NOTE

Spontaneous multicentric myxoma of the dermal nerve sheaths in farmed European eels *Anguilla anguilla*

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ABSTRACT: This report describes a peripheral nerve sheath tumour in 8 European eels *Anguilla anguilla* L. from a fish farm located in Croatia. The newborn tissue appeared as smooth and soft skin nodules without pronounced colour change. Nodules were dome-shaped with a pale crater and were present on different body areas. In general, nodules were located as series of differently sized protrusions extending along the lateral line on both sides of the fish, as well as sensory canals on the head. Cut sections showed a homogeneous, pale white–grey texture. Histologically, the pathological tissue was located in the dermis, occasionally intruding into the hypodermis, and pushing as a space-occupying mass against the underlying muscle tissue without any evident boundaries. The pressure also caused changes in the overlying epidermis, such as atrophy, spongiosis and erosion. In some areas, the epidermis was 1 cell thick and club and goblet cells had completely disappeared. Ultimately, these changes resulted in shallow ulceration. Tumour tissue was characterized by a scant population of spindle or stellate cells, with oval, hyperchromatic nuclei and pale cytoplasm embedded in a copious myxoid matrix. Cells were arranged in fascicles and whorls, extending in a poorly defined manner among the dermal collagen bundles. Occasionally, adipose cells were also detected, mainly in the central portion of the bulges. Myxoid areas appeared rich in metachromatic and alcanophilic mucous ground substance. Reticular fibres and collagenous connective tissue were scarce. Immunohistochemistry (IHC) using antibodies against S-100 and glial fibrillary acidic protein caused a slight positive reaction in neoplastic dendritic cells. High magnification showed the immunostaining to be cytoplasmic in all tumour cells. IHC with anti-calretinin antibody gave only negative results. Macroscopic, histological, histochemical and immunohistochemical findings were consistent with a diagnosis of multicentric myxoma of the dermal nerve sheaths, a tumour not yet reported in fish.

KEY WORDS: Myxoma · *Anguilla anguilla* · Peripheral nerve sheath tumour · PNST

INTRODUCTION

Peripheral nerve sheath tumours (PNSTs), which are neoplastic growths of the peripheral nerves, have been reported in many freshwater and marine fish species. These tumours affect both wild and farmed teleosts, and they can be differentiated into benign peripheral nerve sheath tumours (BPNSTs) and malignant peripheral nerve sheath tumours (MPNSTs). Benign forms include schwannoma (neurilemmoma) and neurofibroma, which have been described in many teleost species (Picci 1933, Lucke 1942, Schlumberger 1952, 1957, McArn & Wellings 1967, Duncan & Harkin 1969, Wellings 1969, Mawdesley-Thomas 1972,

BPNSTs also include myxoma of the dermal nerve sheaths. Myxomas are embryonic tumours of fibroblast origin that produce mucin (Smith et al. 1972, Goldschmidt & Hendrick 2002) regardless of the origin of the fibroblast. Although myxomas such as fibromyxoma and myxosarcoma have been well described in numerous animal species, they have rarely been described in fish (Plehn 1906, McIntosh 1908, Johnstone 1926, Schlumberger & Lucke 1948, Stotk 1958, Honma 1965, Dawe & Harshbarger 1975, Manera & Biavati 1995, Keller et al. 2011).

The present report describes a PNST in 8 farmed European eels Anguilla anguilla.

MATERIALS AND METHODS

Eight juvenile European eels, 11 to 23 cm in length, were obtained over a 1 yr period from a commercial eel farm in Croatia. All eels collected for this report had multiple, unusual external nodules on different body areas; otherwise, collected eels appeared healthy and in good body condition. In the farm studied, no other fish were affected and no increased mortalities during the survey were observed. The source farm had a standing stock of approximately 700 000 fish, and farming was based on glass eels imported from France. The farm used a recirculation culture system supplied by borehole fresh water. Water temperature in the system was between 22.5 and 23.2ºC, and pH was between 5.9 and 6.3.

The collected fish were killed in a separate tank by an overdose of MS 222 or by a blow to the head followed by severing of the spinal cord. Necropsy was performed under a stereoscope. Following necropsy, tissues from afflicted fish were fixed in toto in 10% formalin fixative for 24 h and then paraffin-embedded using routine procedures. Tissues were analyzed by histopathology, histochemistry and immunohistochemistry (IHC).

