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Adenovirus in Parrots: a Case Study

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Introduction:

A wide range of lesions have been associated with adenovirus infection in birds including conjunctivitis, splenitis, hepatitis, enteritis and a wide variety of other organs such as the central nervous system through the damage caused to endothelium (Mori et al., 1989; Gomez Villamandos et al., 1995; Ritchie, 1995; Schmidt et al., 2003; Katohi et al., 2010). Adenoviruses are non-enveloped, double-stranded, linear, DNA viruses in the family *Adenoviridae* which all replicate with high fidelity in the host nuclei of vertebrates (Ritchie, 1995). Adenovirus pathogens of commercial poultry and humans have been well characterised in terms of their genetic diversity, pathogenicity and the diseases they cause. The International Committee on Taxonomy of Viruses (ICTV) recognises five genera in the family with the majority of avian adenoviruses so far characterised belonging to the genus *Aviadenovirus* for which the type species is *Fowl aviadenovirus A.* This genus mostly affects gallinaceous birds with diseases of commercial significance in quail, poultry, ducks, geese, pheasants and turkeys. The predominance of avian adenoviruses in gamefowl and waterfowl has hinted towards an ancient host coevolution with the Galloanserae, however there is widespread serological evidence in many wild birds such as albatross (Padilla et al., 2003), cormorants (Travis et al., 2006) and other avian Neoavian lineages including pigeons, and parrots points to a more widespread host diversity (Hess et al., 1998a, 1998b; Mackie et al., 2003; Marlier et al., 2006; Katohi et al., 2010) . Indeed avian hosts also dominate the related genus *Siadenovirus*, for which the type species is *Frog siadenovirus A,* but includes species from passerines, raptors and turkey (Katoh et al., 2009; Wellehan et al., 2009; Joseph et al., 2014). Psittacine bird species are known to be susceptible to adenovirus infection but the ICTV has so far not recognised any psittacine adenovirus species. Psittacine adenovirus 1, 2 and 3 have been coined as a potential species based on limited adenovirus DNA sequence data including the adenovirus hexon and DNA polymerase genes recovered from birds that have succumbed to fatal infection (Luschow et al., 2007; Katoh et al., 2009). However, only one full genome of a putative psittacine adenovirus, *psittacine adenovirus 3*, is currently available in NCBI GenBank (To et al., 2014). In this paper we present an investigation of mortality in a mixed aviary flock of parrots and lorikeets that caused mortality only in red-bellied parrots (*Poicephalus rufiventris*).

Case Histories

Case 1: Red-bellied Prrot (*Poicephalus rufiventris*) - Male 1

In the first occurrence of disease there was sudden death of a male red-bellied parrot (5th October 2013). The owner had purchased two pairs of apparently mature (4 years old) red-bellied parrots from another breeder four months prior in June 2013. There had been no problems prior to this. This bird had been apparently normal the previous night. It looked ill that morning and died on the way to the veterinary clinic. There was a very small amount of blood soiling the vent and from this the owner was convinced to allow a post-mortem examination. Necropsy revealed significantly dilated and haemorrhagic intestines and mild pallor to liver. The presence of undeveloped testes and a well-developed bursa of Fabricius indicated that the bird was immature and therefore unlikely to be four years of age. Samples of visceral organs were fixed in formalin.

Histopathology of tissues demonstrated widespread severe congestion and necrosis with focal areas of enterocyte necrosis. In the liver and spleen there were multifocal areas of necrosis associated with

karyomegaly and basophilic intranuclear inclusions. The kidney and testicles (immature) appeared congested but otherwise normal. The bursa of Fabricius was congested, oedematous and had moderate follicular lymphoid depletion. Accordingly a diagnosis of acute viral hepatitis, splenitis and enteritis was made. Since no fresh samples were saved from the bird for PCR testing the lesions were considered most likely to be due to *avian polyomavirus* or *psittacine adenovirus* infection. The advice given at the time was to provide careful monitoring of in-contact birds and to destroy contaminated equipment such as nest boxes and disinfect potentially contaminated environment. The histopathological report noted that the immature testicle and presence of the bursa of Fabricius indicated that this bird was less than 12 months of age.

Case 2: Red-bellied Parrot - Male 2

Some 13 days after the first male bird died the second male red-bellied parrot of the two pairs of birds became acutely lethargic. Akin to the first case this bird had been purchased as a three year old mature bird four months prior along with a hen as a breeding pair. It was presented to the clinic on the 18th October 2013 with acute onset of illness consisting of severe lethargy, mild dyspnoea and watery liquid on the cage floor. It was difficult to determine if the liquid was vomit or abnormal droppings. The owner reported that the bird was clinically normal four hours earlier.

