Adenovirus Hepatitis in a Boa Constrictor (*Boa Constrictor*)

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months of age, is slowly progressive, and is characterized by truncal ataxia, increased tone of the extensor musculature, and nystagmus.[1,2,4] Lesions are confined to the cerebellum and consist of atrophy and loss of Purkinje cells and granule cells.[1,2,4] Although some of the gait and postural abnormalities observed in affected pups suggest possible cerebellar involvement, neuronal changes in the cerebellum were not identified, and the age of onset and clinical course was distinct from CCA.

Autosomal recessive disorders may emerge in a population as the prevalence of a deleterious gene or degree of consanguinity increases. Pedigree analysis and breeding trials demonstrate that this disorder is an autosomal recessive trait, which marks both parents and two thirds of surviving offspring as carriers. A test to identify carriers has not been developed. In order to decrease the prevalence of this gene in the breed, the parents and siblings of affected pups should be excluded from the breeding population unless test matings are used to determine that individual siblings are not carriers.

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Adenovirus hepatitis in a boa constrictor (*Boa constrictor*)

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Abstract. A boa constrictor was submitted for postmortem evaluation. At necropsy, there were no substantial lesions except in the liver. Light microscopy revealed severe multifocal to coalescing coagulative necrotic hepatitis, with basophilic and eosinophilic intranuclear inclusions in hepatocytes within the necrotic foci. The histopathological findings suggested a viral hepatitis. An adenoviral infection was diagnosed by means of transmission electronic microscopy and in situ hybridization techniques.

In reptiles, adenovirus infections have been described in Nile crocodiles (*Crocodylus niloticus*),[7] several species of lizards,[4,6,9,10,12,13] and snakes. Among snakes, adenovirus hepatitis has been described in a boa constrictor (*Boa constrictor*)[8] and 2 rosy boas (*Lichamura trivirgata*).[17] In the boa constrictor, viral inclusion bodies were reported only in the liver,[8] whereas in the rosy boas, they were observed in renal epithelial cells, endocardium, and epithelial cells of the lung also.[17] This report describes the clinical, pathological, and ultrastructural findings in a boa constrictor (*Boa constrictor*) diagnosed with adenovirus hepatitis.

A 2-kg, young, male, captive-bred boa constrictor (*Boa constrictor*) was submitted for postmortem evaluation. The snake had been acquired approximately 3 months before submission and had been housed alone in a wooden cage. The snake had been anorexic for 2 months. Twenty-four hours before death, the snake was force fed, but it regurgitated the meal. At necropsy, there were no significant lesions except the liver was slightly enlarged with swollen borders and small pale areas scattered throughout the parenchyma. Tissues from major organ systems (stomach, intestine, lung, heart, liver, spleen, and kidney) were fixed in 10% neutral buffered formalin solution and processed for light and electron microscopic evaluation.

Paraffin-embedded sections of tissues were stained with hematoxylin and eosin (HE) and Gram stain. Replicate tissue sections of liver were placed on charged and precleaned glass microscope slides[a] and processed for DNA in situ hybridization to demonstrate adenovirus nucleic acid. Three different viral-specific probes for avian adenovirus were used as previously described: FN-23 (a 36 base oligomer), FN-96 (a 40 base oligomer), and FN-48 (a 40 base oligomer).[14] A work station[b] was used to simplify manual procedures, control reaction temperatures, and minimize reagent consumption, following published techniques.[14] The incubation temperature was lowered from 37 to 30 C, and incubation time was increased from 45 to 60 minutes, decreasing hybridization specificity of the union of the probes to the putative adenoviral target sequence (R. Campagnoli, per-

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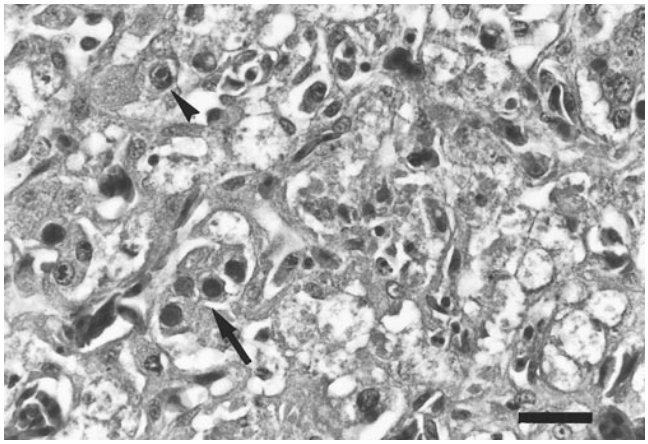

Figure 1. Liver. Boa constrictor. Necrotic hepatocytes showing eosinophilic intranuclear inclusions and margination of chromatin with (arrow) or without (arrowhead) halo. HE. Bar = 25 μm.

