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Duration of protective antibodies and correlation with survival in Nile tilapia *Oreochromis niloticus* following *Streptococcus agalactiae* vaccination

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ABSTRACT: *Streptococcus agalactiae* is a major piscine pathogen that causes significant morbidity and mortality among numerous species of freshwater, estuarine and marine fishes. Considering the economic importance of fishes susceptible to *S. agalactiae* throughout the world, an efficacious *S. agalactiae* vaccine was developed using an extracellular product (ECP) fraction and formalin-killed whole cells of *S. agalactiae*. A vaccine study was conducted by intraperitoneal (i.p.) injection in Nile tilapia *Oreochromis niloticus* in order to determine the duration of protection and its correlation to antibodies specific for this pathogen. After 47, 90 or 180 d post-vaccination (DPV), the fish were i.p. challenged with approximately 2.0×10^4 *S. agalactiae* colony-forming units (CFU) $fish^{-1}$ to determine the duration of protective immunity. The percent survival in control fish i.p.-injected with sterile TSB was 16, 16, and 4% on 47, 90 and 180 DPV, respectively, while the percent survival for the vaccinated fish was 67, 62 and 49%, respectively. The specific mean antibody concentration of the vaccinated fish was significantly higher than that of the control fish, with significant correlation between the ELISA optical density (OD) and protection. These results indicate that the specific antibody has a correlation with protection following immunization with the *S. agalactiae* vaccine and that the vaccine can confer protection against *S. agalactiae* up to 180 DPV.

KEY WORDS: Vaccine · Specific antibody response · *Streptococcus agalactiae* · Nile tilapia

INTRODUCTION

The Group B streptococcal fish pathogen *Streptococcus agalactiae* affects numerous freshwater, estuarine and marine fish species, including golden shiners *Notemigonus crysoleucas* (Mitchill) (Robinson & Meyer 1966), menhaden *Brevoortia patronus* (Goode) (Plumb et al. 1974), sea trout *Cynoscion regalis* (Bloch & Schneider), striped bass *Morone saxatilis* (Walbaum) (Baya et al. 1990), seabream *Sparus auratus* L., tilapia *Oreochromis niloticus* L., mullet *Liza klunzingeri* (Day) (Evans et al. 2002, Glibert et al. 2002) and silver pomfret *Pampus argenteus* (Euphrasen) (Duremdez et al. 2004). Several streptococcal isolates have been reported in recent years, including isolates from the United States (Plumb et al. 1974), Israel (Eldar et al. 1994) and Kuwait (Evans et al. 2002). Some of these isolates were initially unspeciated or misidentified as *S. difficile*, but were subsequently characterized as *S. agalactiae* (Wilkinson et al. 1973, Vandamme et al. 1997, Kawamura et al. 2005). Additionally, different isolates of *S. agalactiae* show significant homogeneity according to biochemical characteristics, whole-cell protein profiles, and certain nucleic acid sequences (Elliott et al. 1990, Vandamme et al. 1997, Berridge et al. 2001, Kawamura et al. 2005).

A number of environmental factors, including warm water temperatures, increased ammonia levels and

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low dissolved oxygen levels, play an important role in *Streptococcus agalactiae* outbreaks (Glibert et al. 2002, Evans et al. 2003). Clinical signs of disease include anorexia, 'C'-shaped body posturing and erratic swimming, and many outbreaks cause considerable mortalities (Evans et al. 2002). Because of the economic importance of fishes affected by this pathogen, an effective vaccine could help decrease related fish losses. Evans et al. (2004a,b) assessed a killed vaccine composed of concentrated extracellular products (ECP) and formalin-killed *S. agalactiae* whole cells. Tilapia injected with this vaccine were significantly protected against *S. agalactiae* challenge. However, the protective effects were assessed only through 28 to 64 d post-vaccination (Evans et al. 2004a,b). To achieve a more comprehensive analysis of the duration of protective immunity following *S. agalactiae* vaccination, we have analyzed the duration of serum antibody responses and survival after *S. agalactiae* challenge at 47, 90 and 180 d post-vaccination (DPV) following a single intraperitoneal (i.p.) immunization. The production cycle of farmed tilapia in tropical regions can be completed in 4 to 6 mo (Stickney 2000), and the vaccine must provide significant long-term protection against *S. agalactiae*. Thus, our objective was to determine the duration of immunity conferred using this *S. agalactiae* vaccine for 180 DPV following a single i.p. injection.

