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Effect of parasitism on vaccine efficacy against *Streptococcus iniae* in Nile tilapia

Maurício L. Martins [a,*], Craig A. Shoemaker [b], Dehai Xu [b], Phillip H. Klesius [b]

[a] AQUOS-Aquatic Organisms Health Laboratory, Aquaculture Department, Federal University of Santa Catarina (UFSC), Rod. Admar Gonzaga 1346, 88040–900, Florianópolis, SC, Brazil
[b] USDA-ARS, Aquatic Animal Health Research Laboratory, 990 Wire Road, 36832–4352, Auburn, AL, USA

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ABSTRACT

Limited information is available on vaccine performance in parasitized fish. The objective of this study was to determine if parasitism of fish affected vaccine efficacy. Antibody level, hematology and survival of Nile tilapia vaccinated with a modified *S. iniae* bacterin were compared among non-parasitized fish, fish parasitized by *Trichodina heterodentata* and *Gyrodactylus cichlidarum*, and fish parasitized by *T. heterodentata*, *G. cichlidarum* and *Ichthyophthirius multifiliis* (Ich). Among vaccinated fish, fish free from parasites (*Trichodina*, *Gyrodactylus* and Ich) had the highest antibody level (0.43, SE = 0.14). Significantly ($p<0.05$) lower anti-*S. iniae* antibody was noted in parasitized vaccinated fish (0.30, SE = 0.08). Among the vaccinated treatments, fish parasitized by *Trichodina*, *Gyrodactylus* and Ich showed the lowest survival (80.0%, SE = 10.0), significantly ($p<0.05$) lower than vaccinated fish free from parasites (97.5%, SE = 2.5) or parasitized by *Trichodina* and *Gyrodactylus* (95.0%, SE = 5.0). Following challenge with *S. iniae*, non-vaccinated fish free from parasites showed the higher survival (47.5%, SE = 2.5) than non-vaccinated fish parasitized by *Trichodina* and *Gyrodactylus* (37.5%, SE = 2.5). Non-vaccinated fish parasitized by all 3 parasites showed the lowest survival (27.5%, SE = 2.5) post challenge. Relative percent survival (RPS) demonstrated a decrease in vaccine performance for the group of fish that were parasitized with *Trichodina* and *Gyrodactylus* and Ich. RPS was 72% compared to 95 and 92%, respectively, in the other vaccinated treatments following challenge. This study demonstrated a reduction in vaccine performance in parasitized tilapia and highlights the importance of monitoring or controlling parasite levels in the aquaculture setting to optimize vaccine efficacy.

1. Introduction

Species of the ciliated protozoan *Trichodina* and monogenoidean platyhelminths are common parasites of cultured fish (Martins et al., 2008; Ghiraldelli et al., 2006; Thoney and Hargis, 1991). These parasites usually do not cause fish mortality except in the case of heavy infestations (Madsen et al., 2000; Huh et al., 2005; Martins et al., 2010a). However, parasitism may facilitate the development of systemic bacterial infections (Busch et al., 2003; Pylkko et al., 2006; Xu et al., 2009a). Rainbow trout (*Oncorhynchus mykiss*) parasitized by *Gyrodactylus derjavini* were more likely to develop bacterial infection (Busch et al., 2003). Tilapia (*Oreochromis niloticus*) coinfected with *Streptococcus iniae* and *G. niloticus* had a higher mortality rate than tilapia infected with *S. iniae* alone (Xu et al., 2007). Evans et al. (2007) infected two groups of channel catfish (*Ictalurus punctatus*) fingerlings, parasitized and non-parasitized by *Trichodina* sp. with *S. iniae* and *S. agalactiae*. The mortality rate was significantly higher in fish that were coinfected. Increased mortality of tilapia to *S. iniae* infection was also observed after experimental parasitism with *Ichthyophthirius multifiliis* (Xu et al., 2009a).

Host–parasite interactions and the fish immune system may be affected by inadequate handling, suboptimal water quality, inadequate nutrition, stress, and parasite infection (Boshra et al., 2006). Therefore, it is necessary to understand host–parasite relationships and the mechanisms whereby parasites influence the fish immune system (Sitja-Bobadilla, 2008). Fish possess innate immunity that is modulated by pathogen recognizing receptors found on the skin and gills that can limit the parasite load (Alvarez-Pellitero, 2008). Adaptive immunity has been demonstrated by the presence of T and B cells in teleosts (Alvarez-Pellitero, 2008). Leukocyte migration in response to *I. multifiliis* parasitism and induction of interleukine-1 expression during the primary monogenoidean parasitism has been demonstrated (Alvarez-Pellitero, 2008).

