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Papillary fibroelastoma with melanin deposition in Atlantic salmon (*Salmo salar* L.) heart: A case report

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Abstract

Papillary fibroelastomas (PFE) are rare tumours in fish. In this report, a PFE tumour with melanin pigmentation in the atrium of farmed Atlantic salmon (*Salmo salar* L.) is described. The tumour was identified in the atrium of an otherwise non-diseased fish. The tumour was present in the close vicinity of the sino-atrial junction, where a few ganglion cells of the cardiac pacemaker were seen and adjacent to the atrio-ventricular junction. The tumour consisted of papillary-like vegetations extending from myocardial fibres. The vegetations were composed of a central avascular fibroelastic core covered by large amounts of myxoid stroma with overlying endocardium. Periodic acid-Schiff (PAS)-Alcian blue (AB) (pH 2.5) staining showed acid mucopolysaccharides in the myxoid stroma and Perl's staining excluded the presence of haemosiderin pigment. No cellular atypia or mitotic activity was observed in the tumour area and this indicated a benign nature of the tumour. Melanin deposition in the tumour area may represent a fish immune response although this needs further investigation. Despite this, no other inflammatory cells or evidence of immune response was observed. To the best of our knowledge, histopathological features of such lesions in salmonids have not been previously described and extend the repertoire of heart diseases reported in fish.

Papillary fibroelastoma (PFE) (also called Lambl excrescence) is a primary cardiac tumour of humans that typically involves one of the valves of the heart. Cardiac tumours are rare with more than 75% of them benign. PFE are the third most common type of benign tumours of the heart, with the commonest being myxomas and lipomas. Cardiac tumours are mostly reported in the atrium, and are rare in the ventricles in humans (Burke and Virmani, 1996; MacGowan et al., 1993; Padera and Schoen, 2008; Silverman, 1980). Histopathological diagnosis of PFE includes branching papillary fronds with a

central avascular core of myxoid tissue covered by endothelial cells (Visser et al., 2008; Saxena et al., 2010). Melanin is a pigment contained within macrophage aggregates (Mas) and appeared brown to black in haematoxylin and eosin (H.E.) staining (Wolke 1992; Aguis and Roberts, 2003). The increase in pigment is suggestive of catabolic, toxic, infectious or stress response (Wolke, 1992; Aguis and Roberts, 2003).

To the best of our knowledge, similar lesions have not been described in detail in fish. However, similar lesions described as a proliferative

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nodular process on semi-lunar valves have been observed in farmed Atlantic salmon (Poppe and Ferguson, 2006). Here we report an individual case that showed PFE-like lesions and to the best of our knowledge no such lesions have been reported previously in Atlantic salmon. The fish showing PFE was one of the apparently healthy and non-diseased fish in a project involving study of salmon heart diseases.

The fish in question was apparently healthy S0 smolt that has been reared within one year using photomanipulation with artificial light to accelerate growth rates and induce smoltification and kept in a research facility (University of Nordland, Mørkvedbukta Research Station, Bodø, Norway) in 2 m³ tanks with fresh ambient sea water (temperature 7-8 °C) and fed 0.5% feed of their body weight three times weekly. The heart was fixed in 10% neutral buffered formalin and sagittally bisected, and processed for paraffin embedding and histology. The 3µm serial sections were cut and stained by standard haematoxylin and eosin (H.E.) staining and studied by light microscopy. Periodic acid-Schiff (PAS)-alcian blue (AB) (pH 2.5) staining was done according to Bancroft et al. (1994). Perl's Prussian blue staining was performed according to Bancroft et al. (1994). Briefly test and control slides were brought to distilled water. This was followed by a mixed solution of equal parts of 2% aqueous potassium ferrocyanide and 2% hydrochloric acid to cover the sections. Slides were incubated at room temperature for 30 min by changing a fresh solution after 15 min; slides were washed with several changes of water for several min and counterstained with neutral red solution for 5 min, washed in water, dehydrated and differentiated in alcohol, cleared and mounted.

