

# Some histopathological aspects of streptococcosis in cultured rainbow trout (*Oncorhynchus mykiss*)

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## Abstract

Streptococcosis in cultured rainbow trout was first reported in northern Iran and subsequently in Fars province, southern Iran. Diseased fish showed darkening of the skin, abscess on the skin, exophthalmia with haemorrhages and typical septicaemic lesions inside body organs. Histopathological investigations showed abscess formation inside the abdominal muscles with coagulation necrosis in the centre; skin tissue with proliferation of mucus cells and spongiosis; eye tissue with hyperaemia, sub-retinal haemorrhages, inflammation in the vitreous and haemorrhages in the posterior chamber of the eye; kidney with glomerulo-nephritis and thickening of the basement membrane of glomerulus capsule; intestine with oedema in tunica submucosa and sloughing of the intestinal mucosa, and liver tissue with focal necrosis, hyperaemia and lymphocyte infiltration.

## Introduction

Streptococcosis is a systemic disease affecting both marine and freshwater fish worldwide. Streptococci were isolated from Persian Gulf grouper with severe skin lesions cultured in sea cages (Peighan *et al.*, 1996) and the disease was first reported in rainbow trout broodstock cultured in Mazandaran province, northern Iran (Ghiasi *et al.*, 2000). Since then it has been observed regularly in Fars province, southern Iran in cultured rainbow trout of both growing and market size (Akhlaghi and Keshavarzi, 2002). The causative organism was identified as *Streptococcus iniae*-like bacterium. Streptococcosis occurred mainly in summer and autumn in freshwater aquaculture sites with elevated water temperatures (in average 18° C), and it was characterized by haemorrhagic septicaemia, bilateral exophthalmia with haemorrhages in eyes,

ocular opacity, skin blackening and petechial haemorrhages on body sides and fin bases. Internally, congestion and wide haemorrhagic enteritis were apparent.

The disease has previously been reported in the region: Saudi Arabia (Austin and Austin, 1999), Turkey (Diler *et al.*, 2001), Bahrain (Yuasa *et al.*, 1999) and Kuwait (Evans *et al.*, 2002). Little is known about the histological lesions in enterococcal infection of fish. The purpose of this study was to look at some histopathological aspects of streptococcosis in organs such as the muscle, skin, eye, kidney, intestine and liver of the diseased rainbow trout cultured in Iran.

## Materials and methods

Diseased moribund fish with clinical signs of skin darkening, exophthalmia and haemor-

rhages and some with furuncle type lesions of the skin on the lateral side of the body (Figure 1) were transported in large plastic bags to the fish disease laboratory.

After a blow on the head, fish were sampled. Small pieces of the liver, intestine, eye, kidney and skeletal muscle were first immersed in physiological saline, then in 10% buffered formalin for 2 days. Samples were dehydrated, sectioned and stained by haematoxylin and eosin (Roberts, 2001). Tissue sections were studied microscopically. Kidney samples were dissected with sterile equipment and plated on sheep blood agar to isolate the bacteria.

### Results

Histological survey of the muscle tissues revealed abscess formation inside abdominal parts. In addition, coagulative necrosis in the centre of the abscess with accumulation of neutrophils was seen (Figure 2).

Skin tissue showed proliferation of mucus cells, mild spongiosis of epidermis and increase in melanophore numbers (Figure 3).

Eye tissue sections revealed hyperaemia of the choroid, sub-retinal haemorrhaging, inflammation in the vitreous, presence of melanomacrophage cells in the retina, haemorrhages in the posterior chamber of the eye, lenticular fibre disruption, vacuolar formation accompanied by infection with numerous streptococci inside eye tissues and chambers were observed (Figure 4).

In kidney tissue of diseased rainbow trout with streptococcosis, the following changes were observed: glumero-nephritis with thick-

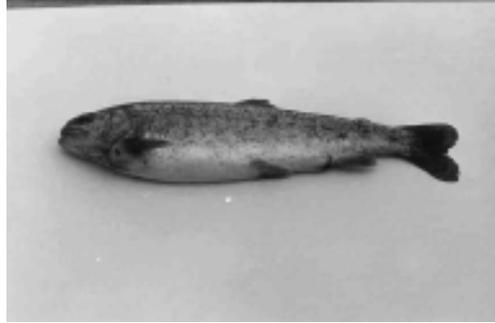


Figure 1. Lateral view of a rainbow trout, naturally infected with streptococcosis and showing abscess in muscles beside pectoral fin.

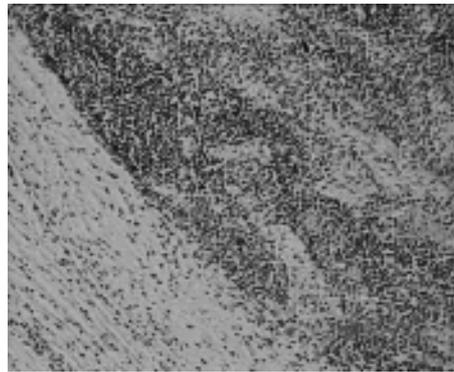


Figure 2. Myositis and abscess with coagulation necrosis (x 400).

