Pathomorphology of experimental *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*)

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**ABSTRACT**

In the present study, an experimental infection with *Streptococcus iniae* was conducted with tilapia (*Oreochromis niloticus*). The bacterial agent was injected to the fish by intraperitoneal inoculation at a dose of 1 × 10^7 cfu/mL. Grossly, haemorrhages on tail and pectoral fins, hyperaemic and prolapsed anus, darkening and bleeding in the gills and exophthalmos in the eyes were identified in fish. Multifocal whitish foci in the liver, spleen and kidneys was observed with ascites. Histopathological changes of tissues of tilapia were studied and evaluated. Gill tissues of infected fish showed intense lymphocyte and macrophage infiltrations. In addition, epithelial cell separation and lamellar fusion with edema, lamellar curling and aneurysms were detected in gill tissue. Sinusoidal dilatation, lymphocyte infiltrations, congestion and degeneration in liver hepatocyte cells have been identified. In kidney degeneration of glomerulus and tubule cells and dense lymphocyte infiltrations were observed. Dense bacterial clusters were observed in the sinusoids of the spleen, while lymphocytes and macrophage infiltrations were found in the heart and intestinal tissues.

**Key words:** Histopathology, Infection, *Oreochromis niloticus*, *Streptococcus iniae*.

**INTRODUCTION**

Streptococcosis is one of the most important bacterial infections in terrestrial animals and humans. In the aquatic animals and especially in the farmed fish species, these infections, which are seen with the same name, are formed by bacteria of *Streptococcus*, *Lactococcus* and *Vagococcus* groups. It is reported that this infection is causing many commercial losses in the aquaculture sector all over the world (Suanyuk *et al.*, 2010, Shoemaker *et al.*, 2000, Amal and Zamri-Saad, 2011). It was first identified in the fish species of the *Seriola quinqueradiata* species grown in Japan in the 1950s (Bromage, 2004). Over 50% of mortality levels have been reported in farms where the infection occurred. Trout, mullet, snake fish, carp and sea bass are other fish species that are often identified with the disease. This infection has been identified in countries such as Australia, South Africa, Singapore, Kuwait, Israel, Brazil, Japan, Thailand and Turkey (Perera *et al.*, 1998, Rodkhum *et al.*, 2012, Diler *et al.*, 2002, Çağırgan 2004, Özer *et al.*, 2008). It is stated that the highest loss of tilapia farms is caused by streptococcus infections. Streptococcus species were more likely to occur in tilapia than other cultured fish (Dodson *et al.*, 1999). The disease is septicemic and has also been observed in wild fish species. It is considered to be one of the aggressive pathogenic bacterial groups in the aquatic environment and is not considered an opportunistic pathogen (Raissey *et al.*, 2012). *S. iniae*, one of the important species causing Streptococcosis, was first isolated from *Inia geoffrensis*, a freshwater dolphin in the Amazon River in 1976. It was later found in many fresh, brackish and salt water fish (Pier and Madin, 1976, Amal and Zamri-Saad, 2011). It was also isolated from humans in the north of the United States in 1991 (Sun *et al.*, 2007). It is considered to be a zoonotic factor leading to infections such as bacteremia, cellulitis, meningitis and osteomyelitis in humans (Amal and Zamri-Saad, 2011). There have been investigations on the formation of experimental infections using strains of streptococcus group in tilapia using *S. iniae*, *S. agalactia*, *S. dysgalactia*, *S. pluranimalium*, *S. anginosus*, *L. garviae*, *Enterococcus gallinarum* and *E. faecalis* species (Shoemaker *et al.*, 2000, Evans *et al.*, 2000, Chen *et al.*, 2004, Rattanachaikunsopon ve Phumkhachorn, 2009, Pretto-Giardano *et al.*, 2010, Abdullah *et al.*, 2013, Badr *et al.*, 2012, Fawzy *et al.*, 2014).

Histopathological examination is a valuable tool in determining the effects of some toxic substances and pathogens in various organs of living organisms. At the same time, it is an important diagnostic method for the identification of bacterial diseases. The examination of histopathological findings in the diagnosis of clinical diseases of aquatic organisms is limited compared to other animal species (Alsalid *et al.*, 2013). In this study, histopathological findings in the tissues of Nile Tilapia (*Oreochromis niloticus*) experimentally infected with *S. iniae* were investigated.

