First Report of Clinical Disease Associated with Canine Distemper Virus Infection in a Wild Black Bear (Ursus americana)

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ABSTRACT: An approximately 1-yr-old black bear was discovered on the porch of a rural residence in southwestern Pennsylvania on October 26, 2011, where it remained during the day in spite of efforts to frighten it away. The bear exhibited periods of somnolence and sporadic tremors and seizures. It was euthanized by gunshot that evening. Immediately after euthanasia it was observed to have footpads that exuded fluid when compressed. It was submitted for necropsy the next day where roughened footpads were noted. Histologic examination of the brain demonstrated nonsuppurative encephalitis with eosinophilic intranuclear and intracytoplasmic inclusion bodies in neurons. The footpads were thickened and hyperkeratotic. Canine distemper virus (CDV) was detected by immunohistochemistry (IHC) in the brain and footpads, and by reverse transcription polymerase chain reaction (RT-PCR) from the brain tissue. Phylogenetic analysis indicated that the CDV cDNA from the bear had 98.2% nucleotide identity to the Rockborn-Candur vaccine and a canine isolate from 2004 in Missouri, USA, and 97.3% nucleotide identity to a raccoon CDV isolated in 2011 from Tennessee, USA. This represents a first report of CDV as a cause of encephalitis or footpad hyperkeratosis in a wild black bear.

Key words: Black bear, canine distemper, clinical disease, Pennsylvania, Ursidae.

Canine distemper virus (CDV) is an enveloped, single-stranded, negative-sense RNA virus belonging to the Morbillivirus genus in the family Paramyxoviridae. The lesions associated with CDV infection are variable and can affect the respiratory, cardiac, gastrointestinal, ocular, integumentary, and nervous systems, alone or in combination (Appel, 1970). It has a broad host range and has caused clinical disease in a wide range of mammal species (Deem et al., 2000). Disease due to CDV infection can be a significant mortality factor in some species (Origgi et al., 2012). Serologic surveys have indicated that black bears are exposed to CDV (Dunbar, 1998), but there are no reported cases of clinical disease in black bears. We describe CDV infection in a yearling female black bear with a history of seizures and abnormal footpads.

The carcass of a yearling female black bear in adequate nutritional and good postmortem condition was presented to the Animal Diagnostic Laboratory at The Pennsylvania State University, University Park, Pennsylvania for necropsy 14 hr after euthanasia by gunshot. Gross examination of the bear was normal, with the exception of the footpads, which were abnormally roughened. Tissue samples from all major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 3–5 μm, and stained with hematoxylin and eosin. Samples of brain were negative for rabies virus by fluorescent antibody assay. Additional tissues were processed for bacteriologic culture.

Histologically, there was multifocal perivascular cuffing by lymphocytes, plasma cells, macrophages, and rarely eosinophils, within the cerebral cortex (Fig. 1A). The neuropil was disrupted by multifocal proliferations of astrocytes and microglial cells; necrotic neurons or astrocytes were common within affected foci (Fig. 1B), and a small number of neurons or astrocytes in the inflamed foci contained eosinophilic intranuclear or intracytoplasmic inclusion bodies. The epidermis of the footpads was thickened by irregularly
fused rete pegs and the stratum corneum was thick with orthokeratotic hyperkeratosis with deep fissures (Fig. 1C). Mixed populations of bacteria were present on the skin, including long chains of parallel rows of bacteria (consistent with *Derma- tophillus congolensis*), some of which invaded the cornified epithelium. The superficial dermis had minimal perivascular infiltrates dominated by lymphocytes with fewer neutrophils. No significant microscopic changes were detected in the other tissues examined. Bacterial culture from brain samples resulted in

![Image](image-url)

**Figure 1.** Histology and immunohistochemistry from a Pennsylvania black bear (*Ursus americanus*) with canine distemper virus infection. (A) Perivascular inflammation in the cerebrum. The image is a tangential section of a vein. The lumen contains erythrocytes and a few leucocytes, but the perivascular space is expanded by leucocytes (brackets), predominately lymphocytes and macrophages as well as a small number of eosinophils. Hematoxylin and eosin stain. Bar=20 μm. (B) Inflammation in the cerebral cortex. Cortical neurons are variably infiltrated by inflammatory cells (gliosis) including lymphocytes and smaller numbers of macrophages. Hematoxylin and eosin stain. Bar=50 μm. (C) Neurons with lesions attributable to canine distemper virus (CDV) infection. One neuron has a marginalized nucleus and three, eosinophilic, intracytoplasmic, inclusion bodies (arrows). Another neuron (arrowhead) is shrunken with hyper eosinophilic cytoplasm and a darkly stained eosinophilic nucleus, all consistent with necrosis. The necrotic neuron also has an eosinophilic, intranuclear inclusion presumably due to CDV infection. Bar=10 μm. (D) Viral antigen in a section of cerebrum. Brown chromogen indicates the presence of specific antibody binding to CDV antigens mostly in neuronal soma and axons. Immunohistochemistry stain with hematoxylin counterstain. Bar=250 μm. (E) Viral antigen in footpad epithelium. A few cells in the stratum spinosum are intensely stained by an immunohistochemical technique with the use of anti-CDV antibodies. This demonstrates abundant viral antigen in the cytoplasm and nuclei of the affected cells. Immunohistochemistry stain with hematoxylin counterstain. Bar=20 μm.
scant to light growth of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Escherichia coli*. The same bacteria, as well as *Streptococcus* sp., were cultured from samples of other tissues with light to scant growth.

