NOTE

European Eel Stomatopapillomatosis in the south Tyrrhenian sea: surgical excision and post surgical recovery

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Abstract

Stomatopapillomatosis is the most known epithelial neoplasm in fish. Although such papilloma has been described in eels in almost all European countries, it is considered extremely rare in the Mediterranean basin. This study represents the first report of this tumour in Italy, the first immunohistochemical characterization, the first attempt of radiographic exam, surgical therapy and demonstration of post-surgical recidiva.

Eel stomatopapillomatosis is one of the most common benign epithelial tumour of teleosts. Such neoplasia has been observed in both marine and fresh water teleosts and it is always located around the mouth, the nostrils and/or over the head. This tumour is classified as papilloma and it is constituted of epithelial cells hyperplasia in a papillomatous matrix. Stomatopapillomatosis was first reported in 1910 in the Jasmund Bay, Germany, by Schaperclaus (1953), who named this Cauliflower Disease. Several reports (Christiansen and Jensen, 1950; Stich et al., 1977) described the spreading of this neoplastic disorder from the Baltic Sea to all the Nordic and Central European countries, until it represented 40% of some eel populations (Peters et al., 1978). The incidence of stomatopapillomatosis may show seasonal fluctuation (Peters, 1975). Tumours described in eel can show differences in size, shape, consistency, colour and connection with the skin. The causative agent of stomatopapillomatosis remains to be elucidated, even if the hypothesis of the presence of a virus (Schwanz-Pfitzner et al., 1984) that increase the susceptibility of fish to chemical carcinogens, is the most promising one (Deys, 1979). Up to now no data have demonstrated the experimental induction of such neoplasia.

The aim of this paper is to show the radiology, surgical removal, post-surgical recovery, histopathology and immunohistochemical findings of a clinical case occasionally observed.

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An adult European eel (*Anguilla anguilla*), 2 years old, male, coming from an experimental farm in Messina, Italy was sent for the presence of a neoformation close to the mouth. None of the eels reared in the same tank had similar changes. The fish was transferred in a tank to the Centro di Igiene e Malattie Animali Sperimentale della Sicilia (C.I.S.S.) of the University of Messina, where it was maintained for about two years at the temperature of 20°C. The excessive increase in size of the mass, which was invading partially the mouth cavity, making normal feeding not possible, as well as progressive deterioration of body condition, gave rise to the suggestion for the surgical excision. To evaluate the tendency of the neoplasm to invade the surrounding and under-laying tissues, a radiological exam, with the fish laying in lateral and dorsoventral projections, was carried out.

In the summer 2005, the eel was anaesthetised by immersion in MS-222 solution (0.3 ml/10 litres of water). The surgery was performed out of water, placing the fish in ventral recumbancy on wet papers. Complete excision of the growth with large margins around the tumour was made. The tumour was removed in three steps: excision of the external part of the mass; excision of the intra buccal portion; scarification of residual neoplastic tissue. During removal of the neoplasm, there was no evident adhesion nor tumour infiltration in closer surrounding tissues, but the mass showed a sharp margin with slight oedema. The surgery started with partial disconnection of the skin, with haemostatic pincer, from the caudal part of the mass, where neoplastic tissues appeared friable. The tumour was disconnected from under laying muscles by smooth dissection with surgical scissors, whereas dissection and excision were performed using scalpel and surgical scissors. Due to the large area excised, tissues were allowed to heal by second intention.

To maintain the gills wet, marine water added with anaesthetic was pumped regularly in the mouth. When the fish showed partial wakening, it was immersed in anaesthetic solution for few seconds.

