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Epidermal Papilloma on the Snout of Cultured Eel, *Anguilla japonica* (Temminck and Schlegel, 1847) in Korea

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Abstract: A case of epidermal papillomatosis is reported in samples of cultured eel, *Anguilla japonica* obtained from an eel farm in Korea. The affected eel showed reddish tumors on the dorsal or ventral aspect of snout. Hisotologically, the lesions appeared very similar to epithelial tumors, with many complex papillae in the epithelial fold. In the dermis, there was a weak fibroblastic proliferation of the connective tissue and diffusive congestion of blood vessels. Although epidermal papillomatosis is rather common in fish but this is apparently the first report of epidermal papilloma incidence in farmed eel in Korea.

Key words: Papillomas · Eel · *Anguilla japonica*

INTRODUCTION

Most neoplasms observed on fishes are mainly epidermal papillomas [1]. Epidermal papillomas in fish are easily recognizable lesions and some of them have been known for centuries. Recently, the lesions have frequently been reported in wild and farmed fish species such as flatfish, catfish and goby in Korea and many other countries [2-7]. Various possible causes are proposed for the disease. In many cases, virus or virus-like particles were identified in the skin papillomas of fish. However, oncogenicitic correlation has been clearly demonstrated only for herpesviruse isolated from benign tumors [3, 8]. In contrast, virus or virus particles have not always been found in papillomas [9, 10].

Several studies previous suggested that chemical and biotic pollutants (e. g. toxic chemicals, heavy metals, crude oil, bacteria) and X-cells present in sediments may be responsible for the skin tumors observed in certain fish species [2, 3, 11-14].

In this study, the authors scrutinized clinical and histological symptoms of epidermal papillomas on snout in Japanese eels, *Anguilla japonica*. Fish had been cultured in a re-circulating aquaculture system in a farm situated near the seashore in Gochang, Jeollabukdo, Korea. When the disease outbreak occurred, water had the following characteristics: water temperature (subsurface water) 25-26°C, salinity 0.3-0.4‰, dissolved oxygen 6.5-6.7 mg/L, pH 5.3-5.7 and nitrite 4.5-5.0 ppm. They had been fed daily with formulated commercial eel feed at 1% rate of the biomass. The daily mortality showed variability depending upon body weight and age of fish. In fingerling tanks (20-30 g), 80-90 fish died daily (approximately 0.4% day) per tank whereas 20-30 eels died (approximately 0.1% day) in those of marketable sizes (100-110 g). Affected fish showed a tendency to swim afloat on the water surface compared to healthy ones but with normal appetite. Increased mucous secretion was apparent in the gills of floated fish.

Diseased fish (25-100 g) were transported to the laboratory, individually examined and photographed before biopsy. No parasites were detected in wet preparation of gills and tumor lesions under a light microscope (CH2, Olympus, Tokyo, Japan). Hemorrhages were evident on the fin and operculum in the diseased eels. The affected fish showed reddish tumors on

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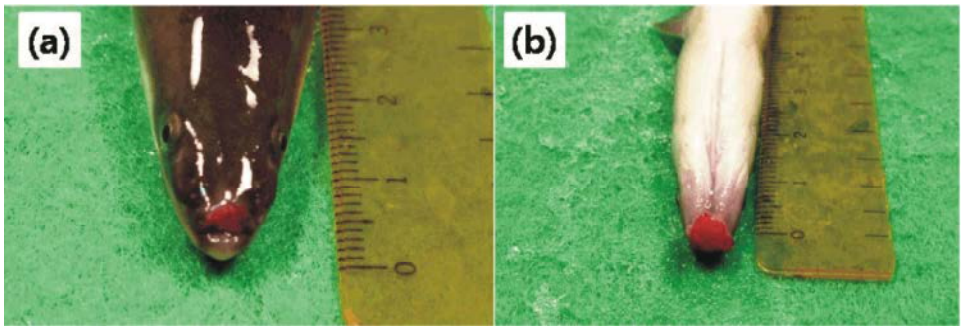

Fig. 1: Diseased eel showing a reddish epidermal papilloma on the dorsal (a) and ventral aspect (b) of snout

