HISTOPATHOLOGY IN AN AVIAN AND EXOTIC PET VETERINARY CLINIC

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PART I

AVIAN HISTOPATHOLOGY

At the Bird and Exotics Veterinarian, clients are offered histology in conjunction with surgery and other tests (including cytology, blood tests and diagnostic imaging), as part of an overall work up of patients. Samples collected are fixed with formalin and forwarded to an external laboratory. Samples may also be obtained after euthanasia or natural death to provide answers for closure, further treatments of other flock members, or human health and safety.

70% of all patients at this clinic are avian, 2.3% belonging to an owner that considers themselves a breeder.

The data in this report were collected to determine what prompted the use of histology in a clinical setting. Similar articles exist in the literature but are focused on specific sample types (eg neoplasia) and are from the perspective of a diagnostic laboratory (Reece, 1992; Garner, 2006). A case report is included (Part II) to highlight the use of histology as part of a diagnostic work up.

RESULTS

Histology results were collated for six years from 234 samples. Of these 152 were avian, 46 mammalian, 30 fish, five reptile, and one amphibian. Results from avian samples only will be discussed. Sample types are categorised based on clinical signs in order to present data from a clinical perspective.

Discrete lumps were the most common sample type forwarded for histopathology, representing 48.0% of all samples. Table 1 shows the different species represented at this clinic that had lumps sampled. Lumps observed or palpated anywhere on the body were grouped together.

Table 1. Histopathologic results for palpable lumps (152 samples) grouped by species.

Species	Number (% of birds with lesions)	Result (number of cases if > 1)
African Lovebird, *Agapornis spp.*	1 (1.4%)	normal cutaneous and subcutaneous tissue
Alexandrine Parrot, *Psittacula eupatria*	1 (1.4%)	dermatitis
Budgerigar, *Melopsitticus undulatus*	19 (26.0%)	myxofibrosarcoma, dermatitis, squamous cell carcinoma (5), keratogenous cyst, ovarian adenocarcinoma, xanthoma (2), possible retroviral pseudo-tumour, sarcoma, lipoma, feather follicle cyst, neuroendocrine or gonadal tumour aberrantly located, haemangiosarcoma, seminoma.
Canary, *Serinus canaria*	2 (2.7%)	Cystic and fibrogranulomatous changes, feather follicle cyst
Cockatiel, *Nymphicus hollandicus*	16 (21.9%)	Stomatitis, squamous cell carcinoma (5), aberrant respiratory tissue, feather follicle cyst (2), spindle cell tumour, hepatic cyst, xanthoma, Shell gland trauma, haemangioma, myxosarcoma, limpoma
Duck, *Anas platyrhynchos domesticus*	*2 (2.7%)*	*Inflammation, cystoadenocarcinoma*
Galah*, Cacatua roseicapilla*	17 (23.3%)	granular cell tumour, xanthoma (5), bacterial dermatitis, lipoma (6), myxofibrosarcoma, cellulitis, myelolipoma, hepatic lipidosis
Indian Ringneck, *Psittacula krameri*	1 (1.4%)	round cell tumour
Pigeon, *Columbus spp.*	3 (4.1%)	xanthoma, hematoma, sarcoma
Princess Parrot, *Polytelis alexandrae*	2 (2.4%)	lipoma, squamous cell carcinoma
Red Collared Lorikeet, *Trichoglossus haematodus*	1 (1.4%)	cutaneous ulcer
Red Tailed Black Cockatoo, *Calyptorhynchus banksii*	1 (1.4%)	cloacal sarcoma
Red Winged Parrot, *Aprosmictus erythropterus*	1 (1.4%)	lipoma
Regent Parrot, *Polytelis anthopeplus*	1 (1.4%)	feather follicle cyst
Sulphur Crested Cockatoo, *Cacatua galerita*	5 (6.8%)	fibrogranulomatous cyst, myelolipoma, haemangioma, lipoma (2)

Obtaining the samples in Table 1 involved using general anaesthesia in all cases - there were only three fatalities during these procedures. Of the birds that did not survive, there was a seminoma in a budgerigar, a squamous cell carcinoma in a budgerigar and a lipoma in a Red Winged parrot.

Samples from deceased animals included 34 cases, 22.4% of all histology cases). Death during a surgical procedure to obtain samples occurred in 5 birds, including the three cases that died during sampling or removal of a lump. The two remaining cases were adenocarcinomas in a sulphur crested cockatoo and a cockatiel. Despite receiving medical treatment, 10 patients died prior to sampling, and 14 died at home. Only five birds were euthanased to obtain samples, three of these were euthanased in order to provide answers to deaths within aviaries, and two were euthanased based on humane grounds and samples taken to confirm the cause of disease. In the latter two birds an intracranial arthropod was identified in a Gouldian finch (*Erythrura gouldiae* - Peacock and Reece, 2010), and evidence of a disseminated viral infection in an Eclectus.