Microscopically papillary vegetations were noted in the atrial cavity (Figure 1A). At various places, the lesions also contained melanin pigment deposition in the stroma (Figure 1B). The vegetations were composed of a central avascular fibroelastic core covered by large amounts of myxoid stroma with overlying endocardium (Figure 1C). These papillary vegetations were resting on myocardial fibres that in places extend between the fibres. The myocardial fibres were located at places in connection with the vegetations (Figure 1D). These vegetations were found on multiple sites such as: (1) close to the sino-atrial junction with few ganglion cells around (2) and adjacent to the atrio-ventricular junction (Figure 1A and 1D). The pacemaker tissue appeared lightly stained and comprised of wavy nerve bundles with oval-wavy elongated nuclei with pointed ends and ganglion cells with cytoplasmic granules (Figure 1E). The cauliflower like myxoid tissue was covered with melanin and at some places with cardiomyocytes. The hypocellular tumour was observed with spindle, stellate and epitheloid shaped cells. There was no cellular atypia or normal/abnormal mitotic activity in the lesion (Figure 2A). Serial sectioning was performed to obtain a complete overview of the tumour. The myxoid tissue and melanin were still visible at deeper levels (Figure 2B). Periodic acid-Schiff (PAS)-alcian blue (AB) (pH 2.5) staining demonstrated alcian blue positivity (acid mucopolysaccharide) in the myxoid stroma and PAS positivity in the fibroelastic core (Figure 2C and 2D). Perl's staining was performed to exclude the presence of haemosiderin in the pigmented area. No positive Perl's staining was observed in the sections (figure not shown).