Tissue samples of the tumour were fixed in 10% buffered formalin solution, embedded in paraffin wax and processed routinely for histopathological exam. 5 μm thick sections were stained with haematoxylin-eosin. Moreover, 5 μm thick sections were transferred on polylysinate slides and stored at 37°C to elicit sample adhesion. To obtain antigenic unmasking, samples were treated with microwaves in citrate buffer 0.01mol/L, pH 6.00, for 10 minutes. Endogenous peroxidase activity was blocked with 0.3% H₂O₂ in methanol for 30 minutes, whereas non specific protein reactions were inhibited via incubation with 5% BSA (bovine serum albumin) for 30 minutes. Samples were incubated overnight at 4°C, with the primary antibody anti-cytokeratin (clone MNF 116) and anti–vimentin (clone V9) (both mouse monoclonal; diluted at 1: 25 – 1: 50, Dako Cytomation). Sections were incubated for 30 minutes at room temperature with the secondary biotinylated antibody (goat anti–mouse biotinylated IgG, dilution 1:100, Vector Laboratories), developed with acetylated avidin (dilution 1:250, Biospa), revealed with DAB (3,3 diaminobenzidine) (Dako) and counterstained with Carazzi Haematoxylin.
Sections were observed at light microscope and the immunoreaction was evaluated considering the cytoplasmic localization.

The mass (2 x 2 x 4 cm), cauliflower shaped, greyish in colour and firm in consistence, was localised at the oral extremity near the right external labial margin (Figure 1). It had a small, well demarcated but irregular, area of insertion, and was expanding over the back of the head and to the inner part of the lips within the mouth. Radiographic examination showed a new irregular growth of soft tissue, overlaying maxilla, without involvement of bone tissue of the head. The tumour was totally removed scarifying the skin around the peduncle (Figure 2). The mass was examined showing a homogeneous and dry cut surface, grey-whitish in colour. After surgery the cicatrisation was complete in 20 days. Six months later, a small growth appeared over the head, close to the surgical field, as expression of neoplasm recidiva, and started to grow up again in a faster way as compared to the primary tumour. The new growth showed intense dark pigmentation. The fish survived one year after surgery.

Histologically, the tumour appeared characterized by polypoidal growth constituted of epithelial cells arranged in sheets. The neoplastic tissue had a lobular appearance, with papillae of hyperplastic epithelial tissue laying over a thin basal membrane placed on connective tissue arising from derma. Strong hyperplasia of the germinative layer was seen. Tumour cells originated from islets of neoformation resembling a vortex or rosette in shape. In more external epithelial layers, glandular epithelial cells, such as goblet cells and alarm cells, showing different level of degeneration were seen. Sometimes, cystic areas

Figure. 1. Anguilla anguilla. The mass was located in the head and partially invaded the mouth.
were observed within epithelial tissue. Near the insertion, peduncle shaped, dermal connective tissue was apparently involved appearing to follow the tumour in its growing to provide a thin stroma to the mass. Within this connective tissue a variable degree of melanic pigmentation was seen. Many vessels were observed in the connective stroma. Histopathology let us identify this tumour as papilloma. On the basis of the macro and histological patterns the papilloma was classified as belonging to the type A (Pilarczyk, 1973).

Immunohistochemically, tumour tissue showed diffuse cytoplasmic positivity when tested with the antibody anti-cytokeratin, while vimentin did not stain the tissue confirming the epithelial origin of the neoplasia.

Stomatopapillomatosis is a neoplastic disorder well known in the eel. Nevertheless, such a tumour has never been reported in Italy and no attempts to undertake surgery have been carried out before. For this reason this study represents the first confirmation of post surgical recovery, permitted by the possibility to know the follow-up for almost one year after surgery. The surgical removal, performed out of the water, was confirmed to be a practical system, provided that particular attention is due to sedation, via repeated immersion in anaesthetic solution and continuous pumping through the gills of the same solution. Tumour excision was carried out to permit a better life quality for the fish, which after surgery started to feed again regularly. Controversially, the tendency to recidivation, on the other hand predictable as for papillomata in higher vertebrates, need a careful monitoring of the animal for all the life.

Figure 2. Anguilla anguilla. Due to the large area of exeresis, tissues were allowed to heal by second intention.
As regards to the presumptive cause of this neoplasm, the viral origin has been proposed but never demonstrated with cell culture isolation nor experimental transmission. In our case, none of the other eels, coming from the same tank, developed tumours over a two years long period.

Finally, on the basis of the morphological characteristics of the neoplastic tissue, the stomatopapillomatosis here reported, have to be included in the type A, i.e. pedunculated masses characterised by sharp invaginations forming high and uneven papillae, with a variable degree of melanotic pigmentation.

References