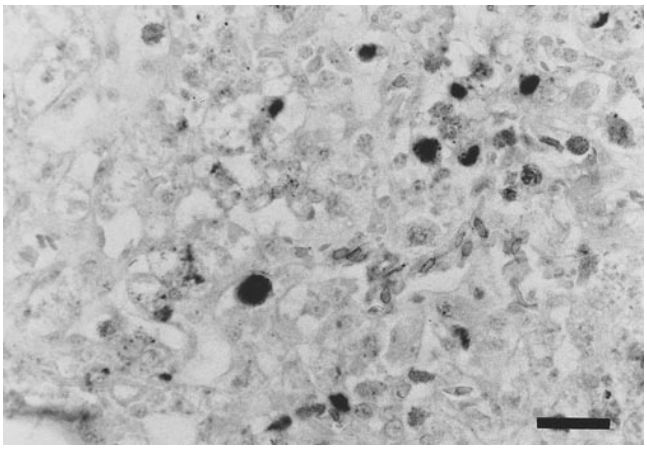

Figure 3. Liver. Boa constrictor. DNA in situ hybridization demonstrating hybrid formation within the nuclei of hepatocytes. Anti–digoxigenin-alkaline phosphatase-NBT system with fast green counterstain. Bar = 25 μm.

sonal communication). Last, both positive and negative control tissue sections were used to validate DNA in situ hybridization procedures. Formalin-fixed liver tissue was routinely osmicated, embedded in epoxy resin, sectioned, and stained with uranyl acetate and lead citrate for transmission electron microscopy (TEM). Bacterial cultures and viral isolation were not performed due to the lack of fresh tissue.

Histologic examination of the liver revealed severe, multifocal to coalescing, coagulative necrosis. Within necrotic foci, numerous hepatocytes contained intranuclear inclusions. Most of the inclusions were basophilic and distended the nuclei; however, some inclusions were centrally located and eosinophilic with peripheral displacement of the nuclear chromatin (Fig. 1). In addition, there was a remarkable lack of inflammatory cell infiltrates associated with necrotic foci. Inclusions were not seen in any of the other tissues examined. There was severe necrosis of the intestinal mucosa, especially at the apices of the villi, where the epithelium had sloughed. The denuded surfaces of the villi were colonized by bacilli, which were Gram positive. In addition, marked edema and heterophilic infiltrates were seen in the submucosa (Fig. 2). DNA in situ hybridization, using probe FN-23, demonstrated adenovirus within the nuclei of hepatocytes containing inclusions (Fig. 3). Staining was weaker than that of the control tissues, which were obtained from

