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

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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.10

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 coinfeckted 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 estimated 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% 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). 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 margined 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.
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 paracristalline 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 paracystalline arrays. Bar = 0.3 μm.
were consistent with adenovirus. All 6 lizards had acute nonenveloped larger viral particles in paracrystalline arrays), a Savannah monitor (Varanus exanthematicus), a Jackson’s chameleon (Chamaeleo jacksonii), a mountain chameleon (Chameleo montium), and Rankin’s dragons (Pogona henrylawsoni). 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. 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. Other viruses that are similar in size are Picornaviridae (24–30 nm) and Circoviridae (15–22 nm). The Picornaviridae viruses are larger than the smaller viruses found in the drag,

Histopathologic findings and the shape and size of the nonenveloped larger viral particles in paracrystalline arrays were consistent with adenovirus. 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 hepaticcellular necrosis.

Adenoviral infection has been identified in several reptiles, including Nile crocodiles (Crocodylus niloticus), rosy boa (Lichanura rosefusca), boa constrictors (Boa constrictor), a 4-lined rat snake (Elaphe quatuorlineata), a gaboon viper (Bitis gabonica), an aesculapian snake (Elaphe longissima), a Savannah monitor (Varanus exanthematicus), and a Jackson’s chameleon (Chamaeleo jacksonii), a mountain chameleon (Chameleo montium), and Rankin’s dragons (Pogona henrylawsoni).


Sources and manufacturers

References


