

NOTE

A case of schwannoma in farmed seabream *Sparus aurata*

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ABSTRACT: A tumour diagnosed as schwannoma with some unusual features affecting a single farmed seabream is described. The fish had a single mass on its back. The mass had well-defined limits and arose from the subcutis. The skin over the tumour was ulcerated. Histologically, the neoplasia consisted of Antoni Type A tissue forming irregularly dispersed bundles, within which a large number of fat cells was detected. No Antoni Type B tissue was observed. There was immunohistochemical evidence indicating a positive reaction of neoplastic cells tested with S-100 and calretinin. As far as we know, this is the first report of schwannoma in seabream.

KEY WORDS: Schwannoma · Tumour · *Sparus aurata* · Fish · Calretinin · Aquaculture

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INTRODUCTION

Tumours are common in fish, as in higher vertebrates and humans, and have been reported in almost all tissues. In fish histopathology, the main problem is the accurate diagnosis of tumour type, especially when specific markers are not available and the morphology of cells is unclear. Schwannomas have frequently been detected in different fish species such as goldfish *Carassius auratus* (Picci 1933, Schlumberger 1952, 1957, Mawdesley-Thomas 1972), snappers *Lutjanidae* (Lucké 1942), coho salmon *Oncorhynchus kisutch* (Masahito et al. 1985), damselfish *Pomacentrus partitus* (Schmale et al. 1983) and rainbow smelt *Osmerus mordax* (Morrison et al. 1993). Generally these tumours are benign, except in damselfish, although Finkelstein & Danchenko-Ryzchkova (1965) reported a single case of malignant schwannoma in perch *Perca fluviatilis*. The high incidence and peculiar distribution of schwannomas in some fish populations suggest a possible viral aetiology, as recently confirmed for damselfish (Schmale et al. 2002) but not in any other fish.

According to Koestner et al. (1999), schwannomas (neurilemmomas) and neurofibromas are neoplastic diseases classified as peripheral nerve sheath tumours (PNST). As both are spindle cell tumours, it can be very difficult to distinguish the two and, in particular, to differentiate them from fibromas (Scarpelli 1969). The differentiation between schwannomas and neurofibromas is still a diagnostic problem, and some authors prefer to treat them as a single morphological entity (Scarpelli 1969, Cordy 1990, Jubb & Huxtable 1993). Ultrastructural features, including the presence of extensive cellular processes and basement membranes, have been proposed to differentiate schwannomas from neurofibromas (Duncan & Harkin 1969). Recently, immunohistochemistry has been used in human medicine (Fine et al. 2004) and in fish pathology (Marino et al. 2007) to differentiate these 2 neoplasms. S-100 and calretinin are calcium-binding proteins that have commonly been used to label cells in both the CNS and PNS and to identify tumours of Schwann cell origin versus other soft tumours. Since calretinin and S-100 appear to be phylogenetically

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well conserved (Germanà et al. 2007, 2008, Levanti et al. 2008), we used these proteins to assess the nature of an unusual histological feature observed in a spontaneously occurring neoplasm in seabream.

MATERIALS AND METHODS

A single gilt-head seabream *Sparus aurata*, 30 cm long, 450 g in weight and with a single mass on its back was sent frozen to our laboratory for examination. The specimen was processed for histopathology, histochemistry and immunohistochemistry following routine procedures. Some pieces of the tumour tissue were processed for cryostat sectioning. The whole fish was fixed in 10% buffered formalin solution and then processed for paraffin embedding. Histological sections (5 µm thick) were stained using haematoxylin and eosin (H&E) and Masson's trichrome stain. For immunohistochemistry, the frozen blocks were cut in serial, frontal, horizontal and sagittal 10 µm thick sections, mounted on gelatine-coated microscope slides, deparaffined, dehydrated and processed for indirect peroxidase immunohistochemistry as described elsewhere (Marino et al. 2007, Germanà et al. 2008). Briefly, sections were rinsed in Tris-HCl buffer (0.05 M, pH 7.5) containing 0.1% bovine serum albumin and 0.2% Triton-X 100. Endogenous peroxidase activity and non-specific bindings were blocked using 3% H₂O₂ and 25% foetal calf serum, respectively, and sections were incubated overnight with rabbit polyclonal antibody against S-100 (Dako, diluted 1:1000) and calretinin (Chemicon, diluted 1:500) (Germanà et al. 2007, 2008). Subsequently, sections were dewashed in the same buffer and incubated for 1 h at room temperature with peroxidase-labelled sheep anti-rabbit IgG (Amersham, diluted 1:100). The specificity of the immunoreactivity developed was tested by substituting the primary antibody with a non-immune serum, omitting the primary antibodies, and incubating the sections with specifically pre-adsorbed sera (5 g of calretinin-blocking peptide [sc 11644P] in 1 ml of anti-calretinin working solution; the pre-adsorbed antibody for S-100 protein was purchased pre-diluted from the Diagnostic Products Corporation, DPC).

