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Adenovirus Hemorrhagic Disease in Moose (*Alces americanus gigas*) in Alaska, USA

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ABSTRACT: In 1993, an epizootic of adenovirus hemorrhagic disease (AHD) caused the death of at least 1,000 mule deer (*Odocoileus hemionus*) in California. Since then, numerous cervid species throughout the United States have had deaths confirmed to be caused by AHD. In 2015, the death of two captive moose (*Alces americanus gigas*) calves marked the first recognized AHD-caused deaths in Alaska, a state in which moose are important economically as well as for food security and cultural identity. Both cases were characterized by systemic vasculitis with endothelial cell intranuclear inclusion bodies, pulmonary edema, petechial hemorrhages, and enterotyphlocolitis.

Key words: Adenovirus hemorrhagic disease, *Alces americanus gigas*, *Deer atadenovirus A*, moose.

Adenovirus hemorrhagic disease (AHD) is caused by a virus, *Deer atadenovirus A*, previously Odocoileus adenovirus (OdAdV-1), which can infect a wide variety of both domestic and wild cervids and is clinically similar to other hemorrhagic diseases, such as epizootic hemorrhagic disease and bluetongue (Miller et al. 2017). The largest known AHD outbreak occurred in northern California in which an estimated 1,000 mule deer (*Odocoileus hemionus*) were infected with OdAdV-1 and died during a 1993 epizootic event (Woods et al. 1996). Before 1993, AHD was not known in cervids, but previous cases may have been misdiagnosed as bluetongue or epizootic hemorrhagic disease (Woods et al. 1996). Additional species in which fatalities occur include white-tailed deer (*Odocoileus virginianus*), black-tailed deer (*O. hemionus columbianus*), moose (*Alces alces*), and elk (*Cervus canadensis nelson*; Woods et al. 2018). We report a case study of two captive Alaska moose (*Alces americanus gigas*) calves that died in 2015 and represented the first recognized cases of AHD in Alaska.

Case 1 was a male, wild calf orphaned at approximately 5 d old in Wasilla, Alaska. It was placed at a captive wildlife facility in south-central Alaska. At 7 mo the calf developed lethargy and inappetence. It fell over when attempting to stand, exhibited seizures, and died within 2 h. Case 2, a newborn male, was confiscated from private property in Willow, Alaska. It was temporarily housed at a zoo before permanent placement at the same facility as Case 1. At 8 mo old, it developed acute diarrhea and weight loss prior to a seizure and death. Necropsies were performed on each calf shortly after death using standard protocols. Tissues were immersed in 10% buffered neutral formalin and processed routinely for histologic examination. Additional fresh tissue was submitted for virology, bacteriology, and parasitology analyses and archived at –80 C.

Both calves had severe pulmonary edema and proteinaceous pleural effusion that clotted on exposure to air (Fig. 1A). Petechial hemorrhages were present in both calves, located in the subcutaneous tissues and serosa of the cecum and ileum of Case 1 and on the serosal surfaces of the kidney, ileum cecum, and colon in Case 2. Case 1 had severe bronchopneumonia with bronchiectasis. Both calves had chronic oral ulcers, Case 1 in the hard palate and Case 2 on the tongue. Both had enterotyphlocolitis most severe in the cecum with multifocal areas of infarction of the mucosa (Fig. 1B). The primary histopathologic finding was acute to subacute vasculitis in multiple organs with intranuclear inclusion bodies within hypertrophied endothelial cells characteristic of adenovirus hemorrhagic dis-

