Histopathological Study on Fin Ulcer Syndrome of Turbot (Scophthalmus maximus) Larvae in China

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Histopathological findings associated with fin ulcer syndrome of turbot larvae in China were reported. Turbot larvae with whitish and ulcerate fins, as well as anorexia with reddish viscera were sampled. By pathological observation, we found that pathological changes occurred in various tissues of the affected fish, such as fin, kidney, spleen and liver, causing focal degeneration and necrosis. Infiltration of red cells was noted within infected tissues, especially the spleen. These findings suggest that the pathogen agent is highly destructive, and capable of developing systemic infection.

Key words: Histopathology, degeneration, turbot.

Turbot (Scophthalmus maximus), a very important commercial species in Europe, was firstly introduced into China in 1992. After achieving technological breakthrough on the fry domestication and artificial breeding, the fish is extensively cultured along the coast in north China. However, with the rapid development of the turbot culture in China, several diseases occur, including bacterial, viral and parasitic diseases1-4.

Epidemiology investigation from three different farms (Laizhou, Penglai, Rushan) located along the coast of Shandong Province shows that fin ulcer disease commonly occurs in turbot larvae, which is caused by a bacterial agent resulting in acute death. The gross signs of the disease include the whitish coloration of dorsal and ventral fins, ulceration with no significant hemorrhage, as well as anorexia and darkened body for moribund animals. The larvae of 25 ~ 40 days is sensitive to the disease and the mortality in 5 ~ 7 days can reach 90%. Etiologically, we confirmed that V. anguillarum was associated with the disease5. In this paper, we give a detailed report on the histopathological changes caused by fin ulcer disease in farmed turbot larvae in China.

METHODS

Fish sampling

Twenty infected and healthy turbot larvae were random collected at July from the farm located in Penglai, Shandong province. Immediately after sampling, fish were transferred to tanks filled with recirculated water prior to dissection. The total length of the fish was measured, and gross signs were noted. Wet mounts from the fish skin scrapings, gills, fin and brain squash of moribund fish were observed under a microscope. After gross examination, individuals were fixed in 10% formalin for histopathological analysis.

Histopathological analysis

Formalin-fixed tissues were dehydrated by transferring them through a series of alcohols of increasing concentrations (70%, 1.5 h; 80%, 1.5 h; 95%, 45 min, two times) up to 100% alcohol (45 min, three times). Next, they were placed into dimethylbenzene for 20 min for two times. Then, they were put into melted paraffin (2×1.5 h, 58°C,
All these operations were performed using automatic tissue processor. The samples were then embedded in paraffin wax (Leica EG1160) and sliced with a microtom Leica RM2145 into 5–6 µm thick sections. The sections were stained using a routine hematoxylin and eosin (H&E) staining method. Examination was done under a Nikon E800 microscope.

RESULTS

Gross sign
In the naturally infected turbot larvae, fins became opaque, whitish, and rough. In addition, the infected fish larvae appeared weak, anorexic, with darkened bodies and reddish viscera (Fig. 1a). The cumulative mortality reached 90% within 5 ~ 7 days, usually without significant hemorrhage. The epidemic disease often occurred during the breeding season and early stocking time. In addition, its prevalence peaked during high temperatures (above 20°C) from May to September.

Histopathology of tissues
By histopathological observation, we found that pathological changes occurred in various tissues of the affected fish, causing focal degeneration and necrosis. Infiltration of red cells was noted within infected tissues, especially the spleen.

Bacteria were observed in the gill under scanning electron microscope (Fig. 1b). Degeneration and necrosis of the fin was observed. Part of the mesenchymal nucleus in the connective tissue was swollen (Fig. 1c). Melanism was widespread in the fin and redundant red blood cells were accumulated in the blood sinus (Fig. 1d).

Inflammatory changes were observed in the kidney. Necrosis of the hematopoietic tissue and hematoblast proliferation were detected. Denaturalization of the renal tubule could be seen. Swelling and mild vacuolation of PCT epithelial cells, edema in the interstitium and inflammatory cell infiltration were also observed (Fig. 2b).

The spleen hematopoiesis tissue showed severe degeneration and necrosis together with red blood cells infiltration. Hemolysis of red blood cell and damage of ellipsoid structure were observed. White pulp decreased significantly (Fig. 2d).

Columnar cells of the gills were destroyed due to congestion of gill lobules. The partial swelling of the gill epithelial cells in fusion with the gill filaments was observed, which may lead to blood circulation obstacle. Swollen salt secreting...
cells were seen, and they were away from the gill filament (Fig. 3b).

Swelling of liver cells was blurred. Expansion of blood sinus cavity was observed. Vacuoles occurred in the cytoplasm of serious lesion cells. Hydroupic degeneration of cells and focal necrosis occurred in some areas (Fig. 3d).

**DISCUSSION**

By histopathological observation, we found that pathological changes occurred in various tissues of the affected fish, and the kidney and spleen lesions were most serious. Renal lesions mainly appeared as the renal tubules and hematopoietic tissue lesions. Renal tubular lesions affected osmoregulation function of the fish, which might result in losing too much water. Hematopoietic tissue necrosis affected hematopoietic function and might cause anemia of fish. The spleen hematopoiesis tissue showed severe degeneration and necrosis together with red blood cells hemolysis and degeneration, which further destructed the hematopoietic function of fish.
the fish. Liver cells showed hydropic degeneration in the earlier stage, and with aggravation of the disease, focal necrosis appeared. As organism's three big metabolisms relied on the regulating of the liver, the lesion of liver could affect material metabolisms. Meanwhile, liver detoxification function would be weakened or even lost, leading to biological toxin accumulation. Multiple organs lesions and necrosis might be the leading cause of death in the fish.

To date vibriosis is known to affect almost 50 species of cultured and wild fish. In China, vibriosis caused by *V. anguillarum* has been one of the most common bacterial diseases in marine fish. There are multiple infection routes of *V. anguillarum* infection, mainly including the skin, gill, lateral line and intestinal tract. Olsson et al. showed that intestinal infection was the most important point of entry of *V. anguillarum* infection. It can survive and reproduce in the intestine, polluting water by waste emissions. In trouts inoculated by *V. anguillarum* immersion, pathological investigations reveal the nature of the gill lesions as well as the increase in eosinophilic granular cells (EGCs) and degranulation in gills. They suggest that gills during immersion may be the primary site of the bacterium entry. In our study, we found the presence of bacteria in the fin, suggesting that the entry route of the bacteria could be the fin lesions and its subsequent spread into the body was perhaps via blood vessels.

Environmental stress was believed to play an essential role for the occurrence of vibrio disease in a variety of marine water fishes held in aquarium. Stress factors including sudden increase in the water temperature and/or antibiotic exposure might be considered as triggers for the present outbreak of the disease. Moreover, high stocking density and poor management might also be associated with the occurrence of the disease. Good health management was essential to control bacterial contamination, dispersion, propagation, and infection. Probiotics was an effective alternative to obliterate the possible contamination.

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