COMPLETED PROJECT CASE STUDY

OPTIMISING VACCINATION FORMULATIONS FOR BALLAN WRASSE

PARTNERS

Mowi Scotland | University of Stirling | Otter Ferry Seafish | Ridgeway Biologicals

AUTHORS

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BACKGROUND

Ballan wrasse play a crucial role in UK aquaculture as biological control agents for sea lice, a significant pest affecting farmed Atlantic salmon. By naturally grazing on sea lice, these cleaner fish help reduce the reliance on chemical and physical treatments, promoting sustainable and environmentally friendly practices in fish farming.

Atypical *Aeromonas salmonicida* is a group of bacterial pathogens that threaten Ballan wrasse populations, commonly causing atypical furunculosis. This disease can lead to high mortality rates, particularly in juvenile wrasse, compromising their effectiveness as biological sea lice controllers in salmon farming. The genetic diversity of atypical *A. salmonicida* complicates vaccine development and disease prevention, making it a persistent challenge for aquaculture operations.

Autogenous vaccines, developed quickly for specific farms, have shown over 90% survival in larger Ballan wrasse (weighing over 25g) when administered via intraperitoneal injection (IP) and are heavily relied on by Scottish producers, as no fully licensed vaccines are currently commercially available.

To further explore vaccines for *A. salmonicida*, researchers studied immune responses in wrasse vaccinated with monovalent and multivalent vaccines via dip and IP injection methods at two sites. The findings aimed to refine vaccine testing methods and optimise formulations for future use. The project was led from the industry side by Mowi Scotland, one of the UK's largest suppliers of farmed salmon. The lead academic partner was the University of Stirling, which hosts the Institute of Aquaculture. Other industry partners include Otter Ferry Seafish, which participated in vaccination and sampling for the experiments, and Ridgeway Biologicals, which develops autogenous vaccines for aquaculture.

AIMS

The WraAs OptiVacc project set out to:

1. Assess the efficacy of multivalent dip vaccination in juvenile Ballan wrasse
2. Use antibody responses to guide rational vaccination strategies for Ballan wrasse in hatcheries
3. Provide tools for developing improved or cost-effective vaccine formulations

EXPLORING VACCINATION REGIMES

At Ballan wrasse hatcheries, multivalent autogenous vaccines target atypical *A. salmonicida* and other pathogens, such as Photobacterium, Vibrio, and Aliivibrio. Lab tests show over 90% protection in larger fish, but juvenile wrasse remain challenging to protect, likely due to their immature immune systems and the pathogen's genetic diversity. Monovalent vaccines (targeting a single antigen) have performed better in lumpfish (the other main cleaner fish species) than multivalent ones, with 73% relative per cent survival (RPS) compared to 60%, suggesting antigenic competition in multivalent vaccines could reduce effectiveness.

In the first work package of this study, trials at two sites tested various regimes, including dip vaccination followed by an IP boost, injection-only vaccination, and a combination of monovalent and multivalent vaccine formulations.

Mucosal skin samples and serum were collected from wrasse at predetermined points before and after vaccination to assess antibody levels using ELISA. In the second work package, tissue samples from vaccinated fish were collected for RNA extraction to study immune gene expression (e.g., MHC II, RAG1, RAG2). Samples were analysed to assess whether adaptive immune responses occurred and to provide

insight into whether wrasse will respond to vaccination. This work package also included ELISA and western blot techniques to examine how antigens uptake in tissue by IHC. Polyclonal antibodies (PAb) were applied to tissue sections to detect vaccine antigens.

The third work package focused on DNA extraction and sequencing from circulating atypical *A. salmonicida* isolates and developing an immunoproteomic profile of these isolates. Researchers also initiated wax moth larvae (*Galleria mellonella*) challenges to test bacterial virulence.

RESULTS

ELISA results showed stronger antibody responses to monovalent vaccines than multivalent ones, possibly due to antigenic competition in multivalent formulations. Fish receiving dip vaccination followed by an IP boost with a multivalent vaccine containing *Photobacterium indicum* showed greater antibody response to *P. indicum* than those receiving IP-only vaccination. This effect was more pronounced with vaccines targeting only *A. salmonicida*.

Immunohistochemistry (IHC) techniques to detect vaccine antigens in tissues were unsuccessful. However, time-matched analysis showed that multivalent vaccines elevated MHC II expression in the spleen, suggesting antigen uptake and an adaptive immune response, as the antigen was actively present in lymphoid tissue after receiving a dip vaccination.

RAG1 and RAG2 expression was also elevated in fish after dip vaccination, indicating lymphocyte activation and maturation. However, IgM expression – a key marker of B-cell activity – was only detected in monovalent-vaccinated fish.

The wax moth model successfully identified virulent *A. salmonicida* strains under different temperature conditions. While promising, the planned bacterial challenges using this model were not completed due to altered project priorities. This work is being developed further in the SAIC-funded project WraAs OptiVacc 2.

IMPACT

While all of the goals of the project have not been met, the WraAs OptiVacc project has led to improved understanding of two vaccine responses and the possibility of developing improved vaccines in future projects. It also advanced our collective understanding of wrasse immunity by identifying antibody responses and immune markers, such as IgM, MHC II, RAG1, and RAG2.

Future vaccine development must address antigenic diversity in *A. salmonicida* and consider differences in immune responsiveness between dip and injection regimes. Completing field trials and bacterial challenges will be essential to validate lab findings and optimise vaccination strategies for commercial aquaculture.