Experimental anal infection of rainbow trout with Flavobacterium psychrophilum: A novel challenge model

Flavobacterium psychrophilum is a Gram-negative psychrophilic bacterium causing rainbow trout fry syndrome (RTFS) in fry and bacterial coldwater disease (BCWD) in older fish. Both diseases challenge fish welfare and economy in hatcheries and in on-growing facilities. The bacteria enter hosts through gills, skin, and the gastrointestinal tract, and transfer horizontally in contaminated water and vertically with sexual products of both male and female fish (Madetoja, Dalsgaard, & Wiklund, 2002; Madsen & Dalsgaard, 1999; Nematollahi, Decostere, Pasmans, & Haesebrouck, 2003). Protection afforded by experimental vaccination (injection or immersion) using bacterins (formalin-killed whole cell) has been described (Hoare, Ngo, Bartie, & Adams, 2017; Madetoja et al., 2006), although no commercial vaccine is presently available for control of RTFS and BCWD. Further research on RTFS/BCWD vaccinology will benefit from an improved challenge method as current methods comprising intraperitoneal (i.p.) injection, bath, and bath exposure after treatment with stressors such as hydrogen peroxide (Henriksen, Kania, Buchmann, & Dalsgaard, 2015; Madsen & Dalsgaard, 1999) remain difficult to reproduce and rely on wounding the structural integrity of mucosal surfaces. The present study compares different infection methods and evaluates systems where the rainbow trout surface (skin, gills, and gut) is kept intact or injured. We compared six different challenge methods comprising anal intubation, i.p. injection, co-habitation, and bath challenge exposing either nontreated intact fish, fish chemically damaged by exposure to hydrogen peroxide or fish mechanically damaged by needle insertion in the tail-fin. Disease development was subsequently recorded for 4 weeks.
Secondary immune response of rainbow trout following repeated immersion vaccination

Teleosts are able to raise a protective immune response, comprising both innate and adaptive elements, against various pathogens. This is the basis for a widespread use of vaccines, administered as injection or immersion, in the aquaculture industry. It has been described that repeated injection vaccination of fish raises a secondary immune response, consisting of rapid, accelerated and increased antibody reaction. This study reports how rainbow trout responds to repeated immersion vaccination against yersiniosis (ERM) caused by the bacterial pathogen Yersinia ruckeri. It was found that rainbow trout does not raise a classical secondary response following repeated immersion vaccination. Serum antibody titres were merely slightly increased even after three immunizations, using 30-s immersion into a bacterin consisting of formalin-inactivated Y. ruckeri (serotype O1, biotypes 1 and 2), performed over a 3-month period. The densities of IgM-positive lymphocytes in spleen of fish immunized three times were increased compared to control fish, but no general trend for an increase with the number of immunizations was noted. The lack of a classical secondary response following repeated immersion vaccination may partly be explained by limited uptake of antigen by immersion compared to injection.

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Antimicrobial peptide CAP18 and its effect on Yersinia ruckeri infections in rainbow trout Oncorhynchus mykiss (Walbaum): comparing administration by injection and oral routes

The antimicrobial peptide CAP18 has been demonstrated to have a strong in vitro bactericidal effect on Yersinia ruckeri, but its activity in vivo has not been described. In this work, we investigated whether CAP18 protects rainbow trout...
Onchorhynchus mykiss (Walbaum) against enteric red mouth disease caused by this pathogen either following i.p. injection or by oral administration (in feed). It was found that injection of CAP18 into juvenile rainbow trout before exposure to Y. ruckerii was associated with lowered mortality compared to non-medicated fish although it was less effective than the conventional antibiotic oxolinic acid. Oral administration of CAP18 to trout did not prevent infection. The proteolytic effect of secretions on the peptide CAP18 in the fish gastrointestinal tract is suggested to account for the inferior effect of oral administration.

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Positive correlation between Aeromonas salmonicida vaccine antigen concentration and protection in vaccinated rainbow trout Oncorhynchus mykiss evaluated by a tail fin infection model

Rainbow trout, Oncorhynchus mykiss (Walbaum), are able to raise a protective immune response against Aeromonas salmonicida subsp. salmonicida (AS) following injection vaccination with commercial vaccines containing formalin-killed bacteria, but the protection is often suboptimal under Danish mariculture conditions. We elucidated whether protection can be improved by increasing the concentration of antigen (formalin-killed bacteria) in the vaccine. Rainbow trout juveniles were vaccinated by intraperitoneal (i.p.) injection with a bacterin of Aeromonas salmonicida subsp. salmonicida strain 090710-1/23 in combination with Vibrio anguillarum serotypes O1 and O2a supplemented with an oil adjuvant. Three concentrations of AS antigens were applied. Fish were subsequently challenged with the homologous bacterial strain administered by perforation of the tail fin epidermis and 60-s contact with live A. salmonicida bacteria. The infection method proved to be efficient and could differentiate efficacies of different vaccines. It was shown that protection and antibody production in exposed fish were positively correlated to the AS antigen concentration in the vaccine.

