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Infectious Canine Hepatitis in a Brown Bear (*Ursus arctos horribilis*) from Alaska, USA

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ABSTRACT: We diagnosed infectious canine hepatitis in a free-ranging brown bear (*Ursus arctos horribilis*) cub from Alaska, US, found dead in October 2015. Intranuclear inclusion bodies were present in hepatocytes, and immunohistochemistry showed reactivity to adenoviral antigens. Sequencing of the hexon protein of adenovirus showed 100% identity to canine adenovirus 1.

A 30-kg, free-ranging, female brown bear (*Ursus arctos horribilis*) cub was found dead in October 2015 in Katmai National Park and Preserve (58°34′N, 155°46′W) near Brooks Falls in Lake and Peninsula Borough, Alaska, US. With over 6,000 brown bears, the Alaska Peninsula is a popular site for bear viewing (Harper and McCarthy 2013) and the US National Park Service (NPS) maintains live streaming webcams at Brooks River and the surrounding areas. Weeks prior to its death, the bear cub was observed with its sow and two siblings, and the week before, with the sow and only one sibling. The sow was identified by unique physical characteristics recorded as part of a long-term monitoring program by NPS. In late October, the River Watch Cam (US National Park Service 2017) captured the cub staggering, collapsing, and subsequently dying. The carcass was recovered by NPS staff, chilled, and submitted whole to the US Geological Survey National Wildlife Health Center (Madison, Wisconsin, USA) for necropsy.

Dark brown to green tarry fecal material covered the fur around the anus of the cub. A small amount of bloody mucus was coming from the nares. The brain was diffusely red, and petechial hemorrhages were observed throughout the parenchyma (Fig. 1). The liver was slightly firm, and the gall bladder was distended with bile. Lungs were mottled red to dark red, and some sections sank in formalin. Intestines contained abundant green mucoid material, and green to brown tarry fecal material was present within the colon. Due to the observed neurologic signs, we tested brain tissue by PCR for canine distemper virus and by direct fluorescent antibody assay for rabies virus at the Wisconsin Veterinary Diagnostic Laboratory (Madison, Wisconsin, USA) and the Wisconsin State Laboratory of Hygiene (Madison, Wisconsin, USA), respectively, and both were negative.

Examination of H&E-stained sections of the liver showed multifocal centrilobular to midzonal necrosis of hepatocytes (Fig. 2A). Hepatocytes occasionally contained intranuclear eosinophilic inclusions that marginated the chromatin peripherally (Fig. 2B). Throughout all brain sections, there were foci of hemorrhage predominantly in gray matter but occasionally within white matter. There were multiple foci of hemorrhage within the gray matter of the cervical spinal cord (Fig. 2C). Within the renal interstitium, there were multiple foci of numerous lymphocytes and plasma cells with fewer eosinophils (Fig. 2D). Alveoli were often filled with hemorrhage and edema fluid.

Immunohistochemical staining was performed at the University of Georgia (Athens, Georgia, USA) by using an automated stainer (Nemesis 3600, Biocare, Concord, California, USA). Protease 3 (Ventura, Tucson, Arizona, USA) was used for antigen retrieval. Primary antibody (monoclonal mouse anti-canine adenovirus antigens, VMRD, Pullman, Washington, USA) at a 1:100 dilution was bound to 4plus Biotinylated Universal Goat Link (goat anti-mouse immunoglobulin G, Biocare) and detected by using 4plus Streptavidin Horse-

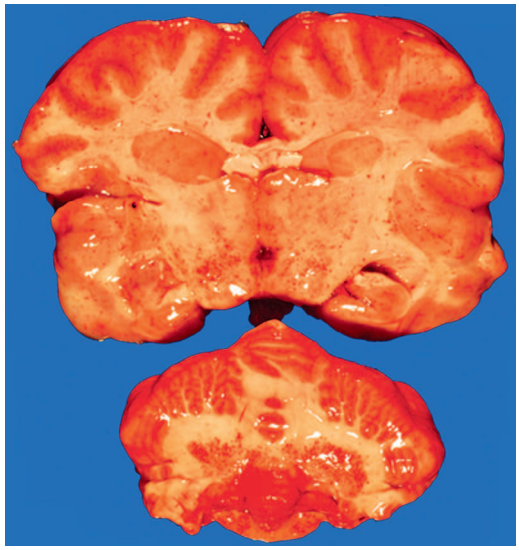

FIGURE 1. Gross image of a brown bear (*Ursus arctos horribilis*) found dead near Brooks Falls in 2015 in Katmai National Park and Preserve in Lake and Peninsula Borough, Alaska, USA. Petechial hemorrhages are present throughout the cut surface of brain sections.

