COMPLETED PROJECT CASE STUDY

EVALUATING IMMUNE RESPONSE OF VACCINATED BALLAN WRASSE (WRAAS OPTIVACC 2)

PARTNERS

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BACKGROUND

Ballan wrasse are widely used as cleaner fish in salmon farming, but like all animals they can be affected by bacterial diseases. Hatchery-produced wrasse reduce reliance on wild stocks and they can also be vaccinated to protect them against disease. Although autogenous vaccines are available, outbreaks caused by pathogens, such as atypical *Aeromonas salmonicida*, can still present a challenge for hatcheries.

The earlier SAIC-funded **WraAs OptiVacc project** explored the immune responses of ballan wrasse to the vaccination strategies used at two commercial production sites. That work showed that fish produced antibodies against several vaccine isolates, but also indicated variation between individuals, differences between vaccination regimens, and possible antigenic competition among isolates.

This follow-on project, led by Otter Ferry Seafish, was designed to deepen that understanding. It focused on whether antibodies raised by vaccinated wrasse can recognise a broad range of *A. salmonicida* strains circulating in the field, and whether those antibodies have any measurable neutralising effect. The project also continued development of an insect alternative model, which can be applied to study bacterial virulence in vivo.

AIMS

The overarching aim was to support the development of improved vaccine formulations for ballan wrasse, specifically by strengthening protection against atypical *A. salmonicida.*

The project addressed two main objectives:

1. Determine the cross-reactivity of vaccine-induced ballan wrasse antibodies with the main circulating and virulent atypical *A. salmonicida* strains;
2. Assess the potential neutralisation effects of these antibodies.

PROJECT OVERVIEW

Fish serum samples were collected from two ballan wrasse production sites. At Site 1, fish received an immersion vaccine followed by an intraperitoneal injection (IP) vaccine; at Site 2, fish received only an IP vaccine. Each site provided serum samples collected pre-IP and at 600 degree days (dd) post-IP. Site 1's vaccines contained three *A. salmonicida* isolates; Site 2's IP vaccine contained five. Analyses focused on commonly occurring Type V and Type VI isolates. Building on earlier findings from the WraAs OptiVacc project, the present study assessed antibody recognition across a wider set of atypical *A. salmonicida* isolates. Western blots with pooled fish sera were performed for all vaccine isolates from both sites plus additional strains absent from the vaccine. Further Western blots were run to investigate differences in antibody responses of individual fish.

To determine whether antibodies detected by western blot could neutralise bacteria, a series of in vitro co-incubation trials was performed using microtitre plates. Conditions were repeatedly optimised for serum and bacterial concentrations, and incubation times, necessary to detect changes in bacterial viability.

To further characterise bacterial virulence, the project expanded on an earlier insect challenge model, the wax moth larva, Galleria mellonella. Type V and Type VI isolates were tested for virulence.

Groups of larvae were injected with bacterial doses and monitored over 120 hours. These trials aimed to compare virulence between isolate types and refine in vivo challenge parameters for future tests.

RESULTS

ANTIBODY RECOGNITION AND CROSS-REACTIVITY
Western blotting confirmed that post-IP sera from both production sites contained antibodies recognising antigens from the vaccine isolates. However, the breadth and intensity of recognition varied between isolates. Type V isolates were generally recognised for a wider range of antigens than Type VI, matching observations from the earlier WraAs OptiVacc work. Cross-reactivity was also evident: post-IP sera recognised antigens from non-vaccine isolates, though again with variation in the range of antigens detected.

There were differences between the two hatchery sites. Fish from Site 1 showed broader antigen recognition than those from Site 2, a pattern providing evidence that an initial immersion vaccination may prime fish to produce a wider systemic antibody repertoire. Supporting this, pre-IP sera from Site 1 contained detectable antibodies against *A. salmonicida* already, whereas pre-IP sera from Site 2 did not.

INTER-INDIVIDUAL VARIABILITY
Testing sera from individual fish reinforced the variability seen in the pooled samples. Most individuals produced antibodies recognising their respective vaccine isolate. However, around 20% of fish tested showed no recognition for either the vaccine or non-vaccine isolate, suggesting the presence of potential non-responders requiring further investigation.

Fish from Site 1 again showed stronger antigen recognition providing supporting evidence that immersion vaccination – a mucosal delivery route – may improve systemic antibody responses and cross-recognition. Still, these observations require experimental confirmation in controlled conditions, as the field setting means natural exposure to pathogens that elicits an antibody response cannot be ruled out.

IN VITRO NEUTRALISATION
After extensive optimisation, trials of pre- and post-IP sera showed they both inactivated bacteria, but there was no significant difference between sera containing or lacking specific antibodies. This suggests either that the antibodies do not exert bactericidal action under test conditions or assay sensitivity requires enhancement, with the observed effects perhaps reflecting the action of non-specific innate immune components.

IN VIVO VIRULENCE COMPARISONS
All *A. salmonicida* isolates tested in wax moth larvae caused mortality in a dose-dependent manner, reaffirming earlier findings that this insect is a suitable alternative model for assessing virulence. Type VI isolates were slightly more virulent than Type V. The presence or absence of the A-layer did not substantially alter virulence, likely due to host-specific factors and the multifactorial nature of pathogenicity.

IMPACT

The project is expected to contribute to improved health and welfare of ballan wrasse by increasing protection against atypical *A. salmonicida.* With up to 400,000 cleaner fish vaccinated annually, enhanced vaccine formulations will reduce disease risk, lessen the need for antibiotic treatments, and support more reliable wrasse production. Reduced antibiotic use brings both economic benefits and important antimicrobial resistance safeguards for Scottish aquaculture.

By improving wrasse health and availability, the project also helps reduce reliance on pharmaceutical and mechanical sea lice treatments in salmon farming, potentially saving tens of thousands of pounds per treatment. Strengthened ballan wrasse production supports the workforce at commercial hatcheries, including roles at Otter Ferry Seafish and Mowi Scotland.

The development of the wax moth larvae model offers a further long-term impact: providing microbiologists and vaccinologists with a new ethical and efficient in vivo system to develop effective vaccines without requiring large numbers of fish in accordance with the principles of the 3Rs.

ADDITIONAL INFORMATION AND FURTHER READING

- Optimising vaccination formulations for Ballan wrasse
- Funding booster for cleaner fish vaccination project
- Mowi Scotland is upping wrasse cleaner fish production by a third