The bird was hospitalised and treated as for critical care. This included a heated hospital cage; fluid therapy (s/c Hartmann's solution with 5% glucose); an oxygen cage PRN; amoxicillin/clavulanic acid 125 mg/kg s/c SID; and Enrofloxacin 30 mg/kg SID.

The bird continued to deteriorate and died the following day, 22 hours after presenting. Necropsy examination revealed a mottled liver and again, undeveloped testes indicating a sexually immature bird. Histopathological examination confirmed a diagnosis of acute necrotising viral hepatitis and enteritis. There was moderate autolysis which interfered with histological interpretation. Nevertheless, throughout the intestine there was widespread congestion and haemorrhage and focal areas of enterocyte necrosis. In the liver there were multifocal areas of necrosis associated with karyomegaly and basophilic intranuclear inclusions. The kidney was moderately congested and scattered tubules had intraluminal uric acid tophi likely associated with terminal dehydration. The testicle was immature and appeared congested but otherwise normal. The bursa of Fabricius was congested, oedematous and had moderate follicular lymphoid depletion. The proventriculus, ventriculus, lung and heart appeared mildly congested but normal. Blood, intestinal swabs and frozen spleen and liver samples tested PCR positive to *psittacine adenovirus 1* (Raue et al., 2005) and negative for *avian polyomavirus* and *beak and feather disease virus* using established PCR protocols (Phalen et al., 1999; Ypelaar et al., 1999). PCR amplicon sequencing confirmed the detection of adenovirus DNA.

Neither in-contact hen birds showed any sign of illness around the time that both cock birds died or after the death of the second male bird. There was also no other unexplained deaths in nearby birds. Accordingly the owner was advised to wait at least three months before sourcing and replacing the male birds. In February 2014 two new cock birds were purchased and placed with the females. There was a small period where the older cock bird bullied the hen when introduced but otherwise the health of the parrots was unremarkable until October 2014.

Case 3 Red-bellied Parrot - Male 3

One of the replacement male red-bellied parrots, two years of age, was presented (7th October 2014) with acute onset of severe lethargy. The owner had noticed that the bird had appeared unwell the previous day where it had been sitting at the food dish and not eating with a progressive worsening of condition. Clinically it was in reasonable body condition but was weak and lethargic with a partially distended crop containing fluid. Non-invasive faecal and a crop wash wet-preparation microscopy were unremarkable. The bird was hospitalised and treated aggressively as for critical care for bird 2 (above). However, the bird continued to deteriorate and passed very bloody droppings a few hours later with haemorrhagic diarrhoea, and died overnight. Post-mortem examination revealed significantly dilated and haemorrhagic intestines with bloody fluid contents in the cloaca. The liver was pale and the spleen was grossly normal size. There were intramuscular haemorrhages in the wall of the proventriculus. Small undeveloped testes were present. The post-mortem findings were very similar to those seen one year earlier in the first two cases. Subsequent histopathological examination demonstrated widespread severe congestion throughout the intestine with massive haemorrhage into the lumen and widespread necrosis of enterocytes with occasional basophilic intranucle-

ar inclusions and karyomegaly, mostly within endothelial cells of the *lamina propria*. In the liver and spleen there were multifocal areas of necrosis associated with karyomegaly and basophilic intranuclear inclusions, mainly in the liver. The lung, heart, brain, kidney and appeared congested but otherwise normal. Akin to the previous cases a diagnosis of acute necrotising viral hepatitis, splenitis and haemorrhagic enteritis with intralesional viral inclusions consistent with acute adenovirus infection was concluded.

