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Inclusion Body Hepatitis in Gambel's Quail (*Callipepla gambelii*)

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ABSTRACT: An acute necrotizing hepatitis in 1- to 3-wk-old Gambel's quail (*Callipepla gambelii*) caused by an adenovirus is described. The infection caused high mortality in captive raised, orphan chicks at two wildlife rehabilitation facilities in Arizona (USA). Gross lesions varied from pale livers to multiple, pinpoint, white foci scattered throughout the livers. Microscopically, scattered foci of hepatocellular necrosis were present. Intact hepatocytes at the periphery of necrotic foci had eosinophilic and basophilic intranuclear inclusion bodies.

Key words: Gambel's quail, hepatitis, necrosis, liver, adenovirus, *Callipepla gambelii*.

Gambel's quail (*Callipepla gambelii*) are indigenous to the desert scrublands of Arizona, New Mexico, west Texas, southeastern California, western Colorado, and local areas of Utah (USA), as well as northern Mexico. Two episodes of mortality in 1- to 3-wk-old, orphaned Gambel's quail chicks were observed. The first occurred in June 1991. Three dead and one live, 3-wk-old, Gambel's quail chicks were presented to our laboratory by a Tucson, Arizona, wildlife rehabilitator. The birds originally were obtained from suburban home owners who found them wandering alone in the desert areas nearby (32°25′N, 111°06′W). Groups of fifteen birds were raised, indoors, in aquariums lined with sand. The sand was obtained from dry desert streambeds (washes) and changed twice weekly. Birds of similar age entered the aquariums together and no additions were made. The aquariums were thoroughly cleaned and disinfected between groups. Mortality occurred in 1- to 3-wk-old, orphan birds over a 1-wk period. Total losses had exceeded 60 birds from a population of 200 birds. Death loss had been as high as 14 of 15 birds in some groups. A few birds exhibited vague clinical signs such as depression and ruffled feathers, however most exhibited no signs prior to death.

The second episode occurred in July 1992. Again, 1- to 3-wk-old, orphaned quail chicks were affected. These chicks were raised by a Phoenix, Arizona, wildlife rehabilitator who described similar husbandry and sanitation methods employed by the Tucson rehabilitator. Approximately 50 of 100 birds died. The birds in this group were obtained from a wider geographic area and included birds from the Sedona (34°53′N, 112°47′W) and Cottonwood (34°44′N, 112°01′W) areas in north central Arizona, the fringes of the Phoenix metropolitan area (33°39′N, 112°00′W) and from the Tucson area (32°25′N, 111°05′W). The birds were mixed shortly after arrival so that the rehabilitator was not able to determine if the disease had originated in birds from a particular source or geographic area. The 1-wk-old birds in this group had few noticeable clinical signs and simply were found dead. The older birds (2- to 3-wk-old) had a 1 to 2 day history of anorexia, depression, and ruffled feathers. A few had greenish diarrhea. Nine dead birds from this group were received for necropsy.

A complete postmortem examination was performed on all birds submitted. Samples of major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E) for light microscopic examination.

For electron microscopy, formalin fixed liver was post fixed in 4% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, Pennsylvania, USA) for 2 hr, embedded (Mollenhauer, 1964) in eponaraldite (Electron Microscopy Sciences),

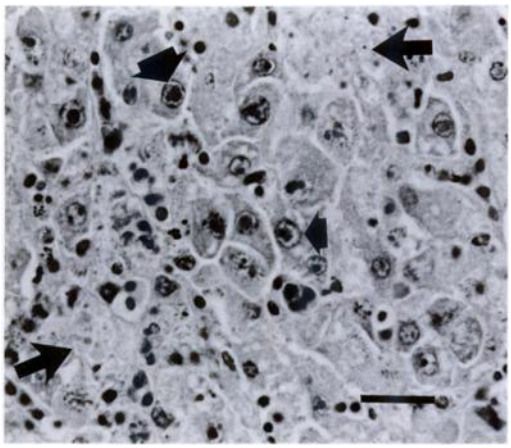

FIGURE 1. Liver. Multifocal areas of hepatocellular necrosis (long arrows) and intranuclear inclusion bodies (short arrows) are present in hepatocytes. H&E. Bar = 30 μm.

sectioned at 100 nm, placed on 300 mesh copper grids and stained with uranyl acetate-lead citrate (Watson, 1958).

Samples of brain, liver and intestine were inoculated onto trypticase soy agar plates with 5% sheep blood and tergitol-7 agar (Microbio Products Inc., Phoenix, Arizona) for bacteriologic analysis. In addition, intestine was inoculated into tetrathionate broth and brilliant green agar (Microbio Products Inc.) for detection of *Salmonella* spp. All cultures were incubated at 37 C for 48 hr and examined for pathogenic bacteria by the techniques of Carter and Cole (1990).

