Failure of Vaccination in Brook Trout *Salvelinus fontinalis* against *Loma salmonae* (Microspora)

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ABSTRACT—The effectiveness of vaccines made of viable spores of two strains of *Loma salmonae* (Microspora) delivered orally to rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis* was evaluated under experimental conditions of re-challenge. Whereas prior exposure to either of two strains of *L. salmonae* led to marked resistance of rainbow trout to a second challenge, brook trout failed to mount an effective protective response.

Key words: *Loma salmonae*, vaccination, *Salvelinus fontinalis*, *Oncorhynchus mykiss*, brook trout, rainbow trout

Microsporidial gill disease (MGD) caused by the presence of spore-laden xenomas forming during the terminal phase of the life cycle of *Loma salmonae* has been described in various species of Pacific salmon *Oncorhynchus* spp. and brook trout *Salvelinus fontinalis*, and in Canada; it is a particular menace affecting the commercial marine netpen culture of chinook salmon *Oncorhynchus tshawytscha*, whereas in other parts of the world it is described as affecting rainbow trout *O. mykiss* and brook trout¹⁾. Previous work has demonstrated that cultured chinook salmon from British Columbia carry two strains of *L. salmonae* denoted as *L. salmonae* OA-strain (which has marked preference for *Oncorhynchus* species), and *L. salmonae* SV-strain (which has marked preference for *Salvelinus* species); *L. salmonae* SV differs from *L. fontinalis*, based on several morphological features²⁾.

The existence of the SV-strain which has low virulence for *Oncorhynchus* species provides an exciting opportunity to use it as the basis for a live vaccine against challenge with the more virulent *L. salmonae* OA-strain, and this has been demonstrated to be highly effective³⁾. However, to date, there has been no investi-

gation of the ability of *Salvelinus* species to mount protective responses against *L. salmonae*. Mindful of the growing significance of Arctic charr *S. alpinus* and brook trout in Canadian aquaculture, the purpose of this trial was to determine the extent to which they can generate protective immune response to *L. salmonae*. The brook trout was used as the proxy species for the genus because of its availability from a local hatchery with disease-free certification.

Materials and Methods

L. salmonae OA-strain and SV-strain were isolated from coho salmon (*O. kisutch*) after passage of infective gill material through rainbow trout and brook trout respectively as previously described²⁾. In Infection Trial 1, 6 groups of 35 10 g fish were isolated into 70 L fiberglass tanks supplied with freshwater at 15°C. Using oral gavage¹⁾ they were exposed to the following inoculum:

Group 1: Brook trout exposed to 200,000 spores of *L. salmonae* OA

Group 2: Brook trout exposed to 200,000 spores of *L. salmonae* SV

Group 3: Brook trout sham exposed (water only)

Group 4: Rainbow trout exposed to 200,000 spores of *L. salmonae* OA

Group 5: Rainbow trout exposed to 200,000 spores of *L. salmonae* SV

Group 6: Rainbow trout sham exposed (water only)

At week 7 post-exposure (PE), five fish from each tank were randomly selected, all of them had xenomas, and the numbers of xenomas per gill arch (XPGA) were counted⁴⁾ (Table 1). The remaining 30 fish in each tank were maintained for an additional 10 wk and periodically examined non-lethally⁵⁾ to ensure that all xenomas had ruptured.

In Infection Trial 2, initiated at week 17, the brook trout (Groups 1–3) were orally re-challenged with 200,000 spores of *L. salmonae* SV-strain. The rainbow trout (Groups 4–6) were orally re-challenged with 200,000 spores of *L. salmonae* OA-strain. Seven wk after exposure the fish were euthanized with an overdose of benzocaine, and the XPGA evaluated for each fish and a group mean and standard deviation calculated (Table 1). Differences between relevant groups were assessed statistically by a comparison of their means through analysis of variance (ANOVA) with significance based on 95% confidence limits.

Results and Discussion

Following the completion of Infection Trial 2, naive rainbow trout had a mean XPGA of 22.3 (Table 1), a result in line with previous trials. In comparison, rainbow trout which had been exposed to either the OA or SV-strain in Infection Trial 1, subsequently developed a

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Table 1. Effects of live *Loma salmonae* vaccines orally administered to rainbow trout and brook trout

Group	Fish	1st challenge	Mean (+/- SD) XPGA Week 7 n=5	2nd challenge Week 17	Mean (+/- SD) XPGA Week 24 n=30
1	BT	<i>L. salmonae</i> -OA	3.2 (2.39)	<i>L. salmonae</i> -SV	18.1 (5.72)
2	BT	<i>L. salmonae</i> -SV	22.8 (6.02)	<i>L. salmonae</i> -SV	21.4 (5.90)
3	BT	sham	0	<i>L. salmonae</i> -SV	20.8 (6.31)
4	RT	<i>L. salmonae</i> -OA	19.6 (6.54)	<i>L. salmonae</i> -OA	6.7* (3.70)
5	RT	<i>L. salmonae</i> -SV	2.0 (1.00)	<i>L. salmonae</i> -OA	5.9* (2.90)
6	RT	sham	0	<i>L. salmonae</i> -OA	22.3 (7.01)

BT: Brook trout, RT: Rainbow trout; XPGA: number of xenomas per gill arch; OA and SV: *L. salmonae* strains isolated from chinook salmon²⁾

*: Significantly different from the control group (Group 6). P<0.05

significantly lower mean XPGA of 6.7 or 5.9 respectively, after Trial 2 (Table 1). The 70% reduction in XPGA associated with prior exposure to either of the *L. salmonae* strains is similar to the degree of protection demonstrated in a previous infection-reinfection study³⁾; agreement between studies confirms the ability of rainbow trout to generate protective immunity, a phenomenon most likely due to cell mediated immune mechanisms⁶⁾.

In contrast to the benefits noted in rainbow trout associated with vaccination, the results in the brook trout groups were disappointing. Following the completion of Infection Trial 2, naive brook trout had a mean XPGA of 20.8 (Table 1). In comparison, upon completion of Trial 2, those brook trout which had been exposed to the SV strain in Infection Trial 1 also had a very similar mean XPGA (Table 1), whereas those exposed to the OA strain in Infection Trial 1 had a mean XPGA of 18.1 (Table 1). From this, we conclude that neither of the *L. salmonae* strains used in Infection Trial 1, elicited protective response in the brook trout such that their vulnerability to *L. salmonae* was equal to that of naive brook trout in the re-infection trial.

Whereas the generation of protective immune responses to *L. salmonae* in members of the genus *Oncorhynchus* holds the promise that a successful commercial vaccine will be produced^{7,8)}, the same cannot be

said for members of the genus *Salvelinus*, although, to date only 1 species within the genus has been examined. The results may point to a failure of the brook trout's cell mediated immune system to either recognize or respond to *L. salmonae*, a hypothesis which will be investigated in future studies.

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