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SHORT PAPER

Hepatic Myelolipoma and Amyloidosis with Osseous Metaplasia in a Swan Goose (*Anser cygnoides*)

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Summary

An adult swan goose (*Anser cygnoides*) kept in a zoological garden had gross hepatic enlargement with extensive ill-defined white foci. Microscopically, the hepatic lesions were composed of a mixture of adipocytes and myeloid cells. The goose was also affected with systemic amyloidosis and there were areas of osseous metaplasia associated with deposition of amyloid within the liver.

Keywords: amyloidosis; liver; myelolipoma; osseous metaplasia; swan goose

Myelolipomas are unusual neoplasms that are composed of a mixture of mature lipocytes and haemopoietic cells and usually occur in the adrenal gland, spleen and liver in man, non-human primates, Felidae, cattle and other animals (Lack, 2000; Capen, 2007; Stalker and Hayes, 2007; Valli, 2007). There are only three case reports describing myelolipomas in birds (Andreasen *et al*., 1995; Latimer and Rakich, 1995; Ozaki *et al*., 1996) and hepatic myelolipoma has been reported in one saffron toucanet (*Baillonius bailloni*) (Latimer and Rakich, 1995).

Amyloidosis is a disorder that is characterized by extracellular deposition of amyloid, which is composed of non-branching fibrils that form β-pleated sheets as identified by X-ray crystallography and infrared spectroscopy (Snyder, 2007). Amyloid appears as an amorphous hyaline material in haematoxylin and eosin (HE)-stained sections and is specifically stained by Congo red. Amyloid stained with Congo red also exhibits a characteristic apple-green birefringence by polarized light microscopy. Cartilaginous and osseous metaplasia within deposits of amyloid has been reported to occur in man and dogs (Ramos-Vara *et al*., 1998), but never in birds. The present report documents the pathological features of myelolipoma and amyloidosis with osseous metaplasia in the liver of a swan goose (*Anser cygnoides*).

An adult female swan goose kept in a zoological garden became debilitated and was later found dead. On necropsy examination the carcass showed no appreciable autolysis. The liver was diffusely enlarged, firm in consistency and had mottled, ill-defined locally extensive white foci in both hepatic lobes (Fig. 1). There were small deposits of chalky white material on the serosal surface. The spleen was moderately enlarged and firm and the cut surface had a waxy appearance. The kidneys were also mildly enlarged, pale and firm and their cut surfaces were glossy, with diffuse accumulation of white viscous material. The pericardium had a light covering of white chalky flakes consistent with visceral gout. Obstruction by an egg, rupture of the oviduct and ulcerative pododermatitis (''bumblefoot'') of the right footpad were also found. The organs, including the liver, spleen, kidneys, heart, lung, brain, gastrointestinal tract, pancreas, thyroid glands, parathyroid glands, adrenal glands, ovaries and right footpad were fixed

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Fig. 1. Diffuse enlargement of the liver with ill-defined, locally extensive white foci.

in 20% neutral buffered formalin and embedded in paraffin-wax. Sections (4 μm) were stained with HE. Immunohistochemistry (IHC) was performed on selected sections using the streptavidin-biotin (SAB) peroxidase method with a commercial kit (Nichirei Corp., Tokyo, Japan). The primary antibodies were anti-human muscle actin (HHF35; diluted 1 in 10, ENZO Diagnostics Inc., NY) and anti-human amyloid A (mc1; diluted 1 in 1000, DakoCytomation, Denmark).

Microscopically, hepatic lesions consisted of a locally extensive proliferation of mature adipocytes with an accumulation of haematopoietic cells, including erythroblasts, myelocytes, eosinophilic myelocytes and heterophils (Fig. 2A, B). These foci showed infiltrative growth, with an indistinct border and often interdigitated with the surrounding hepatic cords. Intracytoplasmic granules and non-segmented round nuclei of eosinophilic myelocytes and heterophils were clearly observed by Giemsa stain. Osseous metaplasia was observed within the foci of myelolipoma (Fig. 2C). Immunohistochemically, hepatic stellate cells and a few proliferative adipocytes were positively labelled by antibody HHF35 (Fig. 2D).

Additionally, amorphous eosinophilic material was deposited throughout the liver, spleen, kidneys, heart, lung, proventriculus, duodenum, ileum, colon, pancreas, thyroid glands and parathyroid glands. In the liver, these deposits were mainly found in the connective tissue of hepatic triads, vascular walls, subcapsule and the space of Disse. The eosinophilic material stained weakly with Congo red. Direct fast scarlet (Muto Pure Chemicals, Tokyo, Japan), which has been used previously for amyloid staining in man and a vervet monkey (Fujita *et al.*, 2006; Nakamura *et al.*, 2008), stained the deposits orange-red (Fig. 2E) and showed apple-green birefringence under polarized light. In a further section pre-treated with potassium permanganate, the deposits were not stained with Congo red and lost birefringence under polarized light. Immunolabelling with anti-human amyloid A antibody was negative. In addition, occasional foci of osseous metaplasia associated with the regions of amyloid deposition occurred in the liver (Fig. 2F).

Marked deposition of amyloid was also observed in the splenic cord, interstitium and trabeculae of the spleen. Mild to moderate amyloidosis was found in the interstitium and arterial walls of the kidneys, proventriculus, duodenum, ileum, colon, thyroid glands, parathyroid glands and adrenal glands. A small amount of amyloid was sometimes deposited in the walls of arterioles of the heart and in the interstitium, arterial wall and subcapsule of the pancreas. The lesion of the right footpad was composed of mild heterophilic and lymphoplasmacytic infiltration, fibrovascular proliferation, acanthosis and hyperkeratosis with ulceration.

Myelolipomas in mammals are composed of well-differentiated adipocytes with a variable admixture of myeloid cells (Capen, 2007; Stalker and Hayes, 2007). The tumour frequently has an irregular edge, with adipocytes interdigitating with normal hepatocytes (Head *et al.*, 2003). Metastasis of myelolipomas to other organs has not been reported, and the tumours are typically found as incidental lesions unassociated with clinical signs. All three previous avian cases of myelolipoma had heterophil predominance in the haematopoietic component of the tumour (Andreasen *et al.*, 1995; Latimer and Rakich, 1995; Ozaki *et al.*, 1996). The findings in the liver of the present case were consistent with those of hepatic myelolipoma. Human adrenal myelolipoma is usually associated with endocrine disorders such as Conn's syndrome, 21-hydroxylase deficiency, hormonally active adrenal neoplasms and adrenocortical hyperplasia (Weiss and Goldblum, 2001a). Myelolipoma is considered to be a hormonally induced metaplasia of the adrenal stromal cells or primitive mesenchymal cells (Bishop *et al.*, 2006; Weiss and Goldblum, 2001a). Chang *et al.* (2002) reported that adipocytes and myeloid cells in human adrenal myelolipoma show the same clonal cytogenetic abnormality, which suggests that adipocytes and myeloid cells may