radish Peroxidase (Biocare) and Warp Red Chromogen (Biocare). Immunoreactivity was observed in intranuclear inclusions in hepatocytes (Fig. 2B, inset) and endothelial cells in the kidney and brain.

With DNA extracted from the frozen brain, PCR was used to amplify the basal part of the hexon protein of the adenovirus with the HexAdB and HexAdJ primers and methods of Kiss et al. (1996). The 300-base pair PCR fragment was resolved on a 1% agarose gel, and the PCR product was sequenced at the University of Wisconsin at Madison Biotechnology Center (Madison, Wisconsin, USA) on an ABI 3730xl sequencer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Primer sequences were trimmed, and the sequence was deposited in GenBank (MF621581) and used in the BLAST to interrogate the GenBank database (National Center for Biotechnology Information 2017). The amplified sequence shared 100% identity to the canine adenovirus 1 574-2013-RS hexon gene (GenBank KP840549.1).

Infectious canine hepatitis, caused by canine adenovirus 1 (CAdV-1), is a member of the species *Canine mastadenovirus A*, genus *Mastadenovirus,* and family Adenoviridae. First observed in dogs in 1930, infection now occurs worldwide (Decaro et al. 2008) and can infect members of the families Canidae, Mustelidae, and Ursidae (Woods 2001). Transmission is by direct contact or by exposure to infected secretions. Viral replication occurs in vascular endothelial cells, hepatocytes, and renal epithelial cells (Decaro et al. 2008).

Pursell et al. (1983) reported the first confirmed mortality from CAdV-1 in captive black bears (*Ursus americanus*), and Collins et al. (1984) reported an epizootic with high mortality. Clinical signs included anorexia, lethargy, salivation, vomiting, diarrhea, nystagmus, ataxia, seizures, and flaccid paralysis. Gross examination showed lymphadenopathy, ascites, vascular congestion, and disseminated petechial hemorrhages. Microscopically, there was hemorrhage and mild inflammation in the brain and mild hepatic necrosis. Intranuclear inclusions were observed in the hepatocytes, urinary bladder, and endothelial cells in multiple organs. In 1965 in a Budapest Zoo, seven brown (*Ursus arctos*) and Tibet bear cubs (*Ursus arctos pruinosus*) died of CAdV-1 after being housed with an infected dingo puppy (*Canis lupus dingo*; Kapp and Lehoczki 1966). Findings were similar to the black bears with the addition of hepatosplenomegaly, pulmonary edema, and catarrhal enteritis. Clinical signs and gross and histologic findings in the current case are consistent with reports in bears.

Although there are no reports of clinical disease and subsequent mortality from CAdV-1 in free-ranging brown bears, several surveys have documented the serum antibody prevalence in free-ranging Alaskan brown bears. The statewide prevalence was 12% from 1973 to 1987 (Zarnke and Evans 1989) and 14% from 1988 to 1991 (Chomel et al. 1998). In both studies, prevalence was highest on Kodiak Island. Prevalence on the Alaska Peninsula, the site of this cub's mortality, was 16% in the first study and decreased to

