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

De-Hai Xu*, Phillip H. Klesius, Craig A. Shoemaker

USDA, Agricultural Research Service, Aquatic Animal Health Research Laboratory, 990 Wire Road, Auburn, AL 36832-4352, USA

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.

© 2008 Published by Elsevier Ltd.

**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
Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization

125

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 ichthyophthiriosis 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 ± 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 e to retain fish skin and mucus. Trophonts were harvested by pouring through a sieve with a pore size of 45 μm. For IP injection, theronts were concentrated by centrifugation at 500 × 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 × g (Model Microfuge 18, Beckman...
Coulter, Palo Alto, CA) for 5 min. Sera were aspirated, collected into microcentrifuge tubes, heat inactivated at 56 °C for 30 min and then stored at −20 °C. Antibodies against Ich in serum were measured using the theront immobilization assay [8]. Theront mobility was determined with an inverted microscope and immobilization titer was defined as the highest dilution in which all theronts lost mobility and aggregated.

**Challenge with theronts**

A preliminary trial with three challenge doses of 20,000, 40,000 and 80,000 theronts per fish was conducted by exposing 10 fish to each dose of theronts overnight to determine a suitable challenge dose for each immunization trial. The doses of 40,000 and 80,000 theronts per fish were selected for challenge in trial I and trial II, respectively, which resulted in fish infected with 100–200 trophonts per fish. After water was adjusted to 10 l in each of 12 tanks with 15 fish per tank, theronts were added to each tank 21 days post-immunization at a dose of 40,000 theronts per fish for trial I. In trial II, 15 tilapia (14.6 ± 1.5 cm in length and 50.4 ± 16.7 g in weight) in each tank were exposed to 80,000 theronts per fish 180 days post-immunization. The fish were exposed to theronts overnight. Then flowing water was resumed at 0.4 l/min and mortality of fish in each tank was recorded daily for 3 weeks after theront challenge.

**Statistical analysis**

Median days to death (MDD) were calculated by SAS Lifetest procedure (Kaplan–Meier method) to express the survival time-span in fish following challenge. Antibody levels against Ich and fish survival in different immunized groups were compared with the Duncan’s multiple range test [9]. Probabilities of ≤0.05 were considered statistically significant.

**Results and discussion**

Tilapia produced a strong antibody response following exposure to live theronts by immersion or IP injection. The IP injection of sonicated trophont also induced anti-Ich antibody. Antibody titers (Tables 1 and 2) were significantly higher (P < 0.05) in fish immunized with live theronts or sonicated trophonts by IP injection than control fish (BSA injection or non-immunized) in both trial I and trial II. Anti-Ich antibody in tilapia immunized with live theronts or trophonts by IP injection had reduced greatly by day 180 compared to day 25 post-immunization (Table 2).

Immunized fish in trial I were challenged 21 days post-immunization to evaluate the short-term protection. All fish immunized with live theronts by immersion or IP injection survived theront challenge (Table 1). The survival (76.7%) was significantly higher in fish immunized with sonicated trophonts by IP injection than fish immunized with sonicated trophont by immersion, BSA by IP injection, or non-immunized controls. The median days to death ranged from 9.8 to 11.5 days in trial I.

In trial II, fish were challenged 6 months post-immunization to evaluate the long-term protection. Most of the fish survived theront challenge in groups immunized with live theronts by immersion (93.3%) or by IP injection (86.7%). Tilapia immunized with sonicated trophonts were partially protected with a 56.7% survival (Table 2). Fish survival in these three groups was significantly higher than fish administered trophonts by immersion (0%), BSA by IP injection (6.7%), or non-immunization (3.3%). The median days to death ranged from 9.2 to 9.9 days.

In a previous Ich infection study, Xu and Klesius [10] noted that channel catfish suffered a two to three times higher loss in the first cycle of Ich infection after theront exposure than tilapia using the same theront concentration. In this study, a dose of 40,000 theronts was used to challenge immunized tilapia in trial I. The challenge dose was based on the result from preliminary trials in which tilapia exposed to 40,000 theronts per fish overnight would become infected with more than 100 trophonts per fish (data not shown). When fish grew bigger in trial II, the challenge dose was increased to 80,000 theronts per fish. This challenge dose is five times higher than the doses used in the challenge of channel catfish [8].

This study demonstrated that tilapia immunized with live theronts by bath immersion or by IP injection elicited immune protection. The immunization provided not only short-term but also long-term protection for the immunized fish.
Protective immunity of Nile tilapia against *Ichthyophthirius multifiliis* post-immunization

**Table 2** Survival of immunized tilapia following challenge by exposure to theronts of *Ichthyophthirius* and immobilization titer in serum of tilapia 12, 25 and 180 days post-immunization against the parasite in trial II

<table>
<thead>
<tr>
<th>Immunization groups</th>
<th>Number of dead fish</th>
<th>Survival (%)</th>
<th>Antibody titer post-immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 12</td>
</tr>
<tr>
<td>Live theront immersion</td>
<td>2</td>
<td>93.3 ± 9.7a</td>
<td>380a</td>
</tr>
<tr>
<td>Live theront IP injection</td>
<td>4</td>
<td>86.7 ± 3.2b</td>
<td>420b</td>
</tr>
<tr>
<td>Sonicated trophont IP injection</td>
<td>13</td>
<td>56.7 ± 4.7b</td>
<td>140c</td>
</tr>
<tr>
<td>Sonicated trophont immersion</td>
<td>30</td>
<td>0 ± 0c</td>
<td>10d</td>
</tr>
<tr>
<td>BSA intraperitoneal injection</td>
<td>28</td>
<td>6.7 ± 9.4c</td>
<td>13d</td>
</tr>
<tr>
<td>Non-immunized control</td>
<td>29</td>
<td>3.3 ± 4.7c</td>
<td>13d</td>
</tr>
</tbody>
</table>

Thirty tilapia were challenged 6 months post-immunization and survival was determined for each immunized group. Immobilization titer was the reciprocal of the highest dilution in which all theronts were immobilized. Each value is the mean of four samples. Within a column, means followed by the same lower case letter are not significantly different (*P > 0.05*) by Duncan’s multiple range test.

Serum anti-Ich antibody titers were lower in the immunized tilapia 180 days post-immunization in trial II compared to antibody titers at the end of trial I in fish immunized with live theronts or trophonts by IP injection. When challenged with a lethal dose of theronts, the immunized tilapia still acquired high survival in trial II. The high survival in the immune fish with lower serum antibody titers suggests the involvement of other immune protective factors, such as cutaneous antibody or cellular immune response or a combination of both. The exact mechanism of long-term protection needs further exploration.

**Acknowledgments**

The authors wish to thank Dr. Mark Meade, Biology Department, Jacksonville State University and Dr. Richard Shelby, USDA, ARS Aquatic Animal Health Research Laboratory, for critically viewing the manuscript.

**References**


