J Vet Diagn Invest 14:332–334 (2002)

An outbreak of adenoviral infection in inland bearded dragons (*Pogona vitticeps*) coinfected with dependovirus and coccidial protozoa (*Isospora* sp.)

Dae Young Kim, Mark A. Mitchell, Rudy W. Bauer, Rob Poston, Doo-Youn Cho

Abstract. Thirty of 200 (15%) hatchling inland bearded dragons were found dead after a short period (48 hours) of weakness and lethargy. The most common clinical signs were head tilt and circling. Six bearded dragons with neurological signs were euthanized, and postmortem examination revealed no gross abnormalities. Microscopically, severe, randomly distributed hepatocellular necrosis with large basophilic intranuclear inclusion bodies in numerous hepatocytes was noted. Small-intestinal enterocytes contained intracytoplasmic coccidial protozoa (*Isospora* sp.) and occasional enterocytes had basophilic intranuclear inclusion bodies. Transmission electron microscopy revealed both 80- and 20-nm-diameter viral particles, which were consistent with adenoviruses and dependoviruses, respectively. Adenoviral outbreaks in groups of animals are uncommon. An adverse synergistic effect of the coccidiosis with the adenoviral infection may have played a critical role in the high morbidity and mortality in this case.

Adenoviruses (Family Adenoviridae) are well-known pathogens in several mammalian and avian species. Adenoviruses are double-stranded DNA viruses, 70–90 nm in diameter, and have a characteristic nonenveloped, icosahedral structure. Generally, adenoviruses are host specific and are transmitted by the fecal-oral route or direct contact via oronasal secretions. Often, mammalian infections are subclinical, except for infectious canine hepatitis. Adenoviral disease generally occurs in immunocompromised or young animals. Outbreaks in groups of animals are uncommon. Recently, adenoviral infections also have been reported in several reptilian species, including crocodiles, snakes, and lizards.[3,4,6–12,15,16]

The Australian inland bearded dragon, *Pogona vitticeps* (Pogon: bearded in Greek), is one of the most popular reptiles in the pet trade. Two isolated cases of adenoviral infection in 4 neonatal inland bearded dragons have been reported, but the infections were limited mainly to the individuals affected.[10] No outbreak of adenoviral infection in a group of reptiles has been reported. This report describes an outbreak of adenoviral infection in a group of captive-bred inland bearded dragon hatchlings coinfected with dependovirus and *Isospora* sp. coccidia.

Two hundred hatchling captive-bred inland bearded dragons, which had been purchased at different times by a reptile importer from different captive breeding populations, were placed in a holding facility. The ages of the dragons were uncertain; however, based on their weight (<5 g), the estimate age was less than 1 month. The bearded dragons were maintained at an environmental temperature of 29–32 C and were fed commercially obtained crickets and lettuce. Thirty of the 200 (15%) bearded dragons were found dead after a short period (48 hours) of weakness and lethargy. The most common clinical signs were head tilt and circling. Physical examination of tympanic bullae revealed no significant findings. Six of the bearded dragons with neurological signs were euthanized and submitted for necropsy to the Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, Louisiana.

At necropsy, no significant gross abnormalities were noted in any of the 6 bearded dragons. Sections from major organs were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 4 μm thick, and stained with hematoxylin and eosin. For electron microscopy, 2 methods were used. At first, the liver specimens from 3 bearded dragons were pooled together, homogenized, and centrifuged at 800 × *g* for 30 minutes. The supernatant was collected and centrifuged at 98,000 × *g* for 1 hour. The formed pellet was harvested after discarding the supernatant. The pellet was suspended in 100 μl of phosphate-buffered saline (PBS) solution, stained with 100 μl of 4% phosphotungstic acid, placed on Formvar- and carbon-coated grids, air-dried, and examined with a transmission electron microscope (TEM). Second, formalin-fixed liver tissue was trimmed, dehydrated, postfixed in 1% phosphotunstic acid (w/v), embedded in LR White,[a] sectioned, and stained with uranyl acetate and lead citrate for TEM. The contents of the large intestines were submitted for parasitologic examination.

Significant microscopic changes were similar in all 6 dragons and were limited to the liver and small intestine. Severe, randomly distributed hepatocellular necrosis involving >50% of the parenchyma was accompanied by mild to moderate infiltration of lymphocytes and histiocytes. Numerous hepatocyte nuclei were expanded (2–4-fold) by large, basophilic, glassy, intranuclear inclusion bodies and marginated chromatin (Fig. 1). Small intestinal villi were atrophied, and many enterocytes contained variable stages of coccidia in the cytoplasm. Enterocytes occasionally had basophilic intranuclear inclusion bodies similar to those seen in the liver (Fig. 2). Electron microscopy revealed viral particles of 2 distinct sizes. Larger viral particles were approximately 80 nm in diameter, nonenveloped, and had hexagonal outlines and electron-dense or electron-lucent cores.

