Cystadenocarcinomas of airsac origin in four cockatoos*

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Introduction

Primary respiratory tract neoplasia is uncommon in companion birds or at least there are insufficient reports of cases to make judgements on their risk factors or biological behaviour (Latimer 1994, Campell 1986, Schmidt 2003)[1-3]. In Moluccan cockatoos (*Cacatua moluccensis*) a primary mucinous adenocarcinoma within the right humerus has been reported in a 13-year-old male[4] and a cystadenocarcinoma protruding from the right axilla and also involving the humerus has also been described[5]. A primary bronchial carcinoma with haematogenous metastasis to the right humerus has been reported in an African grey parrot (*Psittacus erithacus*)[6] and, as in other animal species, metastatic spread of carcinomas to the lung is possible in birds but is probably even rarer than primary respiratory neoplasia[7-9]. In this paper we describe the clinicpathological, radiographic and histological features of 4 cases of air sac cystadenocarcinoma that presented as primary causes of respiratory distress in 3 galahs (*Eolophus roseicapilla*) and a sulphur crested cockatoo (*Cacatua galerita*). The pathogenesis and diagnosis of these lesions are discussed.

Case 1

An 18 year-old male galah was presented with a history of severe dyspnoea that was reported to have developed over several days. Clinical examination and radiography demonstrated a relatively large soft, tumour extending caudally and ventrally from the left axilla. Otherwise the bird was in good body condition and had no other clinical abnormalities. A fine needle aspirate was taken from the mass and yielded 4 mL of serosanguineus, turbid fluid with a PCV of 6 L/L and a protein concentration of 45 g/L. Cytology demonstrated large numbers of vacuolated macrophages with erythrophagia and a few heterophils consistent with a haemorrhagic effusion. The bird was anaesthetized with isoflurane in oxygen and a semi-pedunculated, botryoid, fluid-filled, cystic mass extending from the left axilla was biopsied and debulked for histological examination. The dyspnoea resolved immediately after the surgery but gradually recurred over the course of 4 weeks postoperatively when the bird was reexamined and the tumour was found to have recurred. At this time the bird was euthanased but a necropsy examination was declined.

Histological examination of the biopsied tissue demonstrated a proliferation of simple cuboidal to squamous epithelium supported by a loose and in places thin (<10 μm) fibrovascular network. In other areas there were patches of haemorrhage and or aggregations of foamy lipid-filled macrophages and occasional giant cells with acicular cholesterol-like clefts.

Case 2

Biopsies from a mass extending from the left axilla beneath the wing of an adult female galah of uncertain age were submitted for histopathological examination. The bird had a similar history and primary presentation to Case 1 above and histologically the tumour was composed of dilated, fluid and air-filled sacs with a thin connective tissue support. There were also focal areas of haemorrhage, with haemosiderin-laden macrophages and occasional giant cells forming granulomas. The main proliferative cell type were plump cuboidal cells which in some areas were exfoliating and in other areas were attenuating to squamous. The bird was lost to further investigation because the owner declined any further investigations or necropsy examination several months later when it was learnt that the bird had died after developing respiratory distress.

Case 3

A 23 year-old male sulphur crested cockatoo was presented because the owner had noticed a change in its voice. The bird was in good body condition and weighed 790g and had an end-expiratory wheeze. Clinical examination detected a mass surrounding the left humerus and a fine needle aspirate of this tissue yielded a very low cell harvest with the majority of cells present being adipocytes. Accordingly, a tentative diagnosis of lipoma was made and the bird was taken home on a revised diet but re-presented 2 days later due to severe respiratory distress and the owner authorized general anaesthesia for tracheal endoscopy, thoracic radiography and surgical biopsy. The bird was anaesthetized with isoflurane and oxygen and lateral and ventrodorsal radiographs demonstrated a large homogenous mass over the proximal left humerus and extending down the thoracic wall. The left humerus had an increased radiodensity and a stippled pattern. Endoscopic examination of the trachea failed to demonstrate significant lesions or obstructions. A biopsy of the tumour in the left axilla was performed by making an incision over the medial aspect of the humerus. An impression smear of this tissue demonstrated abundant red cells, occasional epithelial cells and heterophils but no microbial agents. Biopsies of the tumour were fixed in formalin and submitted for histological examination. The bird was then treated with Amoxicillin 100 mg/kg tid at home for 10 days. Histopathological examination of the biopsied tissue demonstrated a papillary and cystic proliferation of cuboidal to squamous epithelium supported by a thin fibrovascular network.

Three weeks after the surgery the bird was represented to check the surgical wound which was mildly inflamed and presumed to be infected. At this time the bird was in good condition and had lost 25g in body weight. The expiratory wheeze was still present but the bird was not dyspnoeic. Amoxicillin was recommenced however, 20 days later the bird's respiratory noise had increased and the mass involving the axilla and proximal humerus had grown larger. The bird was noticeably weaker and was gasping when stressed. General anaesthesia and radical surgical excision was attempted but the bird arrested shortly after sectioning of the humerus was attempted. Sections of cortical bone from the humerus and the tumour were fixed in formalin and submitted for histological examination.

Histologically the submitted cortical bone tissue appeared normal but sections of the tumour were composed of cystic spaces, lined by single layers of proliferative cuboidal to squamous epithelium as for the original biopsy sample. There were also patchy areas of haemorrhage and haemosiderin-laden macrophages. In focal areas the epithelial cells had prominent nucleoli and a high mitotic rate.

