Enteritis associated with adenovirus-like particles in captive lorikeets

J T MACKIE — IDEXX-Veterinary Pathology Services Pty Ltd, PO Box 1119, Coorparoo, Queensland 4151

D BLACK — Moama Veterinary Clinic, 61 Meninya Street, Moama, New South Wales 2731

H PRIOR — Department of Primary Industries, Animal Research Institute, 665 Fairfield Road, Yeerongpilly, Queensland 4105

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Avian adenoviruses infect a wide variety of avian species and consist of three serologically distinct groups.[1] Group I avian adenoviruses are widely distributed throughout the world and have been associated with inclusion body hepatitis in chickens, pigeons and other species, bronchitis in quail, and a number of other conditions in chickens and turkeys, including respiratory disease and growth retardation.[1,2] There is conflicting evidence regarding the primary pathogenicity of adenovirus in many of these conditions.[1] Group II avian adenoviruses are widely distributed throughout the world and are the primary aetiological agents of haemorrhagic enteritis in turkeys, marble spleen disease in pheasants and splenomegaly in chickens.[3] Group III avian adenoviruses are the cause of egg shell defects in chickens (egg drop syndrome).[1] Infection with adenovirus or adenovirus-like organisms has been reported in a number of psittacine species,[1,4-7] and in Australia adenovirus-like particles have been reported in a cockatiel, peach-faced lovebirds and a princess parrot (R Doneley, personal communication).[1,8] This report describes necrotising enteritis associated with intranuclear inclusion bodies and adenovirus-like particles in captive lorikeets.

In February 2002 a well-managed pet shop in northern Victoria noted feed refusal in a group of 10 lorikeets following the introduction of a new batch of commercial lorikeet feed. Three died within 36 to 84 h and three others became ill but responded to supportive treatment. Clinical signs reported by the owner were inappetence, depression and weakness. No other birds in the shop (including 9 other species of psittacine birds) were offered the same food as the lorikeets and all remained healthy.

The three dead birds were submitted for necropsy: bird 1 was an adult female scaly breasted lorikeet (*Trichoglossus chlorolepidotus*); bird 2 was an adult female rainbow lorikeet (*Trichoglossus hematodus*); and bird 3 was an immature male rainbow lorikeet that had been stored frozen. Body condition varied from fair to poor. The pertinent gross lesions in birds 1 and 2 were dilation of the small intestine, with reddening of the mucosa and blood-stained watery contents, and enlargement and pallor of the spleen. Damage from freezing limited the examination of bird 3. For histological examination tissues were collected into 10% neutral buffered formalin at necropsy.

No protozoa or helminths were seen on microscopic examination of wet preparations of duodenal or jejunal contents. Normal intestinal bacterial flora were isolated from swabs of duodenal and jejunal contents cultured aerobically on routine media (Gribbles Veterinary Pathology, Clayton, Victoria). Selective cultures for *Salmonella*, *Yersinia* and *Campylobacter* were negative.

Histological lesions in birds 1 and 2 were similar. There was a severe, acute, necrotising enteritis with mucosal congestion and sloughing of crypt and villus epithelium. There was very little associated cellular inflammatory response. Large, basophilic to amphophilic, intranuclear inclusion bodies were present in numerous crypt and villus epithelial cells (Figure 1). Similar inclusions were present in low numbers in hepatocytes and Kupffer cells and, rarely, in pancreatic acinar cells. In the spleen there was proliferation of mononuclear cells around sheathed capillaries and depletion of lymphoid tissue. In bird 3 the koilin layer of the gizzard was disrupted and contained myriad yeasts morphologically consistent with *Candida* sp and Gram positive cocci, but no intestinal lesion or inclusion bodies were seen. Rare intranuclear inclusions similar to those described above were present in epithelial cells in the bursa of Fabricius. In all three birds there was abundant haemosiderin (positive to Perl's Prussian blue stain) in hepatocytes and Kupffer cells, to a lesser extent in splenic macrophages, and to a minor extent in renal tubular epithelial cells. No significant microscopic lesion was seen in the heart, lung, thoracic air sac, crop, proventriculus, large intestine, skin or feathers.

Electron microscopic examination of ultrathin sections of formalin-fixed duodenum from bird 1 revealed hexagonal viral particles in the nuclei of many epithelial cells (Figure 2). These particles had a mean diameter of about 70 nm and were sometimes in crystalline arrays. In some of the affected cells, the nuclear membrane was fragmented and clusters of viral particles were present in the cytoplasm. There was no evidence of a viral envelope. The size and morphology of the particles and the tendency to form crystalline arrays are characteristics consistent with adenovirus.[1] A sample of formalin-fixed duodenum of bird 1 was macerated with sterile sand in a small volume of distilled water and the supernatant was examined by negative staining electron microscopy. Numerous icosahedral viral particles, approximately 70 nm in diameter, with typical adenovirus morphology were seen (Figure 3).[1] There was no opportunity to attempt virus isolation from these cases.

