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A Pathological Study of Rainbow Trout Organs Naturally Infected with Enteric Redmouth Disease

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ABSTRACT

Enteric redmouth disease is a serious infectious disease in salmonids that causes important economic losses in many countries. Since last few years there were reports on the occurrence of the disease in Iran based on the clinical signs. The disease caused great losses in rainbow trout farms in Fars Province, Iran. Biochemical test of the isolated *Yersinia ruckeri* resembled high degree of similarity between the isolate and the *Yersinia ruckeri* cited in the literature as serotype O1. Diseased fish showed typical hemorrhages, blackening of skin, exophthalmia and wide hemorrhages on internal organs. By histopathological examination, diseased fish showed generalized bacteremia with inflammation, glomerulonephritis and necrotic foci in kidney, necrosis and mild telangiectasis in liver, telangiectasis in spleen, hemorrhages and hyperemia in the intestinal mucosa, myocardial degeneration, atrophy and edema in heart, atrophy of pancreas tissues and mucus cell and melanophores hyperplasia in skin of the infected fish.

Key words: *Yersinia*, pathology, fish, redmouth disease

INTRODUCTION

Enteric red mouth disease caused by *Yersinia ruckeri* was reported initially among hatchery reared rainbow trout in Idaho, USA. The subsequent dissemination of this disease throughout the United States was attributed in part to the transport of infected fish which may have become asymptomatic carriers shed bacteria from their intestines and established reservoirs for recurrent infections (Cipriano *et al.*, 1986).

Recently, the production of rainbow trout in Fars province is between the third main export items after wheat and corn. Fars province, one of the 28 provinces in Iran situated nearly in South with numerous springs provided aquaculture industry to flourish. Also production of rainbow trout has been developed during last few years in different areas in Iran. Recirculation systems for intensive fish culture are increasing now days. Infectious diseases are being emerging due to imposed stress factors. Enteric Redmouth Disease (ERM) has been one of the most significant diseases in salmonid aquaculture with cumulative losses of more than 50% of fish population in many countries (Arias *et al.*, 2007; Austin *et al.*, 2003; Tobbäck *et al.*, 2010). ERM has been diagnosed for more than 30 years ago and commercially vaccines are available in European countries (Fernandez *et al.*, 2007). Since 2002 there were unpublished reports on the occurrence of the disease in Chahar-Mahal Bakhtiary province in the center of Iran and then in Fars. The number of mortalities was significantly greater in water recirculation than flow-through system

farms. The occurrence of the disease in 2002 and 2003 in cold and warm seasons are estimated to be the same, however, adverse environmental conditions have intensified the severity of the disease. Since last few years, Soltani *et al.* (1999) reported a yersiniosis-like infection in farmed rainbow trout. Also Soltani and Tarahomi (2002) reported on the isolation of highly virulent *Yersinia ruckeri*-like bacterium from diseased rainbow trout cultured in Tehran province with clinical signs of the disease. Roozbahanim *et al.* (2009) has designed a diagnostic kit for yersiniosis in the trout fish in Iran.

The aim of this study was to look at some of histopathological changes in rainbow trout organs during the course of enteric redmouth disease. Such study in the literature is scarce and could elucidate the pathogenicity of the disease.

MATERIALS AND METHODS

Diseased fish from a north-western fish farm of Fars province with typical sign of ERM resembling heavy hemorrhages on lips, mouth and pharynx as well as the skin darkening of body and a septicemic hemorrhages on internal organs (Fig. 1, 2) were transported in large plastic bags to the fish disease laboratory.



Fig. 1: Diseased rainbow trout with heavy hemorrhages on lips, mouth and pharynx as well as in eyes

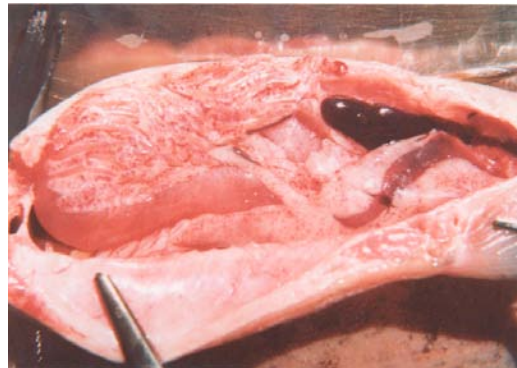


Fig. 2: Diseased rainbow trout with typical ERM signs on viscera, petechial hemorrhages affecting stomach, pyloric caeca and intestine

After a blow on the head, fish were sampled. Small pieces of the liver, spleen, intestine, kidney, heart and skin were first immersed in physiological saline then inside 10% buffered formalin for 2 days.

