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Infectious Canine Hepatitis in a Gray Fox (*Urocyon cinereoargenteus*)

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ABSTRACT: A free-ranging adult male gray fox (*Urocyon cinereoargenteus*) with moderate diarrhea and thick ocular mucus discharge was examined postmortem. Microscopically, the fox had intranuclear inclusion bodies within hepatocytes. Canine adenovirus-1 was identified by polymerase chain reaction (PCR) and nucleotide sequencing. To our knowledge, this is the first report of clinical infectious canine hepatitis in a gray fox.

Key words: Canine adenovirus, gray fox, infectious canine hepatitis, *Urocyon cinereoargenteus*.

Canine adenovirus-1 (CAV-1), also known as infectious canine hepatitis virus (ICHV), has caused mortality in domestic canids and wild mammals in the families Canidae, Mustelidae, and Ursidae. Reviews of the host range of CAV in wild mammals can be found in Woods (2001) and Cabasso (1981). Although serological surveys have disclosed that 9–86% of gray foxes (*Urocyon cinereoargenteus*) are seropositive for CAV, clinical ICH has not been reported in free-ranging gray foxes (Amundson and Yuill, 1981; Cabasso, 1981; Davidson et al., 1992; Woods, 2001; Riley et al., 2004). We report a case of apparent acute fatal hepatitis due to CAV-1 in a free-ranging gray fox.

On 30 January 2005, an adult male gray fox was found dead approximately 1 km from the 12,500 ha Jones Ecological Research Center in Baker County (31° 19'N, 80°20'W), Georgia, USA, where it was originally captured and radio-collared on 13 November 2004. This fox was part of a radio telemetry project conducted by personnel of the Louisiana State University School of Renewable Natural Resources. The fox was last monitored on 28 January 2005, and its movements over the 76-day period had no noticeable change. The carcass had fecal staining of

the perianal region and thick ocular mucous discharge was noted. The carcass was skinned, frozen, and submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS), College of Veterinary Medicine, The University of Georgia, Athens, Georgia, USA, for diagnostic examination.

Portions of the brain, trachea, lung, skeletal muscle, heart, kidney, adrenal gland, testis, liver, spleen, esophagus, stomach, pancreas, small intestine, large intestine, thyroid gland, and urinary bladder were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 3 µm, and stained with hematoxylin and eosin for light microscopy. Portions of the brain, trachea, lung, heart, kidney, testis, liver, spleen, esophagus, stomach, pancreas, small intestine, large intestine, skeletal muscle, and urinary bladder were collected and frozen at –20 C.

A portion of brain was submitted to the Athens Diagnostic Laboratory for rabies virus and canine distemper virus (CDV) testing by fluorescent antibody assay. Additionally, frozen sections of the liver were submitted to the SCWDS virology laboratory for virus isolation. The liver sample (~0.5 cm³) was mechanically homogenized in 1 ml of maintenance media (1X minimal essential media, 2.2 g/L NaHCO₃, 3% fetal bovine serum, 200 units/ml penicillin, 200 µg/ml streptomycin, 0.5 µg/ml amphotericin B). Homogenized tissue was centrifuged (6,700 × G for 10 min) to pellet debris, and multiple aliquots (100 µl each) of clarified supernatant were used to inoculate three-day-old Madin-Darby canine kidney (MDCK) cells in a 12-well-plate format. For wells exhibiting cytopathology, viral DNA was

extracted from culture supernatant using a QIAamp® DNA Mini-Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. Canine adenovirus-specific primers used for polymerase chain reaction (PCR) were 5'-GAGCAGGTAGTATGGAC-3' (sense primer) and 5'-TCAGTAAAAGRAGCAAC-3' (antisense primer). These primers amplify a 235 base-pair (bp) product covering a portion of the E1B 19K/small T antigen gene in both CAV-1 and CAV-2. The PCR was set up using 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl₂, 250 µM deoxynucleotide triphosphates, 0.5 µM of each primer, and 1 U of *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA). Cycling parameters were: denaturation at 94 C for 3 min, followed by 40 cycles of denaturation at 94 C for 1 min, annealing at 50 C for 1 min, and extension at 72 C for 1 min. Reaction products were examined by electrophoresis in a 2% agarose gel and subsequently purified using a QIAquick® Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. Sequencing of DNA was performed at the Integrated Biotechnology Laboratories at the University of Georgia using an Applied Biosystems Inc. (Foster City, California, USA), 3100 Genetic Analyzer.