Enteritis was probably the cause of death in birds 1 and 2 and the failure to isolate or identify any other infectious agent suggests that adenovirus was the proximate pathogen. Bird 3 had rare adenovirus-like inclusions in the bursa of Fabricius but the cause of death was most likely gastrointestinal dysfunction associated with severe ventricular candidiasis and Gram positive bacterial infection.

To our knowledge, this is the first report of adenovirus infection in lorikeets. Infection with adenovirus or similar organisms has been reported in a number of other psittacine species, most often associated with necrotising hepatitis and inclusion bodies in hepatocytes, and less frequently with infection of the gastrointestinal, respiratory or other organ systems.[1,5-8] In the present study, the intestinal epithelial tropism suggests group I avian adenovirus infection, similar to that reported in African grey parrots,[6] though hepatic necrosis was notably absent in our cases. Disease due to group II avian adenovirus infection (such as haemorrhagic enteritis of turkeys) is characterised by viral

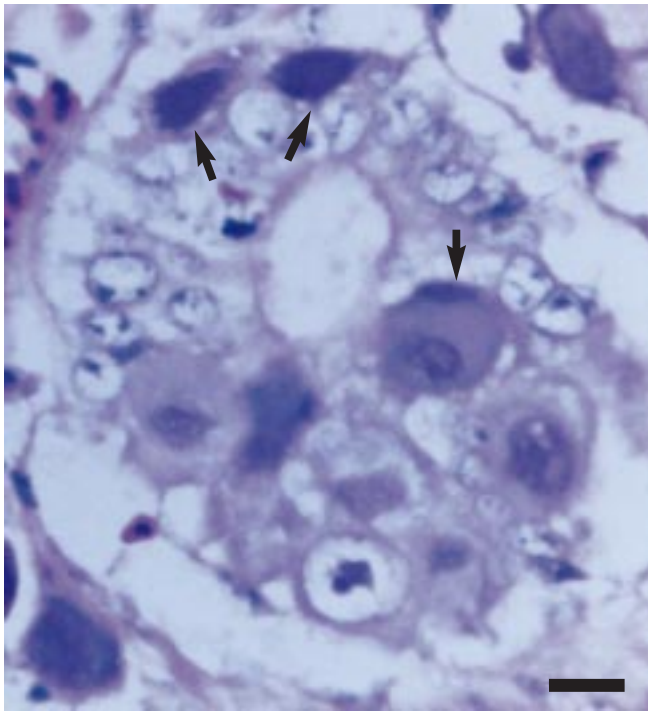

Figure 1. Duodenum; bird 1. Note large intranuclear inclusion bodies in crypt epithelial cells (arrows). Haematoxylin and eosin. Bar = 10 μm.

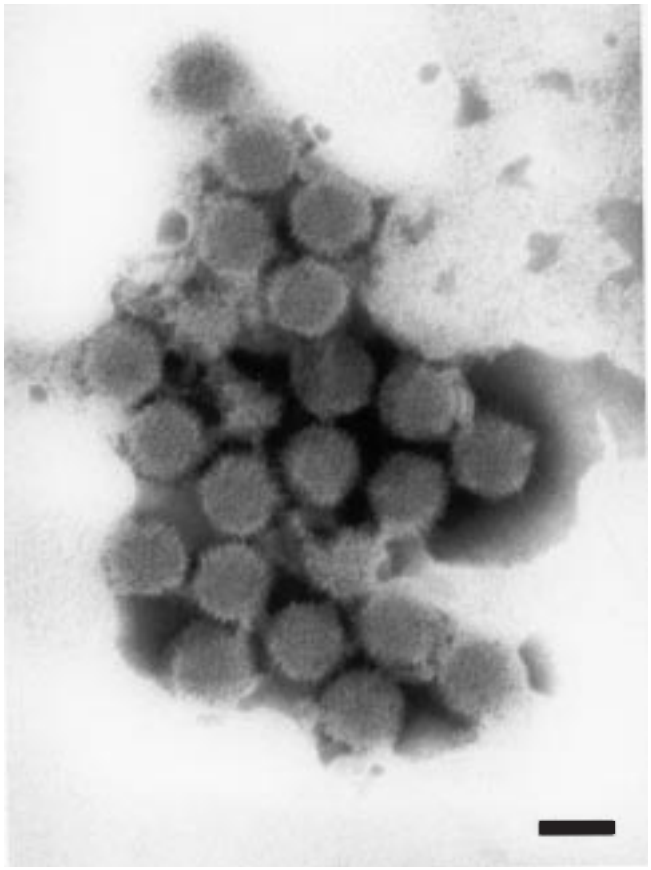

Figure 3. Particles resembling adenovirus, from duodenum, bird 1. Negatively stained, bar = 55 nm.