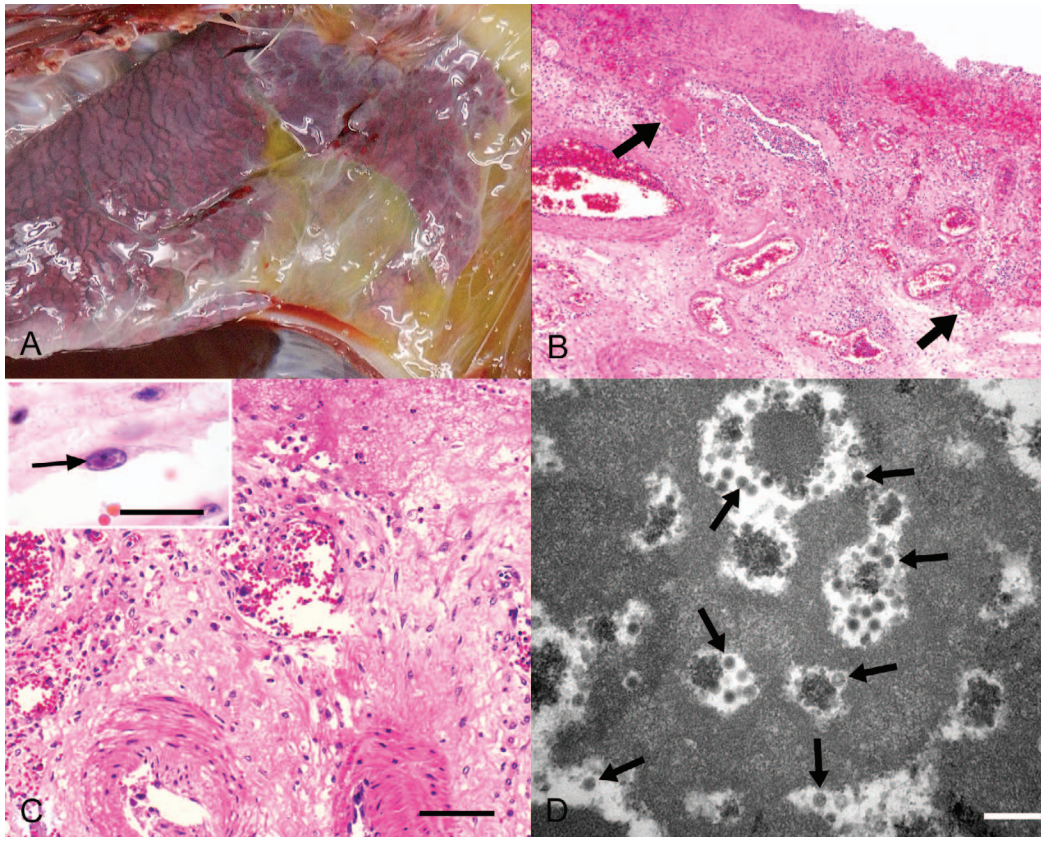

FIGURE 1. These figures represent the significant gross and histopathologic findings for adenovirus infection in moose (*Alces americanus gigas*) in Alaska. (A) There was massive pleural effusion with straw-colored fluid that formed large clots on exposure to air. (B) Several areas of the cecal epithelium and lamina propria are infarcted, characterized by loss of detail and coagulation necrosis. There was inflammation in the submucosa and lamina propria characterized by perivascular cuffing by lymphocytes and macrophages as well as diffuse distribution of neutrophils and fibrin. Some vessels contained fibrin and neutrophilic thrombi (arrows). (C) Scattered blood vessels in the submucosa had fibrinoid necrosis of the wall. There were scattered endothelial cells in multiple tissues, which have margination of the chromatin and distinct eosinophilic intranuclear inclusion bodies (arrow). Bar=20 µm. Magnification=1,000×. (D) Thin section electron microscopy of an intranuclear inclusion body in an endothelial cell demonstrated icosahedral virions 56.7–67.9 nm in width with electron dense central cores. Bar=200 nm. Direct magnification=46,460×.

ease of ruminants. These inclusions were large, amphophilic to eosinophilic, and surrounded by marginated chromatin (Fig. 1C). These were present in both cases in the lung and gastrointestinal tract and in single cases in heart, brain, lymph node, serosa, and tongue. The inclusions were associated with edema within the intima, hyperplasia of the media in some arterioles, and occasional fibrin plugs. Occasional large arteries had marked thickening of the intima infiltrated by lymphocytes and plasma cells; others had fibrinoid necrosis and infiltration by mixed inflammatory cells throughout the wall. Electron microscopy demonstrated particles consistent with adenovirus (Fig. 1D). These particles, formed within nuclei of endothelial cells, were icosahedral virions of approximately 56.7–67.9 nm in width with electron-dense central cores consistent with previous reports (Woods et al. 1996). Attempts were made to isolate virus from lung and nasal swabs using two cell lines: white-tailed deer umbilical vein cells (primary cells) and cattle pulmonary endothelial cells

(American Type Culture Collection, product CCL-209) for three blind passages (Miller et al. 2017). All attempts were unsuccessful, possibly due to issues with deterioration during shipping, level of decomposition, or low viral loads at the time of sampling.