The paraffin blocks were cut into 6 µm thick serial, frontal, horizontal and sagittal sections, mounted on gelatine-coated microscope slides, deparaffinized, dehydrated and processed for routine haematoxylin and eosin, periodic acid-Schiff (PAS), alcian blue-PAS (pH 2.5), Orcein, Van Gieson and Masson’s trichrome stainings; slides were also processed for indirect peroxidase IHC. Frozen sections stained with Sudan III were used to visualize fat. For IHC, sections were rinsed in Tris–HCl buffer (0.05 M, pH 7.5) containing 0.1% bovine serum albumin and 0.2% Triton-X100. Endogenous peroxidase activity and nonspecific bindings were blocked, using 3% H2O2 and 2.5% serum albumin respectively. Sections were incubated overnight with rabbit anti-S-100 polyclonal antibody (1:200; Dako); rabbit polyclonal antibody anti-glial fibrillary acidic protein (GFAP), a marker of astrocytes (1:200, code GFAPR40; GeneTex); and anticalretinin (prediluted; Chemicon). Sections were then washed in the same buffer and incubated for 1 h at room temperature with biotine-labelled goat anti-rabbit IgG (1:200; BIOSPA). IHC specificity was tested in separate trials by substituting buffer for each primary antibody or for the secondary antibody, and by using specifically preabsorbed antibodies.

RESULTS AND DISCUSSION

At gross examination, the newborn tissue of European eels (0.8 to 3.2 × 0.2 to 2.3 mm) appeared as smooth and soft skin nodules without pronounced colour change. Nodules were dome-shaped with a pale crater and were present on different body areas. In general, nodules were observed as series of differently sized protrusions extending along the lateral line on both sides of the fish, as well as sensory canals on the head (Fig. 1). Cut sections showed a homogeneous, pale white-grey texture.

Histologically, the pathological tissue was located in the dermis, occasionally intruding into the hypodermis, and pushing as a space-occupying mass against the underlying muscle tissue without any evident boundaries. The pressure also caused changes in the overlying epidermis, such as atrophy, spongiosis and erosion. In some areas, the epidermis was 1 cell thick and club and goblet cells had completely disappeared (Fig. 2). Ultimately, these changes resulted in shallow ulceration.

Tumour tissue was characterized by a scant population of spindle or stellate cells, with oval, hyperchromatic nuclei and pale cytoplasm embedded in a
IHC using antibodies against S-100 and GFAP caused a slight positive reaction in neoplastic dendritic cells (Fig. 3). High magnification showed the immunostaining to be cytoplasmic in all tumour cells. IHC with anti-calretinin antibody gave only negative results.

CONCLUSIONS

Macroscopic, histological, histochemical and immunohistochemical findings in European eels were consistent with a diagnosis of multicentric myxoma of the dermal nerve sheaths, a tumour never reported to date in fish. Schwannoma and neurofibroma are the most common PNSTs in higher as well as in lower vertebrates such as teleostean fish. Although schwannoma develop from Schwann cells and neurofibroma from fibroblast cells of peripheral nerves, distinguishing these 2 BPNSTs was difficult before the development of more sophisticated diagnostic techniques based on IHC (Marino et al. 2007, 2008, 2012) and ultrastructure (Duncan & Harkin 1969). Myxoma can be diagnosed only on the basis of tumour histopathology, because the loose tissue does not give clear and reliable IHC results. The histological findings of the cases in this study, such as the presence of stellate, reticular, PAS-positive cells lying in a metachromatic, alcianophilic mucoid matrix, are consistent with myxoma (Sirtori 1954, Pulley & Stannard 1990, Manera & Biavati 1995). In fact, the tumours described here resemble those reported in the carangid fish, the yellowtail amberjack *Seriola lalandi* (Keller et al. 2011), as well as in domestic animals and humans.
The location, close to or around the lateral line, and the histological features of the nerve sheath neoplasm described here, such as loosely arranged cells enclosed in a myxomatous stroma sometimes resembling Antoni type B tissue, suggest that this tumour originated from the peripheral nerves related to the lateral line system. Such an origin is also supported by the fact that tumour cells stained positive for S-100 and GFAP, although the scarce cellularity of the tumour does not permit an easy detection of positive tumour cells.

Most studies seem to attribute PNSTs in fish to genetic causes, although toxins (Gardner & Yевич 1988) and viruses (Schmalé et al. 2002) have also been suggested. Recently, a temperature-sensitive mutant fish line offering a vertebrate tp53-inducible system that can be regulated in vivo, by simply controlling the water temperature, has been developed (Berghmans et al. 2005). Thus, it is possible that changes in water temperature, especially during early developmental stages, may play a role in inducing PNSTs, which may have triggered tumour transformation in the fish in the present study, assuming that they were genetically predisposed.

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