Research Article

Histopathological features of infection by *Streptococcus iniae* in Persian sturgeon, *Acipenser persicus*

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Abstract

The present study evaluated the susceptibility of juvenile Persian sturgeon, *Acipenser persicus*, to *Streptococcus iniae*. Fish were intraperitoneally injected with 4.7×10^4 CFU fish^{-1}. The control fish received sterile normal saline (PBS) intraperitoneally. The fish were monitored for clinical signs, and mortalities were recoded daily for two weeks post-challenge. Moribund and/or freshly dead fish were immediately removed for biopsy and/or necropsy, gross evaluation, and histopathological examinations, and the cause of mortality was confirmed by re-isolation of the *S. iniae* from kidney or spleen using standard microbiological tests. Mortality rates were in ranged from 20.83 to 83.33% within 48 to 120 h post challenge. Hepatocyte vacuolization, focal necrosis and melano-macrophage aggregation were dominant histological findings in affected fish. Severe meningitis with massive inflammatory cells infiltration and vacuolation were also detected in the brain layers. In addition, the kidney tissues exhibited severe necrosis, diffused tubular degeneration, hyaline exudates accumulation in tubular lumens, shrinkage of glomeruli, dilation of Bowman's space, cellular infiltration and interstitial tissue necrosis. Hyperemia and inflammatory cells infiltration in the base of primary lamella were the most common histopathological signs of gills. In the eye sections, congestion, hemorrhage and degeneration of the retinal layers were also observable. These histopathological findings show that juvenile Persian sturgeon is a highly susceptible species to *S. iniae* thus, raising a high risk of streptococcosis outbreaks in sturgeon aquaculture either in freshwater or marine environments.

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Introduction

Streptococcosis is one of the most common and important bacterial diseases accounting for variety of problems and economic losses in aquaculture sector (Soltani *et al*., 2008; Agnew and Barnes, 2007, Azad *et al*., 2012; Chen *et al*., 2020). The disease can be caused by various species of Gram-positive cocci of genus *Streptococcus* including *S. iniae, S. agalactiae, S. dysagacatiae, S. uberis,* and *S. parauberis,* among them *S. iniae* has been considered as one of more dominant pathogens in both freshwater and marine aquaculture species worldwide (Soltani *et al*., 2005; Agnew and Barnes, 2007; Russo *et al*., 2006; Chen *et al*., 2010). Sturgeons are valuable fish species with a great potential as valuable aquaculture species especially in case the offspring/population of these ancient species is under danger due to high illegal catching of their natural resources. In some regions such as Iran sturgeon aquaculture has been developed in different parts of the country where the water temperature is suitable e.g., behind the freshwater dams or in cages in the coastal areas of the Caspian Sea. Under such cultural conditions, thus, the chance of some disease outbreaks including streptococcosis can be dramatically increased. Although there are some reports related to sturgeons infection with *Streptococcus spp*, such as *Streptococcus dysgalactiae in Acipenser schrenckii* (Yang & Li 2009) or *Streptococcus iniae* in *Siberian sturgeon*, *Acipenser baerii* (Deng *et al*., 2017; Chen *et al*., 2020), Chinese sturgeon, *Acipenser sinensis* (Muhammad *et al*., 2020) and white sturgeon, *Acipenser transmontanus*, (Soto *et al*., 2017), There is no report of the susceptibility of Persian sturgeon to streptococcosis caused by *S. iniae*. Thus, the aim of the present study was to evaluate pathogenicity of *S. iniae* and its histopathological signs in juvenile Persian sturgeon.