Five micron paraffin embedded slides were prepared and stained by hemotoxyline and eosin. Tissue sections were studied microscopically. Sterile kidney and spleen samples were taken and plated on sheep blood agar to isolate the bacteria.

RESULTS

Gross pathology of rainbow trout affected by ERM showed darkening of the skin, reddening of the tongue, throat and mouth due to subcutaneous hemorrhages. Hemorrhages on body surfaces such as around the mouth, on the operculum, on the base of fins and in eyes were seen in all of the fishes. Internally, congestion of the blood-vessels throughout the peritoneum and petechial hemorrhages affecting visceral organs were also noted.

Histopathological survey of liver revealed focal necrosis, telangiectasis and congestion with presence of bacterial colonies and infiltration of inflammatory cells in the liver tissue. In spleen telangiectasis was prominent (Fig. 3).

Histopathology of the intestine showed haemorrhages in the tunica muscularis and serosal surface, hyperemia and hemorrhages in tunica mucosa, presence of sloughed epithelial cells and RBC in the intestinal lumen, bacteria in lumen of congested vessels, congestion of tunica serosa and tunica muscularis and hemorrhage in fat around the intestine (Fig. 4).

Kidney tissues in histopathology showed severe haemorrhage, hyperplasia of interstitial hematopoietic tissue, tubular necrosis and glomerulonephritis (Fig. 5).

Study of histopathological slides of the heart of rainbow trout's infected with ERM revealed a myocardial degeneration, hemorrhage, infiltration of leukocytes, edema and atrophy (Fig. 6).

The heart of rainbow trout naturally infected with ERM showing infiltration of leukocytes, edema, atrophy and myocardial degeneration (Fig. 6).

Histopathological study of pancreas showed hemorrhages in peripancreatic lipid tissues and atrophy of pancreas tissues (Fig. 7).

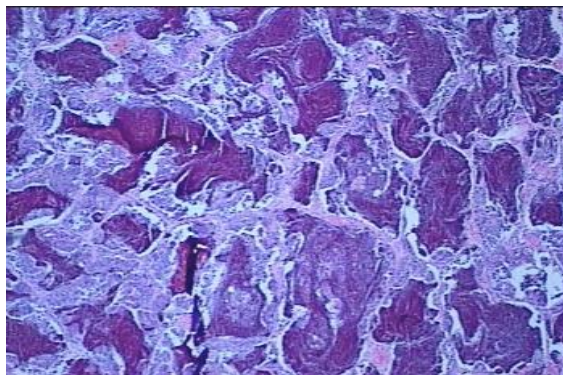


Fig. 3: Telangiectasis in the spleen of rainbow trout naturally affected by ERM (X 400)

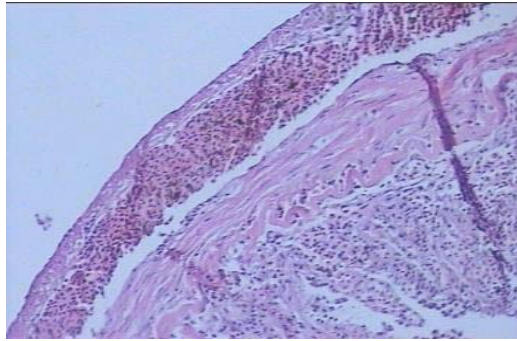


Fig. 4: The intestine of rainbow trout naturally infected by ERM with severe congestion of tunica serosa and tunica muscularis

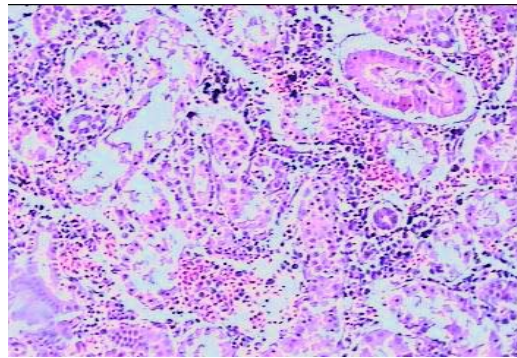


Fig. 5: The kidney of rainbow trout infected by ERM showing interstitial hemorrhage hyperplasia of interstitial hematopoietic tissue and tubular necrosis

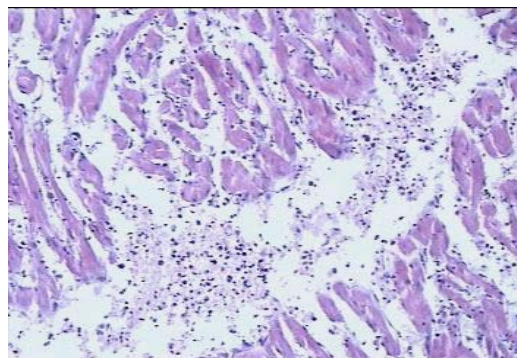


Fig. 6: The heart of rainbow trout naturally infected with ERM showing infiltration of leukocytes, edema, atrophy and myocardial degeneration

Pathological examination of the skin of the rainbow trout naturally infected by ERM showed an apparent increase in mucus cells and melanophores (Fig. 8).

