Experimental evaluation of pathogenicity of Streptococcus iniae in Silver Shark and Rainbow Shark

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Streptococcus iniae is a main cause of streptococcosis causing the disease in a wide variety of fresh and salt water fish species. This study was aimed to evaluate the pathogenicity of S. iniae in two aquarium fish species, silver shark and rainbow shark. Apparently healthy fish were obtained from a local ornamental fish farm and were injected intraperitoneally with 1×10^7 cfu of the bacteria after one week of acclimatization. Classical signs of streptococcosis appeared in some silver sharks in less than 10 h after bacterial challenge and the mortality was recorded 16 h post infection. Although, no mortality and no clinical sign was observed in rainbow shark after 3 weeks, S. iniae was collected from internal organs of fishes challenged with the bacteria and was approved by PCR. It is concluded that silver shark is more susceptible to streptococcosis than rainbow shark, although both species can play an important role in transmission of the disease to other fish species and also to human.

Key words: Streptococcus iniae, silver shark, rainbow shark.

INTRODUCTION

Streptococcosis is a septicemic disease affecting both cultured and wild populations of freshwater and marine fish species throughout the world (Kitao, 1993; Austin and Austin, 1999). Streptococcus iniae is a hemolytic, gram-positive coccus was first reported in 1957, affecting cultured rainbow trout in Japan (Hoshina et al., 1958). Since then, S. iniae has been associated with many outbreaks in different species of fish, including rainbow trout (Oncorhynchus mykiss) (Eldar et al., 1995); hybrid striped bass (Stoffregen et al., 1996; Shoemaker et al., 2001); Nile tilapia (Oreochromis niloticus) (Shoemaker et al., 2001); hybrid tilapia (Oreochromis niloticus×O. aureus) (Perera et al., 1994); red drum (Sciaenops ocellatus) (Eldar et al., 1999); rabbit fish (Siganus canaliculatus) (Yuasa et al., 1999); sea bass (Dicentrarchus labrax) (Colorni et al., 2002); Japanese flounder (Paralichthys olivaceus) (Nguyen et al., 2002); and barramundi (Lates calcarifer) (Bromage et al., 1999).

There are a few reports of S. iniae infections in ornamental fish (Russo et al., 2006), and wild fish (Zlotkin et al., 1998; Ferguson et al., 2000; Colorni et al., 2002).

Depending on the environmental factors, streptococcosis can cause high mortality, sometimes more than 50%, over a period of 3 to 7 days. Streptococcus does not seem to be a truly opportunistic pathogen, as it can be more aggressive than many other environmental bacteria. Ferguson et al. (1994) reported that populations of zebra danios and white cloud mountain minnows exposed to high concentrations of Streptococcus experienced 100% mortality within 2-4 days of exposure. Some outbreaks, however, are more chronic in nature and mortalities may extend over a period of several weeks, with only a few fish dying each day.

This disease is now responsible for significant economic losses in the world aquaculture industry.
resulting in economic losses estimated at US $150 million annually (Shoemaker et al., 2001). The first confirmed streptococcal infection in cultured fish in Iran was reported in 2002 in rainbow trout (Akhlaghi and Keshavarzi, 2002). Since then the infection has spread rapidly throughout the country causing considerable mortality in rainbow trout stocks with significant economic effect (Raissy and Ansari, 2011). It also should be considered that S. iniae is emerged as a threat to public health due to its zoonotic agent, being isolated from humans infected due to injuries during handling of fresh fish (Weinstein et al., 1997; Lau et al., 2003; Koh et al., 2004; Facklam et al., 2005; Lau et al., 2006; Sun et al., 2007). Despite the fact that there are many reports indicating occurrence of the disease in Iran and other countries, Streptococcosis is less studied in ornamental fish. The aim of this study is to evaluate the pathogenicity of S. iniae in two aquarium fish species, silver shark and rainbow shark.

MATERIALS AND METHODS

Fish species

A total of 20 apparently healthy silver shark (n=10) and rainbow shark (n=10) were obtained from a local ornamental fish farm in Esfahan. Fish were randomly examined to ensure that they were pathogen free. The fish average weight was 2.4 g with average length of 7.4 cm. All fish were maintained in 400 L aquarium with aeration and acclimatized for one week before they were used for experiments. Fish were divided into control and test groups with 5 fish in each group. The experiment was conducted in 100 L aquariums supplied with good aeration. The water temperature was 28±1°C, Dissolved Oxygen (DO) was 4.9±0.2 mg l⁻¹ and pH was 7.6±0.1.

Bacterial strains and experimental design

The strain of S. iniae used was an isolate from naturally infected fish. The isolate was identified according to standard microbiological tests and PCR. Single representative colonies from the original plates were utilized to prepare a stock culture. The bacteria were first purified by subculture in TSA at 30°C for 24 h. A few of the resultant purified colonies were grown for 24 h at 30°C in two 250 mL brain heart infusion (BHI) Broth. The isolate was adjusted to McFarland turbidity standard No. 1 which was equivalent to 1×10⁷ cfu ml⁻¹. The test and control groups were injected intraperitoneally with 1×10⁶ cfu of Streptococcus iniae and sterile BHI (0.1 ml), respectively. Clinical signs and mortality were recorded for 3 weeks after challenge. The internal organs of dead fish were collected for detection of Streptococcus iniae by culture and PCR methods.

Identification of the isolated bacteria

Samples of liver, kidneys and heart were placed on a 5% sheep blood agar (Oxoid, Germany) with 1% yeast extract agar (Merck, Germany) plates and then incubated at 24 and 37°C for 2 to 3 days under aerobic conditions. Standard physiological and biochemical tests recommended by Austin and Austin (1999) and Chang et al. (2002) were performed at 25°C.

