

Short communication

Gill metaplasia in a goldfish, *Carassius auratus auratus* (L.)

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The aetiology of gill tissue enlargement is varied. Generalized hyperplasia and hypertrophy of gill epithelial cells has been attributed to gill damage caused by vitamin C deficiency (Ashley, Halver & Smith 1975), toxic metals (Daoust, Wobeser & Newstead 1984; Mazon, Cerqueira & Fernandez 2002), poor water quality, bacteria and parasites. Focal hyperplasia has been induced by the parasites *Ichthyophthirius* sp. and *Amyloodinium* sp. at their site of attachment (Noga 1996) and, rarely, by tumours. Basal cell papillomas have been reported to cause mass lesions on the gills of rainbow trout, *Oncorhynchus mykiss* (Walbaum), and coho salmon, *Oncorhynchus kisutch* (Walbaum), (Roberts 1989) and a gill adenoma has been reported in a trout, *Salmo fario* = *trutta* L. (Sarkar & Dutta-Chaudhuri 1964). Ectopic thyroid and thyroid hyperplasia protruding into the region of the gill has also been mistaken for a neoplasm (Ferguson 1989).

An approximately 4-year-old, female goldfish, *Carassius auratus auratus* (L.), presented to the North Carolina State University, College of

Veterinary Medicine, with abdominal distension and a subopercular mass. The fish was kept in a 160 L aquarium with a fantail goldfish that was obtained together from the same pet store. The fish were fed a mixture of frozen krill, bloodworms and mysid shrimp, along with Omega® flake (Omega Sea Ltd, Sitka, AK, USA) and Ocean Nutrition® pelleted food. The owner first noticed the mass 5 months previously. At this time, the fish was placed by itself in a 75 L quarantine tank, and after consultation with a veterinarian, 0.3 g L⁻¹ iodized salt was added to the water for 3 weeks. No change in the mass was noted. Four months later, abdominal distension developed and the fish was brought to the veterinary college. Upon presentation, the fish was anaesthetized in 150 mg L⁻¹ of buffered tricaine methanesulphonate (MS222; Finquel®, Argent Chemical Laboratories, Redmond, WA, USA) for examination. There was generalized abdominal enlargement that was more severe on the right side and a pink cauliflower-like mass protruded approximately 5 mm from underneath a laterally displaced right operculum (Fig. 1). This tissue appeared to be associated with the gill arch and a 4 mm wedge biopsy of the mass was performed to ascertain the origin of the mass. The tissue was firm, gritty, difficult to cut and fractured when bisected. The biopsies were immediately fixed in 10% neutral-buffered formalin, decalcified in formic acid, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin and eosin (H & E) for examination by light microscopy.

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Figure 1 Gill mass; right operculum removed. The red to white lobulated mass, later found to be osseous metaplasia, protruded from underneath the right operculum. It effaced the majority of the normal gill architecture (arrows define extent of lesion).

Histopathological analysis identified the tissue as a suspect myxoma with inflammation of associated gill lamellae. The examined section of the mass was comprised of bone fragments, blunted gill fragments, and spindle cells with indistinct borders and uniform hyperchromatic nuclei surrounded by marked mononuclear inflammation. Whole body dorsoventral and right lateral radiographs were taken (Love & Lewbart 1997) to determine the degree of mass infiltration. There was a diffuse, heterogeneous mineral opacity confined to the region of the right gills. The fish was administered an enrofloxacin ($2.5 \text{ mg L}^{-1} \text{ H}_2\text{O}$, 1 h, Baytril 2.27%; Bayer Corporation, Shawnee Mission, KS, USA) bath for prophylactic purposes because of moderate haemorrhage following the branchial biopsy. It was then placed in a 20 L tank of fresh dechlorinated water with an aeration stone and

0.1 g L^{-1} sodium chloride added. The following morning the fish was found dead and was immediately placed in a refrigerator at 7°C pending post-mortem examination 1 h later. At necropsy, the animal weighed 24 g and exhibited a fluctuant swelling in the right portion of the abdominal cavity because of a fluid effusion. A $1.5 \times 1 \times 1 \text{ cm}$ poorly demarcated, firm, irregular, white, tan and pink mass arose from the right fourth gill arch. The left gills were multifocally pale, and a wet mount of gill taken from this side revealed abundant *Dactylogyrus* sp. (Monogenea). The kidney was pale. No other gross abnormalities were noted.

