Development of an Effective Whole-Spore Vaccine To Protect against Microsporidial Gill Disease in Rainbow Trout (Oncorhynchus mykiss) by Using a Low-Virulence Strain of Loma salmonae

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In determining the effective vaccine spore dose of a low-virulence strain of Loma salmonae to limit microsporidial gill disease in trout, we found that fish receiving 10^4 to 10^6 killed spores had the best protection against experimental infection, with 85% fewer xenomas in their gills than in the controls. Intraperitoneal delivery of the vaccine was effective, and the addition of adjuvant did not improve vaccine performance against this disease-causing microsporidian.

Microsporidial gill disease of salmonids (MGDS) is among the most significant infectious diseases affecting aquaculture-raised chinook salmon in Canada; the disease most often affects salmon in their second summer of marine cultivation when they are nearing market weight, and outbreaks can lead to a cumulative mortality rate exceeding 30% (3). Microsporidians are a diverse group of unicellular, obligate intracellular, highly reduced parasites of many types of animals. Once considered to be the most primitive eukaryotes, microsporidians are now considered to be highly specialized fungi whose life cycles include a proliferative merogenic stage, followed by a sporogonic stage resulting in small environmentally resistant infective spores (2). During MGDS, sporogony occurs within the pillar and endothelial cells of the gill. These cells undergo hypertrophy, forming a xenoma composed primarily of parasite spores. The rupture of the xenoma and the release of spores cause severe and persistent gill inflammation; affected fish display signs of anoxia (3). Clinical disease is correlated with the numbers of xenomas within the gills (14). Therapeutic drugs against microsporidia are rarely successful, and none are considered to be the most primitive disease-causing microsporidian.

Certified-disease-free rainbow trout (280 fish, 20 to 30 g each) were tagged and placed in eight treatment groups, including the control group (which received only a saline injection) and groups receiving various vaccine doses of 10^2 to 10^6 spores per fish with or without FIA (Table 1) administered by intraperitoneal injection. SV strain spores for the vaccine were collected from the gills of experimentally infected brook trout and killed by freezing (8). Six weeks following vaccination, the fish were exposed to L. salmonae (the virulent OA strain) by oral exposure to xenoma-laden gill material acquired from a group of previously infected trout (6). Following this challenge, the trout were randomly sampled at weeks 6, 7, 8, and 9; they were euthanized, and the second gill arch on the right side of each fish was removed. This fresh gill material was examined with a dissecting microscope, and the xenomas were counted (15). The mean xenoma counts per gill arch (XCPGA) were compared between groups by use of one-way analysis of variance followed by Bonferroni’s multiple-comparison test (STATA version 8; Stata Corporation, College Station, TX). Differences were considered significant at the 0.05 level of probability. In addition, vaccine-associated XCPGA reduction was expressed in proportion to the XCPGA of the control fish as follows: % reduction = 1 – [(XCPGAvaccinated)/(XCPGAcontrol)] × 100. Separate control groups were used to confirm that the frozen spores were no longer viable and that the water flowing into the experimental system was not contaminated with L. salmonae.

Following exposure of the vaccinated trout to the challenge OA strain of L. salmonae, the XCPGA peaked at week 6 and declined in all groups by week 9 (Fig. 1), making week 6 the best point of comparison to assess the vaccine’s effects. Signifi-

TABLE 1. XCPGA reduction in vaccinated trout compared to control (saline-injected) trout at various weeks postexposure to the OA strain of L. salmonae

<table>
<thead>
<tr>
<th>Vaccine group dose</th>
<th>Vaccine-associated XCPGA reduction (%) at wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>10^2 spores</td>
<td>37</td>
</tr>
<tr>
<td>10^3 spores</td>
<td>93</td>
</tr>
<tr>
<td>10^4 spores</td>
<td>88</td>
</tr>
<tr>
<td>10^6 spores + FIA</td>
<td>91</td>
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<td>10^2 spores</td>
<td>95</td>
</tr>
<tr>
<td>10^3 spores</td>
<td>69</td>
</tr>
<tr>
<td>10^6 spores + FIA</td>
<td>86</td>
</tr>
</tbody>
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a Eight fish were examined at each sampling period for each treatment group.
icantly fewer xenomas developed in those fish vaccinated at several spore dose ranges ($10^3$ to $10^5$ spores without adjuvant and $10^5$ with adjuvant) (Fig. 1). Higher doses with or without adjuvant were less effective. The vaccine-associated XCPGA exceeded 85% in groups vaccinated with $10^3$ to $10^5$ spores per fish (Table 1).

To date, although parasitic diseases are common in aquaculture, no parasite vaccines are commercially available. Instead, antiparasitic pharmaceuticals are used, and this raises concerns about drug residues as these fish enter the human food supply (13). Although some recent advances in discovering chemotherapeutic agents against MGDS have been made, none have shown the degree of XCPGA reduction noted herein for vaccination (16). The current study demonstrates the efficacy of a killed-whole-spore vaccine preparation with a known spore dose against MGDS utilizing a recently discovered low-virulence strain of *L. salmonae*. Previous work fore-shadowed the efficacy of this approach. Salmonids which have recovered from experimental MGDS infections, as well as those immunized with live spores of either the virulent or low-virulence strains of *L. salmonae*, develop a cell-mediated immune response which becomes protective against infection by 4 weeks after exposure (8). Immunity appears to block the transfer of the parasite to the gill (11) by ablating the infection during merogony within subendocardial macrophages. In the current trial, although low numbers of xenomas did form on the gills of fish vaccinated with killed spores, the dramatic reduction in xenomas is important and would markedly reduce the likelihood of clinical expression of this disease even when the fish are reared in regions where MGDS is endemic. Given that *L. salmonae* is endemic within the marine coastal environment where salmon are raised in net pens (4), a vaccine administered during the freshwater hatchery phase of cultivation is recommended.

Until recently, despite safety concerns, conventional killed or modified pathogens were the only vaccines licensed for commercial use with farmed salmon (1). The use of whole spores as the basis for vaccine preparation is a relatively simple approach which is also being pursued for active immunization against *Encephalitozoon* microsporidial infections of mammals (12). In the case of MGDS, spores are easily harvested and purified from the gills of infected fish, and the use of a killed low-virulence strain of this parasite as the basis for the vaccine reduces the risks associated with its use. Although several studies have demonstrated humoral responses of fish and mammals to various microsporidian infections (5, 12), the MGDS vaccine is the first developed against a disease-causing microsporidian.

**REFERENCES**

Oncorhynchus mykiss (Walbaum), is dependent upon the method and timing of exposure. J. Fish Dis. 24:453–460.