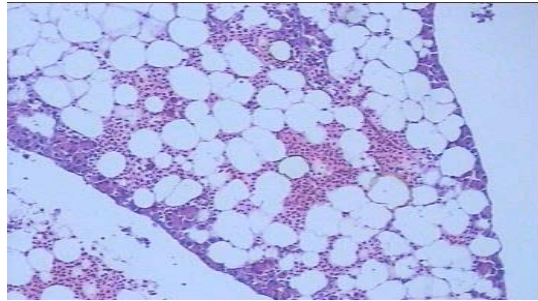


Fig. 7: The pancreas of diseased rainbow trout with ERM showing hemorrhages in peripancreatic lipid tissues and atrophy of pancreas tissues

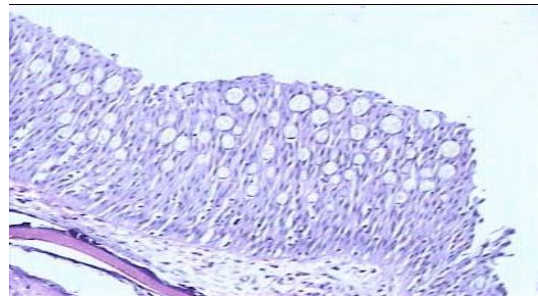


Fig. 8: The skin of rainbow trout naturally infected with ERM showing mucus cells and melanophores hyperplasia

DISCUSSION

Yersiniosis has a worldwide distribution and is considered endemic in most trout producing countries and in natural hosts in marine habitats (Khalil *et al.*, 2006; Surendraraj *et al.*, 2009; Akinjogunla *et al.*, 2011). Enteric redmouth disease is an acute or chronic fish disease which has caused significant losses in aquaculture associated with intensive culture and poor water quality. It seems that younger rainbow trout are more susceptible due to their undeveloped immune response. Diagnosis for a preventive strategy is important. Not much case reports have been published on the pathology of this bacterium for rainbow trout. However, a consistent feature of enteric redmouth disease mentioned by Roberts (2001) is generalized hemorrhagic petechia over abdominal organs with swelling and reddening of the kidney and spleen that is similar to our findings. Present results showed necrotic foci and infiltration of leukocytes with associated hemorrhage which is similar to the finding of Tobback *et al.* (2007) and Roberts (2001).

The disease happens in temperature more than 10°C and in a stressed condition (Surendraraj *et al.*, 2009). In different species of fish such as common carp, a chronic disease characterized by prominent peritonitis and enteritis, exhaustion of the erythroid, granuloid and lymphoid components in haematopoietic kidney tissue as well as focal degeneration and necrosis in organs that indicated a high sensitivity of carp to intraperitoneal infection with a relatively low dose of *Y. ruckeri* has been documented (Berc *et al.*, 1999).

Yersiniosis is clinically characterized by bleeding in the base of fins, inside and around the mouth, bilateral exophthalmia with or without hemorrhage, peri ocular and peri oral hemorrhage. Internally, small hemorrhages are noted in liver, pancreas, large intestine, swim bladder and lateral muscles. In diseased fish, the spleen becomes enlarged and dark and the intestine is inflamed and filled with purulent material. Hemorrhagic inflammation in hindgut, accumulation of fluid in stomach and intestines, enlargement of hematopoietic organs, kidneys and splenomegaly are among the pathologic landmarks (Avci and Birincioglu, 2005; Roozbahanim *et al.*, 2009).

Noga (1996) noted that bacterial colonization of vascularized tissues caused telangiectasis of gill, kidney, liver, spleen and heart that can lead to necrosis of the hematopoietic tissue causing anemia. Necrosis and sloughing of the gastrointestinal tract were also noted that is in coordination with our findings. Severe necrosis of hematopoietic tissue of kidney is also reported by other workers (Furhman *et al.*, 1983; Horne and Barnes, 1999).

Infected fish with *Y. ruckeri* (O1 serotype) usually estimated up to 70% mortality. Diseased fish showed typical hemorrhages, blackening of skin, exophthalmia and wide hemorrhages on internal organs with typical histopathological changes demonstrated in this study. Serotype O1 has a worldwide distribution while serotype O2b is restricted to North America and Baltic Countries (Welch *et al.*, 2011).

In the expansion of aquaculture industry in different provinces of Iran with moderate geographical climates, it seems more work is required to estimate the infected farms. There is also a need to develop a vaccine against enteric redmouth disease for the prevention of the disease.

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