P-088 **Oral immunity against infectious salmon anaemia in Atlantic Salmon (Salmo salar).**

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INTRODUCTION AND OBJECTIVES

Infectious salmon anemia (ISA), caused by the infectious salmon anemia virus (ISAv) is a highly contagious disease of Atlantic salmon (*Salmo salar*) that was first reported in Norway (Kibenge et al., 2004). The disease was subsequently reported from Scotland, Canada and the United States. In 2007, the disease was reported in Chile and since then it has caused a major economic loss estimated at US $100M.

In Chile, besides biosafety measures including early harvest and compulsatory slaughter of infected stock, there are several commercially available vaccines against ISA, which are applied by injection in freshwater stage. Although this procedure ensures vaccine application, it has several limitations including immunosuppression and reduction in feeding rate after vaccine application. Furthermore, vaccine protection usually does not cover the entire farming period, rendering fish susceptible to infection at higher sizes. Although a revaccination (booster) could overcome this problem, to inject fish at higher sizes exposes them to infectious agents and rises up vaccine-associated costs. Thus, the availability of an alternative delivery strategy such as an oral vaccination represents attractive candidates to evaluate.

We report here a novel oral ISAv vaccine from Centrovet Laboratory (Chile) using their unique ISAv-derived antigen expressed in yeast, and encapsulated with a proprietary encapsulation technology, MicroMatrix™, of Advanced BioNutrition Corp., Columbia, Maryland, USA. We showed that vaccinated fish produced specific ISAv antibodies, and the antibody titer is correlated with protection upon challenge with a virulent ISAv strain.

MATERIALS AND METHODS

Oral Vaccine and Vaccination protocols

Oral vaccine consisted on containing HA and NA recombinant antigens from ISAv injectable vaccine (Centrovet, Chile) encapsulated using MicroMatrix™ formulation technology (Advanced BioNutrition Corp., Columbia, Maryland, USA). A total of 180 fish (*S. Salar*, 10 g weight) were separated into three experimental groups of sixty fish each. The groups received injected vaccine, oral vaccine, or no vaccine (Control).

Antibody ELISA and Histology

Fish serum was evaluated for anti-ISAv antibody presence by ELISA in Maxisorp plates, previously coated with HRP7b ISAv strain obtained from SHK-1 cell line. Secondary and tertiary antibodies used in the ELISA included anti-Salmon IgM and anti-mouse HRP. Reaction was developed with TMB and evaluated at 450 nm as recommended by provider. For histology analysis, a portion of the second segment of the endgut was fixed in 4% formalin, and processed for hematoxillin-eosin stain according Baeverfjord et al., 1996.

ISAV Challenge and fish survival.

ISAV challenge was performed following a cohabitation challenge model (Gregory et al., 2009). Briefly, at 310, 650 and 830 day-degrees after vaccination, sentinel fish with no previous vaccine were injected IP with 0.2 ml of pathogenic ISAv (HRP7b) and cohabitated with control or vaccinated fish. Fish mortality was evaluated daily and dead fish were evaluated by ISAv presence by necropsy, cell culture and real-time RT-PCR (not shown).

RESULTS AND DISCUSSION

Oral ISA-vaccine induces ISAv-specific antibodies with ISAv-neutralizing abilities.

The data showed that antibodies against ISAv were detected as soon as 300 dd after vaccination in blood. The antibody titre reached a peak between 450 and 600 dd and then began to fall. However, anti-ISAv antibodies were still detectable until at least 900 dd post-vaccination (Figure 1A). Anti-ISAv antibodies were virus-specific and were able to neutralize the infectious virus, since pre-incubation of virulent HRP7b ISAv with a serum dilution obtained from a vaccinated fish looses the ability to infect and induce cytopathic effect (CPE) in fish cell line SHK-1 (Figure 1B).

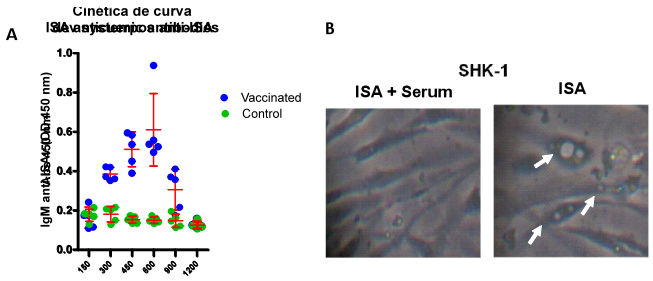

Figure 1. Determining the anti-ISAv antibody response in Atlantic salmon following oral vaccination.