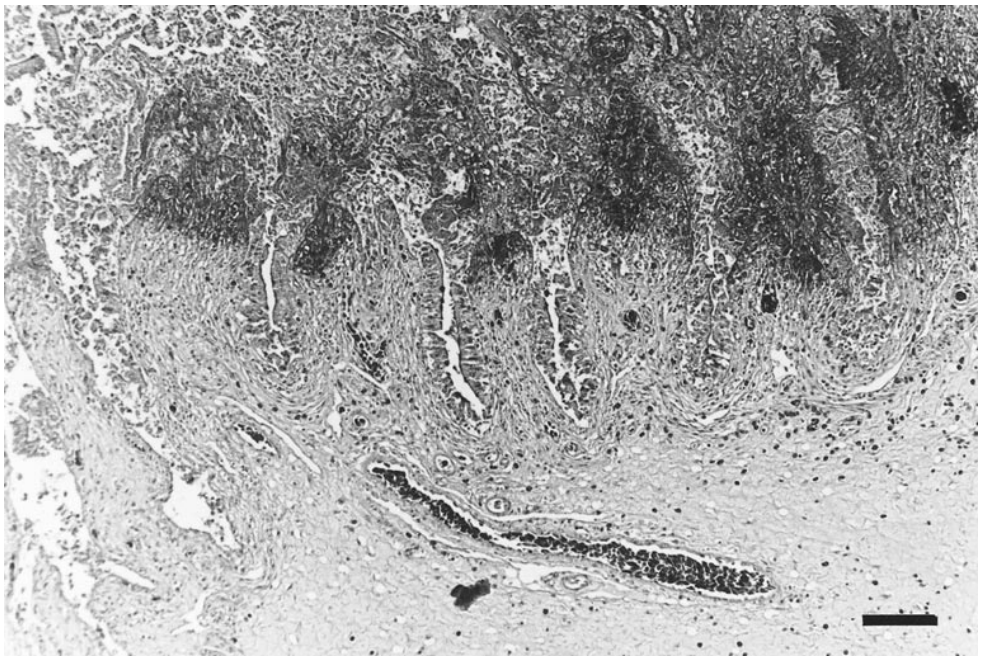

Figure 2. Small intestine. Boa constrictor. Enteritis with severe coagulative necrosis of apices of the villi and edema and heterophilic infiltrate in the submucosa. HE. Bar = 80 μm.

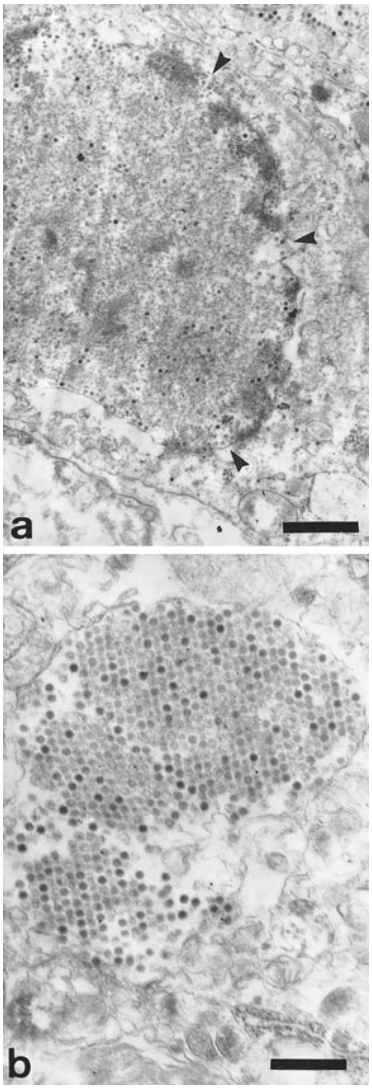

Figure 4. Liver. Boa constrictor. **a**, Scattered icosahedral virions in the inner side of the nuclear envelope. TEM. Bar = 1 μm. **b**, Cytoplasmic vacuole containing viral paracrystalline arrays. TEM. Bar = 500 nm.

an outbreak of adenoviral hepatitis in a group of Eclectus parrots (*Eclectus roratus*). Probes FN-96 and FN-48 did not hybridize to the replicate liver sections.

Electron microscopic examination of the liver revealed numerous degenerative hepatocytes with intranuclear inclusions. In nuclei of affected cells, scattered, icosahedral viral particles measuring 70 nm in diameter were identified. Some viral paracrystalline arrays were found within cytoplasmic vesicles also (Fig. 4a, 4b).

The coagulative hepatocellular necrosis and the associated basophilic intranuclear inclusions in HE-stained liver sections suggested a viral etiology, as confirmed by hybridization and TEM.[11] Viruses known to produce nuclear inclusions within hepatocytes in boas include herpesvirus and adenovirus.[16] Herpesvirus typically forms eosinophilic inclusions and adenovirus forms basophilic inclusions, although adenoviral infections may also produce eosinophilic inclusions.[6] In this report, the location and morphology of virions was compatible for members of the Adenoviridae family.[3]

Although adenoviruses have been identified in reptiles, virus isolation has only been accomplished in tissues from one boa constrictor with hepatitis.[8] Furthermore, published sequence data are not available for reptilian adenovirus. Despite the lack of information on reptile adenovirus, it is believed that they should be grouped in a third genus within the Adenoviridae family, separate from the mammalian (Mastadenovirus) and avian (Aviadenovirus) genuses.[17] The observation that reptilian adenovirus hybridized to probe FN-23 but not to probes FN-96 and FN-48 suggests that probe FN-23 is less selective in detecting adenoviral nucleic acid.[14] Furthermore, probe FN-23 probably detects homologous sequences of reptilian and avian adenoviruses.