The fox was classified as an adult based on weight (Wood, 1958), pelage markings, and pelage wear (Lord, 1961). The fox weighed 3.75 kg, was in fair nutritional condition, and the carcass was moderately autolyzed. Approximately 40 ml of unclotted blood was in the abdominal cavity. The serosal surfaces of all abdominal organs were reddened. No ingestus was present in the gastrointestinal tract, but a few nematodes were found in the lumen of the stomach.

Microscopically, large eosinophilic intranuclear inclusion bodies were scattered throughout the liver and displaced the chromatin within hepatocytes. Inflammatory cells were not evident, but the liver had extensive freeze-thaw artifact. Fluo-

rescent antibody testing for rabies virus and CDV was negative. Canine adenovirus was isolated from MDCK cells inoculated with supernatant harvested from liver homogenate. Nucleotide sequence analysis of the primerless 201 bp PCR product (GenBank® accession number EF611185) revealed that the isolate had a 100% identity to CAV-1 and a 79% identity to CAV-2 as compared to the Genbank® database accession number sequences Y07760 and U77082, respectively.

This is the first report of apparent fatal ICH in a gray fox. The degree of autolysis and freeze-thaw artifact could have masked lesions associated with other disease processes that may have predisposed the fox to clinical ICH. Additionally, the autolysis and freeze-thaw artifact may have precluded us from visualizing lesions associated with the CAV infection. Although free blood was in the abdominal cavity, there was no evidence of hemorrhage or trauma.

Several serologic investigations of wild gray fox populations have disclosed that 9–86% of surveyed animals have antibodies to CAV-1 (Amundson and Yuill, 1981; Cabasso, 1981; Riley et al., 2004). However, caution must be used when comparing these studies due to the variations of seropositive cutoff dilutions, which have ranged from $\geq 1:4$ to $\geq 1:10$. Serological testing performed on confiscated red foxes (*Vulpes vulpes*), gray foxes, and coyotes (*Canis latrans*) from a commercial canid distributing facility in Ohio, USA, revealed that 100% of the red foxes ($n=47$) and 56% of gray foxes ($n=10$) were seropositive for CAV-1 at a dilution of $\geq 1:10$, and 57% of the red foxes and 39% of gray foxes were seropositive for CAV-1 at a dilution of $\geq 1:800$ (Davidson et al., 1992). Additionally, the investigators found that 100% of the confiscated coyotes ($n=13$) were seropositive for CAV-1 at a dilution of $\geq 1:100$. It was reported that these animals were not vaccinated, as occurs at some wild canid distributing or propagating facilities. Ca-

nid propagation and rehabilitation facilities may serve as a source of exposure of wild canids to CAV. It would be interesting to monitor canids from these facilities by serology and virus isolation attempts to determine if these practices increase the risk of exposure of CAV in gray foxes and other wild canids.

Currently, the seroprevalence of CAV-1 in the gray fox population from Baker County, Georgia, is unknown. However, it is worth investigating given this case and the abundance of feral dogs that have been observed in close contact with wild foxes in and around the study area. It may be hypothesized that CAV-1 exposure in wild canids would be observed more frequently in animals that are in close proximity to domestic dogs; however, gray foxes in urban zones in Golden Gate National Recreation Area, California, USA, have been found to have a similar seroprevalence of CAV-1 to foxes from the rural zones of the park (Riley et al., 2004). Records of morbidity and mortality in gray foxes have not previously documented CAV-associated disease. Given this, it would appear unlikely that the finding of CAV in this fox will have a significant impact in the fox population; however, future monitoring of this population may be warranted.

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