Therapeutic and prophylactic immunization against *Streptococcus iniae* infection in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*)

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**Abstract**

Vaccination strategies have traditionally been used as preventative or prophylactic measures against disease (prophylactic immunization) in uninfected fish. Alternatively, therapeutic or remedial measures, such as antibiotic administration, are commonly employed to treat disease in infected fish. Vaccination as a therapeutic measure (therapeutic immunization), however, has not been adequately explored in sub-clinically infected fish. Therapeutic and prophylactic immunization with three *Streptococcus iniae* vaccines, formalin-killed whole *S. iniae* cells (FKC vaccine), concentrated *S. iniae* extracellular products (greater than 2 kDa) (ECP vaccine) and a combination of killed cells and extracellular products (FKC+ECP vaccine), were tested in hybrid striped bass, *Morone chrysops* × *Morone saxatilis*, previously naturally infected with *S. iniae*. Fish (mean weight 1.00 g) were injected intraperitoneally (IP) or intramuscularly (IM) with one of each of the vaccines, tryptic soy broth (TSB-control) or non-injected (non-injected control) to evaluate therapeutic effects (Trial 1). Survivors of the natural infection and ECP and FKC+ECP vaccine immunization and another lot of non-injected control fish were immersion challenged with 1.47 × 10⁶ CFU of *S. iniae* mL⁻¹ at 44 days post-immunization (Trial 2). Survival of fish injected with TSB or immunized with FKC vaccine was significantly lowered and ranged from 12 to 13 by IP injection and 40 to 50 by IM injection and thus, the FKC vaccine had no therapeutic effect. The survival of hybrid striped bass IM immunized with *S. iniae* ECP vaccine in field Trial 3 was 91 and the RPS was 83. These results demonstrate that therapeutic immunization using *S. iniae* ECP and FKC+ECP vaccines can control a natural *S. iniae* infection. Furthermore, *S. iniae* ECP or FKC+ECP vaccines can also be used prophylactically to protect hybrid striped bass against subsequent pathogen challenge.

**Keywords:** vaccines, *Streptococcus iniae*, therapeutic immunization, natural infection, hybrid striped bass

**Introduction**

Historically, fish vaccines have been considered in the context of prophylaxis or stimulation of protection against subsequent exposure to a pathogen by immunization. However, the therapeutic merit of a vaccine or remedial treatment of asymptomatic diseased fish can be equally important as its prophylactic value. Because therapeutic immunization of fish is a novel concept, limited literature is available and no
literature is available on *Streptococcus iniae*. Recently, Rhodes, Rathbone, Corbett, Harrell and Strom (2004) reported therapeutic effects of vaccination with a combined cellular vaccine that consisted of killed-*Renibacterium salmoninarum* cells (MT239) and *Renogen* (commercially available attenuated *Arthrobacter* spp.) against preexisting bacterial kidney disease in chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). Vaccination with this preparation significantly increased survival among fish naturally infected with *R. salmoninarum* before vaccination and decreased bacterial antigen levels in the kidney providing infection therapy. Evans, Klesius, Shoemaker and Fitzpatrick (2004) reported increased survival and a significant reduction in stress associated with infection in Nile tilapia, *Oreochromis niloticus* L. vaccinated with a killed *Streptococcus agalactiae* vaccine.

Hybrid striped bass, *Morone chrysops* × *Morone saxatilis* (Walbaum) production is impacted by heavy economic losses because of mortality caused by *S. iniae* (Shoemaker & Klesius 1997). The need for a vaccine is paramount in the control of streptococcal disease. Several killed, *Streptococcus* vaccine preparations are reported to protect rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Akhlaghi, Munday & Eldar 1997; Eldar, Horovitz & Bercovier 1997; Nakanishi, Kiryu & Ototake 2002), turbot, *Scophthalmus maximus* L. (Toranzo, Cutrin, Nuñez, Romalde & Barja 1995; Romalde, Margaríños & Toranzo 1999), yellowtail, *Seriola quinqueradiata* (Sako 1998) and tilapia species, *Oreochromis* spp. (Klesius, Shoemaker & Evans 1999; Evans, Shoemaker & Klesius 2004; Shoemaker, Vandenberg, Desormeaux, Klesius & Evans 2006). The reader is referred to a review of streptococcal technology (Klesius, Evans, Shoemaker & Pasnik 2006) for the effects of fish species, vaccine, vaccine type and route of vaccination on vaccine efficacy against streptococcal infection. *Streptococcus iniae* vaccine efficacy has not been reported in hybrid striped bass.