Microscopic examination of the gills revealed bilateral osseous metaplasia with the right more severely affected (Figs 2–4). The right fourth gill arch was disrupted by a multilobulated mass composed of immature woven bone, and a mild

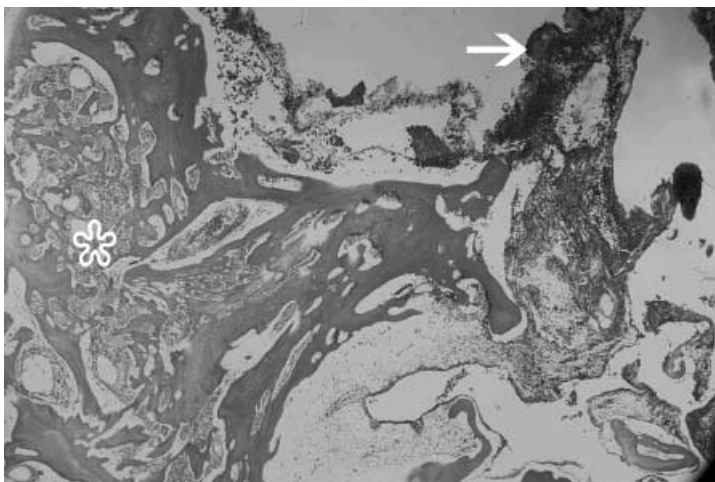


Figure 2 Right gill. Attenuated epithelium is present at one margin (arrow). The subepithelial space is expanded by oedema and mixed inflammatory cells. There is proliferation of bony trabeculae (asterisk) lined by epithelial cells and eosinophilic granular cells (H & E, $\times 40$).

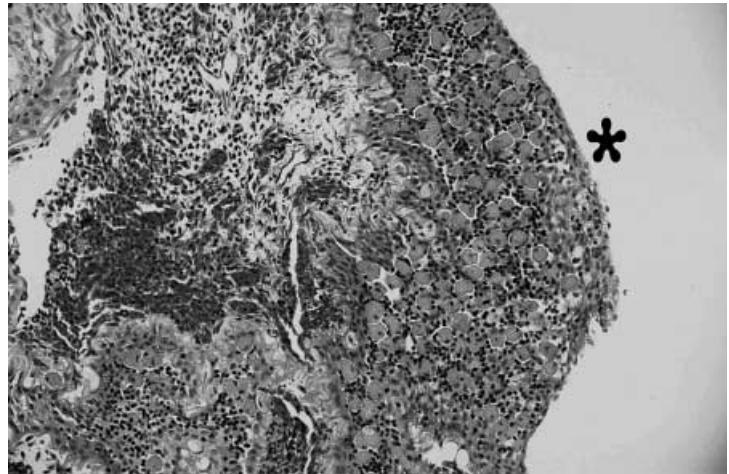


Figure 3 Right gill. Gill epithelium is blunted (asterisk) and the subepithelium is expanded by oedema and inflammatory cells (H & E, $\times 200$).

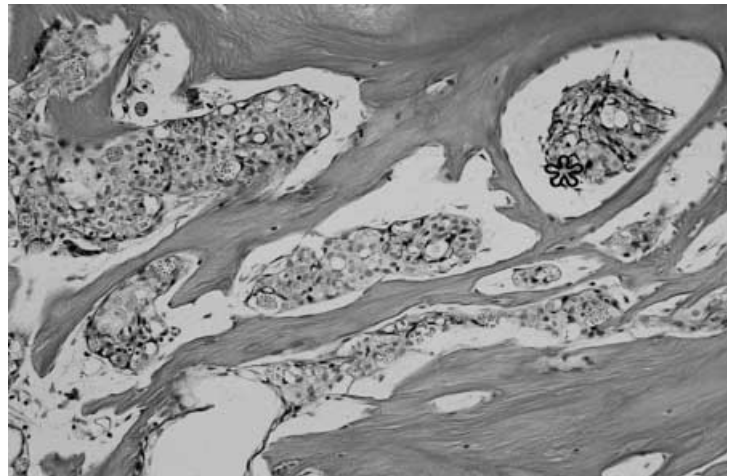


Figure 4 Right gill. Gill epithelium (asterisk) forms small aggregates lining bony trabeculae of varying thickness (H & E, $\times 400$).

amount of mature lamellar bone that contained scattered lacunae and formed irregularly arranged trabeculae lined by a single layer of attenuated gill epithelium overlying moderately oedematous submucosa. To aid in proving the lining was of epithelial origin, immunohistochemical stains for epithelial (cytokeratin) and mesenchymal (vimentin) cells were applied with intense and uniform positive staining for the former and negative staining for the latter. In addition, mucous cells stained positively with Alcian blue. Mild to moderate numbers of heterophils, eosinophils and fewer macrophages were present within the subepithelial space. Parasites were seen in wet mounts of gill, but were not present within the initial and additional sections of gill examined histologically. The findings in the left gill arch were similar, but less severe.