MATERIALS AND METHODS

Fish. Nile tilapia *Oreochromis niloticus* with a mean weight of 45.5 ± 12.2 g were housed at the United States Department of Agriculture/Agriculture Research Service Aquatic Animal Health Laboratory in Chestertown, Maryland. The fish were kept in 57 l glass aquaria supplied with flow-through dechlorinated tap water and were maintained on a 12:12 h light:dark period. The fish were fed daily to satiation with Aquamax Grower 400. Daily water temperature averaged 30.83 ± 1.32°C, mean daily dissolved oxygen was 3.16 ± 0.83 mg l^{-1}, mean pH was 7.03 ± 0.13, and mean total ammonia concentration was 0.79 ± 0.68 mg l^{-1}.

Vaccination. The vaccine was prepared as previously described (Evans et al. 2004a,b). Briefly, the polysaccharide-encapsulated *Streptococcus agalactiae* was grown in tryptic soy broth (TSB; Difco Laboratories) at 27°C for 72 to 125 h. The resulting cultures were treated with 3% neutral buffered formalin for 24 h and then centrifuged to separate the cell pellet and culture fluid. The vaccine ECP fraction of the vaccine was prepared by concentrating the cell-free culture fluid containing ECP on a 3 kDa Amicon column (S3Y3) using a Millipore Proflux M12 (Millipore), and sterilized using a 0.22 µm 1 l microbiological filter (Corning).

Triplicate groups of 15 fish each were injected intraperitoneally (i.p.) with 0.1 ml of the *Streptococcus agalactiae* vaccine, and additional triplicate groups of 15 fish each were injected i.p. with 0.1 ml sterile TSB to serve as control groups. After injection, all fish were sequestered in groups of vaccinated or control fish (N = 15) in separate aquaria and maintained as previously described.

Experimental challenge. Fish from the vaccine and TSB control groups were challenged i.p. with 1.93×10^4, 1.89×10^4 or 2.11×10^4 *Streptococcus agalactiae* colony-forming units (CFU) $fish^{-1}$ on 47, 90 or 180 DPV, respectively. During each challenge period, fish were monitored daily for clinical signs of disease and mortality for 25 d post-challenge. Moribund and dead fish were removed twice daily, and bacterial samples were aseptically obtained from the naris, brain, head kidney and intestine of 10% of morbid and dead fish to confirm the presence of *S. agalactiae*. Samples were cultured at 35°C for 24 h on 5% de-fibrinated sheep blood agar (SBA; Remel), and isolate identity was confirmed as *S. agalactiae* using the BIOLOG MicroLog Microbial Identification System according to the manufacturer's instructions. Positive cultures were beta-haemolytic, oxidase-negative, catalase-negative, and Gram-positive cocci (Evans et al. 2002).

ELISA. On 0, 47, 90 and 180 DPV and 25 d post-challenge, tilapia inoculated with TSB or *Streptococcus agalactiae* vaccine were bled from the caudal vein and the sampled blood was held at 25°C for 1 h. Serum was separated by centrifugation at 400 × *g* for 6 min and then stored at −70°C until use. The tilapia serum was tested for antibodies against sonicated whole *S. agalactiae* cells by indirect ELISA based on the methods of Shelby et al. (2002). The ELISA antigen was prepared by sonication of whole encapsulated *S. agalactiae* cells followed by centrifugation at 4000 × *g* for 20 min and removal of the supernatant. The total protein content of this fraction was determined by the bicinchoninic acid method and adjusted to 500 µg protein ml^{-1}.

One hundred µl of antigen was added to each well of a 96-well microtiter plate, which was incubated at 25°C for 2 h. The wells were blocked with a 3% bovine serum albumin (Sigma) at 25°C for 1 h, and the plates were washed with phosphate-buffered saline plus 0.05% Tween-20 (PBS-T). Nile tilapia serum samples were diluted 1:100 in PBS-T, and 100 µl of the resulting solution was added to 3 replicate wells of the microtiter plate. The plate was incubated at 25°C for 1 h and washed with PBS-T. Mouse anti-tilapia IgM heavy chain-specific monoclonal antibody 1H1 (Shelby et al. 2002) was diluted 1:5000 in PBS-T and 100 µl of this

solution was added to each well. The plate was incubated at 25°C for 1 h and washed with PBS-T. Peroxidase-conjugated rabbit anti-mouse IgG (Pierce Biotechnology) was diluted 1:5000 in PBS-T and added to each well. The plate was washed again and 100 µl of One-Step Ultra TMB-ELISA (Pierce) was added to each well. The ELISA reaction was stopped after 20 min with 50 µl 3 M H_2SO_4, and the optical density of the reactions was read at 450 nm with a Bio-Tek Automated Microplate Reader (Bio-Tek Instruments).

