Fish & Shellfish Immunology (2008) **25**, 124–127

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Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization with live theronts and sonicated trophonts

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Received 16 November 2007; revised 18 March 2008; accepted 19 March 2008
Available online 28 March 2008

KEYWORDS
Protective immunity;
Ichthyophthirius multifiliis;
Live theront;
Nile tilapia;
Antibody titer;
Fish survival

Abstract Two immunization trials were conducted to evaluate host protection of Nile tilapia, *Oreochromis niloticus* against *Ichthyophthirius multifiliis* (Ich). Immunizations were done with live theronts or sonicated trophonts by bath immersion and intraperitoneal (IP) injection. The immunized fish were challenged with theronts 21 days post-immunization in trial I and 180 days post-immunization in trial II. The serum anti-Ich antibody and cumulative mortalities of tilapia were determined after theront challenge. Serum anti-Ich antibody was significantly higher ($P < 0.05$) in tilapia immunized with live theronts by immersion or IP injection or with sonicated trophonts administered by IP injection than tilapia immunized with sonicated trophonts by immersion, with bovine serum albumin by IP injection, or non-immunized controls. Host protection was acquired in fish immunized with live theronts by immersion or IP injection. Tilapia immunized with sonicated trophonts by IP injection were partially protected with a 57–77% survival in both trials. At 180 days post-immunization, serum antibody titers had declined in immunized fish yet they were still able to survive challenge. The protection appears not to be solely depending on serum antibody response against Ich.

Introduction

Tilapia production has increased greatly in the past two decades and world production of farmed tilapia exceeded two million metric tons in 2004 [1]. Tilapia are currently raised in different types of production systems ranging from pond, tank, cage, flowing water and intensive water reuse culture systems. Intensification of tilapia culture requires methods to prevent and control diseases to minimize the loss. *Ichthyophthirius multifiliis* is one of the most virulent ciliated parasites of freshwater fish and causes serious problems in intensively cultured fish [2]. The life stages of the parasite include

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1050-4648/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.fsi.2008.03.012

a reproductive tomont, an infective theront, and a parasitic trophont [3].

Currently, the control of Ich depends largely on the use of chemicals. But chemical control is not effective after the parasite penetrates into fish skin and gills. Chemical treatment is also expensive, may actually harm the fish, and raises concern among the public regarding food and environmental safety. Vaccination against the parasite is an alternative to chemical treatment since fish that survive an Ich infection, or are immunized with Ich antigens, acquire immunity against the parasite [2,4,5]. Very little is known about the immune response of tilapias against Ich. Sin et al. [6] studied the protective immunity for tilapia fry against Ich using passive immunization. Subasinghe and Sommerville [7] investigated the immunity of *Oreochromis mossambicus* against Ich with 20 fish to study antibody level and parasite infection in a small-scale trial. Tilapia suffer losses from ichthyophthiriasis as do most other cultured species [7]. The objective of this study is to evaluate immune protection of Nile tilapia (*Oreochromis niloticus*) against Ich after immunization with live theronts or sonicated trophonts.

Materials and methods

Fish, parasite and water quality

Nile tilapia were spawned in a pond. Fry collected were reared in tanks using filtered recirculated water at the USDA, Agricultural Research Service, Aquatic Animal Health Research Laboratory, Auburn, Alabama. *I. multifiliis* was isolated from an infected channel catfish obtained from a fish farm located in Alabama. The parasite isolate was maintained by serial transmission on channel catfish held in 50-l glass aquaria as previously described by Xu et al. [8].

The dissolved oxygen (DO) and temperature were measured using a YSI 85 oxygen meter (Yellow Spring Instrument, Yellow Springs, OH). The pH, hardness, and ammonia were determined using the CEL/890 Advanced Portable Laboratory (Hach, Loveland, Colorado). During the trials, the mean $\pm$ standard deviation of DO was 5.9 ± 1.7 mg/l, temperature was 22.6 ± 1.3 °C, pH was 7.0 ± 0.2, and ammonia was 0.3 ± 0.1 mg/l.