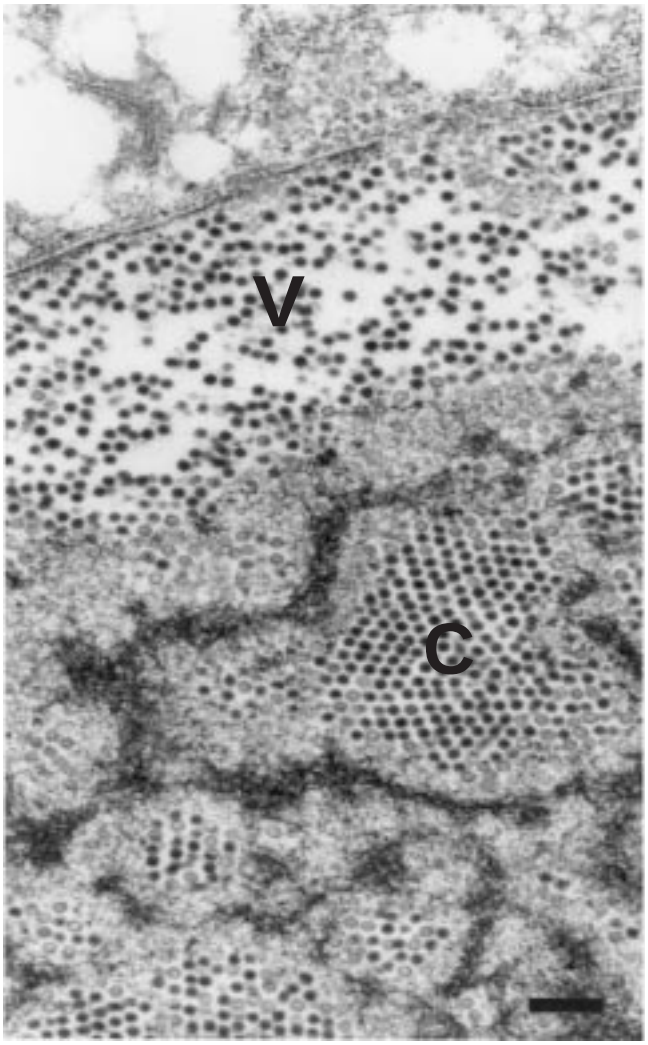

Figure 2. Duodenum; bird 1. Portion of nucleus of crypt epithelial cell. Note the large number of virus-like particles that occur as single, loosely packed particles (V) or as crystalline arrays (C). Electron micrograph, bar = 0.35 μm.

replication and intranuclear inclusion bodies mainly in lymphocytes in the spleen, intestine and other organs, and there is splenic lymphoid hyperplasia followed by necrosis. The haemorrhagic enteritis in group II adenovirus infection appears to be due to capillary endothelial disruption and leakage.[3] A similar disease also featuring viral replication and inclusion bodies in capillary endothelial cells has been reported in macaws, Amazon parrots and cockatoos.[7]

Group I avian adenovirus infection is usually subclinical and antibodies are frequently present in healthy chickens, turkeys and other avian species.[1] Adenoviral disease may involve expression of a latent infection under conditions of stress and/or in association with immunosuppressive diseases, and there is evidence that the group I adenovirus responsible for inclusion body hepatitis in chickens is a secondary pathogen associated with immunosuppressive viruses such as chicken infectious anaemia virus and infectious bursal disease virus.[1] In the present study there was no macroscopic or histological evidence of infection with psittacine circovirus, a known immunosuppressive virus common in psittacines in Australia. Nutritional stress following feed refusal may have contributed to reduced immune competence in these birds, predisposing them to opportunistic

infection with adenovirus or clinical disease. There is also growing evidence that certain genotypes of group I avian adenoviruses may be primary pathogens.[1]

Since some avian adenovirus serotypes can cross species boundaries in nature,[4] the potential for this virus to infect commercial poultry flocks needs to be borne in mind.

