestically acquired arboviral disease in humans (Reimann et al. 2008). The virus circulates in enzootic cycles between *Culex* mosquito vectors and certain competent avian hosts, and causes epidemics/epizootics when it infects large numbers of disease-susceptible hosts, such as humans and horses. Passerine birds are the major WNV amplification hosts and humans serve as incidental hosts, incapable of serving as an infection source for mosquitoes. However, some mammals may play a role in WNV maintenance or amplification. Few WNV isolates have been obtained from sampling wild mammals (Root 2013). Here we describe the isolation of WNV from a Virginia opossum (*Didelphis virginiana*; order Marsupialia) collected in northwestern Missouri.

Free-ranging mammals and birds were sampled in northwestern Missouri, USA, as part of a larger arbovirus surveillance study, which will be described elsewhere. In August 2012, blood samples were collected from 16 Virginia opossums trapped using Tomahawk live traps (81×25×30 cm; Tomahawk Live Traps, LLC, Hazelhurst, Wisconsin, USA). Opossums were anesthetized with isoflurane in customized anesthesia chambers similar to those described by Bentler et al. (2012) or by intramuscular injection of ketamine/ xylazine

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(60 mg/kg of 5:1 mixture), bled from a peripheral vein, individually marked with ear tags, and subsequently released at their locations of capture. Blood samples were centrifuged for separation of serum, frozen on dry ice, and transported to the Centers for Disease Control, Division of Vector-Borne Diseases, in Fort Collins, Colorado, USA. Samples were thawed and serum tested for virus isolation by plaque assay on Vero cells (Beaty et al. 1995). One of 16 serum samples (sample MO12-W059 from an adult opossum in Nodaway County, Missouri, USA, 10 August 2012) produced viral plaques (2.5 $\log_{10}$ pfu/mL); several plaques were harvested and RNA was extracted for RT-PCR analysis using a One-Step RT-PCR kit according to manufacturer's instructions (Qiagen, Valencia, California, USA). Full-length genome sequencing using Illumina (San Diego, California, USA) next-generation sequencing technology and genetic analysis demonstrated that the isolate was a member of lineage 1a viruses, related to other WNV02 strains as determined by screening for diagnostic residues and comparing to DNA sequences in GenBank. The closest match was an isolate from mosquitoes in Texas in August 2012 with 99.6% shared nucleotide identity and 99.7% amino acid identity (Duggal et al. 2013). All other serum samples were screened for prior WNV exposure using a plaque reduction neutralization test and none were positive for WNV-neutralizing antibodies (Beaty et al. 1995).

Missouri experienced extreme drought conditions in August 2012 (US Drought Monitor 2012), and very few mosquitoes were present during the sampling period. Following collection by CDC light traps (BioQuip Products, Rancho Dominguez, California, USA) baited with dry ice, mosquitoes were identified, sorted, and pooled by species. The RNA was extracted from pools using a BioRobot Universal (Qiagen) and quantitative reverse transcriptase–PCR was used for WNV RNA detection. A total of 679 mosquitoes were collected in the study region during early August 2012, of which the dominant species (n=570) was *Culex erraticus.* All pools were negative for WNV RNA (Savage et al. 2013).

Sylvatic transmission of WNV is poorly understood, but serologic evidence suggests that multiple species of vertebrates are exposed and therefore are potentially involved in viral maintenance or amplification. The roles of mammals other than humans and horses are less clear. Passerine birds of the Missouri rural forests, such as Northern Cardinal (*Cardinalis cardinalis*), Blue Jay (*Cyanocitta cristata*), and American Robin (*Turdus migratorius*), are probable amplification hosts (Bowen and Nemeth 2007). However, many other species of birds, mammals, and even ectotherms are exposed to WNV by a variety of vectors and could play roles in the epizootiology of WNV, or be at risk of developing disease. Large wild carnivores and mesocarnivores, including striped skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*), and black bear (*Ursus americanus*), have been found to be antibody positive for WNV (Bentler et al. 2007; Root 2013). Experimental infection of raccoons has demonstrated moderately high viremias and viral fecal shedding up to 10 days postinfection (Root et al. 2010). Similarly, some species in the order Rodentia, including fox squirrel (*Sciurus niger*), eastern gray squirrel (*Sciurus carolinensis*), and eastern chipmunk (*Tamias striatus*), as well as eastern cottontail (*Sylvilagus floridanus*; order Lagomorpha), can develop viremias $>10^5$ pfu/mL serum. This level indicates that some mosquitoes could become infected through viremic blood meal acquisition from these mammals (Tiawsirisup et al. 2005; Root et al. 2006; Platt et al. 2008).

In spite of a very low mosquito presence in the region at the time of collection, WNV was isolated for the first time from a Virginia opossum, although neutralizing antibody has been detected in this species previously (Dietrich et al. 2005; Bentler et al. 2007). According to the Arbonet reporting system of the Centers for Disease Control and Prevention, 2012 ranked as the second highest year for human WNV cases in the US, with 2003 being the most severe outbreak year, so it is not surprising that sylvatic WNV activity was evident as well. The predominant mosquito present at the time of sampling, *C. erraticus*, is not considered a primary WNV vector, although it appears to be important for WNV transmission in Alabama swamps and is known to feed preferentially on birds (Hassan et al. 2003; Cupp et al. 2007). Of the other mosquito species present, albeit in low numbers, *Culex tarsalis*, *Culex salinarius*, and *Culex restuans* are all potential WNV vectors. Detection of WNV in a Virginia opossum is a reminder that the diversity of hosts infected with WNV is high and that investigations into novel hosts will be needed to fully assess the potential for alternative transmission cycles that could serve to maintain viral circulation during climactic anomalies such as the drought observed in Missouri in 2012.

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