Materials and methods

Fish and maintenance conditions

Persian sturgeon fingerlings weighing 7 ± 2 g, were collected from Shahid Marjani Sturgeon Propagation and Rearing Center (Agh Ghala, Iran). Fish were subjected to 3% NaCl bath (for 1 min) before being transferred to 2 × 2 × 0.5 m fiberglass tanks each containing 1000 l clean water at 80 fish per tank, and water flow rate was 8 L min^{-1}. Fish were fed commercial food (BioMar Co. France) at 3% body weight twice a day. After 60 days rearing, (average weight 17 ± 3 g), fish were stocked in 6 plastic tanks each containing 12 fish and were acclimatized for two weeks before the experiment. Water quality including water temperature, pH, salinity and dissolved oxygen were 24 ± 2˚C, 8.15, 3 ppt and 7.1 ± 0.2 mg L^{-1}, respectively.

Bacteria and challenge

Streptococcus iniae 191B previously isolated from rainbow trout farms (Soltani *et al.* 2005) supplied by Department of Aquatic Animal Health, Faculty of Veterinary Medicine of Tehran University was cultured on nutrient agar (Merck, Germany) with 5% defibrinated sheep blood at 28°C for 24 h. The colonies

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were harvested, and a homogenous suspension was prepared using sterile normal saline (NaCl 0.9%). Initial stocks of bacterial suspension were estimated by spectrophotometer (640 nm and OD = 1) and spread plate count was undertaken to determine the viable bacterial count equal to 4.7×10^7 CFU mL^{-1}. The fish were anesthetized using 100 mg L^{-1} clove oil (Sigma Chemical Co. USA). The treatment group in three replicates each fish was subjected to intraperitoneal injection of bacterium at 4.7×10^4 CFU $fish^{-1}$ and control group fish received 0.1 ml of sterile normal saline intraperitoneally. Fish were transferred to their original tanks and were kept for 14 days.

Histological assay

For histological study, tissue samples were taken from liver, kidney, brain, eye and gill, and were immediately fixed in 10% buffered formalin for at least 24 h before histo-technical processing. Routine histological methods were followed, and 5 µm tissue sections were stained using Harris's hematoxylin and eosin and studied using compound microscope. To confirm the cause of death, samples of kidney and spleen were cultured on blood agar incubated at 28 ˚C for up to 72 h for re-isolation of the bacterium (Roberts, 2012).

Results

Fish lost their appetite after 12 h and become anorexic within 24 h post-challenge. The first mortalities were seen 48 h after the challenge. The affected fish showed signs of lethargy, whirling, and up and down swimming. Petechiae were observed in various parts of the body including at the base of fins, under scales, under snout, and especially at the base of fish barbells. After 72 h, in most of the dead and moribund fish, there was hemorrhage and anal prolapse with vascular congestion or hemorrhage in the distal intestines. Also, bloody fluid was observed within the coelomic cavity without abdominal distention. In some fish, the gills were pale, particularly in hemi-branch and hydronephrosis was evident in the kidney. Fluid-filled intestinal lumen with hemorrhage in the distal part of intestine, and pinpoint hemorrhages in eyes were recorded in fish surviving after 14 days. In two cases, bilateral blindness with corneal opacity in eyes plus sunken eyes was observed, and in some cases, fish exhibited signs of scoliosis and lordosis 10 days post-infection. *S. iniae* re-isolated from kidney and brain of all challenged specimens. There were no gross changes or clinical signs in the control group. Details of clinical signs and determination of LD_{50} are given in Soltani *et al*., (2014). All tissue sections from the moribund or freshly dead fish showed moderate to severe inflammation and necrosis. Histological changes of juvenile Persian sturgeon challenged with *Streptococcus iniae* via intramuscular and intraperitoneal injections at 24 ± 2°C are as follows; Liver histopathology was characterized by hepatocyte vacuolization and focal necrosis, melano-macrophage aggregation, and infiltration of eosinophilic granular cells (EGCs) (Figure 1).