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Subunit vaccine candidates against Aeromonas salmonicida in rainbow trout Oncorhynchus mykiss

Aeromonas salmonicida subsp. salmonicida is the etiological agent of furunculosis and a major fish health problem in salmonid aquaculture worldwide. Injection vaccination with commercial mineral oil-adjuvanted bacterin vaccines has been partly successful in preventing the disease but in Danish rainbow trout (Oncorhynchus mykiss, Walbaum) aquaculture furunculosis outbreaks still occur. In this study we tested the efficacy of experimental subunit vaccines against A. salmonicida infection in rainbow trout. We utilized in silico screening of the proteome of A. salmonicida subsp. salmonicida strain A449 and identified potential protective protein antigens that were tested by in vivo challenge trial. A total of 14 proteins were recombinantly expressed in Escherichia coli and prepared in 3 different subunit vaccine combinations to immunize 3 groups of rainbow trout by intraperitoneal (i.p.) injection. The fish were exposed to virulent A. salmonicida 7 weeks after immunization. To assess the efficacy of the subunit vaccines we evaluated the immune response in fish after immunization and challenge infection by measuring the antibody levels and monitoring the survival of fish in different groups. The survival of fish at 3 weeks after challenge infection showed that all 3 groups of fish immunized with 3 different protein combinations exhibited significantly lower mortalities (17-30%) compared to the control groups (48% and 56%). The ELISA results revealed significantly elevated antibody levels in fish against several protein antigens, which in some cases were positively correlated to the survival.
A New Furunculosis Challenge Method for Evaluation of Vaccine Efficacy in Rainbow Trout

Experimental infection of fish for vaccine efficacy studies is associated with several limitations. Administration of live bacteria with the purpose of causing disease in fish can be performed by co-habitation, immersion or injection. We have developed a new Aeromonas salmonicida challenge method for rainbow trout and have applied it for evaluation of furunculosis vaccine efficacy. The method reveals development of systemic immunity in fish as live bacteria are introduced in the tail fin epidermis distant from the vaccine injection site (peritoneal cavity). This method seeks to mimic natural infection in fish farms where tail biting and therefore bacterial exposure to tail fin ulcers is widespread. By use of a multi-needle device ten epidermal perforations were introduced in the dorsal part of the tail fin of anesthetized rainbow trout (vaccinated or naive). Subsequently 100 μL (3.4 × 108 colony-forming units (CFU) mL−1) of a 48 hour culture of Aeromonas salmonicida subsp. salmonicida strain 090710-1/23 was placed at the perforation site for 60 sec whereafter fish were allowed to regain consciousness in clean freshwater. Immunohistochemistry and scanning electron microscopy illustrated the spread of bacteria from the injection site. Classical furunculosis symptoms associated with a high morbidity rate were observed in control fish whereas vaccinated fish exhibited a significantly higher survival.

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Effects of adjuvant Montanide™ ISA 763 A VG in rainbow trout injection vaccinated against Yersinia ruckeri

Enteric redmouth disease (ERM) caused by the fish pathogen Yersinia ruckeri is a major threat to freshwater production of rainbow trout (Oncorhynchus mykiss) throughout all life stages. Injection vaccination of rainbow trout against Y. ruckeri infection has been shown to confer better protection compared to the traditionally applied immersion vaccination. It may be hypothesized, based on experience from other vaccines, that adjuvants may increase the protective level of ERM injection vaccines even more. Controlled comparative vaccination studies have been performed to investigate effects of the oil adjuvant Montanide™ ISA 763 A VG (Seppic) when added to an experimental Y. ruckeri bacterin (containing both biotype 1 and 2 of serotype O1). A total of 1000 fish with mean weight 19 g was divided into five different groups (in duplicated tanks 2 × 100 fish per group) 1) non-vaccinated control fish (NonVac), 2) fish injected with a commercial vaccine (AquaVac® Relera™) (ComVac), 3) fish injected with an experimental vaccine (ExpVac), 4) fish injected with an experimental vaccine + adjuvant (ExpVacAdj) and 5) fish injected with adjuvant alone (Adj). Injection of the experimental vaccine (both adjuvanted and non-adjuvanted) induced a significantly higher antibody (IgM) level, increased occurrence of IgM(+) cells in spleen tissue and significant up-regulation of several immune genes. Additional experiments using a higher challenge dosage suggested an immune enhancing effect of the adjuvant as the challenge produced 100% mortality in the NonVac group, 60% mortality in both of ComVac and Adj groups and only 13 and 2.5% mortalities in the ExpVac and the ExpVacAdj groups, respectively.

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