Statistics. All statistical analyses were performed using the SAS program (SAS Institute). Survival data and ELISA results (mean ± SE) were compared with the general linear model (GLM) procedure, and significant differences between groups and between tanks within groups were accepted at $p < 0.05$.

RESULTS

Behaviorally, control and vaccinated fish began to display clinical signs of disease 24 h after each challenge, and almost all mortalities in both groups occurred during the first 5 d post-challenge. Over the course of the 3 challenge periods, the control group experienced 84 to 96% mortalities while the vaccinated group experienced 33 to 51% mortalities. Within the 25 d observation period of the challenge study, the mean days of survival ranged from 2.4 to 5.3 d for the controls and 13.3 to 17.3 d for the vaccinates (Table 1). Percent survival and days of survival between control and vaccine groups at each challenge period were significantly different. The percent survival in control fish injected with sterile TSB was 16, 16 and 4% on 47, 90 and 180 DPV, respectively, while the percent survival for the vaccinated fish was 67, 62 and 49%, respectively. While the overall percent survival and mean days of survival for each group generally decreased over time, no significant differences were found when comparing the survival data for each group from the 3 challenge periods. A tank effect ($p = 0.0111$) was observed when comparing the mean days of survival for control fish from the 47 DPV challenge, but no tank effect was detected when comparing the percent survival for this group. No other significant tank effects were noted when analyzing survival data. All sampled organs from all sampled fish from each group were culture-positive for *Streptococcus agalactiae*.

On 47, 90 and 180 DPV, the TSB-injected controls also did not exhibit increased *Streptococcus agalactiae*-specific antibody concentrations, but the fish immunized with the *S. agalactiae* vaccine showed significant increases in mean specific anti-*S. agalactiae* antibody concentrations from 0.019 ± 0.002 to 0.115 ± 0.004 optical density (OD) between 0 and 47 DPV, respectively (Table 2). The highest specific antibody concentrations (0.192 ± 0.017 OD) were observed at 90 DPV, and the mean specific antibody concentrations declined from 0.192 ± 0.017 OD to 0.095

Table 1. Cumulative total survivors, percent survival, and mean days (±SE) of survival following *Streptococcus agalactiae* challenge. Tilapia *Oreochromis niloticus* were injected intraperitoneally with tryptic soy broth as control or *S. agalactiae* vaccine; challenged intraperitoneally with 2.0×10^4 CFU *S. agalactiae* $fish^{-1}$ on 47, 90 or 180 d post-vaccination (DPV); and then monitored for 25 d. Different superscript letters indicate significant differences ($p < 0.05$) between groups within each sampling day

Tilapia group	DPV	No. challenged	Total survivors	% survival	Days survival
Control	47	45	7	16^a	5.3 ± 1.3^a
Vaccinated	47	45	30	67^b	17.3 ± 1.7^b
Control	90	45	7	16^a	5.1 ± 1.3^a
Vaccinated	90	45	28	62^b	16.6 ± 1.7^b
Control	180	45	2	4^a	2.4 ± 0.7^a
Vaccinated	180	45	22	49^b	13.3 ± 1.7^b

Table 2. Specific anti-*Streptococcus agalactiae* antibody concentrations (ELISA optical density, OD) in tilapia *Oreochromis niloticus* post-vaccination (pre-challenge and post-challenge) with *S. agalactiae* (details in Table 1 legend) and correlation (r^2) between antibody concentrations and cumulative percent survival. Pre-challenge serum samples were obtained on day of vaccination (Day 0) or challenge (47, 90 or 180 DPV) prior to injection with vaccine or *S. agalactiae*, and post-challenge serum was obtained from challenge survivors 25 d post-challenge. Different superscript letters indicate significant differences between groups within pre- or post-challenge samples; asterisks indicate significant differences between post-challenge and corresponding pre-challenge samples. r^2 designated as *: significant ($p < 0.05$); ‡: significant ($p < 0.001$); –: not assesed