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MATERIALS AND METHODS

The experiment was carried out in 192 L glass aquarium (40 x 120 x 40 cm). Photoperiod during the experiment were 12L:12D and aeration was provided by using a central ventilation system. Water temperature as 25 ± 1 °C, dissolved oxygen and pH were maintained at 6.3 ± 1.4 and 7.45 ± 0.8 respectively in the aquariums. Fish were obtained from Cukurova University Fisheries Research and Production Facility and adapted to the laboratory environment for a period of three months. During the acclimatization period, the fish were fed twice daily with commercial fish feed at a rate of 2% of their body weight until 2 days prior to experiment. The fish were starved for 24 hours before infection. In the study, a total of 36 Nile Tilapia were used, 18 tests and 18 controls with average weights of 81.33 ± 7.31 g and mean lengths of 17.05 ± 0.57 cm. Fish were divided into 6 groups of 6 fish in each group.

The serotype of \textit{S. iniae} ARS-10 was obtained from the Research Laboratory of Aquatic Animal Health in the United States Agricultural Research Service. The serotype was incubated at 35°C in the Todd Hewitt Broth (THB) media and cultures were stored at 4°C.

The number of bacteria was determined using surface counting method (Gürgün and Halkman, 1990). 1 ml of \textit{S. iniae} (1x10^3 cfu/ml) was administered intraperitoneal to fish. The same amount of sterile physiological water was inoculated to the fish in the control group. Gill, liver, kidney, spleen, brain, eye, heart, gastrointestinal and gonad tissues were collected and fixed in 10% neutral buffered formalin. After 24 h standing, washing, dehydration, transparency and paraffin embedding procedures were made for the tissues. The tissues in the form of paraffin blocks were cut with a thickness of 5 μ and stained with Hematoxylin and Eosin (H&E).

RESULTS AND DISCUSSION

Within 48-96 hours after inoculation of the bacterium into the fish, the entire test group of fish was observed to have died (Table 1). The macroscopic examination of the fish revealed haemorrhage on tail and pectoral fins, hyperaemic and prolapsed anus (Fig. 1A, B), darkening and bleeding in the gills and exophthalmos in the eyes. In the liver, spleen and kidneys, multifocal mild whitish foci was observed with ascites in the abdomen (Fig. 1C).

Histopathological examination showed intense lymphocyte and macrophage infiltrations in the primer and secondary lamellas of the gill tissues of fish. In addition, epithelial cells were separated from the ends of the secondary lamella and edema and lamellar curling and lamellar fusion with aneurysms were detected in gills (Fig. 2A, B). In liver sinusoidal dilatation, lymphocyte infiltrations in the sinusoids, congestion and degeneration in hepatocyte cells have been recorded (Fig. 2C, D, 3A). In kidneys, glomerular degeneration, degeneration of tubular epithelial cells and dense lymphocyte infiltrations in hematopoietic tissue were observed (Fig.3B). The presence of \textit{Streptococcus iniae} clusters located within the spleen sinusoids has been determined (Fig. 3C). Lymphocytes and macrophage infiltrations were found in the heart muscle and intestine (Fig. 3D, E, F).

In fish, different parameters are being investigated to identify the disease in bacterial infections. In this study, the clinical appearance and lesions of infection with \textit{S. iniae} in tilapia were investigated. There are various investigations of histopathological disorders of \textit{S. iniae} serotypes in tilapia (Bowser et al., 1998, Chen et al., 2007, Badr et al., 2012).

Table 1: Mortality rates in experimental \textit{S. iniae} infection in Tilapia.

