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Abstract
Injectable fish vaccines are mainly based on inactivated antigens which require an adjuvant to trigger a strong immune response. Water-in-oil emulsified vaccines are currently used in the aquaculture industry due to their cost effectiveness, stability and ability to confer long term immunity. However, oil-adjuvanted vaccines can be reactogenic and induce side effects in fish. In this study, we analyzed the impact of the oil origin of the adjuvant on *Aeromonas salmonicida* vaccines safety and immunogenicity. Two different adjuvants were tested, one based on a non-mineral oil (Montanide™ ISA 763A VG) and one based on a mineral oil (Montanide™ ISA 761 VG). Following intraperitoneal vaccination of rainbow trout, blood samples were taken at 42 and 53 days post vaccination (dpv) to assess antibody response, and adipose tissue samples were collected at 3, 14 and 28 dpv for RT-qPCR analysis of immune genes implied in the pro-inflammatory and adaptive responses. Side effects in the peritoneum were scored at 7, 14, 28, 42 and 53 dpv. Both vaccines induced a high antibody response against A. salmonicida with a significant increase in titre between 42 dpv and 53dpv. Vaccination-induced adhesion scores for the vaccine groups fell within industry-accepted limits as per Spielberg Standardized Extended Post-Vaccine Scoring. However scores were lower for the fish vaccinated with the non-mineral oil adjuvant. Compared to the control group (antigen alone), a clear upregulation of immune genes occurred in response to both vaccine groups, which persisted over time. This upregulation was higher for fish vaccinated with the mineral oil adjuvant. Furthermore, a strong correlation between gene expression, modulated by the oil origin, and vaccine safety was observed. These results showed that oil origin of fish adjuvants has an important impact on the immunogenicity and safety profile of fish vaccines, and that Montanide™ ISA 763A VG and Montanide™ ISA 761 VG are efficient adjuvants for the formulation of inactivated *A. salmonicida* vaccines.
Keywords: Inactivated vaccine, fish, oil, adjuvant, safety

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O-088.

Long-term, proteome-scale analysis of rainbow trout immune proteins: Implications for aquaculture vaccine development

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Abstract
Infectious diseases pose a significant threat to the economic stability and expansion of finfish aquaculture. Vaccination is widely considered the best prevention strategy, but evaluation of immune protection typically relies on measuring immune gene expression at the mRNA level from terminally-acquired tissue samples. However, mRNA expression does not always correlate with tissue protein levels, providing an incomplete representation of the nature and kinetics of the immune response. In addition, inter-individual variation necessitates the use of large numbers of experimental animals to obtain sufficient statistical power. To overcome these limitations, we used a long-term, proteome-scale approach to identify and quantify changes in immune protein levels in rainbow trout (*Oncorhynchus mykiss*) plasma. These changes provide an indication of fish health and immune status, while also permitting non-lethal sampling. Although all experimental fish mounted an antigen-specific humoral response, the timing and magnitude of this, and the response trajectories of most immune-relevant proteins, differed markedly between individuals. However, certain immunological proteins were found to be more consistently expressed across all fish, and may represent useful biomarkers of the immune response. Together our data emphasise the importance both of judicious selection of immunological biomarkers, and of careful assessment of changes in the expression of such proteins over longer-term study periods, when considering whether or not an effective antigen-specific immune response has been mounted. More generally, this approach offers a useful tool to monitor fish immune responses, while dramatically reducing the number of experimental animals required.
Keywords: Proteomics, vaccination, non-lethal sampling, individual variation, biomarkers.

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O-089.

A live attenuated strain of HY9901ΔvscB provides protection against *Vibrio alginolyticus* in orange spotted grouper (*Epinephelus coioides*) model

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Abstract
Vibrio alginolyticus, a bacterial pathogen in fish and humans, expresses a type III secretion system (T3SS) that is critical for pathogen virulence and disease development. In this study, the T3SS gene vscB was cloned from *V. alginolyticus* wild-type strain HY9901 and the mutant strain HY9901&vscB was constructed by the in-frame deletion method. The HY9901&vscB mutant showed an attenuated swarming phenotype and a 23-fold decrease in the virulence to grouper. However, the HY9901&vscB mutant showed no difference in morphology, growth, biofilm formation and ECPase activity. Finally, grouper vaccinated via intraperitoneal (IP) injection with HY9901&vscB induced a high antibody titer with a relative percent survival (RPS) value of 77.6% after challenging with the wild-type HY9901.Real-time PCR assays showed that vaccination with HY9901&vscB enhanced the expression of immune-related genes, including MHC-I3, MHC-II3, IgM, IL-1β, TNF-3 and CD83 after vaccination, indicating that it is able to induce humoral and cell-mediated immune response in grouper. These results demonstrate that the HY9901&vscB mutant could be used as an effective live vaccine to combat *V. alginolyticus* in grouper.
Keywords: *Vibrio alginolyticus*; T3SS; vscB; live attenuated vaccine; *Epinephelus coioides*

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