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Epizootic Hemorrhagic Disease in Alberta, Canada

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ABSTRACT: Epizootic hemorrhagic disease (EHD) virus serotype 2 was identified by reverse-transcription (RT)-PCR in a white-tailed deer (*Odocoileus virginianus*) found dead in southern Alberta in September 2013. Field observations indicate at least 50 deer, primarily white-tailed deer, and three pronghorn antelope (*Antilocapra americana*) died during a suspected localized EHD outbreak.

Epizootic hemorrhagic disease (EHD) virus is the most widespread pathogenic arbovirus of white-tailed deer (*Odocoileus virginianus*) in North America. The virus is enzootic across a broad range of habitats and ecosystems spanning the continental US from Florida to Montana and California (Nettles et al. 1992). Though less well-documented, the virus may also be present over broader areas of the southwest US (Noon et al. 2002). Epizootic hemorrhagic disease virus is transmitted by biting midges of the genus *Culicoides*.

In the US, mortality due to EHD in white-tailed deer generally has an annual seasonal occurrence, although the extent of the outbreaks differs among years (Howerth et al. 2001). In Canada, EHD occurs rarely and sporadically in southern portions of British Columbia (Dulac et al. 1989), Alberta (Chalmers et al. 1964, Ditchfield et al. 1964), and Saskatchewan (Dulac et al. 1992). These outbreaks have been linked to strong winds from the south that blow infected midges from enzootic or epizootic northern states into southern prairie habitats of the three western provinces (Sellers and Maarouf 1991).

Mortality associated with EHD was observed in southern Alberta in 1962 (Ditchfield et al. 1964). At least 450 white-tailed deer, 20 mule deer (*Odocoileus hemionus*), and 15 pronghorn antelope (*Antilocapra americana*) were found dead during the outbreak. Mortality associated with EHD has not been detected in the province since then, despite ongoing passive surveillance. However, minor outbreaks could have gone unnoticed.

From 5–16 September 2013, a cluster of at least 50 dead deer (primarily white-tailed deer) and three dead antelope were reported to Fish and Wildlife offices in southern Alberta. All carcasses were in the same geographic area within 30–50 km north of the US border and were observed by the public during agricultural harvest activities, often near water reservoirs in farmed fields. Given the unusual number of carcasses, proximity to water, location near the US border, time of year, and an ongoing disease outbreak in Montana and North Dakota, EHD was considered a differential diagnosis. Local Fish and Wildlife staff was asked to record all reports of dead deer or antelope and investigate when possible.

Carcasses generally were found in advanced decomposition. One dead white-tailed deer and an antelope were submitted for postmortem examination at the Alberta Agriculture and Rural Development diagnostic laboratory in Lethbridge. Fresh and formalin-fixed tissues were collected and prepared for virus isolation and histopathologic examination, respectively. Severe autolysis precluded interpretation of gross and histologic lesions in both animals, although increased blood-stained serosanguineous fluid was present in the thoracic cavity and pericardial sac of the deer.

Spleen tissues from the deer and antelope were submitted to Michigan State University Diagnostic Center for

Population and Animal Health in Lansing, Michigan, USA for virus testing using nested PCR targeting the NS1 protein of EHD by standard testing procedure. The white-tailed deer was positive and the antelope was negative for EHD virus.

Lung, liver, kidney, retropharyngeal lymph node, mediastinal lymph node tissues, and fluid from the thoracic cavity of the white-tailed deer were submitted to the National Centre for Foreign Animal Disease Laboratory in Winnipeg, Manitoba, Canada for virus isolation and typing using standard methods. No cytopathic effects were observed following each of three passages through tissue culture. No further attempts to isolate virus were made. Standard protocol enzyme-linked immunosorbent assay (ELISA) for antibodies to EHD was performed on fluids collected from the tissue mixture and fluid from the thoracic cavity. The fluids were negative for antibodies to EHD virus, suggesting per-acute infection in the deer or lack of detectable antibody levels in the specimens. Nucleic acid was then extracted from lung, liver, kidney, mediastinal lymph node, and retropharyngeal lymph node tissue homogenates and tested by RT-PCR using standard protocol targeting the S10 gene segment NS3 of EHD virus. The RT-PCR products from lung tissue were then cloned and sequenced and EHD type-2 virus was confirmed (99% identity). Thus, two independent laboratories using PCR methods confirmed that multiple tissues in the deer contained EHD virus, the first documented case in Alberta in >50 yr.

Mortality related to EHD in white-tailed deer was widespread across the US in 2012 (Southeastern Cooperative Wildlife Disease Study 2012). Outbreaks of EHD in the US also occurred in 2013, although on a smaller scale (Illinois Department of Natural Resources 2013; Iowa Department of Natural Resources 2013). The primary serotype in recent outbreaks of EHD in the northern states was EHDV-2 (Southeastern Cooperative Wildlife Disease Study 2012), which is consistent with the 2013 EHD outbreak in southern Alberta. Concurrent EHD virus activity in northern states may have resulted in infected populations of midges that were carried by wind into southern Alberta in late August and early September. Prevailing winds across Alberta are from the northwest; however, gusting winds out of the south were recorded in the Milk River area at the Environment Canada weather station at Onefour, Alberta on 3 August and 1 September 2013 (Environment Canada 2013). This timeframe is consistent with the rapid onset of EHD in deer and the first detection of mortality in Alberta on 5 September 2013.

Increased occurrence of EHD virus in states of the northern US since 2007 (Southeastern Cooperative Wildlife Disease Study 2012) may indicate the virus is expanding its geographic range northwards, perhaps in conjunction with the effects of changing climate on vector populations as suggested by Wittmann and Baylis (2000) and Purse et al. (2005). Wildlife and livestock managers in southern regions of Canada may need to bolster current passive surveillance efforts to document EHD virus activity in future years.

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