Formalin-fixed paraffin-embedded (FFPE) specimens were sent to the Southeastern Cooperative Wildlife Disease Study, University of Georgia, Athens, Georgia for further analysis. Tissue blocks consisting of FFPE samples of footpad and brain were submitted for immunohistochemistry (IHC) for CDV antigen. The primary antibody in the IHC protocol was a mouse monoclonal IgG specific for the CDV nucleoprotein (VMRD, Inc., Pullman, Washington, USA). The secondary antibody was an equine biotinylated anti-mouse IgG (BA-2001, Vector Labs, Burlingame, California, USA). Dako’s LSAB® 2 Streptavidin conjugated to horseradish peroxidase in phosphate-buffered saline was used (K1016, Dako, Carpinteria, California, USA). Immunohistochemical stains demonstrated CDV antigen in neurons with dense stain uptake throughout the soma and axons (Fig. 1D). The foci with positively stained neurons were correlated with areas of inflammation. A few leukocytes around blood vessels were also positive for CDV antigen. In sections of footpad, specific staining demonstrated CDV antigen in scattered epithelial cells of the stratum spinosum (Fig. 1E) and scattered apocrine glands.

RNA was extracted from samples of the paraffin-embedded tissues using the RNeasy FFPE extraction kit (Qiagen, Valencia, California, USA) in accordance with the manufacturer’s directions. Extracted RNA was reverse transcribed and amplified via single-tube reverse transcription polymerase chain reaction (RT-PCR) with the use of primers designed to amplify a 223–base-pair (bp) region of the hemagglutinin (H) gene (H5’-CCCTATTTGCTGTTTTG and H3’-TTGATYTCGTTTG). Resulting cDNA was visualized on a 1.5% agarose gel stained with ethidium bromide. The products were gel purified with the use of the Gel Extraction Kit (Qiagen) and then sequenced with the use of the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer’s directions.

Our CDV sequences have been deposited in the National Center for Biotechnology Information database and assigned GenBank accession numbers: CC-11-488 (bear): KC315291 and CC-11-19A (raccoon): KC315292. We compared these sequences to corresponding sequences from 24 other CDV isolates published in GenBank (Fig. 2). The final data set was trimmed to a 212-nucleotide sequence fragment of the H gene. The sequences were aligned with the use of the Clustal W of MEGA5 (Tamura, 2011), and a phylogenetic tree was created with the use of the neighbor-joining method. Data were subjected to bootstrap analysis based on 10,000 resamplings of the original data set to produce a majority rule consensus tree. Bootstrap values below 50 were omitted. Based on this method, the topology for the clade grouping of CDV sequences from the black bear, the lesser panda, and the Rockborn-Candur vaccine is weakly supported. The positive-control vaccine strain used in this study had 96% sequence similarity, a result of nine nucleotide changes. The partial H gene segment amplified from the bear had the highest sequence identity of 98.2% to a canine isolate collected in Missouri in 2004 and to the Rockborn-Candur vaccine (Fig. 2). The cDNA from this black bear had 97.3% sequence identity to the CC-11-19A (raccoon) CDV strain isolated in 2011 from Tennessee.

We confirmed classic lesions associated with canine distemper. Canine distemper virus is not known to be endemic among black bear populations, and the source of the virus in this case is uncertain. Canine...
distemper virus is a known pathogen of raccoons and skunks in Pennsylvania, but representative samples from Pennsylvania were not available and therefore were not examined. The power of the phylogenetic analysis was limited by the size of the gene product amplified from the formalin-fixed tissue. However, the partial H gene sequence had the highest similarity to a vaccine strain that has been shown to revert to a pathogenic phenotype, and was withdrawn from almost all markets. Other Rockborn-like viruses have been identified in currently marketed vaccines (Martella et al., 2011). This could indicate that an exchange of virus occurred between vaccinated domestic animals and wildlife. We do not know how current vaccine strains affect wildlife species, including black bears. Conversely, this could indicate persistence of Rockborn-like viruses in nondomestic settings. Additional research is necessary to understand the significance of the similarity between the virus in this bear and the Rockborn-Candur vaccine strain. To our knowledge this is the first report of a CDV causing clinical disease in a wild black bear. CDV should be considered when similar cases in this species are investigated.

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