In a large number of fish production facilities, fish populations are exposed to natural infections and become subclinically infected with *S. iniae* (Shoemaker, Klesius & Evans 2001). Naturally infected hybrid striped bass fry and fingerlings may be asymptomatic carriers and then may become symptomatic with *S. iniae* disease. A few days following the stress of handling and transport or changes in water quality. These conditions can impact vaccination considerations.

The objective of this study was to evaluate the therapeutic and prophylactic value of *S. iniae* vaccines prepared from either formalin-killed cells (FKC vaccine), concentrated extracellular products (greater than 2 kDa) (ECP vaccine) or a combination of both (FKC+ECP vaccine).

### Materials and methods

#### Hybrid striped bass maintenance

Hybrid striped bass were purchased from a commercial hatchery and maintained at the ARS, USDA, Aquatic Animal Health Research Laboratory (Auburn, AL, USA). The fingerlings were stocked at 1500 fish into a flow-through 300 L fiberglass tank (5 fish L\(^{-1}\)) supplied with 0.5 L h\(^{-1}\) de-chlorinated water for 20 days. Hybrid striped bass (mean weight 100 g) were acclimated in flow-through 57 L glass aquaria supplied with 0.5 L h\(^{-1}\) de-chlorinated water for 10 days before experiments. A light and dark period of 12:12 h was maintained and aeration was supplied by an air stone. The fish were fed daily to apparent satiation with Aquamax Grower 400 (Brentwood, MO, USA).\(^1\)

Dissolved oxygen, temperature, pH, salinity, hardness, ammonia and nitrite were measured daily. The dissolved oxygen and temperature were measured using a YSI 85 oxygen conductivity, salinity and temperature meter (Yellow Spring Instrument, Yellow Springs, OH, USA). The pH, ammonia and nitrite were determined using the Fresh Water Aquaculture Kit Model AG-2 (LaMotte, Chestertown, MD, USA).

#### Bacteria isolate and vaccine preparation

A *S. iniae* isolate (ARS-60) isolated from hybrid striped bass with natural streptococcal disease (Shoemaker et al. 2001) was used in the preparation of the vaccines and as the challenge isolate. The isolate was identified as *S. iniae* by standard methods (Shoemaker & Klesius 1997). The primary isolate was grown in tryptic soy broth (TSB, Difco Laboratories, Sparks, MD, USA) for 24 h at 27 °C and then frozen in 0.2 mL aliquots at −70 °C.

Vaccines were prepared similar to Klesius et al. (1999, 2000, 2002). Briefly, *S. iniae* (ARS-60 isolate) was cultured in TSB and incubated in a shaker (70 rpm) water bath at 27 °C for 72 h. Cultures were

\(^1\) Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.
treated with 10% neutral buffered formalin to give a final concentration of 3% at 27 °C for 24 h. The formalin inactivated or killed cultures were centrifuged at 7000 × g for 30 min and cell pellet (FKC) and culture fluid (ECP) separated. The cell-free culture fluid was concentrated 20-fold and dialyzed using a 2 kDa hollow fiber concentrator to remove formalin, filter sterilized (0.2 µm) and used to re-suspend the cell pellet at v/v of 1:1. The final concentration of the FKC+ECP vaccine was 4 × 10^8 CFU mL⁻¹ (determined by previous plate count) or I.9 optical density at 540 nm. To verify the non-viable status of the killed S. iniae in the FKC and FKC+ECP vaccines and absence of live S. iniae cells in the ECP vaccine, samples were obtained and streaked directly on 5% sheep blood agar (SBA) (Remel, Lexena, KS, USA) and then incubated at 27 °C for 72 h. Live S. iniae was not isolated from any of the vaccines or TSB control medium. The vaccines were determined to be inactivated or killed by lack of growth on SBA at 72 h.