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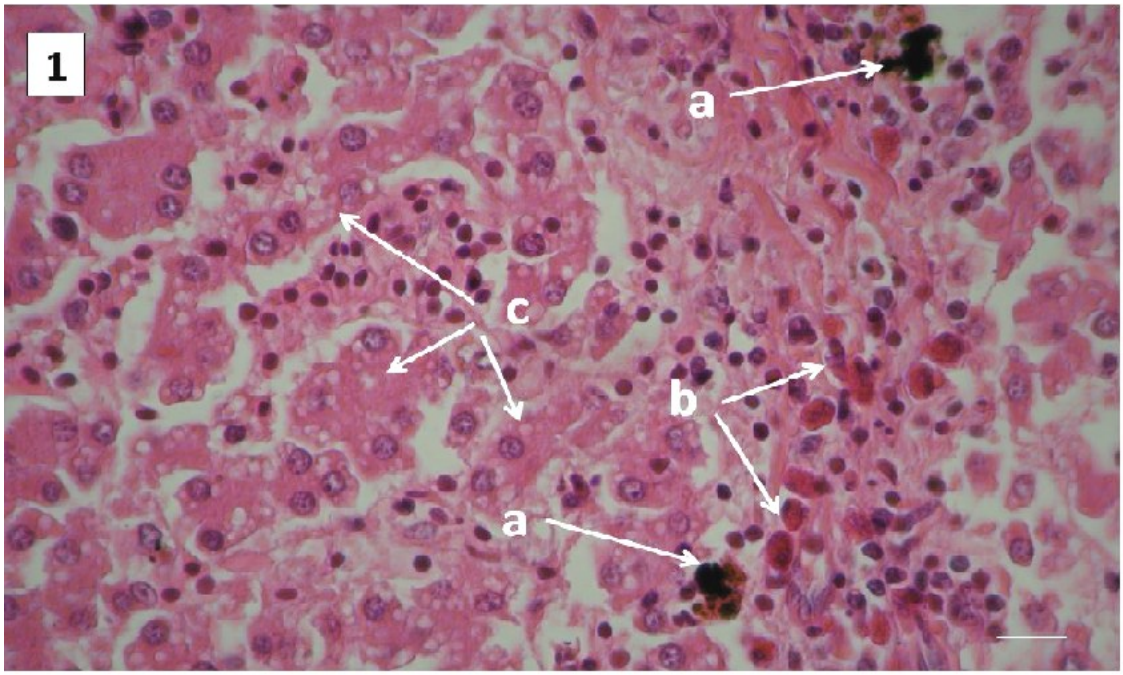

Figure 1. Liver a: melano-macrophage aggregation, b: infiltration of EGCs, c: vacuolation of hepatocytes. (H&E, bar = 100µm).

In the brain sections, massive inflammatory cells infiltration, severe meningitis and vacuolation in brain layers in many challenged fish was detected (Figure 2). The kidney tissue showed severe necrosis, diffused tubules degeneration, accumulation of hyaline exudates in tubules lumen, shrinkage of glomeruli, dilation of Bowman space, cellular infiltration, and interstitial tissue necrosis (Figure 3). Infiltration of EGCs was generally found in some kidney sections, after 72 h (Figure 4).