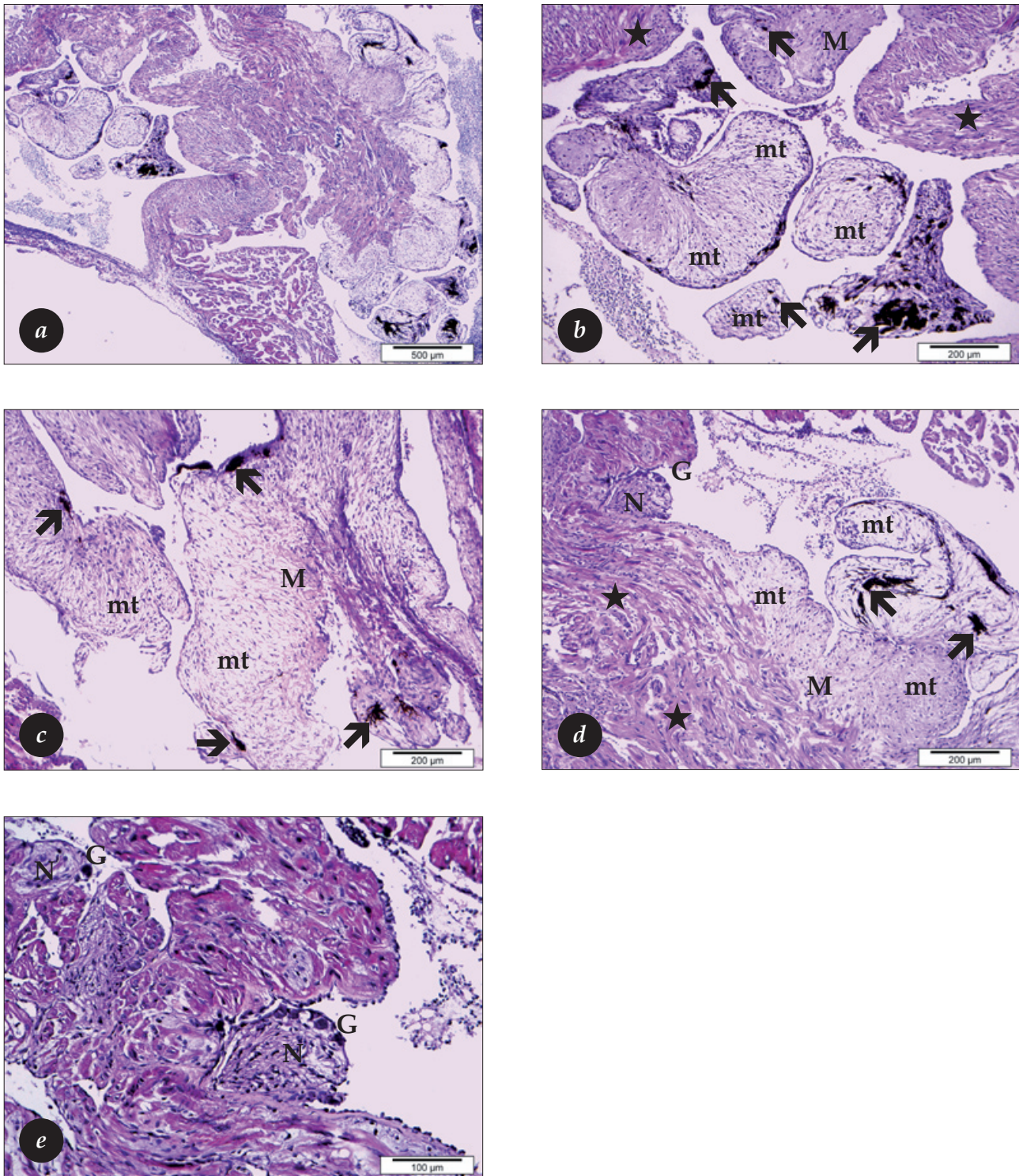


Figure 1. Papillary vegetation with melanin pigment deposition in the atrium of Atlantic salmon (*salmo salar* L.). G: ganglion cells, M: modified cardiomyocytes, N: nerve, star: normal cardiomyocytes, arrow: melanin, mt: myxoid tissue.

(A) complete tumour area in the atrium.

(B) cauliflower and papillary-like appearance of myxoid tissue with melanin.

(C) melanin deposition at atrio-ventral (AV) junction.

(D) papillary myxoid tissue extending in close vicinity of pacemaker at sino-atrial (SA) junction showing ganglion cells and nerve.

(E) Atlantic salmon pacemaker with ganglion cells and nerves at sino-atrial (SA) junction.

