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RESEARCH NOTES/CASE REPORTS

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Isolation of an Adenovirus from Black Bear Cubs

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In May 1979, personnel of the Georgia State Fish and Game Commission trapped two 3-mo-old black bear cubs (*Ursus americanus*) and transported them to the Oatland Island Educational Institute reservation at Savannah, Georgia. The cubs were displayed in portable cages, but were cared for in private homes by workers at the institute. Two wk after they were brought to the institute, they exhibited ataxia, excessive salivation, vomition, convulsions, periodic nystagmus and paddling of legs. One cub (A) died within 24 hr after the onset of clinical signs, and the second cub (B) was euthanitized in extremis 4 days later. Necropsy examination of the cubs revealed enlarged pale mesenteric lymph nodes, congested spleens, and 40–50 ml of clear fluid in the abdomen.

Two timber wolf pups (*Canis occidentalis*) housed in a separate part of the reservation, also exhibited signs of ataxia and convulsions. Blood samples from the pups and tissues from the bear cubs were submitted. Tissues were processed, sectioned and stained with hematoxylin and eosin.

Microscopic changes in the brains were characterized by mild neutrophilic infiltrate within the meninges, vascular walls, perivascular tissue, and adjacent neuropile. There were multifocal minute hemorrhages throughout the brain, and a single focus of gliosis within the brain stem. Hepatic changes consisted of mild, multifocal degeneration and necrosis of hepatocytes. Basophilic intranuclear inclusion bodies were present in hepatocytes (Fig. 1) and renal glomerular endothelial cells. Endothelial cells within the vessels of the neuropile and meninges were hypertrophied and many contained intranuclear inclusion bodies (Fig. 2). Numerous intranuclear inclusion bodies were seen also within the vascular endothelium of the submucosa of the urinary bladder. Reticuloendothelial cell hyperplasia was prominent in the spleen.

A 10% suspension of brain from Cub A was prepared in Earle's balanced salt solution, sonically treated (Branson, Sonifier Cell Disrupter, Heat Systems—Ultrasonic Inc., Plainview, New York 11803, USA) and inoculated onto Madin Darby canine kidney cells (MDCK) in six wells of a 24-well plastic plate. Cytopathogenic effect was observed at 4 days and was typical of an adenovirus.

An adenovirus was also isolated from the brain and liver of Cub B. Isolates from both cubs were identified as infectious canine hepatitis (ICH) by fluorescent antibody (ICH conjugate, Sylvana Chemical Co., Orange, New Jersey 07050, USA) and serum-virus neutralization using ICH specific antiserum. The sera from the wolf pups were tested for antibody to the isolates and to a stock canine hepatitis virus in the microtiter system. The sera neutralized both of the isolates and the stock ICH virus.

Formalin fixed blocks (1 mm^3) of liver were post-fixed in 1% phosphate buffered osmium tetroxide, and were routinely processed and embedded in epon-araldite resin. One micron sections stained with toluidine blue were used to select areas for ultrathin sectioning. Ultrathin sections stained with uranyl acetate and lead citrate were examined with an RCA EMU 4 electron microscope. Tissue culture fluid from infected MDCK cells was sonicated and negatively stained for electron microscopy. Virus particles similar in size (70–90 nm diameter) and structure (icosahedral symmetry, 6 capsomers along each edge) to adenovirus particles were observed in the tissue culture fluid from the virus isolation (Fig. 3).

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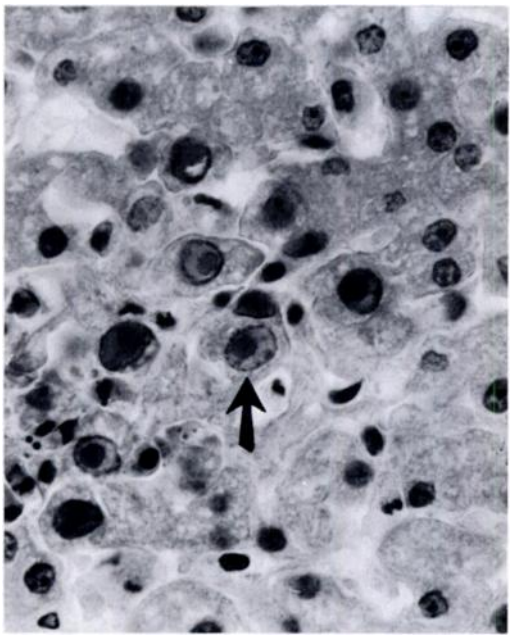

FIGURE 1. Liver from a bear cub with mild hepatocellular necrosis. Note the prominent intranuclear inclusions (arrow) within hepatocytes. H&E; ×128.

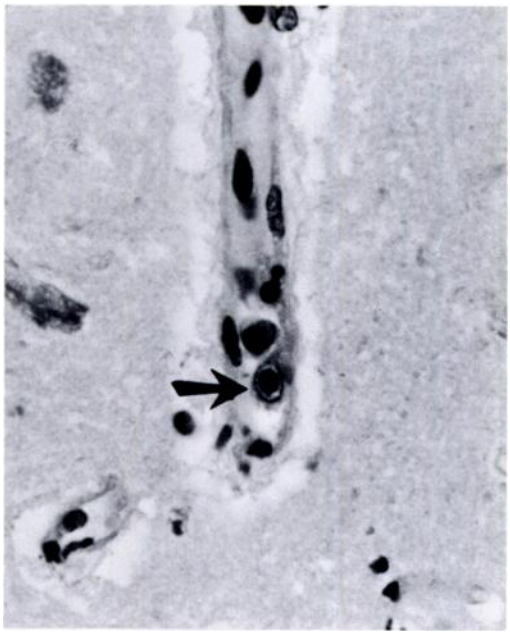

FIGURE 2. Cerebral vessel from a bear cub. Note the prominent intranuclear inclusion within an endothelial cell (arrow). H&E; ×128.