A. The anti ISAv antibody response was measured by ELISA at different points in vaccinated and control fish.

B. In vitro assay to determine the anti-ISAv neutralizing abilities of serum obtained from vaccinated fish. ISAv incubated with serum from healthy fish but not from vaccinated fish could infect SHK-1 cells. The arrows show ISA-derived CPE on SHK-1 cells.

Oral-ISA-vaccine induces mucosal antibodies and recruits lymphocytes to the hindgut.

ISAv-specific antibodies were induced in mucus from fish hindgut (Fig. 2A), and it correlated with the presence of a higher number of inflammatory cells in intestinal tissue (Figure 2B). These observations supports the notion of ISAv-oral vaccine enhances specific immunity at systemic and mucosal level.

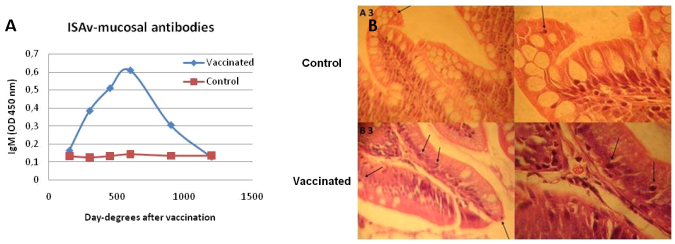

Figure 2.
A. Determination of the levels of anti-ISAv specific antibodies on intestinal mucus induced by oral vaccine.
B. Histological sections (H&E) of control and vaccinated fish taken at 300 dd after vaccination. Arrows show lymphocyte cell in lamina propia of intestinal vili.

The anti-ISAv-specific antibody level correlates with protection degree upon challenge.

The data showed that the ISAv strain used for the virus challenge was highly virulent, since it caused mortality up to 95% in the non-vaccinated fish (Fig. 2A). Moribund or dead fish showed typical ISAv clinical signs, such as haemorrhagic liver and intestine, ascetic fluid, petechia in caecum and visceral fat tissue (Fig. 2B). In contrast, vaccinated fish were successfully protected showing as low as 17% mortality (Figure 2A). Protection degree evaluated with three different vaccine lots showed that it lasted at least 830 dd after vaccination (Fig. 2C) which is consistent with the systemic serum response induced in fish observed at 830 dd (see Fig. 1A).

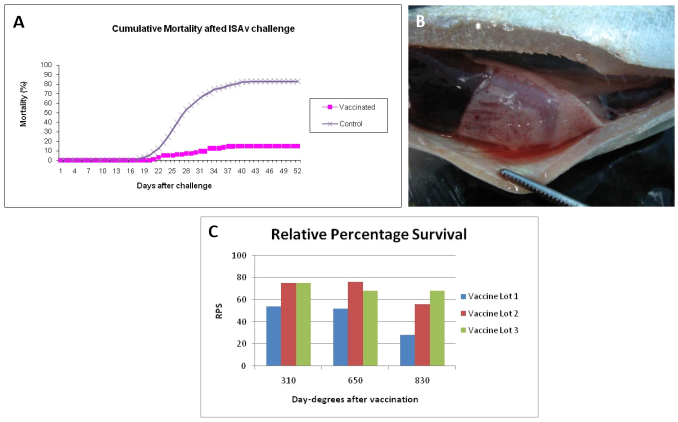

Figure 3.
A. ISAv induced mortality pattern in Atlantic salmon vaccinated via oral route. Vaccinated (pink) and non-vaccinated (blue) fish were challenged by cohabitation using a virulent ISAv strain (HRP 7b region).
B. Typical clinical signs of ISAv infection in dead fish.
C. The relative percent survival (RPS) values of fish vaccinated with three different lots of ISAv-oral vaccine (blue, red and green bars) and challenged at 310, 650 and 830 day-degrees after vaccination.

CONCLUSIONS

- ISAv-Oral vaccine induces both systemic and mucosal immunity starting at 300 dd and remains until 800 dd post-vaccination.
-Serum from vaccinated fish has ISAv neutralizing ability since it inhibited ISAv infection of SHK-1 cells in *in vitro* assay.
- The anti-ISAv antibody response induced by vaccination correlated with the protection upon ISAv challenge with a virulent strain.

ACKNOLEDGEMENTS

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