Case 4. Red-bellied Parrot - Male 4

The second replacement male Red-bellied Parrot was presented to the clinic the following day, after the overnight death of Bird 3 (8th October 2014). This bird was reportedly four years of age and the owner reported that, whilst it had behaved normally during the previous day, it had deteriorated in behaviour alongside with Case 3. Clinically it was in moderate body condition and mildly lethargic. It had an empty crop and the droppings were grossly normal. Non-invasive faecal microscopy and a crop wash were unremarkable. The bird was hospitalised and treated aggressively as for critical care for bird 2 (above). The bird ate well that night but died the following morning. The droppings passed overnight were grossly normal. Post-mortem examination revealed significantly watery fluid filled intestines; food in the crop and some vomit/regurgitation in the oral cavity. The liver was grossly normal and the spleen mildly enlarged. The testes were moderately hypertrophic and sexually mature. Cytological examination of liver impression smears demonstrated many hepatocytes with massively enlarged and strongly staining bluish-purple intranuclear inclusions consistent with an acute DNA viral infection such as *psittacine adenovirus* or *avian polyomavirus*. Subsequent histopathological examination revealed severe congestion throughout the small and large intestine including the cloaca where there was focal to widespread areas of epithelial necrosis. In the liver there were multifocal areas of necrosis associated with marked karyomegaly and basophilic intranuclear inclusions. The lung was markedly congested. The brain, skin, kidney and testicle (immature) appeared congested but otherwise normal. PCR testing was positive for *psittacine adenovirus 1* and negative for avian polyomavirus and beak and feather disease virus.

In both Cases 3 and 4 the pathological findings and test results were very similar to the first two cases seen the previous year. Cases 3 and 4 died one year and two days after the first cases were seen and coincided with the first period of hot weather of the 2014 breeding season. The clinical presentation and histopathology were similar and only the male red-bellied parrots were affected so it seemed plausible that a carrier status may have been present in the flock, perhaps one of the female birds or other species held at the property. The two banks of suspended wire aviaries also housed Senegal parrots and purple-crowned lorikeets.

Attempts to identify carrier birds

In an attempt to identify adenovirus carrier birds, blood and cloacal samples were collected from the in-contact female red-bellied parrots and nearby birds on the 30th October 2014. There had been no further losses at this time. An aviary visit was arranged to assess the management options for the different aviary flock subsets. There were several banks of aviaries housed on a standard house block, some with a single species and others with a mixed open aviary flock. Other species of birds held by the collector included two pairs of Senegal parrots, purple crowned lorikeets, elegant parrots, scarlet parrots, doves, finches and lovebirds. Local wildlife including wild birds that visited the roof of aviaries were also abundant and there were several rainbow lorikeets and sulphur crested cockatoos present on the day of the site visit. Disinfection of aviary walls and substrates was considered not feasible due to difficulty of access below the suspended wire aviaries as well as the use of dirt and sand as a floor substrate in free-flight aviaries. Inter-flock quarantine was considered possible but difficult with relation to the arrangement of aviaries and stock density.

As shown in Figure 1, there were two banks of suspended aviaries opposite each other separated by a narrow aisle in which the male red-bellied parrots which had died had been housed. These are situated on the southern corner of the property. The two pairs of red-bellied parrots and a pair of Senegal parrots were housed separately in the south-western back (A-C). The north-eastern side of these aviaries consisted of four smaller suspended aviaries (D-G), housing purple crowned lorikeets. During the site visit samples were collected consisting of blood and cloacal swabs from both widowed red-bellied parrot hens; blood and cloacal swabs from the pair of Senegal parrots adjacent to the red-bellied parrots; blood and cloacal swabs as well as nest material from a pair of purple crown lorikeets housed in an aviary directly opposite to the affected red-bellied parrots. Cloacal swabs and nesting material were collected from