<table>
<thead>
<tr>
<th>Dose (cfu / mL)</th>
<th>Time (Hour)</th>
<th>Dead fish / Total fish</th>
<th>Mortality (%)</th>
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<tbody>
<tr>
<td>1 x 10^3</td>
<td>48</td>
<td>10 / 18</td>
<td>55.55</td>
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<tr>
<td></td>
<td>72</td>
<td>3 / 18</td>
<td>16.66</td>
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<tr>
<td></td>
<td>96</td>
<td>5 / 18</td>
<td>27.77</td>
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Fig 2: (A) Gill showing lymphocyte infiltration (a) macrophage infiltration (b) epithelial cell separation and oedema (c), lamellar curves (d), aneurysms (e) in the secondary lamella, x200 (B) Gill showing macrophage and lymphocytic infiltration (b), lamellar curves (d), lamellar fusion (f) in the primer lamella, x200 (C) Liver showing sinusoidal dilatations (arrows) and lymphocyte infiltrations in the sinusoids, x200 (D) Liver showing moderate congestion and diffuse moderate vacuolar degeneration of hepatocytes (a, b), x200 (Hx & E) (bar = 10 μm).

Fig 3: Liver showing (A) congestions (a) degeneration of hepatocytes (arrows), x400 (B) Kidney showing glomerular degeneration and necrosis (a), degeneration of renal tubular epithelial cells (b) dense lymphocyte infiltrations in hematopoietic tissue in the kidney, x200 (C) *Streptococcus iniae* clusters (*) located in the sinusoids in the spleen, x1000 (D) Macrophage infiltrations (arrows) in the heart muscle, x200 (E, F) Lymphocyte (*) and macrophage infiltrations (arrows) in the intestinal tissue, x200 (Hx & E) (bar = 10 μm).
Doses ranging from $10^2$ to $10^8$ cfu / mL have been tried in experimental <i>S. iniae</i> infections due to serotype differences (Shoemaker et al., 2000, Bromage and Owens, 2002, Russo et al., 2006, Rahmatullah et al., 2017). In this study, $1 \times 10^3$ cfu / mL of <i>S. iniae</i> ARS-10 serotype was inoculated. The clinical appearance of infected fish was consistent with the clinical appearance of infections with different serotypes (Agnew and Barnes, 2007). In <i>S. iniae</i> infection in tilapia; hydroid degeneration of the hepatocytes, necrosis and lymphocyte infiltrations, tubular vacuolation of the kidney, oedema, necrotic areas and melanomacrophage centers as well as inflammation of brain tissue, lymphocytes and macrophage infiltrations and bacterial clusters have been detected (Badr et al., 2012, Baums et al., 2013). Haemorrhages and macrophage infiltrations have also been identified in the digestive tract (Hossain et al., 2014). The changes that we observe for the liver, kidney and digestive tract were in line with previous studies except for a few.

Perera et al. (1998), reported that histopathological examination of hybrid tilapia revealed brain meningitis, granuloma formation of the liver, epicarditis and myocarditis. Chen et al. (2007), recorded clusters of lymphocytes and macrophage infiltrations and bacterial masses in internal organs. Bowser et al., (1998) found no histopathologic changes in brain and eye tissue. In our study, no histopathological disorders were observed in brain and eyes too. It has been found that <i>S. iniae</i> causes similar histopathological disorders in other fish species except tilapia (Creeper and Buller, 2006, Aamri et al., 2010, Nguyen et al., 2001, Keirstead et al., 2014).

Histopathologic findings are thought to be caused by serotypes and administered dose differences in various tissues. In addition, histopathological changes in the liver have been reported to be caused by dysfunctions in the tissues and defects in the function of detoxification, while changes in the kidney and spleen are caused by the suppression of the lymphoid system of bacterial toxins (Zhu et al., 2015). Biochemical findings of <i>S. iniae</i> infections also suggest that hepatocellular damage and enzymatic degradation may result in these histopathological changes (Oda et al., 2016).

<i>S. iniae</i> also has a bacterial effect with potential to adversely affect human health. Although much work has been done on this species to date, more research is needed to reduce the harmful effects of this bacterium. The development of effective vaccination against multiple serotypes seems to be the most appropriate solution to control <i>S. iniae</i>. For this purpose, further research is needed in order to fully understand the serological diversity and the epidemiology of the disease (Agnew and Barnes, 2007). The histopathological changes recorded in this study would be one of the important tool for diagnosis of streptococcus derived bacterial diseases in tilapia farming.

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REFERENCES


