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The First 10 Years (2006–15) of Epizootic Hemorrhagic Disease Virus Serotype 6 in the USA

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ABSTRACT: Epizootic hemorrhagic disease virus (EHDV) is a *Culicoides* biting midge–transmitted orbivirus (family *Reoviridae*) of wild and domestic ruminants and is an important pathogen of white-tailed deer (*Odocoileus virginianus*). Historically, only two serotypes, EHDV-1 and EHDV-2, have been known to be endemic in the US. However, in 2006, an exotic serotype (EHDV-6) was first detected in the US by a long-term passive surveillance system for EHDV and bluetongue viruses. Here we report EHDV-6 detections made through these passive surveillance efforts by the Southeastern Cooperative Wildlife Disease Study (University of Georgia, Athens, Georgia, USA) and the National Veterinary Services Laboratories (US Department of Agriculture, Ames, Iowa, USA) over a 10-yr period (2006–15). The results demonstrated that EHDV-6 was detected from ruminants every year since 2006 and was widespread in the central and eastern US, providing evidence that EHDV-6 is likely now established in the US.

Key words: Epizootic hemorrhagic disease virus serotype 6, hemorrhagic disease, orbivirus, vector-borne disease, white-tailed deer.

Epizootic hemorrhagic disease (EHD) virus (EHDV) is an orbivirus (genus *Orbivirus*, family *Reoviridae*) transmitted between susceptible ruminant hosts by *Culicoides* biting midges (Diptera: Ceratopogonidae) and is a significant pathogen of white-tailed deer (WTD; *Odocoileus virginianus*; Howerth et al. 2001). Seven EHDV serotypes (1, 2, and 4–8) have been identified in various temperate and tropical regions of the world (Anthony et al. 2009; King et al. 2012). Historically, only EHDV-1 and -2 have circulated in North America, causing cyclical outbreaks among WTD. However, in 2006, EHDV-6 was isolated from dead WTD in Indiana and Illinois (Allison et al. 2010). This virus was determined to be a genetic reassortant EHDV between serotype 2 (endemic) and serotype 6 (exotic). Phylogenetic analysis of EHDV-6 strains from the US, South Africa, Guadeloupe, Australia, and Bahrain suggests the EHDV-6 from the US is most closely related to the CSIRO 753 strain first identified in Australia in 1981 (Allison et al. 2012). To date, the parental strain of EHDV-6 has not been detected in the US (Allison et al. 2010, 2012; Anbalagan et al. 2014). In addition to EHDV-6, numerous exotic bluetongue virus (BTV) serotypes have been isolated from wild and domestic ruminants in the US since 1999 (Ruder et al. 2015). Collectively, the detection of these exotic orbiviruses in the US is of concern for wildlife and livestock health (Gibbs et al. 2008). It is not known which, if any, of these exotic viruses have become established. Here we report EHDV-6 detections over a 10-yr period (2006–15) made through passive surveillance efforts by the Southeastern Cooperative Wildlife Disease Study (SCWDS; University of Georgia, Athens Georgia, USA) and the National Veterinary Services Laboratories (NVSL; US Department of Agriculture, Ames, Iowa, USA).

Diagnostic virology data from SCWDS and NVSL were compiled for the 10-yr period (2006–15) since the first isolation of EHDV-6 in the US. Briefly, tissue samples from sick and dead wild and domestic ruminants suspected to be infected with EHDV or BTV are submitted each year during summer

TABLE 1. Epizootic hemorrhagic disease virus serotype 6 (EHDV-6) isolated or identified from white-tailed deer (*Odocoileus virginianus*) and other ungulates by the Southeastern Cooperative Wildlife Disease Study (University of Georgia) and the National Veterinary Services Laboratories (US Department of Agriculture) from 2006 to 2015 in the USA. — = no data available.

State	Year									
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Arkansas	—	—	—	—	1	—	1	—	—	—
Florida	—	—	—	—	—	—	4	1	2	2
Illinois	4	—	—	—	—	—	11[a]	—	—	—
Indiana	2	—	—	—	—	—	3	—	—	—
Iowa	—	—	—	—	—	—	10[b]	—	—	—
Kansas	—	—	1	—	—	—	—	—	—	—
Kentucky	—	—	—	—	—	—	2	—	—	—
Louisiana	—	—	—	—	—	—	3	—	1	—
Maryland	—	—	—	—	—	—	1	—	—	—
Michigan	—	—	—	3	—	—	23	1	—	—
Mississippi	—	—	—	—	—	—	4	—	—	—
Missouri	—	1	—	1	—	—	5	—	—	1
Nebraska	—	—	—	—	—	—	2	—	—	—
North Carolina	—	—	—	—	—	—	—	—	6	1
Oklahoma	—	—	—	1	—	1	—	—	—	—
South Dakota	—	—	—	—	—	1[c]	1	1	—	—
Texas	—	—	7	1	—	—	—	—	—	—
Wisconsin	—	—	—	—	—	—	7	—	—	—
Total	6	1	8	6	1	2	77	3	9	4

[a] Three of 11 EHDV-6 detections were from domestic cattle (*Bos taurus*).