Non-specific response of fish to *I. multifiliis* parasitism is mediated by cytotoxic cells (NCC) analogous to NK cells in mammals (Buchmann et al., 2001). However, there is also evidence for the production of specific antibodies to *I. multifiliis* (Xu et al., 2009b). Sigh et al. (2004) also demonstrated an increase in the anti-*I. multifiliis* IgM on kidney and skin of rainbow trout. Buchmann and Lindestrom (2002) suggest several mechanisms of host recognition in response to monogenoidean platyhelminths. Among them are the presence of lectins, complement system and antibody production. Sea bass

* Corresponding author at: USDA-ARS, Aquatic Animal Health Research Laboratory, 990 Wire Road, 36832–4352, Auburn, AL, USA. Tel.: +1 334 887 3741; fax: +1 334 887 2983.
E-mail address: mlaterca@cca.ufsc.br (M.L. Martins).

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(*Dicentrarchus labrax*) experimentally parasitized with the monogenoidean *Diplectanum aequans* showed an increase in the interleukine-1 in the spleen and gills (Faliex et al., 2008). These authors found that low level parasitism did not induce adaptive immunity but rather this parasite load may have elicited an innate immune response.

The effects of parasitism on vaccination efficacy in fish are little studied. In commercial aquaculture fish often harbor parasites, bacteria and/or viral pathogens concurrently. Anecdotal information from a commercial tilapia farm suggested that heavy parasite loads of trichodinids and dactylogyrids negatively affected the immune response and decreased effectiveness of a killed *S. iniae* vaccine (Shoemaker and Klesius, personal communication). Treatment and subsequent reduction of the parasite load restored vaccine performance. In mammals, vaccine efficacy is affected by a chronic concurrent infection and subsequent treatment to decrease the parasite load has been shown to improve vaccine performance (Borkow and Bentwich, 2008). Urban et al. (2007) discussed the negative effect of protective immunity in mice experimentally parasitized with *Heligmosomoides polygyrus* to model the condition of gastrointestinal parasitism in mammalian populations targeted for vaccination. Recent work demonstrated that parasitism with *Ascaris suum* reduced the efficacy of a *Mycoplasma hyopneumoniae* vaccine in co-infected pigs that resulted in vaccine failure (Steenhard et al., 2009).

The objective of this work was to determine if parasitism of fish affected vaccine efficacy. Antibody level, hematology and survival of Nile tilapia vaccinated with a modified *S. iniae* bacterin were compared among non-parasitized fish, fish infected by *T. heterodentata* and *G. cichlidarum*, and fish infected by *T. heterodentata*, *G. cichlidarum* and *I. multifiliis*.

2. Material and methods

2.1. Fish and water quality

Tilapia fry from the same spawning pond mean of 8.4 cm (SE = 0.9) total length and mean of 11.4 g (SE = 3.2) body weight were kept in a holding tank using filter-recirculated water at the US Department of Agriculture-Agriculture Research Service (USDA-ARS), Aquatic Animal Health Research Unit (AAHRU), Auburn, Alabama. Prior to the trial, tilapia moved to the experimental tanks with flowing water were moderately to heavily infested with *G. cichlidarum* and *T. heterodentata*. The prevalence of *Trichodina* parasitism was 100%, with a mean abundance of 858.8 (SE = 233.5). The prevalence of *Gyrodactylus* infestation was 70%, with a mean abundance of 30.6 (SE = 10.6). Fish were divided into two groups. One group was treated twice with potassium permanganate (5 ppm bath treatment) for 1 h on two consecutive days, followed by a 150 ppm formalin bath treatment for 1 h on the third day. After treatment, no parasites were recovered from biopsies of the skin and gills of the treated fish. Fish were acclimated for 7 days post treatment and fed ~3–4% body weight daily (Aquamax Grower 400, *PMI Nutrition International, LLC*., Brentwood, MO). The number of parasites from ten fish in each group was estimated just before the trial.

During the experiment total ammonia nitrogen, nitrite-nitrogen, alkalinity, pH and dissolved oxygen were measured once a week in random tanks. Water temperature was measured every day. The means and standard error (SE) of pH was 6.39 (SE = 0.03), ammonia 0.26 (SE = 0.02) $mg \cdot L^{-1}$, nitrite 0.13 (SE = 0.04) $mg \cdot L^{-1}$, alkalinity 58.14 (SE = 6.9) mg $CaCO_3 \cdot L^{-1}$, dissolved oxygen 6.44 (SE = 0.11) $mg \cdot L$ and water temperature 25.5 (SE = 0.14) °C.