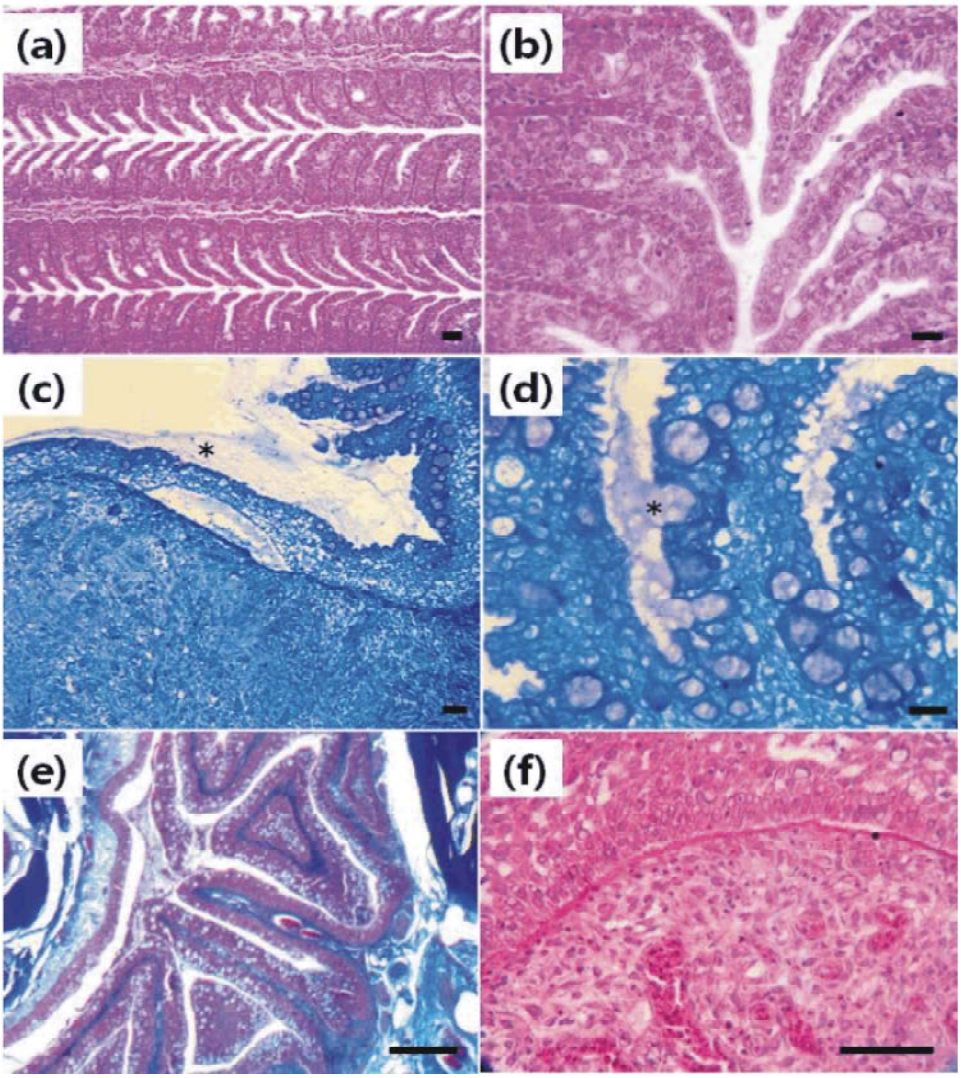

Fig. 2: Histological sections of gills and papillomatous area of Japanese eels. (a) Secondary lamellar fusion and severe proliferation of lamellar epithelia (H and E). (b) Distortion of gill filaments (H and E). (c) and (d) Mucous cells and mucous secretion (*) in the raised skin lesion (MB). (e) Epidermal papillary folds and mucous cell hyperplasia in papillomatous area (MT). (f) Proliferous growth of the cylindrical epithelial cells with oblong, spindle-shaped nuclei in the basal layer of the epidermis (H and E). Bars indicate 50 μm in (a) and (c), 20 μm in (b) and (d), 200 μm in (e) and 30 μm in (f).

the dorsal or ventral aspect of snout (Fig. 1). Erosions were occasionally identified on the dorsal aspect of snout and theses seemed to be the sites from which tumors were cast off. Unlike tumor of European eel, the size of papilloma on the diseased eel was smaller. Temperatures of 15-22°C caused rapid growth of epidermal tumors whereas lower temperatures (5-12°C) inhibited the growth and even caused regression in European eels [15]. From the present observations and the findings by Peters and Peters (1978) [15], it hypothesized that water temperature of 25- 26°C, which are optimal for the culture of Japanese eel, resulted in cessation of tumors growth. We found that fingerlings showed higher daily mortality than marketable sizes in the farm. Similarly, to our findings, it was reported in pleuronectid fish that epidermal papillomas tend to form primarily in young fish, causing significant mortality [16, 17].