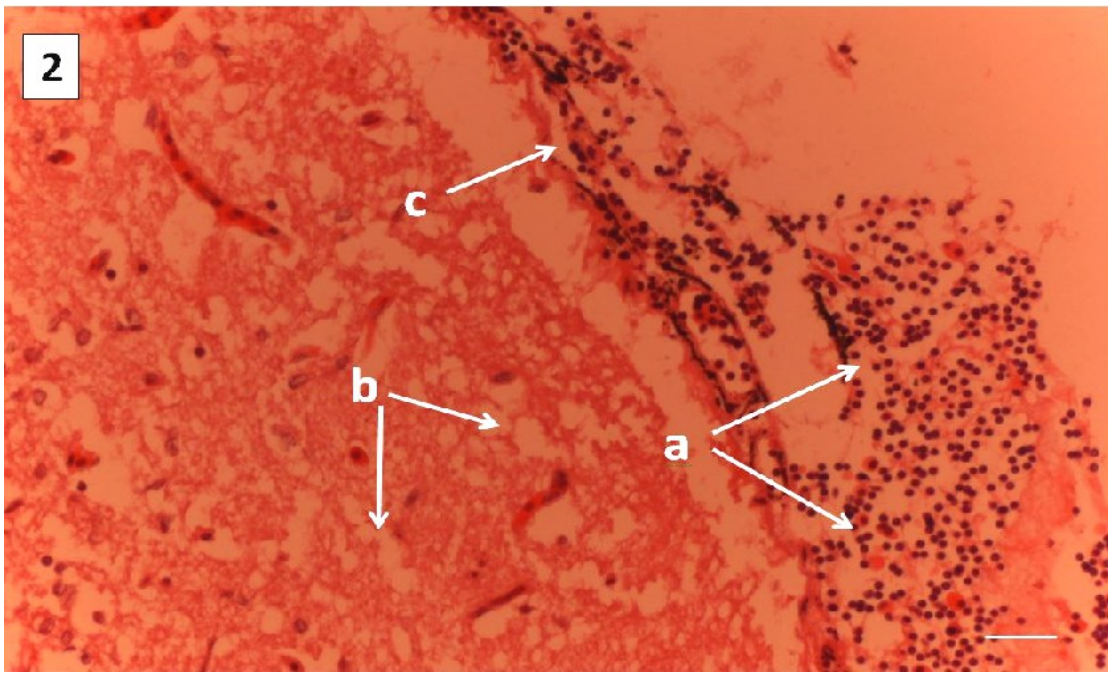

Figure 2. Brain: a: massive cell infiltration, b: vacuolation of brain tissue, c: lifting and cells infiltration in meninges. (H&E, bar = 50µm).

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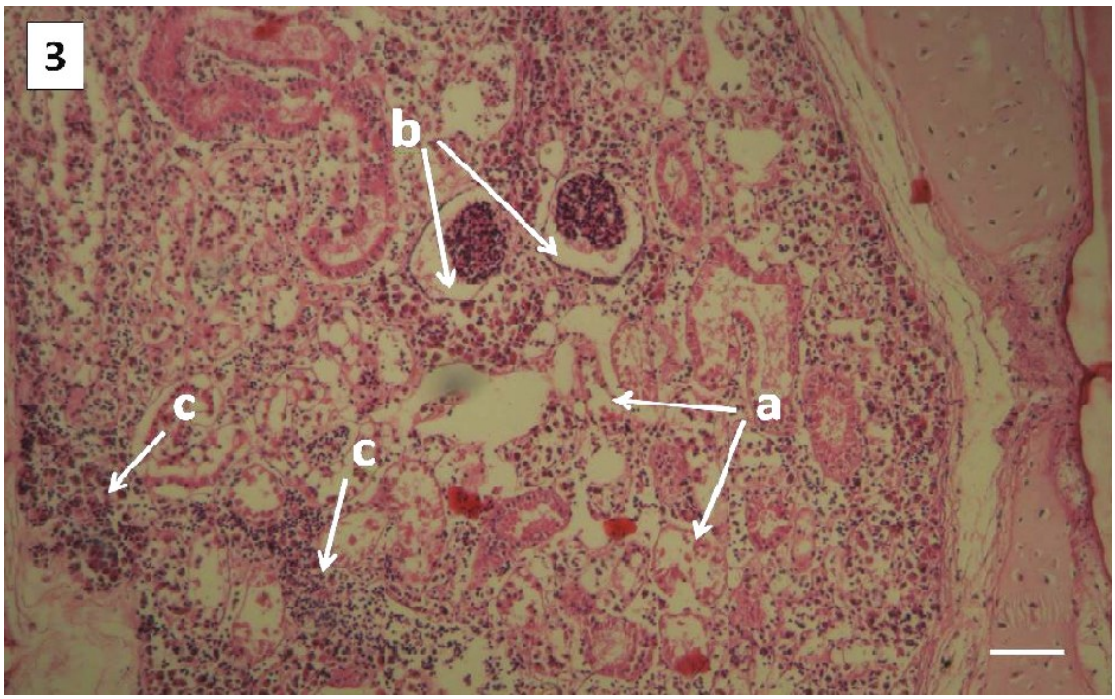

Figure 3. Kidney a: degeneration and necrosis of kidney tubules, b: Dilation of Bowman's space and shrinkage of glomeruli, c: massive cell infiltration (H&E, bar = 50µm).