**Trial 1**

To verify the disease-free status of the fish received from the commercial hatchery, brain and kidney samples from three randomly selected fish were obtained for bacterial culture before initiation of the vaccine trials. Samples were streaked directly on SBA and incubated at 27 °C for 24–72 h. No bacterial growth was observed from any of these samples. Eight hundred fish (10.0 g) were randomly placed in forty 57 L aquaria (five replicate aquaria per treatment) of 20 fish each, and an additional two replicate aquaria were stocked with 25 fish each from the same population to serve as a negative control. Two hundred fish were either intraperitoneally (IP) (100 fish) or intramuscularly (IM) (antior-dorsally) (100 fish) with 0.1 mL of the FKC, ECP or FKC+ECP vaccines (Table 2). The remaining 200 fish were injected either IP (100 fish) or IM (100 fish) with 0.1 mL of TSB (TSB control). The negative control fish were not injected (non-injected control).

Fish were not experimentally infected with S. iniae before vaccination. Furthermore, vaccines and TSB proved to be sterile and free of live S. iniae cells, and fish bacteriological samples were negative for bacterial growth before the onset of the study. Given our inability to culture bacteria or S. iniae, we assumed fish were culture negative. Within 2 days following vaccination, we observed fish behavioral and morphological signs indicative of streptococcal infection which included surface or bottom erratic swimming, refusal of food, lethargy and darkening skin coloration (Klesius et al. 1999) and increased fish mortality. Bacterial culture of morbid fish yielded S. iniae in pure culture indicating natural exposure to S. iniae and sub-clinical S. iniae infection. Trial 1 was continued and fish were monitored for cumulative mortalities because of S. iniae for 44 days and morbid and dead fish cultured bacteriologically. Thirty-four per cent (68/202) and 37% (49/132) of the IP and IM vaccinated morbid and dead fish respectively, were cultured.

**Bacteriologic sample collection and evaluation**

Dead or moribund fish from Trial 1 were removed twice a day for 44 days, and bacteriologic samples were obtained aseptically from brain, eye, nare and kidney sites using sterile inoculating loop. The samples were cultured directly onto SBA at 27 °C for 24–72 h. Beta-hemolytic, catalase-negative and Gram-stained positive coccus colonies were sub-cultured onto SBA and then bacteriologically and biochemically identified as S. iniae according to tests described by Shoemaker and Klesius (1997).

**Trial 2**

Experimental immersion challenge of hybrid striped bass IP and IM immunized with FKC vaccine or injected with TSB medium could not be conducted because of significant mortality of S. iniae infected fish in Trial 1. Vaccinates immunized with ECP vaccine (189 fish) and FKC+ECP vaccine (163 fish) that survived the S. iniae infection from Trial 1 were immersion challenged at 44 days post-immunization. Fish were placed in 2 L of water containing 1.47 × 10^6 CFU mL⁻¹ of S. iniae for 10 min. The surviving fish IP injected with ECP vaccine (96 fish) and surviving fish IM injected with ECP vaccine (93 fish) were immersion challenged in five groups of 18–20 fish each and then placed in separate aquaria following challenge. The IP injected FKC+ECP vaccine survivors (78 fish) and IM injected FKC+ECP vaccine survivors (85 fish) were immersion challenged in five groups of 15–17 fish each. An additional 100 hybrid striped bass from the initial population were used as non-injected controls and immersion challenged in five groups of 20 fish each. The fish were monitored daily for mortalities for 14 days post-challenge.
Trial 3

To further evaluate *S. iniae* vaccine efficacy under more natural conditions, 1020 hybrid striped bass (1.0 g) were IM injected with ECP vaccine at an aquaculture production facility. An equal number of non-injected fish served as controls. Approximately 200 fish separated into the two treatment groups (100 non-injected controls and 100 IM injected fish with ECP vaccine) were shipped overnight by the production facility to the AAHRL in Auburn, AL. No mortality was noted following transport of the fish from the commercial producers. Upon arrival, fish were placed into 14°C water to acclimate for 2 weeks. Fish were then placed in three replicate tanks of 25 fish each per treatment and water temperature gradually increased to 25°C over 5 days before initiation of immersion challenge. All fish (mean weight 5.6 g) were immersion challenged 12 weeks post-immunization as in Trial 2.

Statistical analysis

Survival data (total number of fish surviving per treatment) were analyzed by general linear procedures (GLM), one way-analysis of variance and Duncan’s multiple-range test (SAS Institute 1997). Significant differences were determined at \( P < 0.05 \). The efficacy of the vaccine was calculated as the relative percent survival (RPS) according to Amend (1981). The RPS was calculated for each of the vaccines administered by either IP or IM injection using the corresponding TSB control mortality in Trials 1 and 3 and using non-injected controls in Trial 2. The RPS calculations for Trials 1 + 2 are based on cumulative per cent mortality over the duration of the vaccination and account for fish lost after both immunization (Trial 1) and challenge (Trial 2).