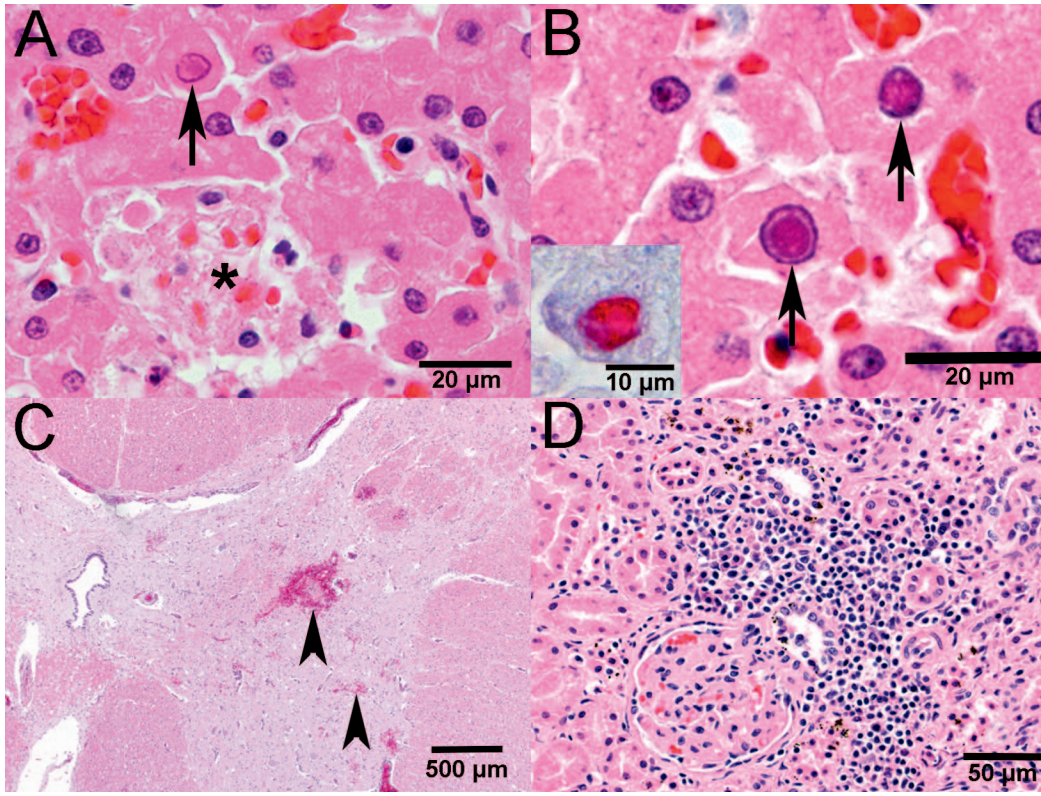

FIGURE 2. Photomicrographs of tissues from a brown bear (*Ursus arctos horribilis*) found dead near Brooks Falls in 2015 in Katmai National Park and Preserve in Lake and Peninsula Borough, Alaska, USA. (A) Liver with a focal area of hepatocyte necrosis (*). An eosinophilic intranuclear inclusion is present within a hepatocyte adjacent to the necrotic focus (arrow). H&E stain. (B) Eosinophilic intranuclear inclusion bodies within hepatocytes marginate nuclear chromatin (arrows). H&E stain. Inset: positive immunoreactivity to adenoviral antigen within an intranuclear inclusion in a hepatocyte demonstrated with the 4plus Streptavidin Horseradish Peroxidase technique. (C) Multiple foci of hemorrhage in the gray matter of the right dorsal and ventral horns of the spinal cord (arrowheads). H&E stain. (D) A focus of numerous lymphocytes and plasma cells within the renal interstitium. H&E stain.

9% in the second. Antibodies were not detected in bears under 2 yr old in either study, suggesting that mortality occurs if a bear is infected (Zarnke and Evans 1989). A likely source of infection in bears is infected individuals in sympatric canid populations, such as gray wolves (*Canis lupus*) or foxes (Zarnke and Evans 1989). Watts and Benson (2016) reported a 90% CAdV antibody prevalence in gray wolves sampled from the Alaskan Peninsula and Unimak Island during 2006–11 and suggested the virus might be endemic in this population.

Although adenoviruses can cause epidemics and high mortality in some species, clinical disease usually occurs sporadically in neonates or immunocompromised hosts (Woods 2001). A 1977 to 1982 study in the western Brooks Range, Alaska, US, reported brown bear cub mortality as high as 47% (Reynolds and Hechtel 1984), but it remains unknown what role CAdV-1 infections may play in cub mortality or what impact it may have on Alaskan brown bear populations overall.

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