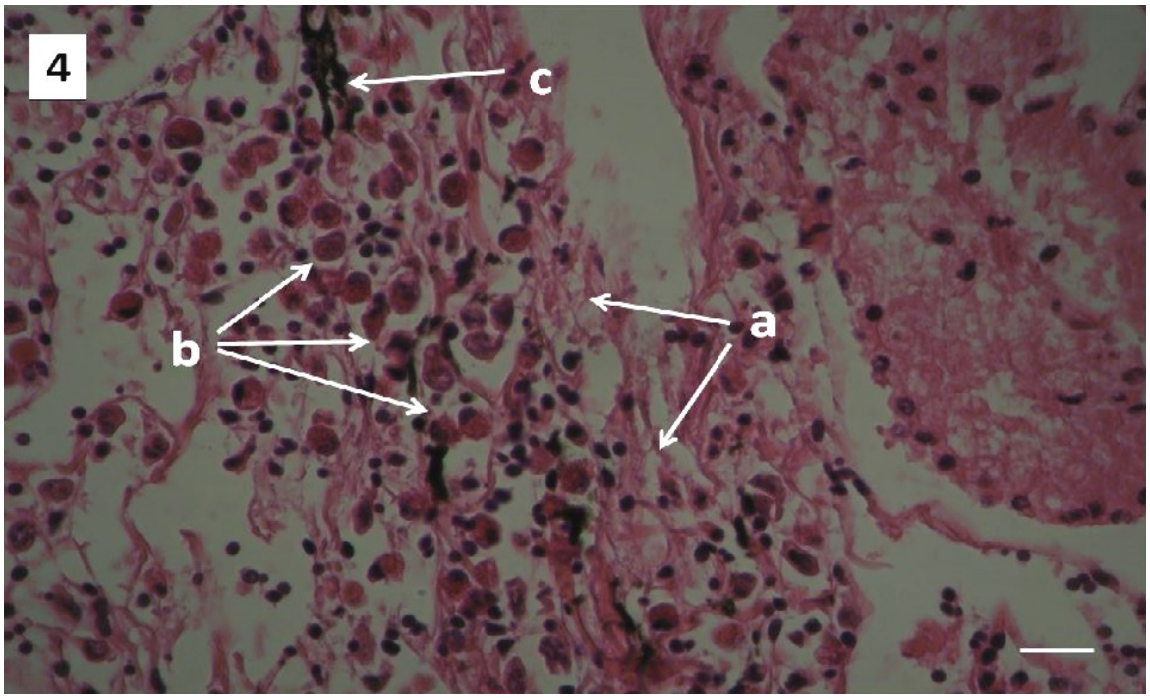

Figure 4. Kidney a: interstitial necrosis with cell infiltration, b: infiltration of EGCs, c: melano-macrophage aggregation (H&E, bar = 100µm).

Hyperemia and cellular infiltration in the base of primary lamella were the most common histopathological signs of gills (Figure 5). Degeneration, epithelial lifting and edema in gill secondary lamella were generally found after 3 days in most of the dead fish (Figure 6).

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In eyes sections, congestion, hemorrhage, and degeneration of retinal layers was observed (Figure 7).