Samples submitted to Wyoming State Veterinary Laboratory (Laramie, Wyoming, USA) for PCR testing included lung, lymph node, bronchus, and nasal swabs. Validation for this assay included determination of analytic sensitivity (standard curve based on 10^{-1}–10^{-8} dilutions with a calculated efficiency of 91.8%) and specificity tested by determining no cross-reactivity with bovine respiratory viruses including BAdV-1 and BAdV-5. Briefly, DNA was extracted using MagMax technology (Qiagen, Valencia, California, USA) according to the manufacturer's direction. We performed PCR amplification using Apex 2x Taq Master Mix (Genessee Scientific, San Diego, California, USA), 10 μM of each primer Ad Aa F (5′ CCGCATTCAGGCCACTTCCATT 3′), Ad Aa R (5′ TGCTGCGTCTGAACACTAATA 3′), and thermocycler conditions of 94 C 2 min (94 C, 15 sec; 62 C, 30 s; 72 C, 1 min) × 40, 72 C 2 min. Genotyping PCR was similarly preformed using primers Ad 6L F (5′ TTACCCAAAATTCCCTATCCA 3′) and Ad 6L R (5′ TTGCGTGTTACTAATGCTGTTCG 3′). Amplicon sequences were verified using Sanger sequencing (GENEWIZ, South Plainfield, New Jersey, USA; Miller et al. 2017). Lung from both calves and the nasal swab from Case 2 were positive for OdAdV-1 identified as genotype B, the genotype most frequently found in mule deer and white-tailed deer in the Rocky Mountain states (Miller et al. 2017).

Lung and fecal samples were submitted to Colorado State Veterinary Diagnostic laboratory (CSVDL; Fort Collins Colorado, USA) and University of Georgia Athens Veterinary Diagnostic laboratory (AVDL; Athens, Georgia, USA) and analyzed for respiratory and enteric pathogens. Analyses were performed using routine methods. Aerobic culture of lung from Case 1 was positive for *Mannheimia haemolytica*, *Pasteurella* sp., and *Pseudomonas* sp. Aerobic cultures of lung from Case 2 yielded no growth. The mixed culture of potential respiratory pathogens and severe bronchopneumonia in Case 1 suggested that these pathogens were secondary invaders after the primary adenovirus infection or that they could have been involved as a comorbidity as has been suggested in outbreaks in elk (Fox et al. 2017). *Mycoplasma* sp. was detected by PCR at CSVDL and AVDL in the lungs of both calves and in the nasal swabs for Case 2. Sequences generated at AVDL were similar to but had imperfect homology with *Mycoplasma dispar* (GenBank accession gi764070914) and *Mycoplasma ovipneumoniae* (GenBank accession gi650087101), which was consistent with an uncharacterized mycoplasma. Histologic lesions consistent with *Mycoplasma* pneumonia as described in other species of cervids characterized by lymphoplasmacytic cuffing of the bronchi and bronchioles with lymphoid nodules were not present (Dyer et al. 2004). Recent studies have identified *M. ovipneumoniae* (Highland et al. 2018) and an uncharacterized *Mycoplasma* sp. unique to Alaska in healthy, free-ranging ungulate populations. Thus, it is unlikely that this *Mycoplasma* sp. was the primary cause of the pneumonia, but it cannot be ruled out as a contributing factor.

Fecal samples in both cases were tested for *Salmonella* sp. and other enteric organisms by culture at CSVDL and AVDL and were negative. Case 2 was tested for *Campylobacter* sp. and *Mycobacterium avium* sp. paratuberculosis by PCR. Both cases were tested for *Cryptosporidia* sp. and *Giardia* sp. by a direct fluorescent antibody test (IFA) and PCR. Case 1 was tested for bovine viral diarrhea virus and bovine coronavirus by direct IFA, and both cases were tested for bovine rotavirus by enzyme-linked immunosorbent assay and PCR. Case 1 was positive for *Cryptosporidium parvum* by IFA and for capillarid nematodes by fecal flotation, and Case 2 was positive for *Nematodirus* sp. (1 egg per gram [EPG]) and strongyles (150 EPG). All other fecal pathogens were negative.

While cases have previously been detected in captive moose in Ontario, Canada (Shilton