Tilapia group	DPV	OD pre-challenge	OD post-challenge	% survival	r^2
Control	0	0.017 ± 0.002^a	–	–	–
Vaccinated	0	0.019 ± 0.002^a	–	–	–
Control	47	0.023 ± 0.002^a	$0.196 \pm 0.009^{a*}$	16^a	–
Vaccinated	47	0.115 ± 0.004^c	$0.218 \pm 0.017^{a*}$	67^b	$0.7923^‡$
Control	90	0.025 ± 0.004^a	$0.246 \pm 0.049^{a*}$	16^a	–
Vaccinated	90	0.192 ± 0.017^d	$0.255 \pm 0.041^{a*}$	62^b	$0.5712^‡$
Control	180	0.013 ± 0.002^a	$0.164 \pm 0.014^{a*}$	4^a	–
Vaccinated	180	0.095 ± 0.005^b	$0.182 \pm 0.006^{a*}$	49^b	$0.8828^‡$

± 0.005 OD between 90 and 180 DPV. No significant differences were observed between post-challenge specific anti-*S. agalactiae* antibody levels during the 47, 90 and 180 DPV time points, but all post-challenge antibody levels were significantly higher than the corresponding pre-challenge antibody levels during the 47, 90 and 180 DPV time points. A significant ($p < 0.05$) or highly significant ($p < 0.001$) correlation between increased specific antibody levels and survival of vaccinates was noted during each challenge trial: Day 47 ($r^2 = 0.7923$; $p = 0.0004$), Day 90 ($r^2 = 0.5712$; $p = 0.0261$), and Day 180 ($r^2 = 0.8828$; $p < 0.0001$) post-vaccination. No significant tank effects were noted when analyzing serum antibody data.

DISCUSSION

The aim of this research was to correlate the specific anti-*Streptococcus agalactiae* antibody concentrations in immunized tilapia with their ability to resist experimental challenge with highly virulent *S. agalactiae*. Klesius et al. (2006) reviewed experimental vaccines for streptococcal disease in fishes, revealing that only a limited number of the reviewed studies assessed the long-term protection conferred by vaccination and that no prior studies have evaluated the correlation between antibody concentrations and survival after *S. agalactiae* challenge. Eldar et al. (1997) i.p. injected rainbow trout *Oncorhynchus mykiss* with a formalin-killed preparation of *S. iniae* and determined that the vaccine conferred protection against *S. iniae* challenge up to 6 mo post-vaccination. Specific anti-*S. iniae* antibody levels, however, decreased over time after vaccination from a titer of 1:20 to 1:1. Romalde et al. (1999) studied a toxoid-enriched *Streptococcus* sp. bacterin and found that protection conferred by the vaccine declined with time. Percent survival rates among vaccinated fish were 90, 75 and 50% after challenge at 6, 12 and 24 mo post-vaccination, respectively. Despite significant protection, Romalde et al. (1999) failed to find a correlation between specific antibody concentrations and protective immunity. Klesius et al. (2000) evaluated an *S. iniae* vaccine (US Patent No. 6 379 677 B1; 2002) with formalin-killed cells and concentrated extracellular products, and this vaccine conferred significant protection against challenge for at least 6 mo. Protection appeared associated with increased specific antibody levels, but the correlation was not reflective of protective immunity.

According to the percent survival and mean days of survival, the *Streptococcus agalactiae* vaccine studied herein provided significant protection against experimental challenges with *S. agalactiae* between 47 and 180 DPV. This finding extends the conclusions previously indicated by Evans et al. (2004b), who observed that protection conferred by this vaccine was comparable at 30 and 64 DPV and suggested that longer duration of protective immunity may be possible. In another study with this *S. agalactiae* vaccine, Evans et al. (2004a) observed a 90% survival rate among vaccinated tilapia (mean weight = 39.0 g) held at 30°C and challenged with 1.5×10^4 *S. agalactiae* CFU fish^{-1}. The percent survival in the current study was generally lower than survival observed previously, but our experimental challenge dose (approximately 2.0×10^4 *S. agalactiae* CFU fish^{-1}) was higher than those in previous studies, which may account for the lower percent survival. Indeed, Evans et al. (2004a,b) found that control fish administered 1.5×10^4 *S. agalactiae* CFU fish^{-1} had 24 to 40% survival rates, while control fish administered 2.6×10^4 *S. agalactiae* CFU fish^{-1} had a 0% survival rate. The challenge dose used in the present study fell between these 2 previously used challenge doses, and our control fish accordingly had 4 to 16% survival rates. The ultimate decrease to a 47% survival rate among vaccinated fish at 180 DPV is probably due to the use of a challenge dose greater than the *S. agalactiae* LD_{50} dose (1.9×10^3 *S. agalactiae* CFU fish^{-1}; Evans et al. 2002).