Cutaneous biopsies were collected for 18 feather-picking and self trauma cases (11.8% of all histology cases) and revealed in 14 cases evidence of secondary changes from self trauma. Four cases had specific lesions - xanthoma in a feather-picking cockatiel, a keratocanthoma in a self mutilating cockatiel, a sarcoma in a sulphur crested cockatoo and a fibrosarcoma in a self mutilating long-billed corella (*Cacatua tenuirostris)*.

Results of nine conjunctival samples (5.9% of all histology cases) showed secondary conjunctivitis in a galah and a black-capped lori (*Lorius lory lory).* Other results included conjunctivitis consistent with chlamydophila in a budgerigar, conjunctivitis secondary to foreign matter or bacteria in an eclectus (*Eclectus roratus),* two cases of lymphoplasmacytic conjunctivitis in cockatiels, a squamous cell carcinoma in an African lovebird, a suppurative conjunctivitis in a rainbow lorikeet (*Trichoglossus haematodus),* and a conjunctival polyp in a budgerigar (Rosenwax et al., 2006).

There were six cases of coelomic swellings (4.0% of all histology cases), with adenocarcinoma being diagnosed in five (two cockatiels and three chickens (*Gallus gallus*)), and cystic uterine dysplasia in one budgerigar.

Hepatic problems were investigated in six cases (3.9% of all histology cases) and revealed iron accumulation in a sulphur crested cockatoo (sampled twice), fibrotic hepatopathy in a galah, cholangiohepatopathy in a cockatiel, hepatic lipidosis in a hybrid olive red-collared lorikeet, and no abnormalities in a cockatiel.

Prolapsed tissue protruding through the cloaca was sampled in five cases (3.3% of all histology cases). Normal tissue was sampled in a budgerigar and a goose (*Anser anser)*, a lymphoid tumour in a chicken*,* a cloacal carcinoma in a Budgerigar, and a lymphoplasmacytic, eosinophilic and heterophilic cloacitis in a sulphur crested cockatoo.

The remaining four cases included a polypoid adenoma in the oral cavity of a galah, a viral dermatitis in a sulphur crested cockatoo, a polyfeather and cutaneous polyp in a cockatiel, and a non-viral papilomatous projection in the cloaca of a blue-fronted Amazon parrot (*Amazona aestiva*).

DISCUSSION

These results gave an indication of the use of histopathology in a mixed avian and exotic pet veterinary clinic. Most samples were taken from visible lesions or lumps. This may simply be an indicator of the types of problems owners noticed, as they were generally easily located external lesions. The number of samples taken may also be biased by an owner's willingness to sample visible lesions with the impression of reduced invasiveness and risk compared with internal surgery.

The low number of sacrificial euthanasia cases may be due to the bias towards individual pet bird owners rather than breeders and owners of aviary flocks.

The broad range of results in each type of sample category also gives justification to the use of histopathology as a diagnostic tool in a clinical setting.

PART II

CASE STUDY

A 41 year old pet male sulphur crested cockatoo presented after sudden weakness and died shortly after arrival. The work up involved in this case is a good example of the important role histopathology..

This bird was a regular client at the clinic for seven years, and during this time had been treated for a number of minor health concerns, including hormonal behavioural concerns and intermittent feather picking. The bird was kept indoors, isolated from other birds. Seeds, mixed vegetables, occasional Australian native eucalyptus and bottlebrush branches, some mixed lawn grass, and processed human food was the normal diet for this bird.

Biochemical analysis (IDEXX Sydney) from three years previously revealed an elevated cholesterol 12.5 mmol/L (normal 3.5-7.3 mmol/L). Radiographs at this time had also showed an enlarged hepatic silhouette. Within two years, lumps suspected to be fatty deposits, were palpated in the caudal coelomic cavity. Cutaneous biopsies revealed no obvious cause for the picking. The intermittent feather picking seemed to respond for short periods to treatment with Clavulox (Pfizer) palatable drops, 100mg/kg BID PO but never resolved completely. Throughout all treatments advice regarding appropriate diet and behaviour modification was given.

On final presentation the bird was severely dyspnoeic, weak and was vomiting clear fluid. The faeces were very dark green and well formed with yellow urates. Based on palpation, body condition was determined as markedly overweight. Overall feather quality was good and suggestive of minimal recent feather picking.

Initial differentials for this case included severe hepatopathology (hepatic lipidosis was considered to be likely in this bird), heavy metal toxicity and cardiovascular disease. Consideration was given to the thought that the dyspnoea could have been metabolic, or from a tracheal obstruction (such as aspirated vomitus, food, or an intraluminal granuloma). The clinical presentation gave an impression of being an acute disease process rather than an ongoing, chronic condition.