Results

In all trials, the mean ± standard deviation of dissolved oxygen (mg L\(^{-1}\)) was 5.7 ± 0.6, temperature (°C) was 25 ± 0.7 and pH was 7.2 ± 0.04. Ammonia and nitrite concentrations (mg L\(^{-1}\)) were below the limit of detection.

Trial 1

The number and per cent of symptomatic moribund or dead hybrid striped bass culture positive for *S. iniae* post-vaccination with either of three *S. iniae* vaccines and TSB medium are shown in Table 1. Of the 68 IP immunized fish sampled microbiologically, 88% brains, 67% eyes, 60% nares and 59% kidneys were culture positive for *S. iniae*. Similar bacteriological results were seen in 49 IM immunized fish cultured. Neurological organs, brain and eye, were the organs from moribund fish most frequently culture positive regardless of vaccine administered. Moribund fish from the group of fish immunized with the FKC vaccine were culture positive in at least one of the organs sampled through 29 days (IP) and 35 days (IM). Fish

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Vaccine preparation</th>
<th># and % of fish culture positive by organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP</td>
<td>TSB (control)</td>
<td>Brain # 28/30 93</td>
</tr>
<tr>
<td></td>
<td>FKC</td>
<td>Brain # 30/32 94</td>
</tr>
<tr>
<td></td>
<td>ECP</td>
<td>Brain # 0/5 0</td>
</tr>
<tr>
<td></td>
<td>FKC+ECP</td>
<td>Brain # 1/1 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain # 60/68 88</td>
</tr>
<tr>
<td>IM</td>
<td>TSB (control)</td>
<td>Brain # 20/20 100</td>
</tr>
<tr>
<td></td>
<td>FKC</td>
<td>Brain # 20/22 91</td>
</tr>
<tr>
<td></td>
<td>ECP</td>
<td>Brain # 1/5 20</td>
</tr>
<tr>
<td></td>
<td>FKC+ECP</td>
<td>Brain # 2/2 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain # 43/49 88</td>
</tr>
</tbody>
</table>

injected with TSB were culture positive in at least one of the organs sampled through 19 days (IP) and 29 days (IM). The moribund or dead fish from the FKC vaccine group were culture positive 1 days (IP) and 25 days (IM) post-immunization. Moribund fish sampled from the ECP vaccine group were culture negative in all organs at 29 days (IM) and 35 days (IP).

Sub-clinically infected hybrid striped bass IP or IM immunized with FKC vaccine or injected with TSB had the lowest cumulative mean per cent survival 44 days post-immunization (Table 2) (Fig. 1a and b). Greater than 50% mortality occurred in fish IP immunized with FKC vaccine and TSB injected control fish within 8 days (Fig. 1a). Hybrid striped bass that were not injected had 100% survival rate. The RPS of fish immunized with the FKC vaccine was 0 and 17 for the IP and IM routes respectively. The FKC+ECP vaccines had a RPS of 75 regardless of route of administration. The RPS was greatest in fish immunized with ECP vaccine IP (96) or IM (88) (Table 2).

**Trial 2**

Experimental immersion challenge of the IM and IP ECP vaccine groups (69–86%) and IM and IP FKC+ECP vaccine groups (92–97%) resulted in significantly higher survival than non-injected controls (41%) (Table 2). No significant difference in survival was noted between FKC+ECP vaccinates by either route of administration. However, significant differences were observed between IP and IM ECP vaccine groups and between IP ECP and IP FKC+ECP vaccinates. The RPS values from the IM ECP (76), IM FKC+ECP (87) and IP

### Table 2 Survival of naturally *Streptococcus iniae* infected hybrid striped bass immunized with three *S. iniae* vaccine; formalin killed whole cells (FKC), extracellular products (ECP) or combination of killed cells and extracellular products (FKC+ECP), by intraperitoneal (IP) and intramuscular (IM) injection 44 days post-immunization (Trial 1) and 14 days post challenge (Trial 2).