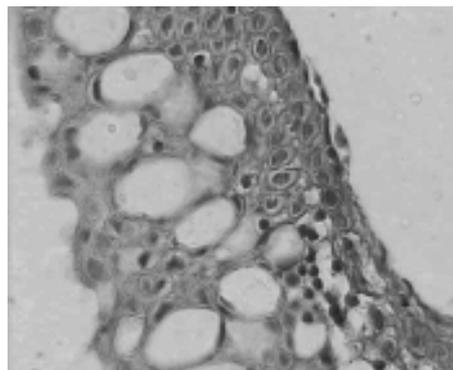


Figure 3. Mucus cell and melanophore proliferation, mild spongiosis (x 1000).

ening of the basement membrane and the glomerulus capsule, proliferation of the mesenchimal cells, hyperaemia, hyperplasia of haematopoietic tissue and bacteria engulfed by a melanomacrophage.

Sloughing of the intestinal mucosa, mild focal necrosis, loss of villar mucosa, exudate in the intestinal lumen and oedema in tunica submucosa of the intestine were also observed (Figure 5).

Liver of a diseased rainbow trout infected with streptococcus sp. showed focal necrosis with lymphocyte infiltration. Streptococci inside the hepatic sinusoid and hyperaemia in the liver tissue were also apparent (Figure 6).

Using the characterization method described by Kusda and Salati (1999), the bacteria isolated from the diseased fish were determined to be biochemically 95% identical to *Streptococcus iniae*.

### Discussion

Streptococcosis is the cause of significant economic losses to the world aquaculture industry and affects marine, brackish and freshwater fish species. Yasunaga (1982) reported the occurrence of *Streptococcus sp.* pathogen of cultured yellowtail (*Seriola quinqueradata*) in the muscle of fish destined for human consumption. Moreover other probable sources such as raw marine inedible fish used as fish-feed may be a route of entry for streptococcosis into the cultured rainbow trout industry (Zlotkin *et al.*, 1998). Various streptococcal/enterococcal species have been identified with the same clinical signs and gross pathology in fish. However, the histopathological features of the disease have not been well

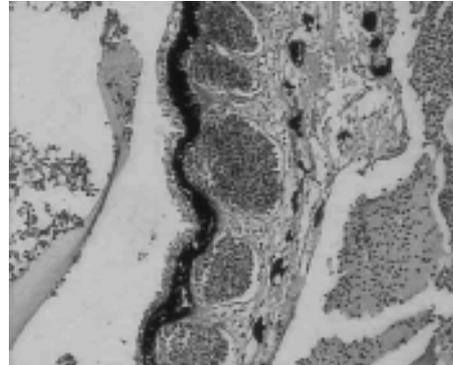


Figure 4. Sub-retinal haemorrhage and presence of inflammatory cells in the optic disc (x 400).

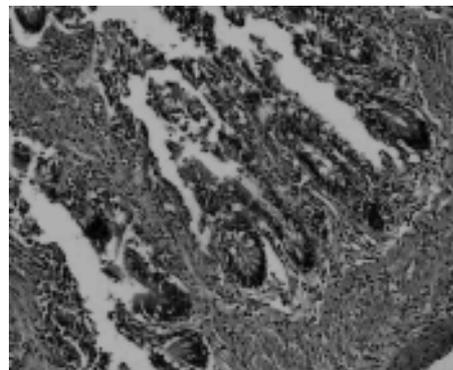


Figure 5. Oedema in tunica submucosa and sloughing of the intestinal mucosa (x 100).

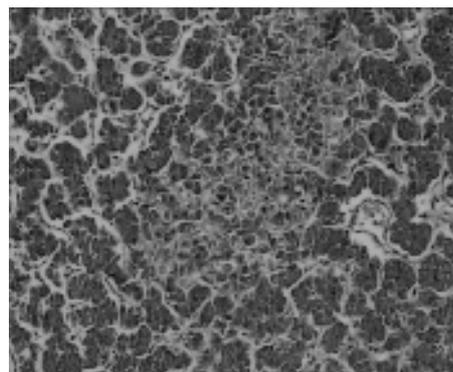


Figure 6. Focal necrosis, hyperaemia and lymphocyte infiltration in liver tissue from rainbow trout with streptococcosis (x 400).



studied. *Streptococcus* reported here was biochemically identical to *S. iniae* (a  $\alpha$ -haemolytic strain with no growth in 6.5% NaCl and pH=9.6) and caused an acute septicaemic disease with typical signs.

Results of this study clearly showed macroscopic abscesses in muscles, which was then confirmed by sectioning. Abscess with coagulation necrosis especially in the abdominal parts may happen when the fish is exposed to streptococci in adverse environmental condition. This was seen mainly in fish with the sub-acute form of the disease and those fish, which tolerated streptococcosis. Our first samplings were somehow confused with furunculosis type lesion or unknown abscesses, but sterile sampling of the abscess content revealed pure growth of streptococci. Skin histopathology revealed many changes in the tissue; proliferation of mucus of melanophore cells dominated, which causes the blackening of the skin during the course of the disease. Eye lesions in gross pathology usually represent extensive haemorrhages, which correspond to sub-retinal haemorrhage observed in the tissue section. This is similar to the inflammatory response associated with clinical exophthalmia. Similar results were reported by Chang and Plumb (1996) in tilapia and channel catfish experimentally infected by *Streptococcus sp.* Intestinal tissues usually show enteritis, in histological sections an oedematous sub-mucosa with sloughing of the intestinal mucosa was observed. Kidney tissues of the diseased fish showed extensive glomerulonephritis and liver tissue of the fish with streptococcosis displayed focal necrosis and hyperaemia with inflammation in liver tissues. Macroscopically, the liver pathology

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appears as a hyperaemic and inflamed tissue, which corresponds to the histopathology findings.

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