[b] One EHDV-6 detection from domestic cattle and one from a captive elk (*Cervus canadensis*).

[c] EHDV-6 detection was from a mule deer (*Odocoileus hemionus*).

and autumn months to SCWDS and NVSL for virus detection and identification. At SCWDS, viruses are isolated in cell culture using cattle pulmonary artery endothelial cells (American Type Culture Collection, Manassas, Virginia, USA) and identified to serotype by virus neutralization (Quist et al. 1997; World Organisation for Animal Health 2016) and/or RT-PCR (Allison et al. 2010; World Organisation for Animal Health 2016). At NVSL, tissues are first screened for EHDV and BTV by RT-PCR prior to virus isolation attempts using cattle pulmonary artery endothelial cells (Hofmann et al. 2008; Clavijo et al. 2010). The majority of the EHDV detections reported here were from wild or penned WTD, although EHDV was also detected in tissues from other wild and domestic ruminants.

From 2006 to 2015, EHDV-6 was detected each year from a total of 117 ruminants, including 111 WTD, four domestic cattle (*Bos taurus*), one mule deer (*Odocoileus hemionus*), and one elk (*Cervus canadensis*; Table 1). Detections of EHDV-6 were widely distributed throughout the central and eastern US and included viruses from 18 different states (Fig. 1). The EHDV serotype most commonly isolated by SCWDS was EHDV-2 (n=647), followed by EHDV-6 (n=84) and EHDV-1 (n=38) over the 10-yr study period (Table 2). During most years, EHDV-6 was detected in low numbers, ranging from one to nine detections annually, typically representing a small portion of overall EHDV detections (EHDV-1, -2, and -6; Table 2). However, during a large-scale outbreak in 2012, EHDV-6 was detected in 77 ruminants from 14 states as far apart as South Dakota and Florida. Furthermore, among all EHD viruses isolated by SCWDS during the 2012

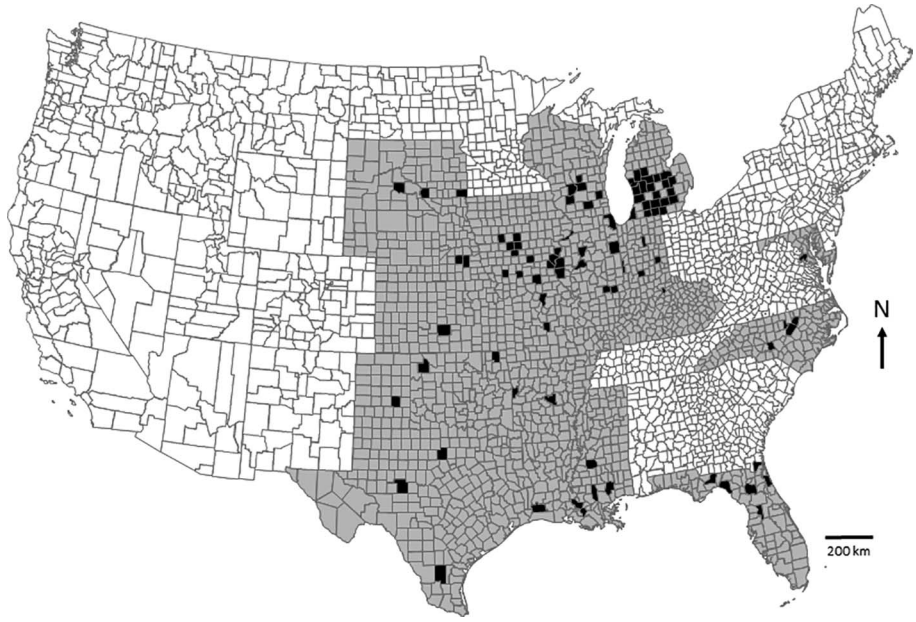

FIGURE 1. Distribution of epizootic hemorrhagic disease virus serotype 6 (EHDV-6) detections from wild and domestic ruminants by the Southeastern Cooperative Wildlife Disease Study (University of Georgia) and the National Veterinary Services Laboratories (US Department of Agriculture) 2006–15. Gray and black shading show where EHDV-6 detections were made in states and counties, respectively.

EHD outbreak, EHDV-6 represented 28% (56/199) of virus isolates (Table 2).