<table>
<thead>
<tr>
<th>Trials</th>
<th>Vaccine preparation</th>
<th># of fish immunized</th>
<th># of fish surviving (% survival)</th>
<th>Relative percent survival (RPS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TSB (control)</td>
<td>100 100</td>
<td>13A (13) 40B (40)</td>
<td>0 17</td>
</tr>
<tr>
<td></td>
<td>FKC</td>
<td>100 100</td>
<td>12A (12) 50B (50)</td>
<td>0 17</td>
</tr>
<tr>
<td></td>
<td>ECP</td>
<td>100 100</td>
<td>96E (96) 93DE (93)</td>
<td>96 88</td>
</tr>
<tr>
<td></td>
<td>FKC+ECP</td>
<td>100 100</td>
<td>78C (78) 85CD (85)</td>
<td>75 75</td>
</tr>
<tr>
<td></td>
<td>Non-injected (control)</td>
<td>50 100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Non-injected (control)</td>
<td>100</td>
<td>41^</td>
<td>IP IM</td>
</tr>
<tr>
<td></td>
<td>ECP</td>
<td>96 93</td>
<td>66E (69) 80E (86)</td>
<td>48 76</td>
</tr>
<tr>
<td></td>
<td>FKC+ECP</td>
<td>78 85</td>
<td>76E (97) 76CD (92)</td>
<td>95 87</td>
</tr>
<tr>
<td></td>
<td># of fish challenged</td>
<td>IP IM</td>
<td>IP IM</td>
<td>IP IM</td>
</tr>
<tr>
<td>1+2</td>
<td>Non-injected (control)</td>
<td>100</td>
<td>41^</td>
<td>IP IM</td>
</tr>
<tr>
<td></td>
<td>ECP</td>
<td>100 100</td>
<td>65E (65) 80E (80)</td>
<td>41 66</td>
</tr>
<tr>
<td></td>
<td>FKC+ECP</td>
<td>100 100</td>
<td>75E (75) 78E (78)</td>
<td>58 63</td>
</tr>
<tr>
<td>3</td>
<td>TSB (control)</td>
<td>ND 75</td>
<td>ND 34E (45)</td>
<td>ND 83</td>
</tr>
<tr>
<td></td>
<td>ECP</td>
<td>ND 75</td>
<td>ND 68E (91)</td>
<td>ND 83</td>
</tr>
</tbody>
</table>

*Vaccinated fish were injected with 0.1 mL vaccine and tryptic soy broth (TSB) control fish were injected with 0.1 mL TSB or not injected. Vaccinates were challenged by 10 min immersion in water containing 2.5 mL 2 L⁻¹ of a 24 h culture of *S. iniae* (1.47 × 10⁸ CFU of *S. iniae* mL⁻¹). Different superscripts denote significant differences (P < 0.05) between vaccine preparations (columns) and routes of administration (rows). ND, not done.
Trials 1 and 2

The cumulative mean per cent survival results for both Trials 1 and 2 are depicted as a continuum for IP vaccination (Fig. 1a) and IM vaccination (Fig. 1b) followed by *S. iniae* immersion challenge. The IP ECP or FKC+ECP vaccine resulted in 65% and 75% cumulative survival of immunized and challenged fish respectively (Fig. 1a). The IM ECP or FKC+ECP vaccines resulted in 80% and 78% cumulative survival of immunized and challenged fish (Fig. 1b). Overall, the RPS for IM administered ECP vaccine (66) and FKC+ECP vaccine (63) was greater than the RPS values for IP ECP vaccine (41) and FKC+ECP vaccine (58) (Table 2).

**Trials 3**

Mean per cent survival was 45% in non-injected controls as compared with 91% in IM injected ECP vaccines. The RPS was 83 (Table 2). Mortality patterns were the same as in Trial 2 with the majority of mortality occurring within 8 days post-challenge with *S. iniae*.

