A PATHOLOGIST'S LAMENT

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INTRODUCTION

Several viruses, including the canary and finch cirocviruses, canary and finch polyomaviruses, passerid herpesvirus, finch papillomavirus, canary poxvirus, and avian paramyxoviruses 2 and 3, infect canaries and other finches and have been associated with disease (reviewed by Schmidt, 2008). These viruses have been studied to some extent in Europe and North America and lesions resembling those caused by polyomaviruses have been described in Gouldian finches in Australia (Black, 2010).

Sadly, in comparison to the often extensive work that has been done on the epizootiology of similar viruses in psittacine birds, very little is known about the biology and behaviour of these viruses in canaries and finches. Progress in this field has been stymied in large part because of the lack of available funding. Aviculturists and veterinarians around the world have donated millions of dollars to better understand viral diseases of psittacine birds, but very little has been donated to assist research into viral diseases of canaries and finches. As a result, there are few commercially available molecular-based tests available for these viruses and our understanding of the disease patterns caused by these viruses and the expected short-term and long-term outcomes of the presence of these viruses in canary or finch collections is limited.

The following cases are presented to further the process of our understanding of the viral diseases present in canaries and finches in Australia. These cases are also presented so that the clinician can better understand the difficulties associated with detecting and determining the impact that viral diseases play in these aviaries and will be better aware of the syndromes that may ultimately have a viral aetiology.

CASE REPORTS

Paramyxovirus Infection in a Canary Flock?

Samples were submitted from five birds from a canary flock spanning a period from January 2010 to February 2011.

Case 1 (January 2010): Initially a bird was submitted because the breeder had lost 30 of his 120 canaries over a few weeks with nonspecific signs including weight loss. Gross post-mortem findings included low levels of coccidia in a faecal smear and free blood was visible in the intestines. Microscopically, there was mild multifocal hepatic necrosis and mild chronic granulomatous hepatitis. In the spleen, a histiocytosis and lymphoplasmacytosis were seen. A

heavy growth of *Macrorhabdus ornithogaster* was observed, associated with crypt dilation in the proventriculus and isthmus. Abundant single-celled parasites, possibly *Hexamita* spp. or *Giardia* spp., were seen in the duodenum and coccidia were found in the jejunum. Brain and other central nervous system tissues were not submitted.

It was concluded that the *M. ornithogaster* and intestinal parasites were the most important lesions and these infections could be causing the observed mortality. An aetiology for the splenic and hepatic lesions was not determined.

Case 2 (March 2010): Additional canaries were showing signs including neurological signs. Grossly there were no visible lesions, but large numbers of *M. ornithogaster* were seen on a scraping of the proventriculus. Microscopically, perivascular extramedullary haematopoeisis and a mild multifocal perivascular cuffing of portal veins with lymphocytes, plasma cells and histiocytes were observed in the liver. Within the brain there was locally extensive regions of necrosis of glial cells. Within these regions it was common to find intracytoplasmic eosinophilic inclusions and rarely intranuclear inclusions within glial cells. Similar changes were found in the spinal cord. The inclusion bodies resembled those caused by a paramyxovirus.

Case 3 (November 2010): Mortalities from the previous outbreak had stopped six months previously. A new bird was presented with unilateral proprioceptive and motor deficits of the left leg. No gross lesions were observed. Microscopically, lesions of the digestive tract included histiocytic cuffing of the vast majority of portal tracts and focal areas of hepatocyte necrosis. Additionally there were widely scattered lymphohistiocytic foci in the submucosa of the proventriculus. Splenic lesions included an expansion in the number and size of lymphoid follicles. Widely scattered histiocytic nodules were found within the interstitium of the cortical regions of the kidney. Multiple lesions were found in the respiratory system including a moderate symmetrical increase in submucosal lymphocytes at the junction of the respiratory epithelium in the nasal pharynx, a moderate diffuse infiltration of the submucosa with lymphocytes and histiocytes within the trachea and a diffuse histiocytic air sacullitis. Axonal degeneration was observed in the cerebrum and cerebellum.

