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Susceptibility of Five Species of Tilapia to *Streptococcus* sp.

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Abstract

Streptococcus sp. was isolated from dead and moribund hybrid tilapia, (*Oreochromis niloticus* x *O. aureus*) in Saudi Arabia. Hybrid tilapia (*O. niloticus* x *O. aureus*), Taiwanese red tilapia (*O. mossambicus* x *O. niloticus*), Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*) and Mossambique tilapia (*O. mossambicus*) were tested for susceptibility to *Streptococcus* sp. at concentrations of 10^4 - 10^8 cells per 0.1 ml. Total mortalities at termination of the experiment were significantly different among the five species with Nile tilapia demonstrating slightly less susceptibility to the pathogen than the other four species. The LD₅₀ of *Streptococcus* sp. was 2.8×10^5 cells for Mossambique tilapia, 3.5×10^5 cells for red tilapia, 1.8×10^6 cells for hybrid tilapia, 2×10^7 cells for blue tilapia and 9.2×10^7 cells for Nile tilapia.

Streptococcus sp. was not isolated from the peritoneal cavity, liver or kidney from any of the five tilapia species that survived challenge (12-d post-infection). Moribund fish of all five species displayed similar clinical symptoms, i.e., erratic swimming, exophthalmia, melanosis (with exception in red tilapia), hemorrhagic areas around the jaws and at the base of the pectoral and dorsal fins, and on the tail, and the presence of ascitic fluid in the abdominal cavity.

Introduction

Tilapias are among the most commercially important freshwater fish species in the tropics. Although comparative growth performance of tilapia species has been investigated, no information is available on their comparative susceptibility to disease. The relative susceptibility of several other fish species to several bacterial pathogens, however, has been investigated (Plumb and Sanchez 1983; Plumb and Hilge 1987; Whittington and Cullis 1988; Sakai et al. 1989; Baxa et al. 1990; Houghton et al. 1991; Sakai et al. 1991; Soltani et al. 1994; Magarinos et al. 1995).

Streptococcus sp. is a serious infectious fish disease, and has been isolated from a number of freshwater and marine fish species (Hoshina et al. 1958; Kusuda et al. 1976; Kitao et al. 1981; Ugajin 1981; Nakatsugawa 1983; Foo et al. 1985; Baya et al. 1990; Ceschia et al. 1992; Austin and Robertson 1993; Eldar et al. 1994; Ferguson et al. 1994; Kim and Lee 1994; Perera et al. 1994).

The disease has also been found to be a major problem in cultured tilapia in the Kingdom of Saudi Arabia (Al-Harbi 1994).

The present paper describes the ability of *Streptococcus* sp. to cause disease in five species of tilapia.

Materials and Methods

Fish

Hybrid tilapia (*Oreochromis niloticus* x *O. aureus*, average weight 63.2 g and total length 15.5 cm), Taiwanese red tilapia (*O. mossambicus* x *O. niloticus*, average weight 50 g and total length 14.8 cm), Nile tilapia (*O. niloticus*, average weight 66.1 g and total length 16.8 cm), blue tilapia (*O. aureus*, average weight 97.7 g and total length 17.5 cm) and Mossambique tilapia (*O. mossambicus*, average weight 83.5 g and total length 16.6 cm) were obtained from the Fish Culture Project, King Abdulaziz City for Science and Technology in Riyadh, Saudi Arabia. The fish were acclimated to the experimental conditions for 2 weeks, and maintained on a commercial pelleted fish feed containing 34% crude protein at the rate of 2% body weight daily.

Bacterial Strain

The *Streptococcus* sp. used in this study was isolated in March 1992 from the kidney of a moribund hybrid tilapia cultured in Saudi Arabia (Al-Harbi 1994). The organism was grown on brain heart infusion agar (BHIA; Oxoid, Basingstoke, England) at 30°C for 24 h. To enhance bacterial virulence prior to each trial, the *Streptococcus* sp. was passaged twice through healthy hybrid tilapia, and then reisolated from the kidney for use in subsequent experiments.

Experimental Infection

Each fish species (in groups of 10 fish) was tested independently for their susceptibility to *Streptococcus* sp. All animals were maintained in fiberglass tanks containing 100 l aerated dechlorinated water at a flow-rate of 0.5 l·minute⁻¹·kg⁻¹ fish. Water temperature was 28-30°C throughout the experimental period. Before injection, fish were anesthetized with MS222 (tricaine methanesulphonate), and injected intraperitoneally (IP) with 0.1 ml each of a 10-fold serial dilution containing 1.2 x 10⁴ to 1.2 x 10⁸ cells in 0.85% NaCl (w/v). Control fish were similarly injected with 0.1 ml of 0.85% NaCl (w/v) solution. Following injection, the fish were observed five times daily for 12 d to record the appearance of clinical signs and mortality. The lethal dose-50% end point (LD₅₀) values were calculated using the method of Reed and Muench (1938). Dead animals were removed and subjected to bacteriological examination. After 12 d when no dead or moribund fish were observed for 3 d, two fish were randomly selected from the surviving population of each species for each dilution and were sacrificed. The peritoneal cavity, liver and kidney samples

from each fish were sampled for the presence of *Streptococcus* sp.