From the Departments of Pathobiological Sciences (Kim, Cho) and Veterinary Clinical Sciences (Mitchell), School of Veterinary Medicine, Louisiana State University, and The Louisiana Veterinary Medical Diagnostic Laboratory (Bauer, Poston), Baton Rouge, LA 70803.

Received for publication August 27, 2001.

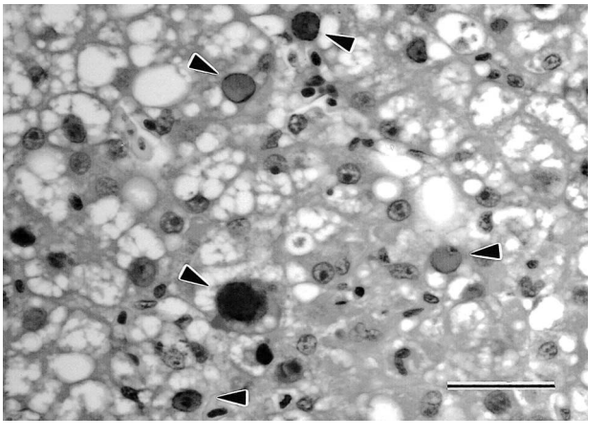

Figure 1. Liver of inland bearded dragon with numerous inclusion bodies within enlarged nuclei of hepatocytes. Bar = 40 μm.

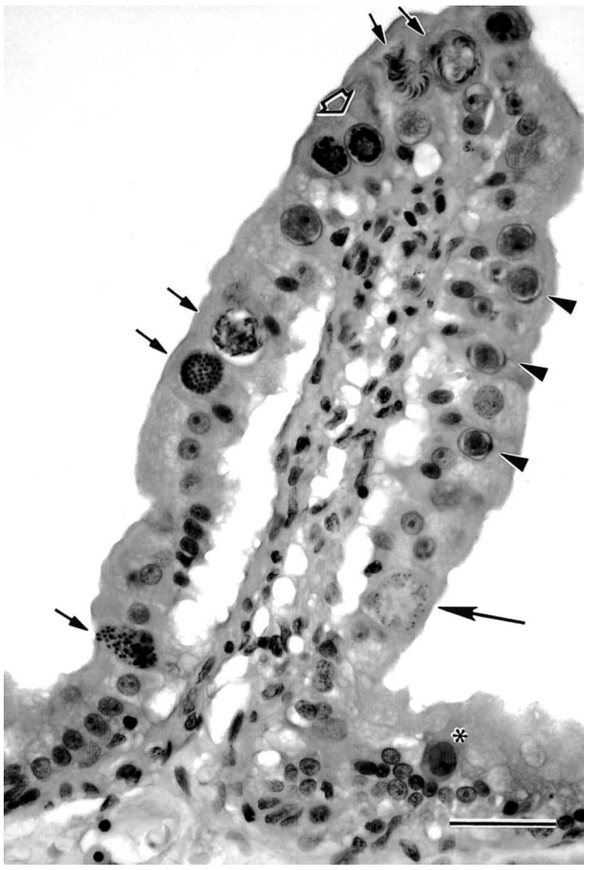

Figure 2. Small intestine of inland bearded dragon with an intranuclear inclusion body (asterisk) and various stages of coccidia in the cytoplasm of enterocytes including microgamonts (short arrows), a macrogamont (long arrow), zygotes (open arrow), and unsporulated oocysts (arrow heads). Bar = 25 μm.

These viral particles were arranged in paracrystalline arrays and occupied most of the nucleus (Figs. 3, 4). Smaller viral particles were approximately 20 nm in diameter, nonenveloped, and had hexagonal outlines and electron-dense cores

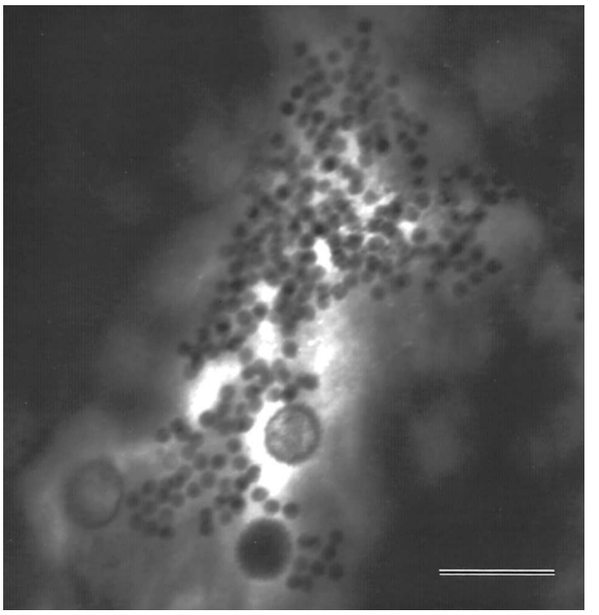

Figure 3. Transmission electron micrograph of viral particles from the pooled liver homogenate. Note viral particles of two distinctly different sizes. Bar = 150 nm.