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References

1. McFerran JB. In: Calnek BW, Barnes HJ, Beard CW, McDougald LR, Saif YM, editors. *Diseases of poultry.* 10th edn. Iowa State University Press, Ames, 1997:607-608, 608-620.
2. Crespo R, Shivaprasad HL, Droual R et al. Inclusion body tracheitis associated with avian adenovirus in turkeys. *Avian Dis* 1998;42:589-596.
3. Pierson FW, Domermuth CH. Adenovirus infections: hemorrhagic enteritis, marble spleen disease, and related infections. In: Calnek BW, Barnes HJ,
4. McFerran JB, Connor TJ, McCracken RM. Isolation of adenoviruses and reoviruses from avian species other than domestic fowl. *Avian Dis* 1976;20:519-524.
5. Desmidt M, Ducatelle R, Uyttebroek E, Charlier G, Hoorens J. Respiratory adenovirus-like infection in a rose-ringed parakeet (*Psittacula krameri*). *Avian Dis* 1991;35:1001-1006.
6. Droual R, Woolcock PR, Nordhausen RW, Fitzgerald SD. Inclusion body hepatitis and hemorrhagic enteritis in two African grey parrots (*Psittacula erithacus*) associated with adenovirus. *J Vet Diagn Invest* 1995;7:150-154.
7. Gomez-Villamandos JC, Martin de la Mulas JM, Hervas J et al. Spleno-enteritis caused by adenovirus in psittacine birds: a pathological study. *Avian Pathol* 1995;24:553-563.
8. Pass DA. Inclusion bodies and hepatopathies in psittacines. *Avian Pathol* 1987;16:581-597.

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Prion-associated spongiform encephalopathy in an imported Asiatic golden cat (*Catopuma temmincki*)

S YOUNG — Melbourne Zoo Veterinary Department, PO Box 74, Parkville, Victoria 3052

RF SLOCOMBE — School of Veterinary Science, University of Melbourne, Werribee, Victoria 3030

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AAHL	Australian Animal Health Laboratory, Geelong
BSE	Bovine spongiform encephalopathy
PrP	Prion protein
SE	Spongiform encephalopathy

On 4 March 2002, a 10-year-old male Asiatic golden cat at Melbourne Zoo was found dead in his enclosure with no preceding clinical abnormalities observed. The cat was born at Allwetter Zoo in Munster, Germany, in 1992. He then spent time at Zoologischer Garten in Berlin and Wassenaar Wildlife Breeding Centre in the Netherlands before being imported to Melbourne Zoo on 15 October 1998. During the three and a half years the cat was housed at Melbourne Zoo his only clinically apparent health problem was unilateral carpal arthritis, resulting from distal antebrachial fractures sustained at some time before importation.

A thorough necropsy was performed on the cat at Melbourne Zoo within several hours after death. A number of significant gross lesions were found, including perforation of the proximal duodenum, with adjacent mesenteric, hepatic and pancreatic adhesions. A range of tissue samples was collected and preserved in 10% neutral-buffered formalin. Unpreserved duplicates of each sample were frozen at -70°C. The formalin-preserved tissues were submitted to a commercial diagnostic laboratory (Gribbles Veterinary Pathology, Clayton, Victoria) for processing and histopathological examination.

Histopathological findings included severe, chronic, necrotising pancreatitis and extensive acute fibrinopurulent peritonitis. Sections of brain showed widespread vacuolation affecting the white matter of the corpus callosum, internal capsule, thalamus and brain stem and to a lesser extent the cerebral cortex, cerebellum, brain stem nuclei and midbrain. The myelinic vacuolation appeared to be the result of acute oedema, most likely due to terminal changes associated with the peritonitis and pancreatitis, or artefactual. There was also widespread, but mild, grey matter neuropil vacuolation in brain sections, including deeper laminae of the cerebral cortex, cerebellum, brain stem nuclei and midbrain. Occasional small and single intraneuronal vacuoles were also noted in the thalamic nuclei, caudal colliculus, dorsal spinocerebellar tract nuclei and spinal tract nucleus of the trigeminal nerve (Figure 1).

The grey matter neuropil and intraneuronal vacuolation was considered suspicious for SE. Formalin-fixed and fresh brain samples were sent to AAHL for prion-associated SE testing. Scrapie-associated fibril extraction and transmission electron microscopy showed positive intracytoplasmic and extracellular PrP antigen staining in the four sections of brain examined: cerebral cortex, basal ganglia, cerebellum and brain stem. Immunohistochemical staining detected PrP antigen in the cerebral cortex, brain stem and cerebellum, with both intraneuronal and grey matter neuropil antigen present. While small amounts of antigen occurred throughout most of the brain tissue, it was abundant in scattered regions of the grey matter. Pooled brain samples showed a weak positive reaction for the presence of a protease-resistant PrP with western blot examination. The extensive testing performed by AAHL confirmed the diagnosis of prion-associated SE.

Spongiform encephalopathies are chronic, invariably fatal diseases characterised by long incubation periods, followed by the development of clinical signs related to progressive loss of central nervous system function.[1] The pathogenesis of these diseases is thought to involve the accumulation of abnormal, protease-resistant isoforms of a single prion protein that is normally coded for and expressed in the central nervous system and other tissues.[2] Knowledge regarding the transmission of SEs is incomplete and some cases in humans arise spontaneously. [1,3,4]