As in other vaccine studies in which protection against other pathogen species was directly correlated with serum antibody concentrations (Gudmundsdottir et al. 1997, Bricknell et al. 1999), significant correlations were found in this study between anti-*Streptococcus agalactiae* antibody concentrations and percent survival. The statistical results of the present study demonstrate that the specific antibody responses significantly correlated with percent survival at 47, 90 and 180 DPV and that the antibody concentrations and percent survival declined at 180 DPV. This correlation between specific antibody titers and protection was substantiated by statistical analysis ($p < 0.05$). Although the percent survival from 47 to 90 DPV decreased, the decrease was not statistically significant; meanwhile, the ELISA OD increased significantly, but may have not been high enough to increase survival significantly. Therefore, the correlation at 90 DPV was not as strong, but was still significant. This study also does not definitively discount the role of other immune factors that are protective against other *Streptococcus* sp., such as nonspecific cytotoxic cells (Taylor et al. 2001). However, our conclusions correspond with previous studies that have shown that mammalian immunity against *S. agalactiae* is based on a specific antibody-dependent phagocytic response by polymorphonuclear cells (Klesius et al. 1974, Mathews et al. 1974).

Post-challenge specific antibody concentrations were significantly increased in response to experimental challenge, even at 180 DPV. Although the post-

challenge levels of control and vaccinated groups were generally equivocal, the vaccinated fish may have generated immune responses more rapidly. This presumptively protected the vaccinated fish against mortalities while the control fish were not able to generate rapid specific antibody responses and experienced high mortalities within 5 d of challenge. In addition, because challenge increased post-challenge specific antibody concentrations in control fish, exposure of vaccinated fish to *Streptococcus agalactiae* present in aquaculture systems would be expected to booster the antibody concentrations. This booster effect would increase the levels of protection over time. The use of adjuvant or immunization booster may be required to protect tilapia during production periods longer than 6 mo, but the reduced degree of immunity at 180 DPV may be sufficient to protect fish exposed to *S. agalactiae* in aquaculture production systems.

In conclusion, the current study found good correlation between specific anti-*Streptococcus agalactiae* antibody concentrations and survival following *S. agalactiae* challenge, despite the decline in both parameters at 180 DPV. Furthermore, because of the positive correlation between specific antibody concentrations and survival, antibody levels could be measured as a non-lethal monitoring tool to assess the potential degree of protection and the efficacy of vaccination.

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LITERATURE CITED

Baya AM, Lupiani B, Hetrick FM, Roberson BS, Lukacovic R, May E, Poukish C (1990) Association of *Streptococcus* sp. with fish mortalities in the Chesapeake Bay and its tributaries. J Fish Dis 13:251–253

Berridge BR, Bercovier H, Frelier PF (2001) *Streptococcus agalactiae* and *Streptococcus difficile* 16S–23S intergenic rDNA: genetic homogeneity and species-specific PCR. Vet Microbiol 78:165–173

Bricknell IR, King JA, Bowden TJ, Ellis AE (1999) Duration of protective antibodies, and the correlation with protection in Atlantic salmon (*Salmo salar* L.), following vaccination with an *Aeromonas salmonicida* vaccine containing iron-regulated outer membrane proteins and secretory polysaccharide. Fish Shellfish Immunol 9: 139–151

Duremdez R, Al-Marzouk A, Qasem JA, Al-Harbi A, Gharabally H (2004) Isolation of *Streptococcus agalactiae* from cultured silver pomfret, *Pampus argenteus* (Euphrasen), in Kuwait. J Fish Dis 27:307–310

Eldar A, Bejerano Y, Bercovier H (1994) *Streptococcus shiloi* and *Streptococcus difficile*: two new streptococcal species causing meningoencephalitis in fish. Curr Microbiol 28: 139–143

Eldar A, Horovitcz A, Bercovier H (1997) Development and efficacy of a vaccine against *Streptococcus iniae* infection in farmed rainbow trout. Vet Immunol Immunopathol 56: 175–183

Elliott JA, Facklam RR, Richter CB (1990) Whole-cell protein patterns of nonhemolytic group B, type Ib, streptococci isolated from humans, mice cattle, frogs, and fish. J Clin Microbiol 28:628–630