Blood samples were immediately taken for biochemistry and blood lead levels without incident and 25ml Hartmann's solution was given subcutaneously (equivalent to 3% body weight/kg). Supplementary oxygen was given by mask during treatments and sampling, afterwards the bird was placed in an oxygen box. Blood lead levels were 4.3ug/dL (>10ug/dL is considered to be lead toxicity on a LeadCare II blood lead analyser (ESA Biosciences, Chelmsford, Massachusetts)).

Sanguinous fluid was noted within the glottis after oxygen supplementation and the bird died within thirty minutes of this observation.

The owner recollected that the bird's behaviour had been subdued for five days preceding death. The

owner also then revealed that she had recently been away for two weeks, during which time the bird had potentially been exposed to aerosolised methamphetamine.

Recent exposure to potentially high levels of natural gas from a kitchen stove in a poorly ventilated room had also occurred.

POST-MORTEM RESULTS

Abnormalities in the gastrointestinal tract, liver and respiratory system were revealed in the post-mortem.

Large fatty deposits were adhering to the gizzard and cloaca. Gizzard contents included glass and plastic beads, costume diamantes, small quartz-like pebbles/grit and seed. Some parts of the intestines had a "ropey" appearance and were filled with dehydrated intestinal matter.

The liver was markedly enlarged, pale with discrete dark areas, and an irregular surface.

The lungs were bilaterally congested, and the immediately adjacent respiratory membranes were thickened. The tracheal mucosa was erythematous but no obstruction was found. It was noted that there was only minimal blood clotting throughout the post-mortem .

Biochemistry (collected in lithium heparin and analysed at IDEXX Sydney) revealed an elevated blood glucose 42.6 mmol/L (normal 7.4-22.7 mmol/L), elevated cholesterol 15.2 mmol/L (normal 3.5-7.3 mmol/L), elevated GLDH 111 IU/L (normal 1-4 IU/L), elevated bile acids 215 µmol/L (normal 0-81 µmol/L). All other results were within normal levels.

Samples for histopathology included liver, kidney, spleen, heart, lungs, trachea, testis, adrenal gland, small intestine, gizzard and pancreas. Hepatic lipidosis and cryptococcal pneumonia were both evident histologically.

DISCUSSION

Testing for methamphetamine exposure was considered in this case. Tests available, however, are not considered to be sufficiently sensitive to detect very low levels of methamphetamine especially given that at least six days had lapsed since potential exposure. The terminal half-life of methamphetamine can range from 5-31 hours in people (Schep et al., 2010). Japanese Quail (*Coturnix coturnix japonica*), that have been given methamphetamine 12-15mg/kg intramuscularly appear physically normal within two hours (Yamazaki et al., 2004).

Clinical signs of methamphetamine toxicity have not been recorded in birds. In humans, signs of overdose include, but are not limited to, tachycardia, hypertension, tachypnoea, gastrointestinal upset, mydriasis, hyperthermia and hyperreflexia. Myocardial ischaemia or infarction, cardiomyopathy, lung injury and hepatic injury can also occur (Westover et al., 2008; Schep et al., 2010). Cardiovascular effects are often observed within 5 to 10 minutes of exposure (Cruickshank and Dyer, 2009).

Cryptococcal infections localised to the upper respiratory tract have been recorded in Australia and have been considered to be primary infections based on clinical presentation of affected birds (Doneley, 1994; Malik et al., 2003). It has been suggested that pulmonary and disseminated

cryptococcosus may not be acting as a primary pathogen. Pulmonary cryptococcosis has also been considered to be an extension of a sinus infection (Schmidt et al., 2003). Histology of upper respiratory tissues was not done in this case, however there was no obvious nasal or choanal discharge, and no obvious upper respiratory tract obstructions. Pulmonary cryptococcosis has been recorded previously in a sulphur crested cockatoo (Welle, 2002) although clinical presentation was more subtle and did not include overt dyspnoea and long term treatments were able to be administered.

Cryptococcus spp. have been associated with plants including eucalyptus and gum species, as well as avian guano (Ellis and Pfeiffer, 1992; Krockenberger, 2002; Ergin et al., 2004; Xue, et al., 2007; Costa, 2009; Hagen, 2010).

CONCLUSION

Cause of death was assumed to be respiratory failure due to cryptococcal pneumonia, an unexpected and uncommon finding. A combination of evidence from the bird's history, blood tests, post-mortem and histopathological analysis provided an overall picture of factors that might have led to the bird's death.

A diet chronically high in energy resulting in hepatic lipidosis is likely to have led to the diabetic state and poor clotting. Immunosuppression resulting from hepatopathology and diabetes were contributing factors to the bird's pneumonia, although a primary cryptococcal infection cannot be ruled out. Recent inhalation of gas and exposure to low levels of lead may also have contributed to some local and systemic immunosuppression in this case.

The source of the infection was not identified. Eucalyptus branches were provided occasionally for this bird as browse and may have been a source of the infection. Cryptococcus spp. is zoonotic and the owner was advised to warn all people that had direct contact with the bird or it's faeces.

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