Suspensions of intestinal contents and scrapings of the intestinal mucosa were suspended in 0.85% saline. The wet mounts were examined for parasitic organisms by phase contrast microscopy.

Homogenates (10% w/v) of pooled livers from the first epizootic were made in Hanks' balanced salt solution (Gibco Laboratories, Grand Island, New York, USA) containing 120 μg/ml Gentocin® (Gemini Bioproducts Inc., Calabasas, California) and 2 μg/ml Fungizone® (Gibco Laboratories). The tissue was finely ground using a 2 ml Tenbroeck tissue grinder (Wheaton, Millville, New Jersey, USA). The resulting mixture was centrifuged at 1,500 rpm for 20 min at 4 C in an IEC Centra-7R refrigerated centrifuge (International Equipment Co., San Juan Capistrano, California). The supernatant was separated from the pellet and 0.2 ml and 0.1 ml of this fluid was injected into the chorio-allantoic membranes and allantoic sacs, respectively, of 9- to 10-day-old chicken embryos for virus isolation. The embryos were incubated at 38 C and candled daily for 1 wk. One blind transfer was performed utilizing allantoic fluid from the embryos.

Liver homogenates from the second epizootic were prepared as described above and filtered through a Costar 0.45 μm syringe filter (Costar, Van Nuys, California). One ml of filtered fluid was added to a primary chicken embryo fibroblast monolayer (Burleson et al., 1992) in a Costar 25 cm^2 flask (Costar) at 37 C with 5% CO_2. After 45 min, the inoculum was aspirated off and fresh Dulbecco's minimal essential media (Gibco Laboratories) containing 10% fetal calf serum (Sigma Chemical Co., St. Louis, Missouri, USA) to which 120 μg/ml Gentamicin® and 2 μg/ml Fungizone® was added. The flask was observed daily for 5 days. One blind passage was performed on the fifth day.

Gross necropsy lesions in birds from the first epizootic included dilated intestines with pasty contents and pale, friable livers. Gross liver lesions in the second epizootic were better defined. The livers of these birds contained numerous, pinpoint white foci of necrosis which appeared to be randomly scattered. These foci coalesced in some areas to form a mottled pattern on the liver surface. All other organs were within normal limits.

Aerobic bacterial cultures yielded no significant isolates from either group of birds. Wet mounts of intestinal contents contained trichomonads and occasional coccidia oocysts for the first group only.

Microscopically, lesions were similar in affected birds of both groups. There were multifocal to coalescing, random foci of hepatocellular necrosis in the livers (Fig. 1). These foci varied from involvement of

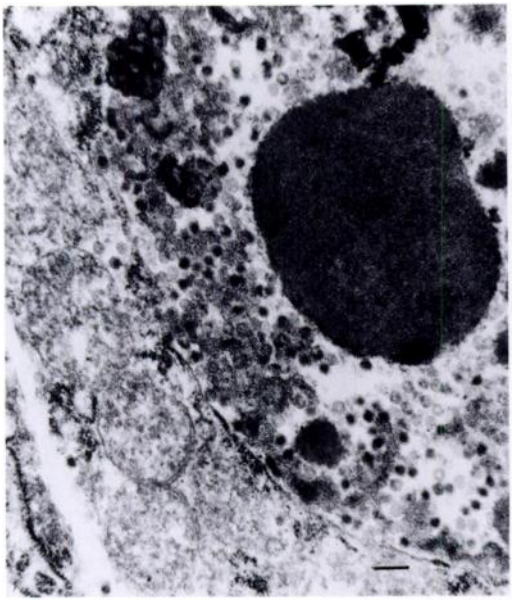

FIGURE 2. Liver. Numerous viral particles are present within the nucleus of a hepatocyte. Uranyl acetate-lead citrate. Bar = 200 nm.