Gills and tumors dissected out from the lesions on the snout were fixed in Bouin's fluid, followed by embedding in paraffin, according to standard procedures. Histological Sections (5 μm) were stained with haematoxylin and eosin (H and E), new methylene blue (MB) and Mallory's trichrome stains (MT). The sections were examined with a light microscope. Gill exhibited lamellar distortion, fusion and epithelial hyperplasia in the base of secondary lamellae (Fig. 2a and b). Hypertrophied cells encased in hyaline capsules (common characteristic in lymphocystis) were not observed (Fig. 2c and d). In the epidermal lesions, thickening and epithelial hyperplasia were clearly observed. The lesions appeared very similar to epithelial tumors, with many complex papillae in the epithelial fold (Fig. 2e). In the region of the epithelial proliferation, the typical basal layer cells were displaced by cells with an oblong, spindle-shaped nucleus and these abnormal cells were disorderly arranged (Fig. 2f). The proliferated epithelial cell showed a large nucleus with a prominent nucleolus. These enlarged nuclei were in vesicular form in some epithelial cells and contained one or two prominent nucleoli. No cytoplasmic or nuclear inclusions were present. Like in the cauliflower diseased eels [15, 18, 19], the epidermal tumors were covered by meandering fold. There were numerous mucous and club cells in the superficial layer of epidermis. Some intercellular spaces between epidermal cells were observable. In the dermis, there was a weak fibroblastic proliferation of the connective tissue and diffusive congestion of blood vessels (capillaries). Melanophores were not seen in the basal cell layer of the epidermis or dermis. Large and small round cells were present in connective tissue supporting the papilloma.

Papillomas are usually composed of local agglomeration of dermal connective tissues covered by a hyperplastic epidermis [1]. Skin papillomas grow as a fold of epidermis, supported and nourished by branching dermal connective tissue. We found that hyperplasia of the epithelial cells begun in the basal part of the epidermis leading to thickened epidermis supported by connective tissues stroma. In cauliflower diseased eels, cauliflower cells remain confined mainly within the epidermis, where a largely undifferentiated tissue is formed in place of a differentiated epidermis [18, 19]. Similarly, numerous undifferentiated epidermal cells in variable shapes were observed in this study. The epidermal papillomas of affected fish were red in color. This phenomenon seems to be caused by dilation and congestion of blood vessels of the neoplastic area.

In the present study, undifferentiated epidermal cells and mucus cells increased in both size and number. In contrast to our findings, papillomas in many fish species exhibit only proliferation of epithelial cells, but not mucous cell hyperplasia [20]. Mucous cell hyperplasia is closely related to bacterial cell loads in the culture water of Atlantic halibut [21]. Turbidity of affected fish tanks was higher due to the increase in organic loads attributable to breakdown of influent water filtering systems. It seems that mucous cell proliferation in this study is a response to protect the epidermis as proposed by other investigators [5, 22]. The spongiotic and hyperplastic epidermis observed in the present study was similar to the papilloma in European eel and rainbow trout [15, 23]. Ottesen *et al.* [5] reported that vacuolar degeneration of the epithelial cells is an indication of epidermal sloughing processes. Similarly, vesicular epithelial cells that we observed may be a preceding event to the tissue erosion in the dorsal snout area.

Viruses play an important role in the induction of skin tumors. Epidermal papillomas are mostly caused by herpesviruses and less frequently adeno-, retro-, birna- or picornaviruses [3]. Roberts [24] reported that outbreaks of epidermal papilloma are influenced by a combination of viruses and environmental pollutants because experimental transmissions of isolated viruses from lesions were unsuccessful. Microbes (parasites, bacteria or evidence of viruses) were not observed in any tissue sections, suggesting a non-infectious etiology. However, as we examined a limited number of samples and did not

attempt culture, we cannot rule out the possibility of an infectious agent playing a role in theses lesions. Hence, further investigation is needed of the pathogen evolved with epidermal papillomatosis of farmed eel in the future. This is the first report of epidermal papilloma occurrence in farmed eel in Korea.

