

Evaluation of Histopathologic Findings in Spix's Macaws (*Cyanopsitta spixii*) with Emphasis on Glomerulosclerosis

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Abstract: The histopathologic findings of 12 Spix's macaws (*Cyanopsitta spixii*) that have died at the Al Wabra Wildlife Preservation were evaluated in a retrospective study. In addition to proventricular dilatation disease (PDD) and its associated findings, the predominant lesions included nephropathy (100%) characterized by glomerulosclerosis (91.7%) and/or hyaline tubular degeneration (83.3%), pathologic alterations in the liver (91.7%), pneumoconiosis (58.3%), hemochromatosis (41.7%), and myocardial degeneration (33.3%). The incidence of glomerulosclerosis, confirmed by special staining, was observed to be independent of age, sex, origin of the birds, incidence of PDD, or exposure to avian polyomavirus and paramyxovirus. Diet and genetics remain as potential predisposing factors to this condition in the Spix's macaw.

Retrospective Study

Introduction

The critically endangered Spix's macaw (*Cyanopsitta spixii*) is presumed to be extinct in the wild (2008 IUCN Red List of Threatened Species. Available at: www.iucnredlist.org. Accessed February 24, 2009). As of February 2009, out of a captive population of 63 Spix's macaws that are part of an international recovery effort, 47 birds, representing almost 75% of the extant population, are kept at Al Wabra Wildlife Preservation (AWWP), Qatar. The significant threat posed by proventricular dilatation disease (PDD) to the captive breeding of the Spix's macaw at AWWP has been well documented.^{1,2} However, to the authors' knowledge, no information has been published regarding other significant pathological findings in this species. A micro-satellite DNA analysis of the captive population in 2007 revealed that every Spix's macaw is very closely related and this is strongly supported by the known pedigree, which traces a majority of the birds back to a single founder.

Since the year 2000, when the Spix's macaw first arrived at AWWP, 12 birds, representing approximately 15% of the extant population, have died. Being the priority species at AWWP, intensive efforts have been made to investigate and establish the cause of death in these birds. The purpose of this study is to report the significant histological findings with a focus on renal disease, particularly glomerulosclerosis, which appears to be a relatively common occurrence in the deceased Spix's macaws. It is hoped that the collective data reported here, along with a brief historic review of the birds, will provide both an insight into the main pathologic processes and a basis for comparison and management of similar findings in other facilities involved in the recovery efforts for the species.

Data/Methods

A retrospective evaluation of histopathologic findings of 12 Spix's macaws that have died at AWWP between 2003 and 2008 was carried out. The sample included 8 males and 4 females, and age at the time of death ranged from 41 months (3.5 years) to 216 months (18 years). The source of the birds included 7 birds from the Philippines, 4 from Switzerland, and 1 hatched and hand-reared at AWWP.

During the necropsy examination of the birds, tissue samples from the crop, proventriculus, ventriculus, small and large intestine, liver, spleen, heart, kidney, lungs, pancreas, skeletal muscle, brain, gonads, thyroid, and adrenal gland were routinely collected and stored in 10% buffered formalin solution. Other tissues, for example skin, were collected only when gross pathologic changes indicated it. Histopathologic examination was carried out on hematoxylin-eosin stained sections of paraffin-embedded tissue by the at least 2, and in most cases 3, pathologists (H.G., G.M.D., and V.S.). Where appropriate, kidney sections were stained with periodic acid-Schiff (PAS) reagent, for detecting glycoprotein, van Gieson method or Heidenhain's azan trichrome (AZAN) for connective tissue, and Congo red, for amyloid.

Results

Figure 1 provides an overview of the incidence of lesions in the 12 dead Spix's macaws at AWWP. The results of the examination revealed abnormal findings in the kidney of 100% (12/12) birds, with varying degrees of glomerulosclerosis recorded in 91.7% (11/12) birds. Histologically the lesions were characterized by protein deposition in the mesangium and thickening, followed by replacement, of the mesangium by connective tissue. The presence of connective tissue was confirmed with specific staining in 5 of the Spix's macaws with glomerulosclerosis (Fig 2). In severe cases, several shrunken glomeruli were observed with adhesions of the parietal membrane. In addition, tubulonephrosis was also observed, either concurrently or independently, in 83.3% (10/12) of the birds with hyaline degeneration being the predominant finding.