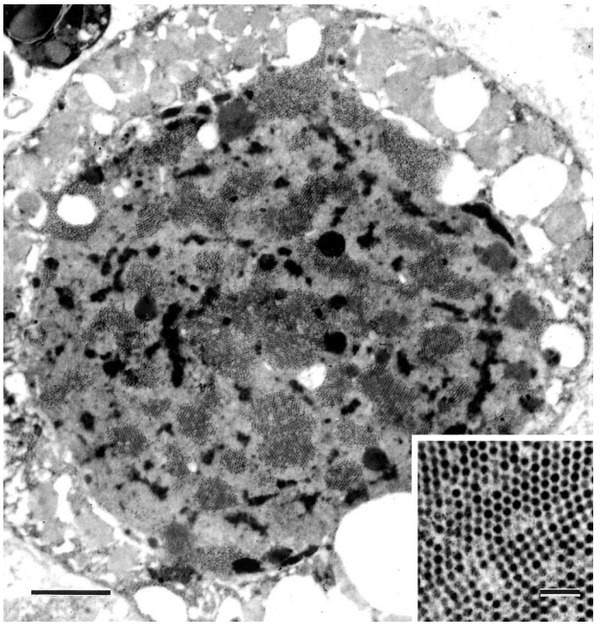

Figure 4. Transmission electron micrograph of a hepatocyte with a nucleus filled with viral particles. The cytoplasm is markedly vacuolated. Bar = 2.5 μm. **Inset**, The viral particles (approximately 80 nm in diameter) are arranged in paracrystalline arrays. Bar = 0.3 μm.

(Fig. 3). Direct smear and flotation of the intestinal contents revealed large numbers of *Isospora* sp.

Histopathologic findings and the shape and size of the nonenveloped larger viral particles in paracrystalline arrays were consistent with adenovirus.[2,5] All 6 lizards had acute severe coccidiosis throughout the small intestines. In the absence of significant morphologic changes in the brain, the neurologic signs (head tilting and circling) in some of the lizards may be attributed to an acute hepatoencephalopathy due to the severe hepatocellular necrosis.

Adenoviral infection has been identified in several reptiles, including Nile crocodiles (*Crocodylus niloticus*),[7] rosy boas (*Lichanura rosefusca*),[16] boa constrictors (*Boa constrictor*),[4,8,15] a 4-lined rat snake (*Elaphe quatuorlineata*),[4] a gaboon viper (*Bitis gabonica*),[4] an aesculapian snake (*Elaphe longissima*),[4] a Savannah monitor (*Varanus exanthematicus*),[9] a Jackson's chameleon (*Chamaeleo jacksoni*),[6] a mountain chameleon (*Chameleo montium*),[12] bearded dragons (*Pogona* [= *Amphibolurus*] *barbatus* and *Pogona vitticeps*),[10,11] and Rankin's dragons (*Pogona henrylawsoni*).[3] Generally, typical large intranuclear adenovirus inclusions were found in the liver or upper gastrointestinal system, including esophagus, or both. With the long replication cycle of 32–36 hours, adenoviruses form large, dense intranuclear inclusion bodies consisting of massive numbers of virions in the infected cells.[5] Common clinical problems were inappetence, vomiting, neurological signs, such as disorientation, head tremor, and opisthotonos, and sudden death without clinical signs.

The smaller viral particles found in the liver homogenate were compatible with dependoviruses based on the morphologic characteristics, especially the virion size (20 nm), and copresence with adenoviruses. No dependoviruses, however, were identified by TEM in the formalin-fixed liver specimens due to possible inadequate prefixation in formalin. Dependoviruses (Family Parvoviridae) are small (18–28 nm), nonenveloped viruses that have icosahedral symmetry.[1,2] Other viruses that are similar in size are Picornaviridae (24–30 nm)[2,14] and Circoviridae (15–22 nm).[14] The Picornaviridae viruses are larger than the smaller viruses found in the dragons, and circoviruses have not been reported in reptiles. Dependoviruses are commonly referred to as adeno-satellite viruses or adeno-associated viruses (AAV) because of their dependence on helper viruses (adenoviruses or herpes viruses) for replication.[1,2] Some of the early-stage genes from adenovirus, as a helper virus, are required for the synthesis, transport, and translation of dependovirus mRNA. Both viruses, however, do not share the same set of viral proteins, such as DNA binding proteins, DNA polymerase, etc., for their replication.[1] Interestingly, recent studies have shown that pretreatment of several cell lines with toxic agents results in dependovirus replication in the absence of a helper virus.[1]