Evans JJ, Klesius PH, Glibert PM, Shoemaker CA and 5 others (2002) Characterization of beta-haemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* (L.) and wild mullet, *Liza klunzingeri* (Day), in Kuwait. J Fish Dis 25:505–513

Evans JJ, Shoemaker CA, Klesius PH (2003) Effects of sublethal dissolved oxygen stress on blood glucose and susceptibility to *Streptococcus agalactiae* in Nile tilapia, *Oreochromis niloticus*. J Aquat Anim Health 15:202–208

Evans JJ, Klesius PH, Shoemaker CA, Fitzpatrick BT (2004a) *Streptococcus agalactiae* vaccination and infection stress in Nile tilapia, *Oreochromis niloticus*. J Appl Aquacult 16:105–115

Evans JJ, Shoemaker CA, Klesius PH (2004b) Efficacy of *Streptococcus agalactiae* (Group B) vaccine in tilapia (*Oreochromis niloticus*) by intraperitoneal and bath immersion administration. Vaccine 22:3769–3773

Glibert PM, Landsberg J, Evans JJ, Al Sarawi MA and 7 others (2002) A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: the roles of disease, harmful algae, and eutrophication. Harmful Algae 12:1–17

Gudmundsdottir BK, Jonsdottir H, Steinthorsdottir V, Magnadottir B, Gudmundsdottir S (1997) Survival and humoral antibody response of Atlanic salmon, *Salmo salar* L., vaccinated against *Aeromonas salmonicida* ssp. *achromogenes*. J Fish Dis 20:351–360

Kawamura Y, Itoh Y, Mishima N, Ohkusu K, Kasai H, Ezaki T (2005) High genetic homogeneity of *Streptococcus agalactiae* and *Streptococcus difficilis*: reclassification of *S. difficilis* Eldar et al. 1995 as a junior synonym of *S. agalactiae* Lehmann and Neumann 1896. Int J Syst Evol Microbiol 55: 961–965

Klesius PH, Zimmerman RA, Mathews TH, Krushak D (1974) Cellular and humoral immune response to Group B streptococci. J Pediatr 83:926–932

Klesius PH, Shoemaker CA, Evans JJ (2000) Efficacy of a single and combined *Streptococcus iniae* isolate vaccine administered by intraperitoneal and intramuscular routes in tilapia (*Oreochromis niloticus*). Aquaculture 188:237–246

Klesius PH, Evans JJ, Shoemaker CA, Pasnik DJ (2006) Streptococcal vaccinology in tilapia aquaculture. In: Lim C, Webster CD (eds) Tilapia: biology, culture, and nutrition. Haworth Press, Binghamton, NY (in press)

Mathews TH, Klesius PH, Zimmerman RA (1974) Opsonin system of Group B streptococcus. Infect Immun 10: 1315–1320

Plumb JA, Schachte JH, Gaines JL, Peltier W, Carroll B (1974) *Streptococcus* sp. from marine fishes along the Alabama and Northwest Florida coast of the Gulf of Mexico. Trans Am Fish Soc 103:358–361

Robinson JA, Meyer FP (1966) Streptococcal fish pathogen. J Bacteriol 92:512

Romalde JL, Magarinos B, Toranzo AE (1999) Prevention of streptococcosis in turbot by intraperitoneal vaccination: a review. J Appl Ichthyol 15:153–158

Shelby RA, Klesius PH, Shoemaker CA, Evans JJ (2002) Passive immunization of tilapia, *Oreochromis niloticus* (L.),

with anti-*Streptococcus iniae* whole sera. J Fish Dis 25:1–6
Stickney RR (2000) Tilapia culture. In: Stickney RR (ed) Encyclopedia of aquaculture. John Wiley & Sons, New York, p 934–941
Taylor SL, Jaso-Friedmann L, Allison AB, Eldar A, Evans DL (2001) *Streptococcus iniae* inhibition of apoptosis of nonspecific cytotoxic mechanism of activation of innate immunity in teleosts. Dis Aquat Org 46:15–21
Vandamme P, Devriese LA, Pot B, Kersters K, Melin P (1997) *Streptococcus difficile* is a nonhemolytic group B, type Ib *Streptococcus*. Int J Syst Bacteriol 47:81–85
Wilkinson HW, Thacker LG, Facklam RR (1973) Nonhemolytic group B streptococci of human, bovine, and ichthyic origin. Infect Immun 7:496–498

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