Antigen preparation

Fish heavily infected with maturing trophonts were anesthetized with 200 mg/l tricaine methane sulfonate (MS 222, Argent Chemical Laboratories, Redmond, Washington), rinsed in tank water and the skin was gently scraped to dislodge the parasites. Isolated trophonts were poured through a sieve with an opening 425 μm (Dual MFG Co., Chicago, IL) to retain fish skin and mucus. Trophonts were counted with a Sedgewick-Rafter cell (VWR Scientific Products, Atlanta, GA, USA) for total numbers and concentrated in 50 ml plastic tubes at $400 \times g$ for 5 min. After the supernatant was discarded, trophont pellet was resuspended with PBS in 1.5 ml plastic tubes and stored at −20 °C. Frozen trophonts were vortexed and then sonicated for 30 s on ice (VirSonic digital 600, VirTis, Gardiner, New York) before immunization. The sonicated trophont solution was adjusted in volume with PBS to approximately 0.1 ml per fish (20 trophonts/g fish or approximately 65 μg trophont protein/g fish) for injection. The protein concentrations in the sonicated solution were measured with NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To collect live theronts, isolated trophonts were placed in Petri dishes and allowed to attach. After replacing the water in the Petri dishes with fresh dechlorinated water to remove contaminating mucus, the trophonts were incubated for 18—20 h at 24 °C. Theronts were harvested by pouring through a sieve with a pore size of 45 μm. For IP injection, theronts were concentrated by centrifugation at $500 \times g$ for 5 min. Theronts were counted in five 20 μl samples of the theront solution with the aid of a Sedgewick-Rafter cell.

Experimental design and immunization procedure

Two immunization trials were conducted. The immunized fish were challenged with theronts 21 days post-immunization in trial I and 180 days post-immunization in trial II. Ten fish from the holding tank were sampled for serum anti-Ich antibody prior to each immunization trial. Twelve tanks with 20 tilapia (10.5 ± 0.7 cm in length and 17.6 ± 3.1 g in weight) per tank were assigned for trial I. These fish were divided into six replicated groups (two tanks per group) and immunized as follows: (1) with live theronts by immersion at the dose of 20,000 theronts per fish; (2) with live theronts by IP injection at the dose of 20,000 theronts per fish; (3) with sonicated trophonts by IP injection at dose of 20 trophonts/g of fish; (4) with sonicated trophonts by immersion at 100 trophonts/g of fish or 325 μg trophont protein/g of fish; (5) with 5% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, Missouri) in PBS by IP injection; and (6) non-immunized control. Fish in the immunized groups 2, 3 and 5 were anaesthetized with 100 mg/l MS 222, weighed and IP injected with antigen in a volume of 0.1 ml per fish. Water was adjusted to 10 l for each tank in the remaining immunized groups. Live theronts, sonicated trophonts or 500 ml tank water (non-immunized control) were added to two of these tanks, respectively. Fish were exposed to the antigen for 1 h and then water flow was resumed. When fish exposed to live theronts showed visible spots on the skin surface, the fish were treated for 1 h daily for 5 days with formalin at 100 mg/l (equivalent to 37 mg/l formaldehyde) until completely free of visible trophonts. All fish were held in 50-l tanks with aeration, flowing water, temperature of 22—24 °C, on a 12 h day/night cycle. Trial II consisted of 12 tanks with 25 tilapia per tank. The procedure for immunization was the same as trial I except fish were challenged with 80,000 theronts per fish.

Blood sampling and antibody measuring

In trial I, two fish per tank (four fish per immunized group) were removed and blood samples collected to determine the antibody level against Ich at 12 and 21 days post-immunization. In trial II, two fish from each tank were sampled at 12, 25 and 180 days post-immunization. The fish were anesthetized with 100 mg/l MS 222 prior to blood sampling. Blood was allowed to coagulate at 4 °C overnight and then centrifuged at $6000 \times g$ (Model Microfuge 18, Beckman