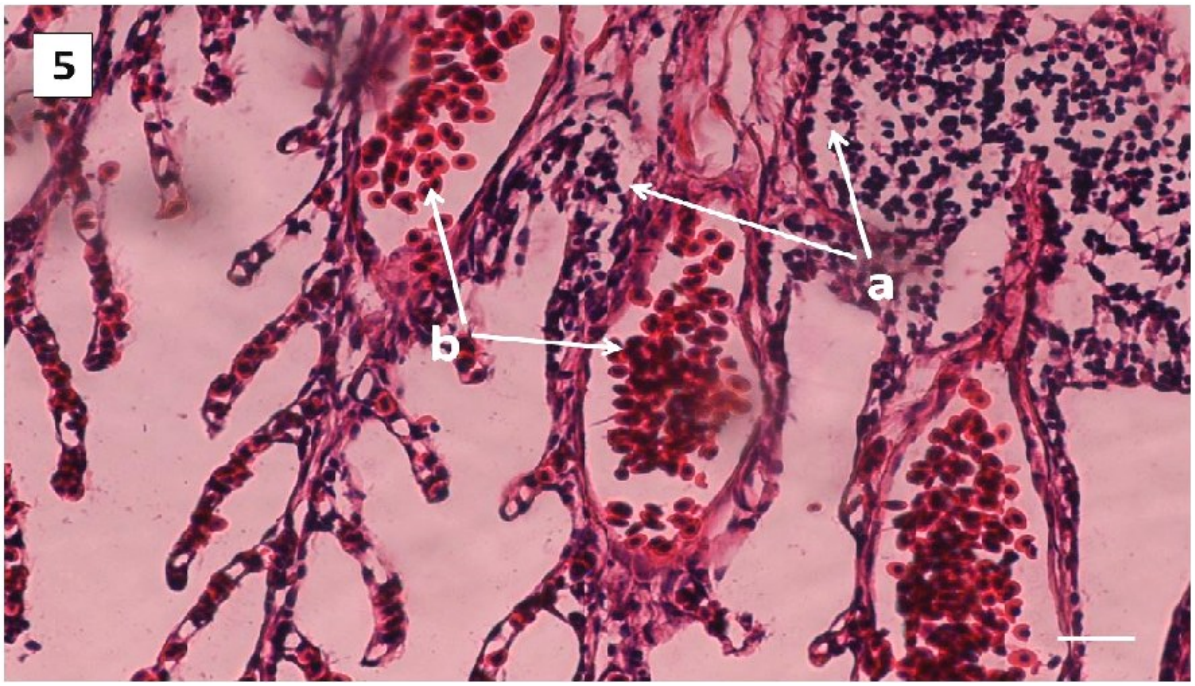

Figure 5. Gill a: inflammatory cells infiltration, b: hyperemia in primary and secondary lamella. (H&E, bar = 50µm).