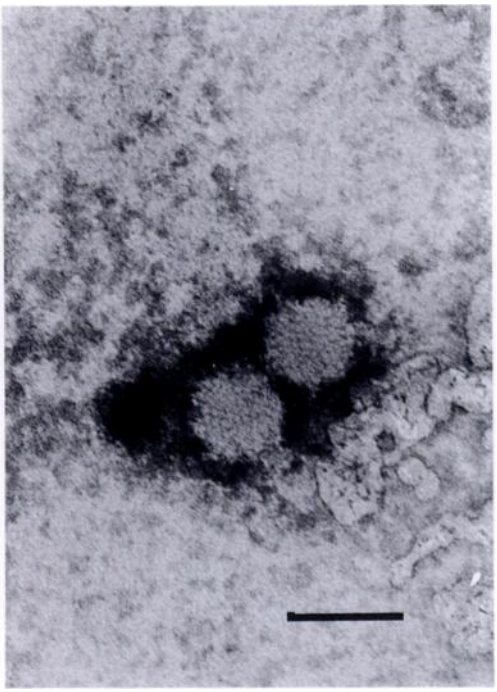

FIGURE 3. Negatively stained viral particles obtained from cell cultures. Bar = 100 nm.

five to ten adjacent hepatocytes to obliteration of entire lobules. Necrosis was characterized by loss of hepatocytes with replacement by eosinophilic debris, nuclear fragments and infiltrates of heterophils, macrophages, and lymphocytes. The numbers of infiltrating inflammatory cells tended to be minimal in the youngest birds (1-wk-old) and moderately intense in the older birds. Few to many intact hepatocytes at the periphery of necrotic foci contained small, eosinophilic, intranuclear inclusions surrounded by clear halos. The nuclear chromatin was marginated. A lesser number of hepatocytes contained larger, more basophilic inclusions which occupied the entire nucleus. No lesions were found in the other major organs except for one bird from the initial outbreak which had a few coccidian parasites in the mucosal epithelium of the small intestine.

There were intranuclear inclusions and individual, non-enveloped viral particles in thin sections of affected livers (Fig. 2). The intranuclear capsids measured 80 nm and had an electron dense core.

Initial attempts at virus isolation using chicken embryos (first group only) were unrewarding. However cytopathic effect was noted in the chick embryo fibroblast cell cultures inoculated with liver homogenates from the second group. This was characterized by rounding up and detachment of the monolayer beginning 48 hr after inoculation. The cytopathic agent was chloroform resistant. Based on electron microscopic examination of negatively stained grids obtained from the cell cultures we observed 80 nm diameter viral particles with icosahedral morphology consistent with adenoviruses (Fig. 3).

Quails are members of the Galliformes and tend to have many diseases in common with other members of this family, including domestic chickens (Barnes, 1987). Inclusion body hepatitis caused by an adenovirus has been described in 3- to 7-wk-old chickens (McFerran, 1990). A related group I adenovirus (quail bronchitis virus) has long been recognized as a cause of respiratory disease and mortality in farm-reared bobwhite quail (*Colinus virgini-*

anus) (DuBose et al., 1958). Inclusion body hepatitis caused by a similar group I adenovirus also has been described in farm reared bobwhite quail without respiratory disease (Jack et al., 1987). And titers to quail bronchitis virus have been found in wild bobwhite quail with hepatic intranuclear inclusion bodies (King et al., 1981).

Adenovirus hepatitis in farmed bobwhite quail causes high mortality (50 to 60%) in birds <3-wk-old and spreads rapidly through the susceptible population with no preceding clinical signs (Jack et al., 1987). The course of the disease in the Gambel's quail chicks closely resembled the disease in bobwhite quail, both in terms of age of onset and morbidity and mortality.

The gross and microscopic lesions in the Gambel's quail chicks also were similar to those described in inclusion body hepatitis of farmed bobwhite quail. Grossly, disseminated, 1 to 2 mm-diameter pale foci of necrosis were seen. The randomly scattered microscopic foci of hepatocellular necrosis with variable infiltrates of leukocytes also was similar in the two diseases. However, large basophilic intranuclear inclusion bodies predominated in hepatocytes at the margins of necrotic foci in bobwhite quail (Jack et al., 1987) rather than the smaller eosinophilic inclusions which were more common in the Gambel's quail chicks.

The adenovirus infection in this group of young Gambel's quail caused significant mortality due to massive hepatocellular necrosis. Other causes of death were not found in any of the 13 birds necropsied; thus the isolated adenovirus probably was the sole agent responsible for the death loss. The birds were orphaned, held in close confinement and likely undergoing significant stress; this may have enhanced their susceptibility to infection and to the development of clinical disease.

The source of the virus infecting these chicks was not determined. According to the wildlife rehabilitators, there was no close contact with birds of other species while in the rehabilitation facilities. Both reported employing diligent sanitation procedures for utensils and for their hands to minimize the possibility of transmitting disease within or between species while in the rehabilitation facilities but interspecies transmission before capture cannot be ruled out.

The ultrastructural morphology of the isolated adenovirus was similar to that of quail bronchitis virus (Dutta and Pomeroy, 1967) but further studies are needed to classify this Gambel's quail isolate and determine its relationship, if any, to adenovirus isolates of bobwhite quail.

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