The respiratory lesions resembled those seen in poultry with mycoplasma infections and in other birds with *Chlamydia* infections. Liver and spleen lesions might have been in response to a systemic bacterial infection. The aetiology of the brain lesions was not determined.

Case 4 (March 2011): Mortality events had persisted in this flock since the last sample was submitted. Gross lesions were not observed. Digestive lesions included periportal lymphoid aggregates in the liver, karyomegaly of basal epithelial cells of the crop with chromatin margination and the presence of lightly basophilic to clear pan nuclear inclusions, multifocal pancreatic necrosis with associated lymphocytic inflammation, and multifocal to locally extensive lymphoplasmacytic enteritis, confined to the duodenum. *M. ornithogaster* was present in the isthmus with no associated lesions.

Within the spleen, there was a marked expansion of the lymphocytic and histiocytic components of the spaces between the sheathed arteries including the development of several lymphoid follicles. There was marked apoptosis occurring within these expanded populations. The occasional cell contained a central eosinophilic inclusion, others have distinct eosinophilic intracytoplasmic inclusions.

A piece of muscle adjacent to the femur showed a marked lymphoplasmacytic infiltration of the facia adjacent to the muscle and this infiltration penetrated into the muscle in a few locations. There was a linear area where the nuclei of the myocytes were moderately enlarged and contained a pan-nuclear eosinophilic inclusion.

Sections through the skull revealed multifocal to locally extensive area of muscle degeneration with lymphocytes and plasma cell infiltration. Within the submucosa of the respiratory epithelium at the junction with the choanal slit was a moderate to marked increase in lymphocytes and plasma cells, many of which were undergoing apoptosis. Brain and spinal cord lesions were not observed.

Based on these findings, it was concluded that the canary was clearly infected with a virus that was causing a chronic lymphoplasmacytic systemic response. The precise nature of the virus could not be determined histiologically. The intracytoplamsmic inclusions found in the spleen were suggestive of a paramyxovirus as was seen in Case 2. The intranuclear inclusions seen in the crop and the muscle could also be paramyxovirus inclusions, but resembled those caused by some DNA viruses including herpesviruses and polyomaviruses.

Case 5 (March 2011): Mortality events in this collection continued. No gross lesions were identified in this canary. Digestive lesions included a superficial colonization of the isthmus with *M. ornithogaster*. There were scattered heterophils within the lamina propria of the isthmus glands and widely scattered lymphoid, and at times, histiocytic, aggregates in the submucosa of the proventriculus. There were many scattered small periportal lymphoid cuffs in the liver.

Changes in the immune system included enlargement of the spleen as the result of an increase in the number of histiocytes and plasma cells. There was a marked increase in the number of cells undergoing necrosis or apotosis, leaving numerous cell-sized spaces diffusely within the spleen. In the bursa there was marked apoptosis of the medullae of the bursa follicles to the point that medullary lymphocytes were absent. Within the bursa were rare lymphocytes that contained eosinophilic intranuclear inclusions. There was also a locally extensive histiocytic and lymphoplasmacytic infiltration of the subcutaneous tissue adjacent to the bursa.

The lesions in this bird, though more subtle than those seen in the Case 4 canary, were consistent with a virus infection that stimulated a lymphoplasmacytic response and targeted immune cells. The *M. ornithogaster* was considered an incidental finding.

Viral enteritis in a long-tailed and Gouldian finch?

Both birds originated from a flock of Gouldian and long-tailed grass finches. The Gouldians were breeding but the chicks, when fledged, lost weight, had diarrhoea and died. Only one grass finch became sick, and this was a young adult with similar signs of weight loss and diarrhoea. This bird and a Gouldian chick were submitted for necropsy.