Necrotic enteritis with Gram-positive bacilli has been associated with clostridial infections in mammals[1] and birds[2,5] classically; however, Clostridial enteritis has not been reported in reptiles. The significance of the enteric lesions is not clear in this case. Most Gram-positive bacteria are not considered pathogenic in reptiles under normal conditions, but they are potentially pathogenic, especially in immunocompromised animals.[15,18] Therefore, necrotic enteritis could represent a secondary bacterial infection in association with primary adenoviral hepatitis.

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b. MicroProbe system, Biomeda Corp., Foster City, CA.

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Serotyping of *Mannheimia* (*Pasteurella*) *haemolytica* isolates from the upper Midwest United States

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Abstract. *Mannheimia* (*Pasteurella*) *haemolytica* biotype A serotype1 (A1) is the primary bacterial agent responsible for the clinical signs and pathophysiologic events in bovine pneumonic pasteurellosis. The goal of this study was to determine the prevalence of other serotypes of *M. haemolytica* biotype A organisms obtained from the upper Midwest diagnostic laboratories. A total of 147 *M. haemolytica* isolates were collected from Minnesota, South Dakota, and Michigan. Isolates were tested against *M. haemolytica* antisera obtained from the National Animal Disease Center, Ames, Iowa. Results indicated that *M. haemolytica* serotype 1 represented approximately 60%, serotype 6 represented 26%, and serotype 2 represented 7% of the total examined isolates. In addition, 7% of the isolates were serotype 9, 11, or untypable. This finding suggests that *M. haemolytica* serotypes other than serotype 1 can be isolated from the lung lesions of diseased cattle and seem to be capable of causing the pathologic changes observed in the lung with pneumonic pasteurellosis.

Mannheimia (*Pasteurella*) *haemolytica* is the primary bacterial pathogen of pneumonic pasteurellosis, or what is commonly called shipping fever in beef and dairy cattle.[12,16] Reclassification of *P. haemolytica* to *M. haemolytica* based on DNA-DNA hybridization studies has been suggested by Angen et al.[2] The disease is characterized by presence of an acute fibrinonecrotizing lobar pneumonia and fibrinous exudate in the plural cavity. *Mannheimia haemolytica* biotype A, serotype 1 (ST1) has been reported to be the most commonly isolated serotype from pneumonic lesions.[1,3,11,14,15,17] Stressful conditions such as a change in climate or previous exposure to viral infection such as bovine herpesvirus 1 are thought to facilitate *M. haemolytica* serotype A1 colonization and shedding.[8,18] However, other *M. haemolytica* serotypes such as 2, 5, 6, and 9 can experimentally induce lesions and can be isolated from pneumonic lungs. Interestingly, serotypes that are untypable do not experimentally produce the disease.[7]

In this study, the goal was to examine the prevalence of *M. haemolytica* serotypes among isolates collected from Minnesota, South Dakota, and Michigan. It is felt that identification of the prevalent serotypes would improve our understanding of the epidemiology of the disease as well as provide information on the most appropriate serotypes to be used for successful vaccination against *M. haemolytica.*

Mannheimia haemolytica strains were collected during the period of 1997–1999. Eighty-eight isolates were obtained from the Veterinary Diagnostic Laboratory, University of Minnesota, St. Paul, Minnesota. Forty-nine isolates were obtained from the Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, South Dakota, and 10 isolates were obtained from the Veterinary Diagnostic Laboratory, Michigan State University, East Lansing, Michigan. All of the isolates, except 5 cultured from nasal cavities or abscesses, were recovered from lung lesions. Upon receipt of the isolates, they were transferred to sheep blood agar plates. The plates were incubated overnight at 37 C, and then the growth was resuspended in 1 ml of Todd Hewitt broth (Difco) plus 20% glycerol and frozen at −80 C for future use.

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