RESULTS

The tumour (6 × 6 × 6 cm) appeared as a subcutaneous firm, smooth, nodular mass (Fig. 1), with a pale grey to fairly pink, homogenous cut surface (Fig. 2). The mass had well-defined limits and arose dorso-laterally from the subcutis, deforming the left side of the body surface. The skin surface over the tumour was



Fig. 1. *Sparus aurata*. Macroscopic appearance of the tumour affecting the dorso-lateral part of the body



Fig. 2. *Sparus aurata*. Section of the tumour mass showing homogenous texture

ulcerated. In H&E-stained sections, the tumour had well-defined borders and was slightly encapsulated. It was arranged in irregularly dispersed tissue bundles among which a large number of fat cells was always present (Fig. 3). Tumour tissue was predominantly composed of elongated cells with ellipsoid, hyperchromatic nuclei and pale cytoplasm. The cells of the neoplastic bundles were usually densely packed and arranged in parallel ranks with distinctive palisading of nuclei, resembling Antoni Type A tissue. Verocay bodies were rarely detected in some areas of the tumour. No Antoni Type B tissue was observed. The tumour showed a very low degree of vascularisation. Neither haemorrhages nor necrosis were seen. A few nerve fibres were observed at the periphery of the tumour. Masson's trichrome-stained sections revealed a general lack of collagen. Immunohistochemistry revealed strong positivity in neoplastic cells when tested with S-100 and calretinin (Fig. 4). Calretinin

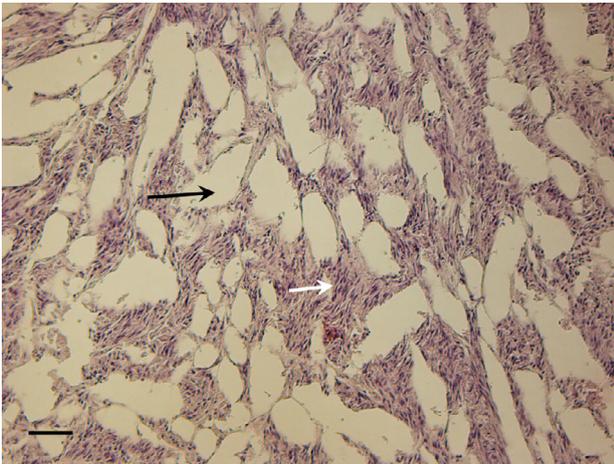


Fig. 3. *Sparus aurata*. Histological section showing a dermal schwannoma with nuclear palisading (white arrow) resembling Antoni Type A tissue among large numbers of fat cells (black arrow) (haematoxylin and eosin staining). Scale bar = 50 μ m

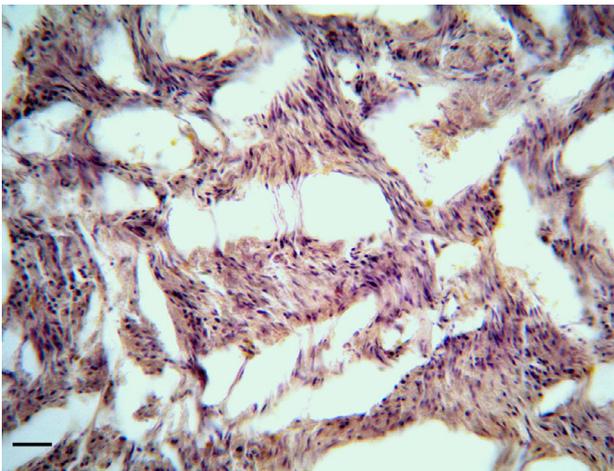


Fig. 4. *Sparus aurata*. Calretinin-stained section showing diffuse positivity in the tumour cells (indirect peroxidase). Scale bar = 20 μ m

immunostaining was homogeneously spread in neoplastic areas, whereas no reaction was detected in the dermis. At high magnification, the immuno-reaction was evident in the cytoplasm of all tumour cells.

DISCUSSION

As far as we know, this is the first report of schwannoma in sea bream. It has been suggested that there may be a relationship between this disease complex in goldfish and von Recklinghausen's neurofibromatosis in humans (Schlumberger 1952, Schmale et al. 1983), which, however, has different histopathology patterns

than the tumour we observed. The macroscopic appearance and the histological findings of the case presented here differ from the schwannoma in *Cyprinus carpio* and schwannomas in humans and domestic animals. Histological features of the nerve sheath neoplasm in the present case, such as the large number of fat cells among the neoplastic tissue, the presence of Antoni Type A and the absence of Antony Type B tissue (characteristic of the human schwannoma), as well as the rarity of Verocay bodies did not allow easy identification of the histogenesis of this tumour. However, the origin of the neoplasia was demonstrated by S-100 staining. The differentiation from neurofibroma was supported only by the positive staining of tumour cells with calretinin, a specific marker used to distinguish schwannoma from neurofibroma in human (Fine et al. 2004) and goldfish (Marino et al. 2007) PNST, and used here for the first time on sea bream.

Since ours is the first description of this tumour in farmed *Sparus aurata*, it cannot be regarded as a serious hazard for public health. However, the new European approach to food safety of farmed fish can create new perspectives with respect to fish pathology. Recent EU Regulations (178/2002, 852/2004, 853/2004, 854/2004, 882/2004) have greatly modified the approach to food safety management. In particular, official controls and the food business operators' rules must now be extended over the entire food chain in order to connect animal health, animal welfare and public health considerations at all stages of production, processing and distribution. Aquaculture products are also progressively undergoing this new assessment procedure. Fish farming is included in primary productions regulated by EU Reg. 852/2004, which specifically also provides for the registration of 'the occurrence of diseases that may affect the safety of products of animal origin'. This innovative accomplishment confirms the careful attention given by European regulators to fish pathology with potential implications for public health. For this reason, semiotic and anatomopathological monitoring could be very useful. The case reported here is an occasional finding that occurred during such monitoring at a local fish farm located in the South Tyrrhenian Sea, Italy.

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