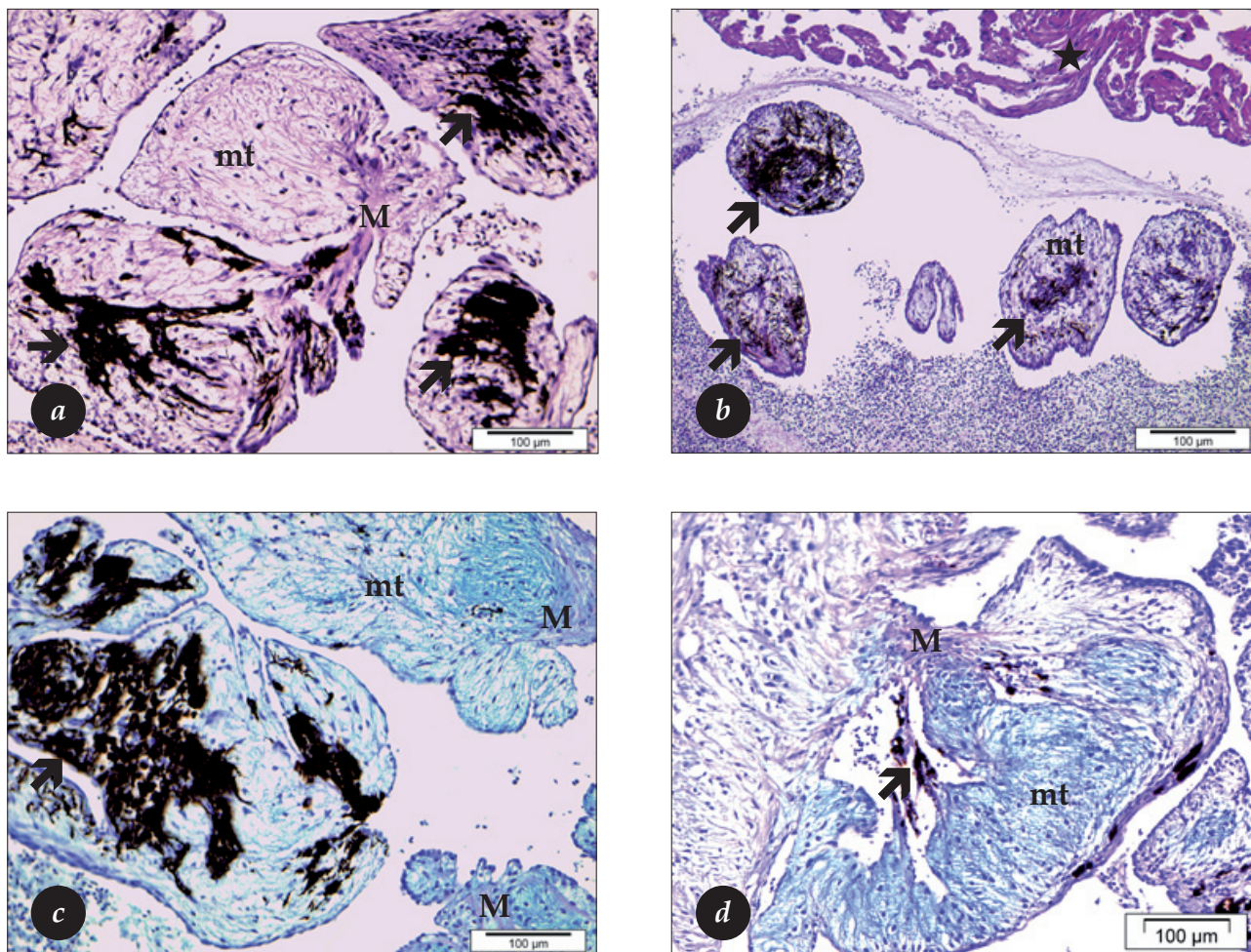


Figure 2. Papillary tumour. Arrow: melanin pigment, arrow head: acid mucopolysaccharide, M: modified cardiomyocytes, mt: myxoid tissue.
 (A) myxoid tissue with spindle, stellate and epithelioid shape cells with endothelial cell lining.
 (B) deeper cut showed myxoid tissue and melanin.
 (C) light photomicrograph of AB-PAS (pH 2.5) staining showed blue acid mucopolysaccharids in papillary region.
 (D) detailed view of AB-PAS (pH 2.5) staining showed acid mucopolysaccharide myxoid tissue and modified cardiomyocytes.

The tumour was found in close vicinity to the sino-atrial and atrio-ventricular junctions, but did not involve atrial valves. The papillary configuration with central fibroelastic core was covered by myxoid tissue rich in acid mucopolysaccharides consistent with PFE as described in humans. Based upon these microscopic observations, the lesions were categorized as a papillary (benign) fibroelastoma of the atrium of fish heart with melanin pigmentation (Fishebein et al., 1975; Darvishian and Farmer, 2001; Yamamoto

et al., 2002; Saxena et al., 2010; Visser et al., 2008; Jallad et al., 2009).

As described in humans, PFE generally are incidental findings and do not normally present any characteristic symptoms, however, larger lesions can lead to cardiac dysfunction leading to myocardial infarction or stroke and even sudden cardiac death (Burke and Virmani, 1996; Darvishian and Farmer, 2001). The presence of PFE in an apparently 'non-diseased'

fish suggests that no external or avert disease signs were observed. This may have also been a consequence of the size of the lesion which was quite small representing approximately 3-5% of the atrial mass.

Melanin deposition in fish organs has been reported previously under a number of different circumstances and some of these are as follows (1) a possible immune response in salmonids although the exact role/function still needs to be confirmed (Thoresen et al., 2006); (2) secondary to parasitic infections like black spot disease where metacercaria of digenetic trematodes encysted within skin or muscle show secondary deposition of melanin around the parasite (Poppe and Ferguson, 2006); (3) is also reported in 30-40 g Atlantic salmon where atrial endocardial degeneration occurred with experimentally induced cardiomyopathy syndrome (CMS) but melanisation was absent in larger (1.1 kg fish) CMS affected fish (Fritsvold et al., 2009; Bruno and Noguera, 2009). It was suggested that large melanin deposits occurred in regions with severe focal endocardial degeneration; an observation we have also observed in hearts from CMS affected Atlantic salmon (Yousaf and Powell unpublished), a feature also reported by Fritsvold et al. (2009); (4) in salmonids, melanin pigmentation is also suggested to be correlated with age and stress in liver, kidney and spleen (Schwindt et al., 2006).

The presence of melanin in the described lesions could represent part of immune response to the lesions but this requires further investigation. Differential diagnosis of PFE can be made from myxomas, lipomas and myxoid degeneration in neural tumours like neurofibroma, neurilemmoma and neurothekeoma (Suh et al., 1992;

Henderson et al., 1997). The description of PFE with melanin pigmentation in salmonids is useful addition to the already described spectrum of heart conditions in fish.

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