Case 4

A 22 year-old female galah was presented to the Murdoch University emergency clinic with a sudden onset of moderate to severe dyspnoea. The bird was also making harsh sounds whilst breathing. The owners reported the bird had had intermittent episodes of moderate dyspnoea with harsh breathing sounds for a number of years previously. They also reported that the bird had a mass underneath its left wing, which had been diagnosed as a likely neoplasm by their usual veterinarian.

On clinical examination a 1.5 cm diameter reddish-brown, multilobulated mass was found in the left axilla. The bird was stabilized in an oxygen box and monitored overnight. The bird's demeanour improved markedly overnight and in the morning the bird was anaesthetized with isoflurane for closer examination of the mass and radiography. This revealed a large soft tissue-opaque mass extending from the left axilla and increased radio-opacity of the thoracic airsacs, left lung fields and left humerus. An air-gun pellet was also noted in the pectoral muscles as an incidental finding.

The extensive nature of the lesion and the high likelihood of invasive neoplasia were discussed with the owners and they elected to have the bird euthanased. Permission was granted for a limited cosmetic necropsy which demonstrated an extensive semi-pedunculated, greenish-brown multiloculated reddish cysts and greenish-brown nodules extending through the axilla and involving approximately 90% of the left cranial thoracic airsac and left lung. Histological examination demonstrated cystic and tubulopapillary proliferations of cuboidal to flattened epithelial cells with a sparse supporting network of fibrovascular connective tissue similar to as described above. Some of the nodules contained haemorrhage and/or foamy macrophages or haemosiderophages with acicular cholesterol clefts. In some sections the cranial part of left lung was partly replaced by these same cystic structures and the remaining lung had diffuse areas of haemorrhage, with congestion of the alveolar capillaries and bronchiolar haemorrhage. Extensive neoplastic invasion of the medullary cavity of the humerus was also present and there were individual neoplastic foci scattered throughout the clavicular airsac system including the contralateral side.

Immunohistochemical (IHC) staining
IHC staining using primary monoclonal antibodies to vimentin and cytokeratin (DAKO®)[1] and the avidin biotin complex (ABC) method were performed on tissue sections from each of the cases, respectively. Antigen-antibody complexes were visualised with the chromagen diaminobenzidine (DAKO® DAB chromagen) and imaging was performed using an Olympus BX 13 microscope and digital camera accessory.

The epithelial component of the neoplasms from all 4 cases were consistently positive for cytokeratin and negative for vimentin. They were also Periodic Acid Shiff (PAS) negative. In those sections were there were haemorrhages, erythrophagocytosis and PAS positive foamy macrophages these cells also stained positively for vimentin but not cytokeratin.

[1] DAKO corporation, Carpinteria, California. www.dakocytomation.com.au

Discussion

The presenting clinical signs, radiographic, gross and histological appearances of the neoplasms presented in these 4 cases were very similar to each other and given the anatomical location and histological features were most likely of respiratory tissue origin. A diagnosis of airsac cystadenocarcinoma was made for each of the cases based on the gross and histological appearance of fluid and air-filled cystic spaces and the differentiation into airsac-like tissue in at least some areas in all of the sections examined. No evidence of ciliated or mucous cell differentiated epithelium as has been reported in other similar cases of neoplasia in cockatoos[4,5] or an African grey parrot[6] were present in any of the sections from our 4 cases.

In all of the cases described in this paper the main proliferative cuboidal to squamous cell types were positive by immunohistochemical staining for cytokeratin and negative for vimentin which provides further evidence that the cell types were epithelial in origin.[10,11] Vimentin is an intermediate filament protein (IFP) found in cells of mesenchymal origin and has a role as a storage network for steroidogenic cholesterol containing lipid droplets[12] so it was not surprising that the foamy macrophages seen accompanying neoplastic proliferative cysts and nodules were positive for vimentin. The associated presence of these cells was most likely a response to haemorrhage that was observed in many places but could also represent a component of the mesenchymal response that often accompanies epithelial neoplasms.

Neoplasms of the air sac are rare in companion birds but of those cases reported so far all have involved the right axilla or humerus.[3] In contrast, our 4 cases were unilateral and involved the left axilla most likely originating from a diverticula of the clavicular airsac.[13] The predominance for the left side in our cases is probably a statistical aberration and may have no biological explanation given that the clavicular airsac system in cockatoos is interconnected from left to right sides and effectively bilaterally symmetrical. The humeri of cockatoos and most other bird species are pneumatised and receive invaginations of airsac epithelium from the clavicular or cervical airsac systems and local spread through this air sac system is the most likely explanation for the occurrence of neoplastic tissue within the humeri in these cases.

An airsac mucinous adenocarcinoma of the right humerus reported in a Moluccan cockatoo[4] was most likely a primary neoplasm given that the mass extended from the humerus into adjacent soft tissue but not proximally to the airsac system or lungs and that the bird made a complete recovery following amputation of the wing at the shoulder. A histologically similar carcinoma was also reported in the right humerus of a 12 year old African grey parrot[6] but haematogenous metastasis from a primary neoplasm in the lungs rather than local invasion through the airsac system was proposed as the method of spread in that case.

All of the case reports of lower respiratory tract neoplasia in large companion psittacine birds have been in relatively old, mature birds. From our observations, and previously published cases, lower respiratory tract neoplasia must be considered as a possible differential diagnosis for cases of subacute dyspnoea in otherwise apparently healthy companion birds older than 10 years of age. Other differentials that should be included are tracheal obstruction, primary pulmonary disease such as exudative or interstitial pneumonia, airsaculitis and primary or secondary cardiovascular disease such as atherosclerosis and pulmonary embolisms. Furthermore, air sac carcinoma should be considered as an alternative differential diagnosis to the relatively more benign diagnosis of lipoma for soft tissue tumours that occur beneath the wing or near the axilla in older cockatoos.

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