2.2. Experimental design

A total of 320 fish with 12.5 (SE = 2.0) cm in length and 20.2 (SE = 0.6) g in weight was distributed in 16 glass aquaria divided in 8 treatments with 2 replicates in each, as follows (see Table 1): 1) fish not parasitized with *Trichodina*, *Gyrodactylus* (No TRICH/GYRO) and *I. multifiliis* (NoICH), not vaccinated (NoVAC), not exposed to *S. iniae* (No *S. iniae*); 2) no parasites or vaccination, but exposed to *S. iniae* (No TRICH/GYRO-NoICH-NoVAC-*S. iniae*); 3) no TRICH/GYRO-NoICH-VAC-*S. iniae*; 4) TRICH/GYRO-NoICH-No VAC-*S. iniae*); 5) TRICH/GYRO-NoICH-VAC-*S. iniae*; 6) TRICH/GYRO-ICH-NoVAC-*S. iniae*; 7) TRICH/GYRO-ICH-VAC-No *S. iniae*; and 8) TRICH/GYRO-ICH-VAC-*S. iniae*.

2.3. Parasites

Trophonts of *I. multifiliis* were originally isolated from a parasitized channel catfish from the USDA-ARS, AAHRU, Auburn, Alabama. Infected channel catfish were kept in 50 L glass aquaria and the theronts obtained as described by Xu et al. (2000). Briefly, fish were humanely euthanized by immersion in 300 mg/L tricaine methane sulfonate (MS-222), mature trophonts were gently scraped to dislodge the parasites, transferred to a 10 L glass aquarium with aeration, and incubated for 24 h at 24 °C. Theront concentrations were quantified with the aid of a Sedgewick–Rafter chamber. Fish were exposed to 40,000 theronts per fish (Xu et al., 2009b).

Prior to the experiment, 10 fish from the holding tank were examined for the parasites *Trichodina* and *Gyrodactylus*. After that, parasite samples were collected from 6 fish of each treatment at day 7 and day 21 post vaccination and 10 fish of each treatment at the end of the trial. For parasite quantification, body surface mucus was scraped into a Falcon tube and fixed in a 5% formalin solution for counting in a Sedgewick–Rafter chamber according to Ghiraldelli et al. (2006). Twenty percent of the sample was counted and estimated from the total volume. The mean abundance of parasites was calculated according to Bush et al. (1997).

2.4. Vaccination

Non-vaccinated fish were intraperitoneally (i.p.) injected with 100 μL of sterile trypic soy broth (TSB). Vaccinated fish were

Table 1
Mean abundance (mean ± standard error) of parasites 7 days post vaccination with *Streptococcus iniae* vaccine. Within a column, means followed by the different lower case letter are significantly different ($p<0.05$).

Treatments	*I. multifillis*	*T. heterodentata*	*G. cichlidarum*	Total*
NoTRICH/GYRO-NoICH-NoVAC-No *S. iniae*	0^a	0^a	0^a	0^a
NoTRICH/GYRO-NoICH-NoVAC-*S. iniae*	0^a	0^a	0^a	0^a
NoTRICH/GYRO-NoICH-VAC-*S. iniae*	0^a	0^a	0^a	0^a
TRICH/GYRO-NoICH-NoVAC-*S. iniae*	0^a	644.7 ± 254.0^b	47.6 ± 13.8^b	692.3 ± 256.3^b
TRICH/GYRO-NoICH-VAC-*S. iniae*	0^a	142.7 ± 80.6^c	39.2 ± 7.6^b	182.0 ± 84.3^c
TRICH/GYRO-ICH-NoVAC-*S. iniae*	14.5 ± 5.4^b	680.5 ± 238.5^b	26.2 ± 11.8^c	721.2 ± 250.4^b
TRICH/GYRO-ICH-VAC-No *S. iniae*	12.7 ± 4.1^b	696.7 ± 83.7^b	28.8 ± 7.3^c	738.2 ± 85.8^b
TRICH/GYRO-ICH-VAC-*S. iniae*	4.0 ± 4.0^a	720.3 ± 305.1^b	9.8 ± 4.5^d	734.2 ± 306.8^b

* Total count of parasites, including *T. heterodentata*, *G. cichlidarum* and *I. multifillis*.