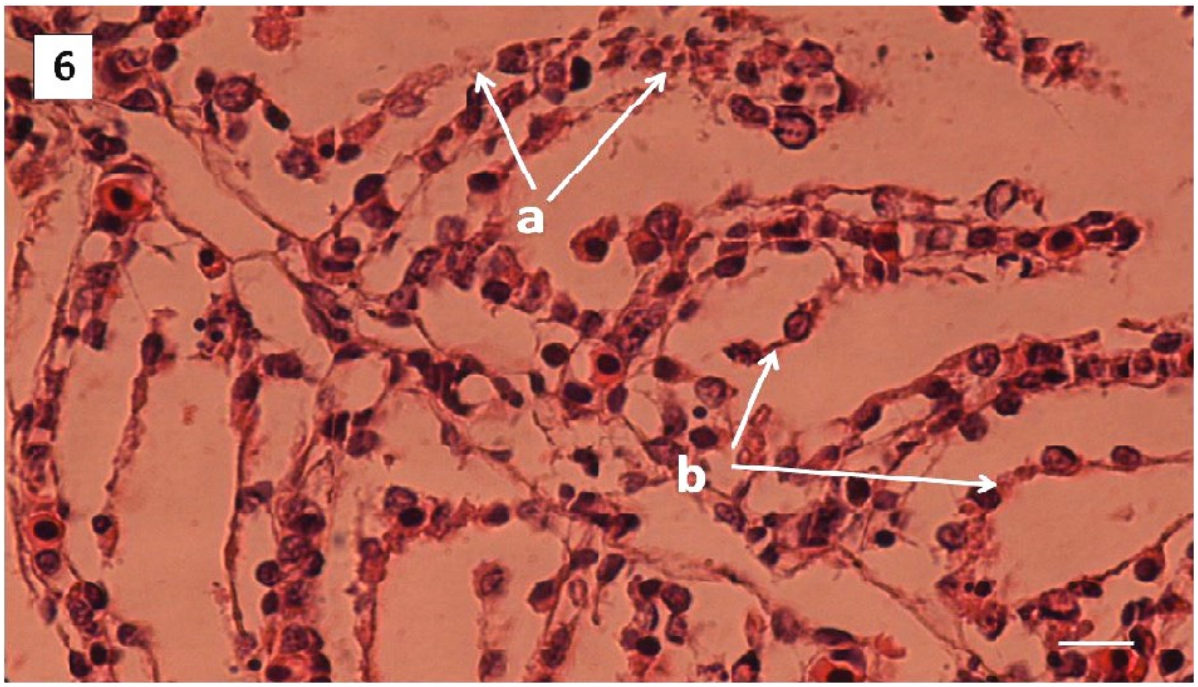

Figure 6. Gill a: degeneration of secondary lamella, b: epithelial lifting and edema in secondary lamella. (H&E, bar = 100µm).

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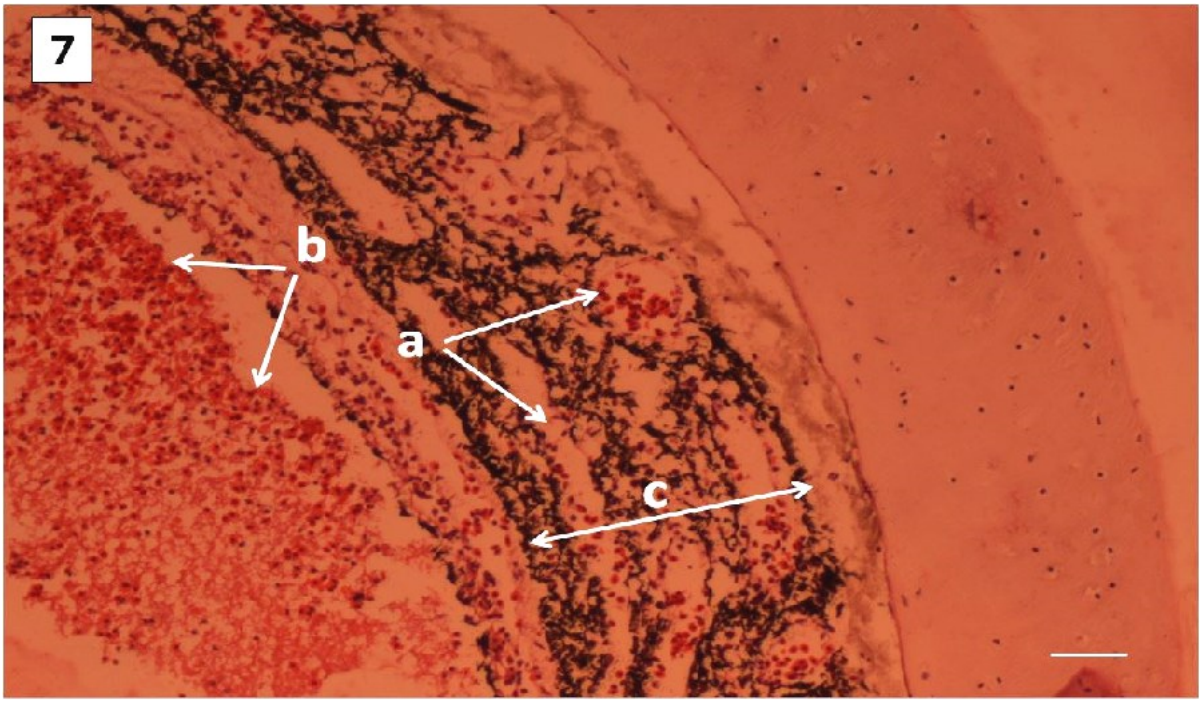