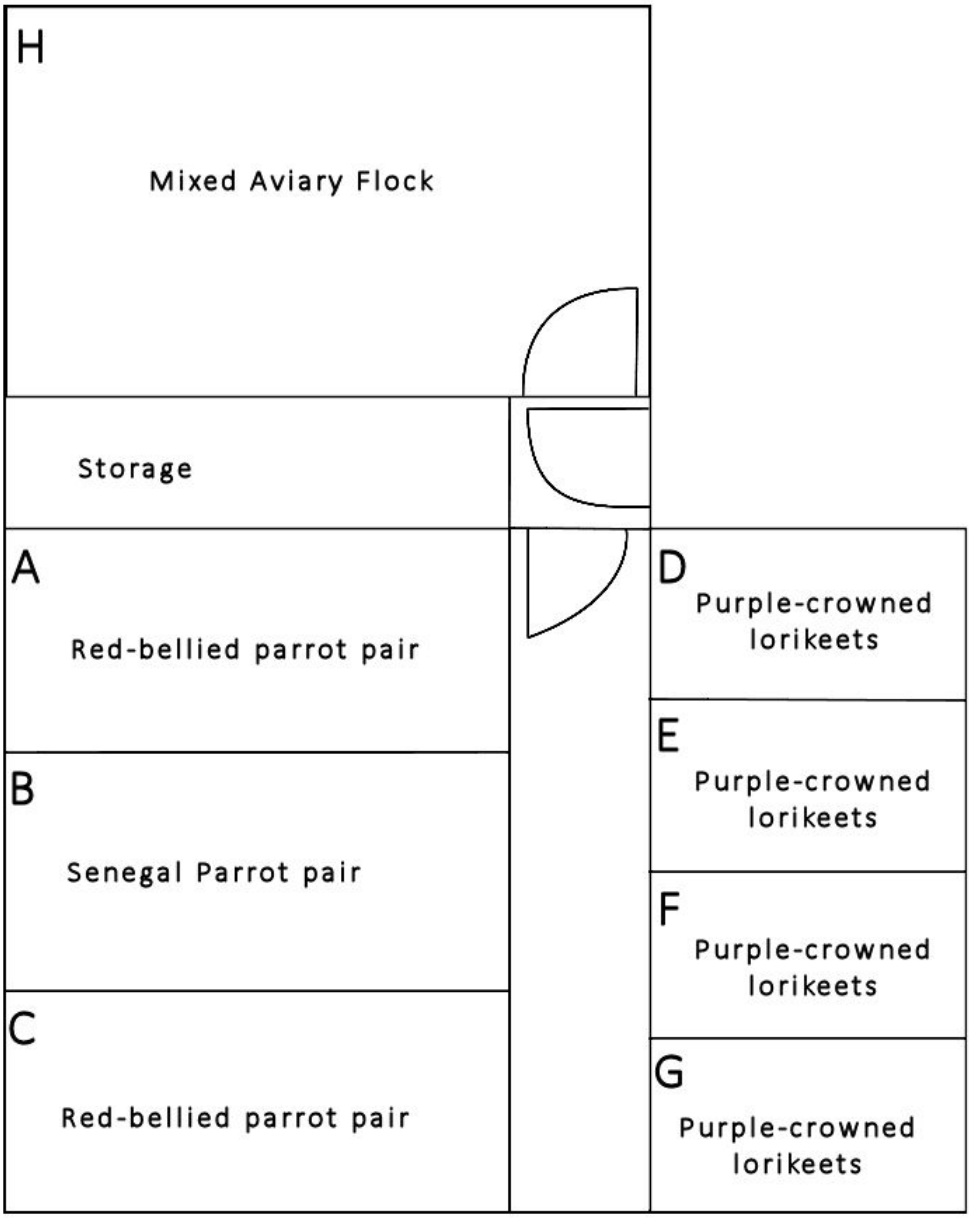

Figure 1. Layout of facility showing location of suspended wire aviaries (A-G) in relation to a larger open flighted aviary with earthen floor (H).

all other nearby purple crown lorikeets (nine birds and three nests). All nest material samples were PCR negative for adenovirus by PCR. Three purple crowned lorikeets were positive (Table 1).

No deaths occurred in the aviary flocks for five months, when on the 20th ch 2015 the owner reported that the female purple crowned lorikeet (ID 10-36 gold) that was housed in aviary E, directly opposite the affected red-bellied parrots had died a little over one week earlier and was lost to investigation. At the owners request the mate of this bird and its single offspring were euthanased for diagnostic testing. These were PCR negative for adenovirus and avian polyomavirus.

Repeat sampling and testing were organised with cloacal swab samples collected from five clinically normal breeding purple crowned lorikeets in November 2014 which identified two birds as PCR positive for adenovirus and were assumed to be shedding the virus. Birds in this colony, and not necessarily the pair

Table 1. Adenovirus PCR Testing (30th October 2014)

Aviary	Bird ID and sex (male/unknown/female)	Sample	Adenovirus
G	Purple crowned lorikeet (u)	Swab	**Positive**
G	Purple crowned lorikeet Captn 78 split (u)	Swab	**Positive**
G	Purple crowned lorikeet Kirk 61 split (u)	Swab	Negative
G	Purple crowned lorikeet Kirk 63 split (u)	Swab	**Positive**
G	Purple crowned lorikeet Yellow Kirk split (u)	Swab	Negative
G	Purple crowned lorikeet CAPTN 77 split (u)	Swab	Negative
F	Purple crowned lorikeet KIRK 55 (m)	Swab	Negative
F	Purple crowned lorikeet 09-02 (f)	Swab	Negative
E	Purple crowned lorikeet 08-01 black (m)	Blood	Negative
E	Purple crowned lorikeet 08-01 black (m)	Swab	Negative
E	Purple crowned lorikeet 10-36 gold (f)	Blood	Negative
E	Purple crowned lorikeet 10-36 gold (f)	Swab	Negative
D	Purple crowned lorikeet CAPTN 08 + 10-49 (f)	Swab	Negative
D	Purple crowned lorikeet 10-06 (u)	Swab	Negative
C	Red-bellied parrot Matt-10-008 (f)	Blood	Negative
C	Red-bellied parrot Matt-10-008 (f)	Swab	Negative
A	Red-bellied parrot Matt-10-005 (f)	Blood	Negative
A	Red-bellied parrot Matt-10-005 (f)	Swab	Negative
B	Senegal parrot (f)	Blood	Negative
B	Senegal parrot (f)	Swab	Negative
B	Senegal parrot (m)	Blood	Negative
B	Senegal parrot (m)	Swab	Negative