According to others,[10] coccidiosis is a common problem in captive-bred inland bearded dragons; however, in that report, only 1 of 4 neonatal dragons coinfected with adenovirus-like and dependovirus-like viruses had coccidiosis. *Isospora amphiboluri* has been identified in the inland bearded dragon,[13] but the pathologic significance of these coccidia has not been fully elucidated. In this current outbreak, all 6 hatchling bearded dragons examined had adenoviral inclusions as well as variable stages of coccidial organisms throughout the small intestines. The coccidia were identified as *Isospora* sp. Common clinical manifestations of coccidiosis in animals are sudden onset of bloody diarrhea with fever, followed by dehydration, emaciation, and occasional death, especially in severely infected young animals. Although no obvious clinical evidence of coccidial infection was observed in these dragons, it is possible that an adverse synergistic effect of the coccidiosis and adenoviral infection resulted in the high morbidity and mortality in this case.

Sources and manufacturers

a. LR White, London Resin Company Limited, Berkshire, UK.

References

1. Berns KI: 1991, Parvoviridae and their replication. *In:* Fundamental virology, eds. Fields BN, Knipe DM, Chanock RM, Hirsch MS, Milnick JL, Monath TP, 2nd ed., pp. 817–837. Raven Press, New York, NY.
2. Doane FW, Anderson N: 1987, Electron microscopy in diagnostic virology. Cambridge University Press, Cambridge, UK.
3. Frye FL, Munn RJ, Gardner M, et al.: 1994, Adenovirus-like hepatitis in a group of related Rankin's dragon lizards (*Pogona henrylawsoni*). J Zoo Wildl Med 25:167–171.
4. Heldstab A, Bestetti G: 1984, Virus associated gastrointestinal diseases in snakes. J Zoo An Med 15:118–128.
5. Horwitz MS: 1991, Adenoviridae and their replication. *In:* Fundamental virology, eds. Fields BN, Knipe DM, Chanock RM, et al., 2nd ed., pp. 771–813. Raven Press, New York, NY.
6. Jacobson ER, Gardiner CH: 1990, Adeno-like virus in esophageal and tracheal mucosa of a Jackson's chameleon (*Chamaeleo jacksoni*). Vet Pathol 27:210–212.
7. Jacobson ER, Gardiner CH, Foggin CM: 1984, Adenovirus-like infection in two Nile crocodiles. J Am Vet Med Assoc 185: 1421–1422.
8. Jacobson ER, Gaskin JM, Gardiner CH: 1985, Adenovirus-like infection in a boa constrictor. J Am Vet Med Assoc 187:1226–1227.
9. Jacobson ER, Kollias GV: 1986, Adenovirus-like infection in a Savannah monitor. J Zoo An Med 17:149–151.
10. Jacobson ER, Kopit W, Kennedy FA, et al.: 1996, Coinfection of a bearded dragon, *Pogona vitticeps*, with adenovirus- and dependovirus-like viruses. Vet Pathol 33:343–346.
11. Julian AF, Durham PJK: 1982, Adenoviral hepatitis in a female bearded dragon (*Amphibolurus barbatus*). NZ Vet J 30:59–60.
12. Kinsel MJ, Barbiers RB, Manharth A, Murnane RD: 1997, Small intestinal adeno-like virus in a mountain chameleon (*Chameleo montium*). J Zoo Wildl Med 28:498–500.
13. McAllister CT, Upton SJ, Jacobson ER, et al.: 1995, A description of *Isospora amphiboluri* (Apicomplexa: Eimeriidae) from the inland bearded dragon, *Pogona vitticeps* (Sauria: Agamidae). J Parasitol 81:281–284.
14. Murphy FA: 1996, Virus taxonomy. *In:* Fields virology, eds. Fields BN, Knipe DM, Howley PM, et al., pp. 15–57. Lippinocott-Raven Publishers, Philadelphia, PA.
15. Ramis A, Fernandez-Bellon H, Majo N, et al.: 2000, Adenovirus hepatitis in a boa constrictor (*Boa constrictor*). J Vet Diagn Invest 12:573–576.
16. Schumacher J, Jacobson ER, Burns R, et al.: 1994, Adenovirus-like infection in two rosy boas (*Lichanura trivirgata*). J Zoo Wildl Med 25:461–465.