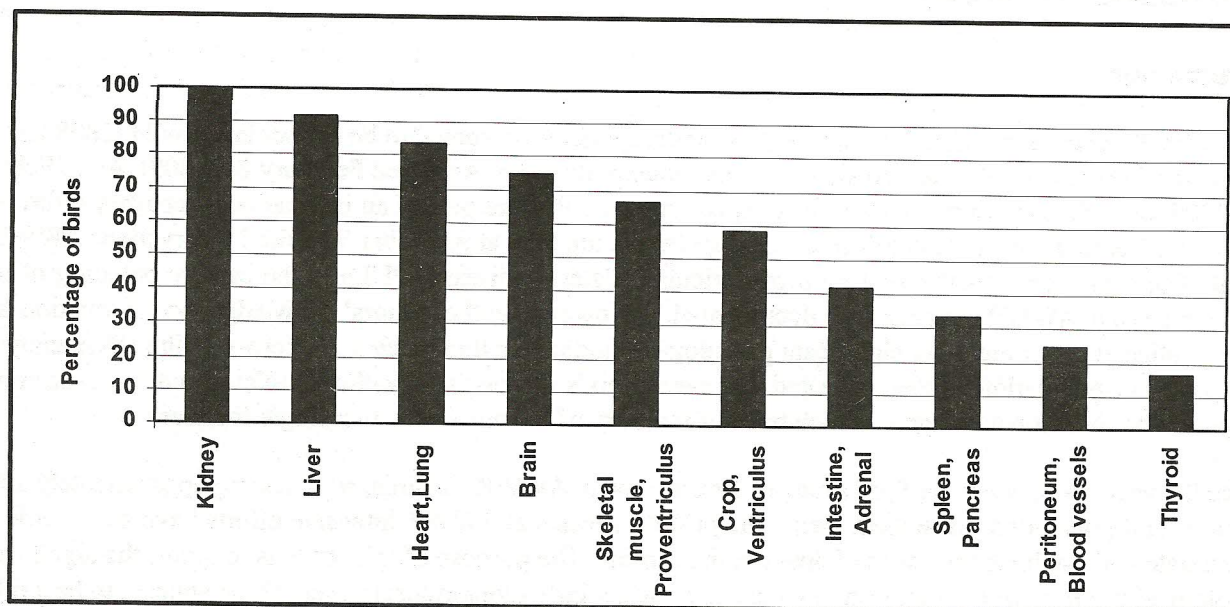


Figure 1. Comparative incidence of histopathologic lesions reported in different organs of 12 Spix's macaws at AWWP.

The incidence of pathologic alteration in the liver was found in 91.7% (11/12) of the birds with changes ranging from hyperemia, hypo- or hypertrophy, and degeneration in most cases to a lymphoplasmacytic infiltration in 1 case. Hepatic iron storage, or hemochromatosis, was reported in 5 birds.

Similarly, 83.3% (10/12) of the birds had lesions in the heart including cardiac myodegeneration in 4, lymphoplasmacytic infiltration in 3, and purulent epicarditis in 1 bird. The same percentage of birds had lung changes; 2 with signs of aspiration of food, 1 with mycotic pneumonia, and 7 with pneumoconiosis or anthracosis. An incidental finding of foci of bone formation in lung tissue was seen in 2 cases, possibly as a result of a developmental disorder.

Lesions in the cerebrum or cerebellum were recorded in 75% (9/12) of the individuals. In 4 cases, perivascular and perineural lymphoplasmacytic infiltration was recorded. Meningeal edema, dilatation of Virchow-Robin's space, and perineural gliosis were some of the other non-specific findings in the brain. Skeletal muscles were degenerated in 66.7% (8/12) of the birds.