Results

The mortality rates of all five species of tilapia increased with increasing concentrations of *Streptococcus* sp. (Table 1). However, Mossambique, red and hybrid tilapia were more susceptible to *Streptococcus* sp. infection than were Nile and blue tilapia (Table 1).

At the highest concentration of *Streptococcus* sp. (1.2×10^8 cells) 80% of Mossambique, red and hybrid tilapia died within 48 h, whereas for Nile and blue tilapia, mortality rates after 96 h were 30% and 50%, respectively. The LD_{50} of *Streptococcus* sp. was 2.8×10^5 cells for Mossambique tilapia, 3.5×10^5 cells for red tilapia, 1.8×10^6 cells for hybrid tilapia, 2.0×10^7 cells for blue tilapia and 9.2×10^7 cells for Nile tilapia (Table 1).

Moribund fishes of all species showed similar clinical signs, i.e., erratic swimming, exophthalmia, melanosis (except red tilapia), hemorrhagic areas around the jaws, at the base of the pectoral and dorsal fins, and on the tail, and the presence of ascitic fluid in the abdominal cavity.

The inoculated strain was isolated from the peritoneal cavity, liver and kidney of all dead fish. The isolates were biochemically similar to the *Streptococcus* sp. challenge strain and were identified as *Streptococcus* sp. with the API 20 STREP (API BioMerieux, France) rapid identification system. However, *Streptococcus* sp. was not recovered from the fish that survived challenge.

Table 1. Cumulative mortalities of five tilapia species 12-d after infection with serial 10-fold dilutions of *Streptococcus* sp.

Cells $\cdot 0.1 \text{ ml}^{-1}$ of inoculum	Number death / Number inoculated				
	Blue tilapia	Hybrid tilapia	Mossambique tilapia	Nile tilapia	Red tilapia
1.2×10^8	5/10	8/10	8/10	3/10	8/10
1.2×10^7	3/10	5/10	7/10	2/10	6/10
1.2×10^6	3/10	4/10	6/10	1/10	6/10
1.2×10^5	2/10	4/10	5/10	1/10	5/10
1.2×10^4	1/10	3/10	5/10	1/10	5/10
Control ^a	0/10	0/10	0/10	0/10	0/10
LD_{50} ^b	2×10^7	1.8×10^6	2.8×10^5	9.2×10^7	3.5×10^5

^aSaline-injected controls.

^bCalculated number of *Streptococcus* sp. required to kill 50% of injected fish (Reed and Muench 1938)

Discussion

The present experiments involved dealing with five species/hybrids of tilapia. Apart from the control and the treatments for each group, I was unable to

duplicate the treatments. However, the consistent trends make me believe that the observations are entirely due to the treatments, and not influenced by tank effects, etc.

From the results of this study it appears that the five groups of tilapia examined have different degrees of susceptibility to *Streptococcus* sp. The *Streptococcus* sp. used in this study was originally isolated from cultured hybrid tilapia (*O. niloticus* x *O. aureus*) (Al-Harbi 1994), but Mossambique and red tilapia were more susceptible to *Streptococcus* sp. than hybrid tilapia indicating that these two species will be more susceptible to natural infections involving *Streptococcus* sp. In another study, common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*) were found to be resistant to *Streptococcus* sp. infection (Al-Harbi 1993). The reason why the five tilapia species have different degrees of susceptibility to *Streptococcus* sp. is not entirely clear. It may be related to different genetic make up of each species. Cook and Lofton (1975) reported different degrees of susceptibility to *Streptococcus* sp. for Atlantic croaker (*Micropogon undulatus*), sea catfish (*Arius felis*), gulf menhaden (*Brevoortia patronus*), striped mullet (*Mugil cephalus*) and spot (*Leiostomus xanthurus*).

Isolation and identification of *Streptococcus* sp. from moribund or freshly dead specimens of five tilapia species during this study, indicated that the cause of death was associated with *Streptococcus* sp.

Streptococcus sp. was not reisolated from the survivors of all five tilapia species 12-d post infection. Similarly, Plumb and Hilge (1987) were unable to isolate *Edwardsiella ictaluri* from European catfish 15-d post inoculation. Thus, all five tilapia species are not capable of harboring the bacterium for a considerable time, or the bacteria existed in a non-cultivable state in all five species, 12 d after injection.

The results suggest that *Streptococcus* sp. is a potential pathogen of tilapias in Saudi Arabia. The implications of these results should be seriously considered by fish farmers. Nevertheless, Nile tilapia was found to be more resistant to *Streptococcus* sp. and appears to be more suitable for intensive culture than the other species of tilapia.

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