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REFERENCES

1. Peters, N. and B. Watermann, 1979. Three types of skin papilloma of flatfishes and their causes. Marine Ecology, 1: 269-276.
2. Fujimoto, Y., H. Madarame, H. Yoshida, R. Moriguchi, H. Kodama and H. Iizawa, 1984. Pathomorphological observations on epidermal papilloma of flatfish (*Liopsetta obscura*). Japanese Journal of Veterinary Research, 34: 81-103.
3. Anders, K. and M. Yoshimizu, 1994. Role of viruses in the induction of skin tumors and tumor-like proliferations of fish. Diseases of Aquatic Organisms, 19: 215-232.
4. Yu, J.H., S.W. Park and D.W. Kim, 2009. Epithelioma of farmed catfish, *Silurus astotus*, in Korea. Journal of Fish Pathology, 22: 107-113.
5. Ottesen, O.H., E.J. Noga and A. Amin, 2010. Histopathology of culture-associated skin erosions and papillary hyperplasia of Atlantic halibut, *Hippoglossus hippoglossus* (L.). Journal of Fish Diseases, 33: 489-496.
6. Park, J.J., J.Y. Byoun, H.S. Choi, M.A. Park, M.G. Kwon and S.R. Kim, 2011. Papillomatosis on the skin of the wild marbled sole *Pleuronectes yokohamae*. Journal of Fish Pathology, 24: 213-223.
7. Park, S.W., J.H. Yu, E.B. Jung and J.H. Song, 2012. Histological observations on skin papilloma in wild Red halibut (*Hippogossoides dubius*). Journal of Fish Pathology, 25: 243-247.
8. Bekesi, L., I. Horvath, E. Kovacs-Gayer and G. Csaba, 1986. Demonstration of hepresvirus-like particles in skin lesions of European eel (*Anguilla anguilla*). Journal of Applied Ichthyology, 2: 190-192.
9. Hayes, M.A., I.R. Smith, T.H. Rushmore, T.L. Crane, C. Thorn, T.E. Kocal and H.W. Ferguson, 1990. Pathogenesis of skin and liver neoplasms in white suckers from industrially polluted areas in Lake Ontario. The Science of the Total Environment, 94: 105-123.
10. Premdas, P.D., T.L. Metcalfe, M.E. Bailey and C.D. Metcalfe, 1995. The prevalence and histological appearance of lip papillomas in white sucker (*Catostomus commersoni*) from two sites in central Ontario, Canada. Journal of Great Lakes Research, 21: 207-218.
11. Brown, E.R., J.J. Hazdra, L. Keith, I. Greenspan and J.B.G. Kwapinski, 1973. Frequency of fish tumors found in polluted watershed as compared to nonpolluted Canadian waters. Cancer Research, 33: 189-198.
12. Kinne, O., 1984. Diseases of Marine Animals. Vol. IV, Part 1. Biologisshe Anstalt Helgoland, Hamburg, FRG: John Wiley and Sons Ltd., pp: 541.
13. Möller, H. and L. Anders, 1986. Diseases and parasites of marine fishes, Möller Publications, Kiel, pp: 365.
14. Maccubin, A.E. and N. Ersing, 1991. Tumors in fish from the Detroit River. Hydrobiology, 219: 301-306.
15. Peters, G. and N. Peters, 1978. Temperature-dependent growth and regression of epidermal tumors in the European eel (*Anguilla Anguilla* L.). Annals of the New York Academy of Sciences, 298: 245-260.
16. Wolf, K., 1988. Fish viruses and fish viral diseases. Cornell University Press. Ithaca, pp: 476.
17. Mellergaard, S. and E. Nielsen, 1995. Impact of oxygen deficiency on the disease status of common dab *Limanda limanda*. Diseases of Aquatic Organisms, 22: 101-114.
18. Koops, H., H. Mann, I. Pfitzner, O.J. Schmid and G. Schubert, 1969. Cauliflower disease of eels. Archiv Fur Fischereiwissenschaft, 20(1): 1-52.
19. Schäperclaus, W., 1992. Diseases caused by pathogen. 3. Cauliflower disease of eels. In Fish Disease Vol II, A.A. Balkema, Rotterdam, pp: 329-338.
20. Huh, M.D., C.D. Thomas, P. Udomkusonsri and E.J. Noga, 2005. Epidemic trichodinosis associated with severe epidermal hyperplasia in largemouth bass, *Micropterus salmoides*, from North Carolina, USA. Journal of Wildlife Diseases, 41: 647-653.

21. Ottesen, O.H. and J.A. Olafsen, 2000. Effects on survival and mucous cell proliferation of Atlantic halibut, *Hippoglossus hippoglossus* L. larvae following microflora manipulation. Aquaculture, 187: 225-238.
22. Shephard, K.L., 1994. Functions for fish mucus. Reviews in Fish Biology and Fisheries, 4: 401-429.
23. Roberts, R.J. and A.M. Bullock, 1979. Papillomatosis in marine cultured rainbow trout, *Salmo gairdneri*, Richardson. Journal of Fish Diseases, 2: 75-77.
24. Roberts, R.J., 2001. Tumors of epithelial cell origin. In Fish Pathology. 3rd edition, W.B. Saunders, pp: 151-155.