Figure 7. Eye a: congestion in retina b: hemorrhage in posterior chamber c: degeneration of retina layers (H&E, bar = 50µm).

Discussion

The pathogenicity of streptococcosis can vary depending on the species and age. So knowing the susceptibility of farmed fish to this pathogen will be very useful in farm health management. The susceptibility of several commercial fish species has been assessed towards infection by *S. iniae* and the results demonstrated various level of resistance to the disease (Soltani *et al*., 2014). Signs of streptococcosis are different in various fish species. Most clinical signs of the disease in this study such as cataract and congestion in eyes, hemorrhage and prolapsed anal and other mentioned clinical signs were similar to other susceptible fish species including rainbow trout, tilapia, yellow tail (Ferguson *et al*., 1994; Perera *et al.* 1997; Prera *et al.* 1998; Neely *et al*., 2002; Russo *et al,*. 2006; Yang and Li, 2009). In some recent studies, the pathogenicity of this bacterium in Siberian sturgeon (Deng *et al*., 2017; Chen *et al*., 2020), Chinese sturgeon (Muhammad *et al*., 2020) and white sturgeon (Soto *et al*., 2017) has been reported too. LC50 value of this bacterium in Siberian sturgeon were 5.1×10^5 to 6.4×10^5 cfu per fish (Chen *et al*., 2020) and in Chinese sturgeon was 1.6×10^6 cells $fish^{-1}$ (Muhammad *et al*., 2020). However, in this study the Persian sturgeon demonstrated severe clinical signs two days post-challenge with the bacterium and caused above 80% mortality within 5 days of infection, suggesting this species is highly susceptible to streptococcal infection. In

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addition, some symptoms such as sunken eyes and hydronephrosis were seen in the affected sturgeon but have not been reported in other susceptible fish species. Distinct histopathological findings were the lymphocytic infiltration in the brain and acute necrosis in kidney, liver and other mentioned tissues that was similar to the reports in other susceptible species such as tilapia, Asian sea bass (*Lates calcarifer*), rainbow trout (Chang and Plumb, 1996; Yuasa *et al*., 1999; Suanyuk, *et al.,* 2010; Avci *et al,*. 2010). On the other hand, the histopathological signs for this disease were similar to other sturgeons. For example, histological signs in the liver and brain of Chines Sturgeon (Muhammad *et al*., 2020), Kidney of white sturgeon (Soto *et al*., 2017), liver and kidney of Siberian sturgeon (Deng *et al*., 2017) in affected fish were more and less similar to our finding in Persian sturgeon. These histopathological findings indicate an acute course of disease for *S. iniae* in Persian sturgeon causing a systematic infection in this fish.

In collusion, the data of this study clearly demonstrated that Persian sturgeon is a highly susceptible species to *S. iniae* infection as the quick and high mortality level occurred in the infected fish. Also, the severity of histopathological findings exhibited that this fish species can be considered as a high susceptible species to infection by *S. iniae*. Therefore, in aquaculture practice it is important to provide necessary prevention and/or protection criteria such as improving of the water quality treatment and vaccination in order to avoid from the disease outbreaks in fish farms. Such protection measures are especially necessary where sturgeons are grown in earthen ponds and using river water that can increase the chance of disease outbreaks.

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Conflict of interest

Authors have no conflict of interest on this work.

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