that tested positive, had bred successfully without nestling or fledgling mortality. On the 25th July 2015 this colony was removed and relocated to another premises in an effort by the owner to de-populate and remove potential adenovirus carriers. The owner was advised to remove all other purple crowned lorikeets from that bank of aviaries and house them much further away. The owner was advised to thoroughly clean the entire bank of aviaries, including those with the red-bellied parrot hens and pair of Senegal parrots, followed by at least two treatments of the disinfectant F10 1:250 dilution over 2 weeks. The owner was also advised to wait at least two months before introducing new male red-bellied parrots which were being held in pre-sale quarantine at the seller's property. In follow-up communications with the owner on the 9th November 2015 two new male red-bellied parrots had settled in well with no deaths or evidence of illness in any of the red-bellied and Senegal parrots, even with six weeks of very warm weather.

Repeat testing of suspect adenovirus carrier purple crowned lorikeets

At the time they were relocated from the owner's property the purple crowned lorikeet colony with the suspect adenovirus carriers comprised two pairs of adult purple crowned lorikeets and their eight offspring, including a recently hatched nestling. They were clinically normal. On the 20th March 2016 they were retested PCR negative for adenovirus.

Adenovirus Genome sequence analysis

Liver tissue from three affected red-bellied parrots was used to extract and analyse viral DNA using Next-Generation sequencing. This produced full length virus genomes with near identical similarity to one another with a genome architecture most closely aligned and 51.8% similar to *Fowl adenovirus C* and only 29.5% similar to *psittacine adenovirus 3*. The hexon gene had 73-82% similarity with fowl, psittacine and pigeon adenovirus hexon genes whereas the DNA polymerase gene had 62% similarity to *Fowl aviadenovirus* C DNA polymerase.

Discussion

In this paper we present an investigation of mortality caused by adenovirus infection in a mixed aviary flock of parrots and lorikeets that caused mortality only in red-bellied parrots (*Poicephalus rufiventris*). A recent survey of captive parrot populations in Victoria found a low prevalence of adenovirus infection (Hulbert et al., 2015), alongside much higher prevalence of *beak and feather disease virus* and *avian polyomavirus* infection with only one bird found to have been co-infected with BFDV and adenovirus. The survey demonstrated a very low prevalence of adenovirus infection with only four from 118 faecal samples recording a positive result, three from one aviary flock including an *Amazona* sp. a Macaw and an Eclectus. The fourth bird found positive in this survey was a budgerigar also co-infected with BFDV.

A 2003 report demonstrated adenovirus as a cause of enteritis in scaly breasted and rainbow lorikeets in a Victorian pet shop (Mackie et al., 2003). The pertinent gross lesions in affected lorikeets were dilation of the small intestine, with reddening of the mucosa and blood-stained watery contents, and enlargement and pallor of the spleen. As in the case for our disease red-bellied parrots histologically there was severe, acute, necrotising enteritis in the affected lorikeets. It was suggested that the intestinal epithelial tropism may be an indication of group I avian adenovirus infection, similar to that reported in African grey parrots (Droual et al., 1995). Group I avian adenovirus infection is often subclinical with antibodies frequently present in healthy chickens, turkeys and other avian species (McFerran et al., 1977, 2000; Mackie et al., 2003).

The significance of infection by a novel adenovirus most closely related to *Fowl adenovirus C* in psittacine birds remains unclear. However, it is evident that psittacine birds should be considered susceptible to a wide range of avian adenovirus species from different host sources (Gomez Villamandos et al., 1995; Ritchie, 1995; Schmidt et al., 2003; Katohi et al., 2010; Joseph et al., 2014). Adenovirus infections have been found in apparently healthy birds and carrier states may exist in certain species or within individually infected birds (Wellehan et al., 2005; Luschow et al., 2007) and it has been suggested that stress may precipitate the overt viral replication in the pathogenesis of necrotising lesions (Droual et al., 1995).

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