**Discussion**

The microbiological culture of organs from symptomatic, dying and dead fish and isolation of *S. iniae* in pure culture indicated that hybrid striped bass were sub-clinically infected with *S. iniae* before immunization. Although bacterial culture of hybrid striped bass for detection of *S. iniae* before immunization did not yield *S. iniae* colonies, this is not an uncommon phenomenon as we have recognized that sub-clinical infections of *S. iniae* may not be confirmed by standard techniques. As such, the sampling of a larger number of fish to establish the prevalence of *S. iniae* sub-clinically infected fish before immunization may have also yielded negative results because of inadequate methodology. It is unclear whether the inability to detect carrier state is a result of very low numbers of bacteria, their potential intracellular status within phagosomes or macrophages, or the poor sensitivity of the culture technique. Recently, improvements in the ability to detect and identify as few as 10 *S. iniae* cells in infected and carrier fish by indirect fluorescent antibody technique have been reported by Klesius, Evans, Shoemaker, Yeh, Goodwin, Adams and Thompson (2006) but this technology was not available before hybrid bass immunizations in this study. However, this technology or expertise is not generally available at hatchery and aquaculture facilities. This study was not originally intended to establish the prevalence of a carrier state before immunization. The realization of the carrier state was only learned after the immunization and culturing of fish with disease signs. Nonetheless, bacterial culture of the organs of moribund symptomatic fish following immunization verified disease status in hybrid striped bass and confirmed that these fish were infected with *S. iniae*. The vaccines and TSB were proven to be uncontaminated and can be ruled out as the source of infection. Although we cannot
rule out the possibility that a low percentage of fish were sub-clinically infected before vaccination and the bacteria may have spread to uninfected fish, this scenario seems unlikely as significant mortality did not occur in all vaccine groups.

The *Streptococcus iniae* ECP and combined FKC+ECP vaccines administered by IP and IM injection increased the survival of hybrid striped bass sub-clinically infected before therapeutic vaccination. These results indicate that immunity is produced by systemic injection (IP or IM) of the ECP and FKC+ECP vaccines. The asymptomatic naturally infected fish became symptomatic for *S. iniae* following handling and administration of the FKC vaccine or TSB control medium. The FKC vaccine had no therapeutic effect. Survival was significantly lower for fish IP injected with TSB or FKC vaccine (12–13%) or IM injected with TSB or FKC vaccine (40–50%) as compared with those injected with either ECP vaccine (> 90%) or the combined FKC+ECP vaccine (78–85%) by either route of administration. The non-injected control fish remained asymptomatic and experienced no mortality because of *S. iniae*. We suspect that a stress response because of handling and injection was responsible for suppressing the innate immunity (Barton & Iwama 1991) and caused mortality. As such, handling and IP injection of FKC vaccine or TSB may serve as a stress test for testing of sub-clinically infected hybrid striped bass. The decreased survival of sub-clinically infected fish IP injected versus IM injected with either FKC vaccine or TSB may be attributed more to the route of administration and distribution to tissues by different pathways or mechanisms rather than vaccine formulation, although we cannot adequately explain this result.

A therapeutic vaccination effect on survival of naturally infected salmonids was not observed until 14 weeks post-vaccination leading Rhodes et al. (2004) to propose that this time period may be necessary for killed whole cell vaccines to stimulate a response that can clear the infection in contrast to combined cell (live and killed) vaccines which reduce levels of bacterial antigens and more rapidly clear bacteria. The injection of TSB medium or the FKC vaccine may enhance bacterial replication. *Streptococcus iniae* was isolated from TSB injected moribund fish through 29 days and through 35 days for fish injected with FKC vaccine. Conversely, the ECP and FKC+ECP vaccines may antagonize bacterial replication or eliminate bacteria thereby reducing mortality. Fish immunized with the FKC+ECP vaccine that became moribund and cultured 25 days post-vaccination were culture positive while those given the ECP vaccine that became moribund and cultured 35 days post-vaccination were culture negative. Although the mechanism of the therapeutic effect is unknown, Rhodes et al. (2004) suggested that combined killed and live cell vaccines may more rapidly clear bacteria or that vaccination itself serves as infection therapy by boosting immunity. Evans et al. (2004) reported vaccination mitigates infection stress, thereby increasing survival.

Despite the ECP vaccine providing superior therapeutic value in naturally infected fish following IM or IP immunization in the absence of *S. iniae* challenge, mean per cent survival and RPS values decreased in Trial 2 after immersion challenge with virulent *S. iniae*. The RPS of the ECP vaccine was 48 and 76 by IP and IM injection respectively. In contrast, mean per cent survival and RPS derived from the FKC+ECP vaccine increased after experimental challenge and were greater than those derived for the ECP vaccine. These results indicate that killed cells (FKC) may impart some protective effect to fish previously exposed to *S. iniae* or reflect a booster effect of the third exposure of these immunized fish to *S. iniae*.