In both birds, the bulk of the histologically significant lesions were confined to the gastrointestinal tract. Lesions of the duodenum and proximal jejunum included a marked diffuse hyperplasia of the mucosal glands. There was also a marked transformation of the intestinal epithelial cells resulting in a 10- to 20-fold increase in the number of goblet cells. The goblet cells themselves considerably larger than normal and there was blunting and fusion of the villi. There was a locally extensive to diffuse lymphoplasmacytic, and to a lesser extent, heterophilic infiltration, within the

submucosa and interstitium. Cryptosporidia were found in scattered glands within the proventriculus, in some glands they were abundant. At the isthmus of the proventriculus, there was a locally extensive erosion of the mucosa with a marked lymphoplasmacytic, histiocytic and heterophilic response and associated hyperplasia of the associated proventricular glands. A diagnosis of a severe diffuse chronic active lymphoplasmacytic enteritis and an locally extensive chronic ulcerative proventriculitis and cryptosporidiosis of the proventriculus was made.

The intestinal changes in these birds clearly explained the clinical signs of diarrhoea and weight loss. The intestinal changes were marked, and were consistent with severe damage to the villi with regeneration, albeit not to a normal villous structure. This change seen in these birds was consistent with that caused by a virus.

What little we know about enteric virus infections in birds comes from studies in poultry, particularly turkeys. In turkeys cornonaviruses, rotaviruses, astroviruses, entro-like viruses, and toroviruses have been found to cause diarrhoeal diseases and corresponding histological changes. The lesions seen in these finches most resemble those described for astroviruses (reviewed by Reynolds and Schultz-Cherry, 2008) and entro-like viruses in turkeys (reviewed by Guy et al., 2008).

Polyomavirus infection in canaries and Gouldian finches?

History: Died at one day of age. This was the first clutch of Gouldian finches, but all canary chicks had died this year at a young age. In the dead chicks, a black spot could be seen through the skin.

Birds originated from an aviary where there had been high mortality in recently hatched canary chicks, some as young as one day of age, and now a similar mortality was occurring in Gouldian finch chicks. Two Gouldian chicks were submitted, one month apart. Histological sections generated by serial sections through the entire bird were examined. In the first chick, which was reportedly one day of age, there was no evidence of an infectious disease.

The age of the second chick was not known, but it was considerably larger than the first one but had not yet developed feathers. Microscopic lesions suggesting a polyomavirus infection were found in the liver of this chick. These included widely scattered hepatocytes that exhibited karyomegaly, chromatin margination and contained clear to lightly basophilic pan nuclear inclusions. Other significant lesions were found in the digestive tract and immune tissues. Digestive lesions included a heavy overgrowth of bacteria on the mucosa of the oesophagus, an overgrowth of spiral bacteria in the oesophageal glands, and mild overgrowth of long filamentous bacteria on the mucosal surface of the duodenum. The spleen and the bursa were severely hypoplastic.

DISCUSSION

The findings from each of these three disease outbreaks provide a number of take-home messages and raise many questions that require further focussed research before they can be answered. The first take home message is that unless a complete set of tissues and, in some cases, entire bodies, are examined, critical lesions will be missed. Secondly, lesions will vary significantly between birds, even when infected with the same agents, and it may be necessary to examine multiple birds from the same collection before a full picture of the disease processes present in the flock can be identified. Thirdly, it is likely that multiple pathogens will be found in most canary

and finch collections and it often challenging to determine which one is the most significant.

These examples demonstrate that there is a critical need to better understand the viral diseases of canaries and finches for the benefit of our clients and to understand what diseases are present in Australia and what impact they might have on other species, including our native species. So much basic information needs to be determined. We need to know how to detect infections in live birds, how long virus shedding is occurring, if viral diseases such as the polyomaviruses can kill day old chicks, and if infections with finch and canary circoviruses might be underlying some of these disease outbreaks.

What then can the avian practitioner do? When possible when suspected viral diseases are detected on histopathology, practitioners should encourage clients to pay for additional testing such as electron microscopy, immunohistochemistry, and virus isolation attempts. In the end, however, the most important element of control for these diseases will be a better understanding of them. This can only be done if funds are provided for new research into these diseases. Therefore, practitioners should encourage their clients and local bird clubs to raise funds for research so we can all have a better understanding of the important diseases that are impacting their animals.

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