Although based only on results of passive surveillance for EHDV and BTV among ruminants by two laboratories, these findings suggest EHDV-6 is likely established in the US and represents a third EHDV serotype actively circulating among ruminant and vector populations. Over the 10 yr since the first isolation of EHDV-6 in the US (2006), this virus has been confirmed during each virus transmission season among sick and dead ruminants (primarily WTD) throughout much of the central and eastern US. In addition to this apparent broad geographic distribution, virus isolation results from SCWDS indicate the number of EHDV-6 isolations (84) surpassed EHDV-1 isolations

TABLE 2. Number of epizootic hemorrhagic disease viruses (EHDV) isolated from dead white-tailed deer and other ungulates by the Southeastern Cooperative Wildlife Disease Study (University of Georgia) from 2006 to 2015.

	Year										
	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total
EHDV-1	5	5	7	0	3	0	8	7	0	3	38
EHDV-2	31	275[a]	12	28	11[b]	48	135[c]	48[d]	17[e]	42	647
EHDV-6	6	1	6	3	1	0	56	2	6	3	84
Total	42	281	25	31	15	48	199	57	23	48	

[a] Seven of 275 EHDV-2 isolates during 2007 were from cattle (*Bos taurus*).

[b] Two of 11 EHDV-2 isolates during 2010 were from elk (*Cervus canadensis*).

[c] Nine of 135 EHDV-2 isolates during 2012 were from cattle and one each from a mule deer (*Odocoileus hemionus*) and an alpaca (*Vicugna pacos*).

[d] One of 48 EHDV-2 isolates during 2013 was from an American bison (*Bison bison*).

[e] One of 17 EHDV-2 isolates during 2014 was from a black-tailed deer (*Odocoileus hemionus columbianus*).

(38) during the 2006–15 study period. This finding is somewhat surprising, because EHDV-1 is historically endemic in the US and was the first EHDV serotype to be identified, with the prototype strain isolated in New Jersey in 1955 (Shope et al. 1960). The underlying mechanisms driving these observations are unknown.

During the study period, intense and geographically widespread EHD outbreaks occurred in 2007 and 2012. The apparent geographic centers varied, with the 2007 outbreak largely centered along large stretches of the Ohio River Valley and Mid-Atlantic states, whereas the 2012 outbreak was focused along the middle and lower Missouri River Valley and upper Midwestern states (Ruder et al. 2015; Stallknecht et al. 2015). Interestingly, EHDV-6 was rarely detected during 2007 (one detection) but became more prevalent during 2012 (77 detections; Tables 1, 2). In fact, most EHDV-6 detections (66%, 77/117) over the 10-yr period were made during 2012, and, given the geographic distribution of that outbreak, the majority of detections (69/117) were from northern states, including Wisconsin, Michigan, South Dakota, Illinois, Indiana, Iowa, and Nebraska. While this is possibly an artifact of a passive surveillance system, the frequent detection of EHDV-6 in this region is intriguing and is consistent with the reported northern expansion of hemorrhagic disease in the Midwest and Northeast (Stallknecht et al. 2015). The potential mechanisms driving the increased number of EHDV-6 detections in the northern Great Plains and upper Midwest during 2012 are not known. A variety of interacting host-vector-virus-environmental factors may have been involved that facilitated transmission in the region. For instance, a novel virus, an immunologically naïve population, and harsh environmental conditions (e.g., heat, drought) potentially favored transmission by *Culicoides* vectors. However, similar conditions existed during the 2007 EHD outbreak, and EHDV-6 nearly went undetected. One possible explanation is this newly emerging virus was not yet well established in vector and ruminant populations at the time of the 2007 outbreak. However, neutralizing antibodies against EHDV-6 were detected in WTD in the southern US as early as 2000, suggesting that the virus may have circulated undetected in the US prior to 2006 (Hecht 2010).

The full story of the introduction and establishment of EHDV-6 in the US may never be completely understood, but the potential for years of silent virus transmission prior to detecting the virus is of interest. Although southern WTD are commonly infected with EHDV and BTV, widespread morbidity and mortality are not common in these populations, and viruses may circulate undetected and evade passive surveillance systems that rely on clinically affected animals. This has important implications for the introduction of novel or exotic viruses and highlights the need for active surveillance or sentinel systems for early detection of novel viruses.

These findings also highlight gaps in our knowledge of EHDV transmission. The only confirmed vector of EHDV in the US, *Culicoides sonorensis*, is not commonly reported throughout much of the upper Midwestern and Eastern US, where EHDV-6 has been commonly detected (Schmidtmann et al. 2011; Ruder et al. 2015). Further, EHDV-6 replicates poorly in colonized *C. sonorensis* (Ruder et al. 2016), and the potential for transmission of EHDV-6 by other *Culicoides* spp. has not been investigated. Experimental studies that aim to incriminate other *Culicoides* spp. as vectors of EHDV, including EHDV-1 and -2, are needed (Pfannenstiel et al. 2015).

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