NOTE

A histopathological study of Hexamitiasis in farmed rainbow trout (Oncorhynchus mykiss) fry in Turkey

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Abstract
The flagellated parasitic protozoan Hexamita salmonis were found in the upper intestine and pyloric caeca in large numbers in the farmed rainbow trout fry. Generally, infected fish fry showed significant level of mortality, exophthalmia and abdominal distension. Histopathologically, invasion of the pyloric caeca or upper intestine epithelium by the parasites and severe necrotizing of enteric mucosal epithelium and perforation of the serosal surface was observed. Inflammatory response was generally lacking in the gut wall, but large granulomatous lesions in some perforated area were found. In the histological sections, characteristic paired anterior nuclei of the parasites were easily recognized.

Hexamitiasis is the internal flagellate parasitosis of fish, notably in young salmonids, also in carp, aquarium species and various marine fish (Amlacher, 1970; Needham & Wootten, 1978; Stoskopf, 1993; Toja & Santamarina, 1998; Noga, 2000). Characteristic signs include anorexia, dark coloration, fecal pseudo cast and pinhead (Sano, 1970; Mo et al., 1990; Poppe et al., 1992) and swollen bellies (Ferguson, 1979, 1992).

This parasite does not seem to occur frequently in farmed rainbow trout in Turkey. There is only one report about the seasonal prevalence and intensity of the parasites in the rainbow trout, aged one year and up (14-181 g) raised in the farms located in the south eastern Black Sea region in Turkey (Ogut & Akyol, 2005).

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In May 2007, increased mortality was noted in USA originated rainbow trout fry (0.5-1 g) raised on a fish farm located in the Marmara region of the north western Turkey. Generally, sick fish (0.5-1 g) showed high mortality (up to 20%). They were noted to remain at the bottom of the raceways near to the side. Characteristic signs included dark coloration of the skin, anorexia and fecal pseudo cast. Most fish usually had exophthalmia and showed an abdominal distension by a clear ascetic fluid. Heavily infected fish were weak, listless, anorexic and emaciated, so that the head appeared large with respect to the body (pinhead fish).

Fifteen rainbow trout fry (Oncorhynchus mykiss) (4-5 cm, 0.5-1 g) from this farm were sampled for bacteriology, parasitology and histopathology. Fish samples were killed by decapitation. Tissues (kidney, liver) from affected fish were inoculated onto TSA (Merck, Darmstadt, Germany), and plates were incubated at 22 °C for 24-48 h. The morphological and physiological characteristics of bacterial colonies from each plate were determined together with their biochemical characteristics using API 20E, and bacteria was identified following Bergys Manual (Holt et al., 1994) and Austin & Austin, (1987, 1999).

Samples of tissues from liver, kidney, spleen, gut, gills, heart and skeletal muscle immediately fixed in 10% buffered formalin and processed for paraffin embedding. Histological sections (5µm) were stained with haematoxylen and eosin, Giemsa stain and examined by light microscopy (Culling, 1963; Bullock, 1978). Gut samples and gall bladder removed and contents examined on a slide by light microscopy. Also both skin smears and squash preparations of the gill samples examined by light microscopy (Bullock, 1978).

Microscopic observations of a wet smear of intestinal contents revealed the presence of large numbers of actively motile, typically pear-shaped Hexamita salmonis with their characteristic paired anterior nuclei within the intestinal contents of the all sampled fish. Large numbers of Costia sp. were also observed in the coverslip covered squash preparations of some gill samples but no protozoan parasite observed in the skin smears.

Bacteriological, only the yellow greenish colored diffusible bacteria colonies was isolated on TSA from only a few infected fish liver. The bacteria colonies were composed of motile, Gram-negative, rod cells. They were resistant to O/129 (10µg, 150µg) and showed fluorescence characteristic under the UV light. According to the morphological, chemical and physiological characteristics of bacterial colonies were and identified as Pseudomonas fluorescens.

Histological examinations revealed the presence of the parasites within mucosal epithelium of the intestine (Figure 1) and the pyloric caeca and quite often, the parasites were found tightly packed together within the sub mucosal tissue of the intestine (Figure 2a) and gastric wall (Figure 2b). The parasites were also observed within the gastric gland mucosal epithelium (Figure 2b). Characteristic, paired anterior nuclei of the parasites were easily recognized in the histological sections. Pyloric
caeca and intestinal mucous membrane was usually necrotic and sloughed into the lumen and necrotizing mucosal epithelium was invaded with the parasites. Necrotic intestinal wall also showed perforation but not all fish. Inflammatory response was lacking in many of the affected areas while others demonstrated a granulomatous response within the sub mucosal tissue of the perforated serosal surface of the intestine (Figure 3a, b). In addition to these findings affected fish included diffuse vacuolar degeneration and necrotic cell areas in the liver, and depletion of the red and white pulp cells of the spleen. The gill epithelium was showing hyperplasia and hemorrhage. Most of the fish had severe skeletal muscular degeneration, but no inflammatory reaction or parasitic cells were found in the skeletal muscle.

In this study the clinical sings, gross pathology and results of a wet smear of intestinal contents revealed large numbers actively moving *Hexamit salmonis* were present in the gut as described by other workers (Sano, 1970; Ferguson, 1979; Mo *et al.*, 1990; Poppe *et al.* 1992; Tojo & Santamarina, 1998). Presence of the parasites in the mucosal epithelium of the gut was confirmed by examination of the H&E and Giemsa stained paraffin sections of the gut where large numbers of the parasites tightly packed together within sub mucosal tissue of the intestine and gastric wall as described by Poppe *et al.* (1992) in sea-caged Atlantic salmon. However, we easily recognized their characteristic paired anterior nuclei in the histological sections.
Although, no acute inflammatory reaction were present in the intestinal mucosa infested with the parasites in rainbow trout fry as described by Ferguson (1979), we observed necrotizing mucosal epithelium sloughed of into gut lumen as described by Sano (1970) in the fingerling rainbow trout with hexamitiasis. Additionally, perforation of the intestinal wall on serosal surface with a large granuloma was also observed in some affected fry as described in Siamese fighting (Ferguson, 1992). But we did not observed any granulomatous lesions in the liver and kidney as described by Mo et al. (1990) and Poppe et al. (1992) in Atlantic salmon in the seawater farms fish in Norway.

However we observed diffuse vacuolar degeneration and necrotic cell areas in the liver. Most affected fish had muscular degeneration as described by Mo et al. (1990).

In conclusion, severe hexamitiasis associated with severe diffuse necrotizing slough of enteric mucosal epithelium invaded with the large numbers diplomonad flagellate parasites, Hexamita salmonis and perforation of enteric wall caused high mortality in the affected rainbow trout fry. The presence of Pseudomonas fluorescens and Costia sp. may have been additional factor in the accelerating the rate of morbidity and mortality in the rainbow trout fry suffering from hexamitiasis.

Reference