The crop and ventriculus in 58.3% (7/12), and proventriculus in 66.7% (8/12), of the birds had lesions with a majority of the changes including varying degrees of non-purulent ganglionitis and lymphoplasmacytic infiltration of the mucosa and glands. In 1 case (stocklist number 5158), the ventriculus had hemorrhages in the lumen accompanied by a necrosis of the mucosa and fibrinous inflammation of the serosa. The small and large intestine, combined, were affected in 41.7% (5/12) of the birds, with 4 of them exhibiting non-purulent ganglionitis.

Further, lymphoplasmacytic ganglionitis was noted in 41.7% (5/12) of the adrenal tissues examined. In contrast, only 33.3% (4/12) of the birds exhibited lymphoid depletion in the spleen. The same percentage had a degeneration of the exocrine pancreatic cells.

Signs of peritonitis were recorded in 25% (3/12) with a non-purulent ganglionitis in 1 peritoneal ganglia being the only indication of a possible PDD infection in another bird (stocklist number 4335). Abnormalities of the large blood vessels with either non-purulent ganglionitis (2 cases) or atherosclerosis (1 case) was seen in 25% of the birds and thyroid hyperplasia was recorded in 16.7% (2/12). No significant pathologic changes were observed on examination of the gonads of the birds. The conclusions from the cumulative histopathologic findings for the birds (Table 1) demonstrate that, in addition to PDD, glomerulopathy is the most significant pathologic process in the deceased Spix's macaws at AWWP.

Table 1. History and histopathologic conclusions of 12 deceased Spix's macaws at AWWP.

Stocklist number	Sex	Age (months)	Clinical history	Histopathologic conclusion
3255	Male	61	Sudden death, found lying on its back in the cage.	Heart - and circulation failure (toxic cause?), nephropathy.
4182	Female	107	Treated for over 2 months for dyspnea and central nervous signs.	PDD, chronic glomerulonephritis
4183	Male	130	Sudden death one month after treatment for mild illness	Acute heart failure with pulmonary hemorrhage, renal mineralization, glomerulopathy, tubulonephrosis
4270	Male	138	Exhibited signs of dyspnea and passage of undigested seeds in feces	Acute shock and atherosclerosis, chronic glomerulonephritis and tubulonephrosis.
4334	Female	216	Treated for 1 week prior to death for inappetance and passage of undigested seeds in feces.	PDD with aspiration of food and severe glomerulosclerosis..
4335	Male	84	High uric acid values in blood, polydypsia and feather plucking reported.	Fungal pneumonia, purulent necrotizing dermatitis and septicemia, nephropathy. Non-purulent ganglionitis in 1 peritoneal ganglion – possible PDD.
4338	Male	180	Partner died of PDD, treated for weight loss and auto-mutilation for over a year	PDD in multiple organs and severe glomerulosclerosis.
4361	Male	108	Unilateral limb paralysis and in coordination for 3 months before it was euthanized.	PDD and underlying glomerulosclerosis.
4368	Female	58	Treated for anorexia and progressive emaciation prior to death	Heart - and circulation failure with underlying PDD, nephropathy.
4378	Male	141	PDD positive on crop biopsy, developed central nervous signs prior to death	PDD, hyperplastic goiter, nephropathy with mineralization.
4380	Male	134	Euthanized due to progressive central nervous signs	PDD and glomerulonephritis.
5158	Female	41	Small wound over left eye reported 2 weeks prior to death. Developed complete anorexia with regurgitation 3 days before death.	Subacute fibrinous serositis caused by proventricular perforation, hepatic necrosis, glomerulosclerosis.

Discussion

The histopathologic examination revealed a high incidence of non-purulent ganglionitis in the digestive tract of the dead Spix's macaws. A non-purulent ganglionitis, characterized by lymphoplasmacytic infiltration of the nerves of the myenteric plexus on histologic examination, is considered pathognomonic for PDD.¹⁻³ PDD appears to be responsible for 66% of the mortalities, being the primary cause in 7 cases and a contributing factor in another (Table 1). Notably, in addition to digestive organs, lesions associated with PDD were recorded in adrenals, heart, brain, large blood vessels (in nerves and ganglia of the wall), and even in peritoneal ganglia of affected birds.