The therapeutic protection conferred to sub-clinically infected hybrid striped bass by the combination FKC+ECP vaccine administered by IP is analogous to what has been previously reported in non-infected fish from prophylactic vaccine trials using similar *S. iniae* FKC+ECP vaccines (Klesius et al. 1999; Klesius, Shoemaker & Evans 2000; Shoemaker et al. 2006) or FKC alone (Sakai, Kubota, Atsuta & Kobayashi 1987; Eldar et al. 1997; Sako 1998; Nakashita et al. 2002). These prior studies indicated that the IP route of administration was the most commonly used and efficacious method of administration in fish. Sako (1998) reported decreased bacterial numbers in the blood, spleen and kidney of IP vaccinated yellowtail but not in the brain and suggested that bacterial clearance following vaccination by this method may be responsible for efficacy.

Trials 1 and 2, collectively, represent the efficacy of different vaccines in sub-clinically infected hybrid striped bass post-vaccination and challenge. These results demonstrate that the IM route of administration provides comparable efficacy by either ECP and FKC+ECP vaccines in carrier hybrid striped bass. Although, no significant differences in survival existed between IP and IM immunization of either ECP or FKC+ECP vaccines in Trial 1+2, survival generally increased by IM immunization. Both ECP and FKC+ECP vaccines administered by IM injection
had RPS values > 60 and overall, were more efficacious than by IP injection in sub-clinical infected hybrid striped bass. The longer antigen retention time in the musculature versus the peritoneal cavity may explain the higher RPS values of the ECP vaccines by the IM over the IP route. Furthermore, the ECP vaccine administered IM provided superior efficacy in field vaccinations yielding a RPS of 83 although infection status was not ascertained in hybrid striped bass vaccinated at the production facility. Nonetheless, our results with the IM injected ECP vaccine in Trial 3 suggest that ECP vaccine alone may provide delayed protection based on higher RPS values obtained 12 weeks post-immunization (83) versus 44 days post-immunization and challenge (66). Knowledge of the effects of S. iniae ECP on macrophage activation is limited. It may be possible that saccharide components of ECP are responsible for the stimulation of innate immunity, i.e. enhanced clearance of S. iniae. This enhanced clearance may be the result of macrophage activation following macrophage receptor recognition of the saccharide components of ECP. Streptococcus iniae ECP are likely key components of either therapeutic or prophylactic vaccines against S. iniae by the stimulation of both innate and acquired immune responses.

Few studies have explored vaccine efficacy in sub-clinically infected fish. Bruno and Munro (1989) examined the efficacy of a Versinia ruckeri vaccine in salmonid fry that were carriers of infectious pancreatic necrosis virus (IPNV) and found no significant differences in mortality between vaccinated and non-vaccinated. Although virus carrier status did not appear to affect vaccine efficacy, no assessment of vaccine efficacy was made of carriers harboring Y. ruckeri. In another study, vaccination of salmonids sub-clinically infected with Y. ruckeri resulted in elimination of sub-clinical infection (Hunter, Knittel & Fryer 1980) but effects of carrier status on vaccine efficacy was not assessed. Likewise, Inglis, Robertson, Miller, Thompson and Richards (1996) did not evaluate the efficacy of a formalin-killed whole cell furunculosis vaccine on salmonid parr sub-clinically infected with Aeromonas salmonicida but demonstrated complete mortality of fish following vaccination, handling, and transport stress. However, administration of the furunculosis vaccine combined with amoxicillin showed therapeutic effects of increased survival and elimination of carrier state (Inglis et al. 1996). Vaccine manufacturers warn against vaccine use in sub-clinically infected fish populations and restrict use for only healthy fish. Our results suggest that vaccination of carrier fish with S. iniae ECP and FKC+ECP vaccines confer therapeutic benefits against S. iniae and that performance of these vaccines is not significantly impaired by use in sub-clinically infected hybrid striped bass. Streptococcus iniae ECP and FKC+ECP vaccines confer prophylaxis for sub-clinically infected hybrid striped bass against immersion challenge with virulent S. iniae. Furthermore, ECP and FKC+ECP vaccines enhance survival of fish with preexisting infection and thus have therapeutic merit. Therapeutic vaccination is an immunization program that needs further exploration as it may prove to be adapted to specific species of fish, pathogens, and conditions of production facilities.

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References