The genomic DNA was prepared using a standard DNA extraction method (Ausubel et al., 1987). Briefly, the bacteria were grown overnight at 30 °C in Tryptic Soy Broth containing 1% sodium chloride. Bacterial culture (1.5 ml) was centrifuged for 10 min at 12,000g, and the cell pellets were resuspended in 567 µl of Tris-EDTA buffer (Merck, Germany) (10 mM Tris–HCl, 1 mM EDTA, pH 8.0), followed by addition of 30 µl of 10% (w/v) sodium dodecyl sulfate (Merck, Germany) and 3 µl of protease K (Cinnagen, Iran) (20 mg/ml) and incubation for 1 h at 37 °C. The samples were treated with 100 µl of 5 M NaCl and 80 µl of hexadecltrimethylammonium bromide (CTAB)/NaCl (Sigma, Germany), and incubated at 65°C for 10 min. The mixture was extracted with an equal volume of phenol-chloroform- isoamyl alcohol (25:24:1, v/v) and DNA was precipitated with 0.6 volume of cold isopropanol (Sigma, Germany) and washed with 1 ml of 70% cold ethyl alcohol. The DNA pellet was dried at room temperature for 30 min and resuspended in TE (10 mMTris–HCl, 100 mM EDTA, pH 7.8) buffer and stored at -20°C. The purity and quantity of genomic DNA in each sample was evaluated by measuring optical densities at 260 and 280 nm wavelengths. The DNA concentration of each sample was adjusted to 50 ng/µl for PCR.

The PCR reaction was performed in a 50 µl reaction system consisting of 2 µl of purified genomic DNA (50 ng/µl), 5 µl of 10×PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 60 mM MgCl₂, 0.1% gelatin and 1% Triton X-100), 1 µl each of the primers (50 pmol/µl), 1 µl each of the 10 mM dNTPs, 0.2 µl units Taq DNA polymerase (5 units/µl) and 40 µl of sterile distilled water. The reactions were performed with a thermal cycler (Eppendorf, Germany).

PCR assay was done using specific primers F (5'-GTC GTA ACAAGG TAA GCC GTA TCG -3') and R (5'-CTT ACC TTA GCC CCA GTC TAA CGAC-3') as described by Mata et al. (2004). The expected 513-bp PCR amplification product confirmed the preliminary biochemical identification.

RESULTS

Clinical signs, behavior and mortalities of the fish were recorded after injection. Lethargy, abnormal behavior, wandering around corners and erect swimming were the first signs observed in challenged silver shark. Classical signs of streptococcosis were observed in some silver sharks in less than 10 h after bacterial challenge and the mortality was recorded 16 h of post infection. Three of 5 silver sharks died about 16 h after injection while no clinical signs were observed in the 2 other fish until 3 weeks. Petechial hemorrhage was also observed on the surface of internal organs of dead fish, although pathological study was not possible to be done. No mortality and no clinical sign were appeared in rainbow shark after 3 weeks. The fish from control group were active during the whole experiment and showed neither mortality nor clinical signs. S. iniae was collected from internal organs of fishes challenged with the bacteria and identified by PCR using specific primers targeting lactate oxidase gene according to Mata et al. (2004).

DISCUSSION

In recent years, Streptococcosis has been the most important bacterial disease in trout culture in Iran. Streptococcosis, as a systemic disease, is a serious
problem for fish farmers causing economic losses every year in aquaculture industry. The disease has been previously reported from rainbow trout fish farms in Iran (Akhlaghı and Keshavarzı, 2002; Soltani et al., 2005). Despite the importance of the disease, it is not studied in ornamental fish. This study was done to evaluate the pathogenicity of S. iniae, as an important causative agent of streptococcosis, in two aquarium fish species, silver shark and rainbow shark following intraperitoneal injection.

After challenge, darkening of the skin and lethargy were the first signs observed in infected fish. Moribund and dead fish presented external hemorrhages especially around the base of the pectoral fins and over the internal organs. These observations are in accordance with the report in other studies (Ferguson et al., 1994; Perera et al., 1998; Neely et al., 2002) where S. iniae is described to cause a systemic disease in different fish species. Developing challenge models is one of the first steps in vaccine or pharmaceutical development for animal diseases. Fish can be infected by bath with a chosen bacterium concentration; by introduction of diseased fish or by intraperitoneal or intramuscular injection. The advantage of the bath method is that they replicate the natural route of the mucosal immunity is involved in protecting fish from the infection. The advantages of challenging fish by intraperitoneal injection is that this technique is more replicable and efficient than the others methods (Perera et al., 1997; Sako, 1998). However, in some studies no difference in mortality rate was observed at the end of the experiments in fish between the intraperitoneal and cohabitation challenge methods (Nordmo and Ramstad, 1997). In this research, the intraperitoneal injection was chosen as challenge route for its replicability. Fish began to show clinical signs and mortality after less than one day post-challenge. Mortality due to S. iniae was confirmed by identification from kidney and liver cultures of dead fish.

It is proved that S. iniae can cause mortality in rainbow shark (Russo et al., 2006) but in this study no clinical sign and no mortality was observed in this fish, although the bacteria was collected from internal organs of the fish. The differences in the results of this study with the results of Russo et al. (2006) could be due to genetic variation or environmental factors that influenced the fish immune system (water temperature, stocking density, feed consumption, and dissolved oxygen).

It is concluded that silver shark is more susceptible to streptococcosis than rainbow shark, although both species can play an important role in transmission of the disease to other fish species and also to human.

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REFERENCES


Russo R, Mitchell H, Yanong RPE (2006). Characterization of Streptococcus iniae from ornamental cyprinid fishes and