The highest incidence of lesions was recorded in the liver, although in most cases the changes were non-specific inflammatory ones. Hemosiderosis or excessive iron storage in the hepatocytes without visible pathologic changes, was detected in 5 birds, of which 3 died of confirmed PDD. The increased gastrointestinal transit time in birds suffering from PDD, allowing increased uptake of dietary iron, has been presumed to be the basis for the association between these 2 conditions.⁴ However, hemosiderosis was seen in 1 Spix's macaw (stocklist number 3255) without PDD. Hemorrhage and circulatory disturbances were recorded in the lung and brain of this bird that possibly lead to an excessive RBC destruction—also reported as an underlying cause of hemochromatosis.⁵

Cardiac myodegeneration was described in 30% of the deceased Spix's macaws. Usually a postmortem finding, the possible differentials for this condition include infections, bacterial toxins, intoxications (lead etc), and deficiency of vitamin E and selenium, although in many cases the etiology has not been established.^{5 (p9)} A deficiency of vitamin E and selenium, especially in the chronically ill and emaciated birds, appears to be a plausible explanation, corroborated by the skeletal muscle degeneration reported in 66.7% of the birds. However, confirmation or rejection of this hypothesis requires further investigation of blood levels of these nutrients.

Ectopic bone is reported to be a common finding in the interstitium of lungs of birds. There is no evidence for a disease etiology for the formation of ectopic bone in the lungs.⁶

The terms pneumoconiosis, or anthrasilicosis, has been used to describe the finding of black-brown refractile pigment in macrophages of the parabronchial septae of the lungs in birds. The findings often suggest exposure to airborne pollutants in birds.^{5(p34)} The incidence appears to be independent of age, with even the juvenile bird, hand-reared at AWWP, showing lesions. A combination of exposure to the excessively polluted environment in Manila and the silica-abundant desert of Qatar could be responsible for the high incidence of these lesions.

Renal disease was a universal finding associated with mortality in the Spix's macaw at AWWP. Eleven out of 12 birds had end-stage renal disease characterized by glomerulosclerosis, described as a thickening and scarring of the glomeruli. Almost no data was encountered in literature specifically about glomerulosclerosis in psittacine birds, and even in humans, it is often idiopathic with specific etiologies established in only 20% of cases.⁷ Diabetes mellitus, hyperlipidemia, and atherosclerosis have been associated with glomerulosclerosis in mammals, along with a genetic predisposition, notably in captive cheetahs (*Acinonyx jubatus*). However, only avian polyomavirus- (APV-) associated PAS-positive membranous glomerulopathy has been documented in psittacine birds,⁸ although any process causing prolonged formation of antigen antibody complexes can presumably lead to this finding. Severity and incidence of the lesions in the Spix's macaw appeared to have no correlation with sex (7 males, 4 females), origin, or age of the birds. In fact, severe multifocal thickening of the basement membrane and capillary wall was observed histologically in the youngest bird (stocklist 5158) that was hand-reared at AWWP which, until 2 weeks before death, had no history of illness or virus exposure. When the status of the birds for APV antibodies was examined, 7 out of 10 birds with glomerulosclerosis had always tested negative, with the negative PAS staining further ruling out a typical membranous glomerulopathy in *C. spixii*. In addition, no convincing correlation was observed to PDD or avian paramyxovirus (APMV) antibody status, precluding a viral basis to the condition.

Dietary factors, especially excess protein, cholesterol, vitamin A, and vitamin D, can cause renal disease in birds.⁹ Although blood levels of vitamin A and D are not routinely measured in the Spix's macaw, no other lesions suggesting toxicity of these elements have been observed consistently. Blood uric acid levels, being the predominant end product of protein metabolism, were examined and found to be within the range specified for the species¹⁰ in 4 of 5 birds, with measurements done within a month prior to death. Similarly, total cholesterol levels in blood were lower or normal in all birds as compared with the reference range. Although the role of dietary factors cannot be completely excluded at this point, the combined nature of the glomerular and tubular lesions along with the severely restricted gene pool that the captive breeding program relies on, suggests that a disorder of protein metabolism, with a familial or idiopathic predisposition, possibly underlies the widespread and indiscriminate incidence of glomerulosclerosis in the Spix's macaw at AWWP.

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