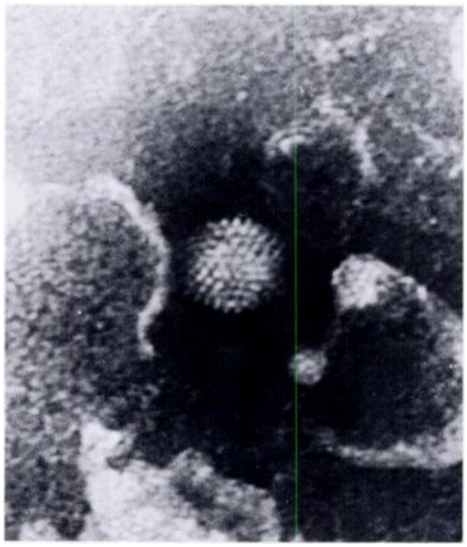

FIGURE 3. Negatively stained adenovirus particle prepared from Madin Darby canine kidney tissue culture cells inoculated with liver suspension from bear cub. ×200,000.

The clinical signs, necropsy findings, and histopathology are consistent with a diagnosis of ICH. This was confirmed by FA and SN tests on the isolated virus and the observation of adenovirus particles in the EM preparations.

The hepatic necrosis and multifocal hemorrhages in the brain, in association with large intranuclear inclusions, are compatible with those described for ICH infections in the dog (Appel and Carmichael, 1979, *In* Canine Medicine, Catcott (ed), Am. Vet. Pub. Inc., Santa Barbara, California, pp. 25–32; Cabasso, 1981, *In* Infectious Disease of Wild Mammals, Davis, Karstad, and Trainer (eds.), Iowa State Univ. Press, Ames, Iowa, pp. 191–195).

Although ICH virus has been isolated from dogs (Strom and Riser, 1947, N. Am. Vet. 29: 751–752; Riser, 1948, N. Am. Vet. 29: 568–573), and foxes (Chaddock and Carlson, 1950, N. Am. Vet. 31: 35–41), to our knowledge this is the first reported isolation from a bear. In 1948, Chaddock reported the probable occurrence of ICH in a polar bear (*Ursus maritimus*) in a zoo (Cabasso, 1981, op. cit.). No virus isolation was attempted but the polar bear, which was nearly prostrate with an unknown condition, recovered after the administration of 150 ml of ICH antiserum. Three wk earlier ICH had been

diagnosed in a grey fox (*Urocyon cinereoargenteus*) at the zoo. It was noted that the same caretaker took care of both the fox and the bear.

The source of the infection in the cubs in this report is unknown, but these cubs and the two timber wolf pups, with clinical signs suggestive of ICH infection, were attended by the same caretaker. The Oatland Island Educational Institute is a wildlife refuge for the preservation and study of wildlife in a natural habitat. Animals are obtained from the Department of Natural Resources, Georgia State Fish and Game Commission, other zoos and the general public. New entries are rarely isolated. Members of the staff play with and handle the young animals prior to their reaching sufficient age to be placed in the natural habitat.

Mammals are on an accepted vaccination program as recommended by Fowler and Theobald (1978, *In* Zoo and Wild Animal Medicine, Fowler (ed), W. B. Saunders Company, Philadelphia, Pennsylvania, pp. 613–617), however the bear cubs and wolf pups had not been vaccinated for ICH. Problems requiring treatment are dealt with by the improvisation and adaptation of known techniques used in domesticated species.

The diagnosis of ICH in bear cubs in this instance suggests that the vaccination of bear cubs housed in zoos and other exhibits should be considered. It must be pointed out however, that the only vaccines currently available are for use in dogs. Until data can be compiled on the use of these vaccines in other species only killed ICH vaccines are suggested for use in bears.

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Serologic Testing of Wild Roe Deer (*Capreolus capreolus* L.) from the Trois Fontaines Forest Region of Eastern France

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With the continuing expansion of agriculture, the range of wild cervids is constantly overlapping with domestic ruminants and the transmission of infectious disease from the latter to the former in epizootic proportions is always a possibility. No surveys to determine the presence of pathogens of domestic animals in wild cervid populations have been made in France. During January and February of 1979 blood samples were obtained from a substantial portion of a wild roe deer population in the Trois Fontaines forest of eastern France. This opportunity was used to investigate the possible presence of antibodies in this cervid population for a number of pathogens of domestic ruminants in France. This paper reports the results of that study.

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The population is protected from contact with herbivores from outside by a fence. In addition to the roe deer, the population consists of one or two red deer (*Cervus elaphus*) and approximately 100 wild boar (*Sus scrofa*). The deer were captured in nets by the use of dogs which constituted the only other animal contact. A 40 ml blood sample from each animal was taken in a sterile Vacutainer. These samples were screened for antibodies to 19 pathogens commonly found in domestic cattle (Table 1). Antibody titers considered to be significant are as follows: *Aspergillus* > 1/320, *Babesia* > 1/16, *Chlamydia* > 1/8, *Coxiella* (Q Fever) > 1/16.

The results of this study are summarized in Table 1, which indicates that of the 19 pathogens tested for, evidence of only four occurred in this roe deer population, namely *Aspergillus fumigatus*, *Babesia capreoli*, *Chlamydia* sp. and *Coxiella* sp.