et al. 2002) and in free-ranging ungulates in Wyoming (Miller et al. 2017), our cases are the first to be confirmed as AHD in Alaska. In the captive moose in Canada, one case had occurred in a dam followed by death of her calf and another in contact calf in 1985; no further cases were detected until 1998 when two young calves died (Shilton et al. 2002). Possible sources for the virus in the Canadian moose included latency in the adult, introduction from infected wild moose, and infection from other captive cervids. Our cases were similar to these preciously described with the exception that our calves were both older at 7 and 8 mo of age. Determining the source of infection is crucial to understanding the potential significance for free-ranging cervids as well as for captive animals. It remains unclear exactly how the two moose calves contracted this virus. The deaths occurred 2 wk apart in late winter. It seems likely that they became infected by an inapparent carrier such as the cohoused adult moose or by transmission by cervids in adjacent enclosures. Less likely would be infection from free-ranging moose through the facility's perimeter fences. The facility encompasses 809,371 m^2 and houses moose, elk (*Cervus canadensis*), reindeer (*Rangifer tarandus tarandus*), and Sitka black-tailed deer (SBTD; *Odocoileus hemionus sitkensis*) as well as muskoxen (*Ovibos moschatus*) and wood bison (*Bison athabascae*). The only new ungulates introduced to the facility around the time frame of the disease occurrence were two muskox calves and one SBTD fawn, none of which showed signs consistent with adenovirus infection nor were tested. Adenovirus was not detected by PCR of nasal swabs from two elk and one of the two adult moose.

Subsequent to the detection of adenovirus in the moose calves, nasal swab, tonsil, and respiratory tissue screening via PCR of 394 samples from live captured or dead moose (154 nasal swabs, one conjunctival swab, 12 pharyngeal swabs, eight lung) and caribou (184 nasal swabs, 30 conjunctival swabs, four lungs, one retropharyngeal lymph node) did not provide any detections. The only PCR detection of adenovirus from a free-ranging cervid in Alaska was from a tonsil from one of two SBTD killed by trauma. Thus, performing opportunistic sampling via nasal swabs for adenovirus is unlikely to identify carriers. Focusing testing lung tissue from mortalities with respiratory signs, lesions consistent with AHD, and mortality events in calves is more likely to yield a detection in free-ranging populations. Testing of archived sera from Alaska cervids for adenovirus antibodies could help to determine whether this is an emerging disease or if it is enzootic. However, validated serologic tests for moose and other Alaskan cervids are not available.

The first detection of moose mortalities attributed to AHD within Alaska raises concerns of previously undetected AHD mortalities as well as the potential for negative population impacts on free-ranging cervids. If adenovirus is enzootic, it is possible that the captive moose were simply the first documented cases. Even if SBTD are the reservoirs, they are not typically sympatric with moose and thus transmission is unlikely. Since there was a SBTD introduced to the facility during this event, and the only other PCR positive test was from a SBTD, it is possible transmission occurred under these circumstances. Because we do not know the source of this virus, reasonable methods to prevent transmission include strict quarantine protocols and preventing any contact of animals between enclosures and from free-ranging cervids by double, high fences with sufficient distance between to prevent direct contact and aerosol transmission. It is unlikely that AHD outbreaks will escalate unless there are additional introductions from immigrating potential carriers such as mule deer or white-tailed deer or increased contact of SBTD with moose or caribou populations. However, if AHD is a recently introduced pathogen to Alaska, developing an AHD surveillance program and validating serologic screening tests to monitor moose populations are potential management objectives.

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LITERATURE CITED

Dyer NW, Krough DF, Schaan LP. 2004. Pulmonary mycoplasmosis in farmed white-tailed deer (*Odocoileus virginianus*). *J Wildl Dis* 40:366–370.

Fox KA, Atwater L, Hoon-Hanks L, Miller M. 2017. A mortality event in Elk (*Cervus elaphus nelsoni*) calves associated with malnutrition, pasteurellosis, and deer adenovirus in Colorado, USA. *J Wildl Dis* 53:674–676.

Miller MM, Cornish TE, Creekmore, TE, Fox K, Laegreid W, McKenna J, Vasquez M, Woods LW. 2017. Whole genome sequence of *Odocoileus hemionus* deer adenovirus isolates from deer, moose and elk are highly conserved and support a new species in the genus *Atadenovirus*. *J Gen Virol* 98:2320–-2328.

Shilton CM, Smith DA, Woods LW, Crawshaw GJ, Lehmkuhl HD. 2002. Adenoviral infection in captive moose (*Alces alces*) in Canada. *J Zoo Wildl Med* 33: 73–79.

Woods LW, Schumaker BA, Pesavento PA, Crossley BM, Swift PK. 2018. Adenoviral hemorrhagic disease in California mule deer, 1990–2014. *J Vet Diagn Invest* 30:530–537.

Woods LW, Swift PK, Barr BC, Horzinek MC, Nordhausen RW, Stillian MH, Patton JF, Oliver MN, Jones KR, MacLachlan NJ. 1996. Systemic adenovirus infection associated with high mortality in mule deer (*Odocoileus hemionus*) in California. *Vet Pathol* 33: 125–132.

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