The kidneys, ovary, small intestine and coelom exhibited multifocal to diffuse granulomatous inflammation with intralésional rod-shaped bacteria. Granulomas were composed of layers of epithelioid macrophages and a central core of debris. The bacteria measured 1–2 μm , were acid fast, Gram and Fites-positive, and were determined to be *Mycobacterium* sp. based on morphology and staining characteristics.

The formation of bone from the morphologic and functional transformation of cells in mature fibrous or non-skeletal soft tissue, or from the stroma in muscle and glands, is termed osseous metaplasia (Pool & Thompson 2002). It is not common and its aetiology is unknown. In humans, osseous metaplasia of epithelial origin has been reported in eye (Vemuganti, Honavar & Jalali

2002), gastrointestinal tract (Haque, Eisen & West 1996), kidney (Perez-Ordonez, Hamed, Campbell, Erlandson, Russo, Gaudin & Reuter 1997; Yokozaki, Ukai, Kawashita, Ikeda, Kuniyasu & Tahara 2000), bladder (Eble & Young 1997), abdominal scar (Apostolidis, Legakis, Gregoriadis, Androulakakis & Romanos 1981), endometrium (Navani, Alvarado-Cabrero, Young & Scully 1996) and breast tissue (Yen, Florentine, Kelly, Bu, Crawford & Martin 2000). It has also occurred in a urachal cyst (Sasano, Shizawa, Nagura & Yamaki 1997) and in an odontogenic squamous cell carcinoma (Bennett, Jones & Speight 1993). In domestic animals, metaplastic bone formation has been reported in the feline (Head 1990), ovine (Barker, VanDreumel & Palmer 1993) and equine intestine (Kirchhof, Steinhauer & Fey 1996), and in canine mammary tissue (Nerurkar, Chitale, Jalnapurkar, Naik & Lalitha 1989; Gartner, Gerald, Cassali, Rema & Schmitt 1999). In humans, horses, sheep, dogs and cats, osseous metaplasia is often associated with neoplastic tissue.

Neoplastic tissue was not noted in the examined sections of the gill mass. There have been reports of non-neoplastic heterotopic bone formation in association with chronic inflammation and mucinous leakage in the human gastrointestinal tract (Groisman, Benkov, Adsay & Dische 1994; Haque *et al.* 1996). The epithelium covering the gill arches of freshwater fish is continuous with the epithelium of the pharynx and buccal cavity and often contains mucous cells near the base of the secondary lamellae (Stoskopf 1992). Histopathological analysis of the gill mass revealed epithelial cells with mucinous differentiation based upon cell morphology and positive staining with Alcian blue. The numerous monogenetic trematodes observed on the wet mount are a probable cause of gill irritation in this case; branchitis, as evidenced by oedema and heterophilic inflammation was noted on histopathological analysis. Although no reports were found relating to fish, it is possible that chronic inflammation led to the metaplastic bone formation evidenced in the gill, much as it does in the human gastrointestinal tract. Mucinous change has also been associated with osseous metaplasia in canine mammary tumours (Nerurkar *et al.* 1989).

In canine mammary tumours, osseous metaplasia has been attributed to the endochondral ossification of cartilage formed by myoepithelial cells (Moulton 1990; Gartner *et al.* 1999). This is another possible

aetiology for the osseous metaplasia found in the fish's gill. Primary lamellae have a central cartilaginous core covered in squamous epithelium that is continuous with the secondary lamellae. As a fish ages, the supporting gill arch becomes less cartilaginous and more osseous (Stoskopf 1992). If the regulation of this process was disrupted or hyperstimulated because of chronic irritation and inflammation, osseous metaplasia could have resulted. In our case, the sections of ossified gill stained negative with Alcian blue indicating the lack of a myxomatous stroma. Over time however, complete ossification could have occurred leaving the cartilaginous matrix no longer present.

Although *Mycobacterium* sp. was not found in the gill tissue, it was found in many organs including the kidneys, ovary, small intestine and coelom. *Mycobacterium* is a Gram-positive, acid-fast rod that causes chronic progressive disease. Classical signs include lethargy, emaciation, ascites and skin ulcerations. The visualization of white to grey nodules in multiple organs is not uncommon. A chronic disease like this is capable of causing immunosuppression; fish having advanced stages of the disease may be less capable of natural parasitic defences. Mycobacteriosis may have contributed in this case by allowing increased parasite infestation leading to chronic gill irritation. Mycobacteriosis has a worldwide distribution and all piscine species are considered susceptible (Stoskopf 1992). The bacteria can survive for up to 2 years in the environment and is probably transmitted by ingestion of bacteria shed from the gastrointestinal tract and infected skin ulcers (Noga 1996).

Osseous metaplasia is rare and its aetiology is unknown. Possible contributors to the formation of bone in gill tissue are chronic irritation, mucinous change and cartilaginous ossification. This case of osseous metaplasia in the gill of a goldfish also had disseminated mycobacteriosis.

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