Nasal vaccination of young rainbow trout (*Oncorhynchus mykiss*) against infectious hematopoietic necrosis and enteric red mouth disease

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ABSTRACT

Determining the earliest age at which farmed fish can be successfully vaccinated is a very important question for fish farmers. Nasal vaccines are novel mucosal vaccines that prevent aquatic infectious diseases of finfish. The present study investigates the ontogeny of the olfactory organ of rainbow trout by histology and aims to establish the earliest age for vaccination against infectious hematopoietic necrosis (IHN) and enteric red mouth (ERM) disease using the nasal route. Rainbow trout (*Oncorhynchus mykiss*) were vaccinated intranasally (I.N) at three different ages: 1050° days (DD) (group A); 450 DD (group B); and 360 DD (group C), or 70, 30 and 24 days post-hatch (dph), respectively. The mean weights of groups A, B and C were 4.69 g, 2.9 g and 2.37 g, respectively. Fish received either a live attenuated IHN virus vaccine, ERM formalin killed bacterin or saline (mock vaccinated). Fish were challenged to the corresponding live pathogen 28 days post-vaccination. IHN vaccine delivery at 360 DD resulted in 40% mortality likely due to residual virulence of the vaccine. No mortality was observed in the ERM nasal delivery groups. Following challenge, very high protection rates against IHN virus were recorded in all three age groups with survivals of 95%, 100% and 97.5% in groups A, B and C, respectively. Survival against ERM was 82.5%, 87.5% and 77.5% in groups A, B and C, respectively. Survival rates did not differ among ages for either vaccine. Our results indicate the feasibility and effectiveness of nasal vaccination as early as 360 DD and vaccination-related mortalities when a live attenuated viral vaccine was used in the youngest fish.

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1. Introduction

Many diseases affect early life stages of fish so it is important to determine the earliest age when fish can be successfully vaccinated (Ellis, 1988). The earliest time to vaccinate salmonids has been determined in a number of disease models such as vibriosis, enteric red mouth disease (ERM) or viral hemorrhagic septicemia (VHS). In rainbow trout (*Oncorhynchus mykiss*), cellular immunity is functional and main lymphoid organ development is completed at ~200° days (DD) (Tatner and Manning, 1983). However, mucosal immunity develops later (Salinas et al., 2011) than systemic immunity in teleosts. As a consequence, and generally speaking, 0.5 g (10 weeks post-hatch) at 10 °C (=700 DD) is considered the earliest time to vaccinate rainbow trout using immersion vaccination (Tatner and Manning, 1983; Obach, 1991; Ellis, 1988). Currently, immersion vaccination of young fingerlings is the industry gold standard for ERM vaccination. On the other hand, injecting vaccines into small fish is impractical and often leads to high mortalities due to the handling. As a result, 1 g is considered the smallest size for injection vaccination of salmonids whereas 2.5 g is considered the optimal size to achieve maximum protection for all species (Johnson et al., 1982; Ellis, 1988).

In fish, traditional mucosal vaccination strategies include immersion and oral vaccination. Recently, nasal vaccines were added to this list. Nasal vaccination can be a very effective way to control

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aquatic infectious diseases in finfish (Tacchi et al., 2014; LaPatra et al., 2015). The use of this delivery route has been tested in small trout but age was not controlled in those studies. Nasal vaccination of young non-aquatic vertebrates such as piglets, newborn mice, calves and rabbits (Guzman-Bautista et al., 2014; Sandbulte et al., 2014; Sabirov and Metzger, 2008; Ellis et al., 2010; Lancaster et al., 1960) is feasible, effective and does not induce tolerance. In fact, the human nasal vaccine FluMist (LAIV) is recommended for use in young infants (6–59 months) since it prevents about 50% more cases than the injected flu vaccine (TIV) at this age (Carter and Curran, 2011).

We currently lack information regarding the age at which rainbow trout fry can be vaccinated nasally. The aim of the present study is to determine the earliest age at which rainbow trout can effectively be vaccinated using the nasal route. Two different vaccine models are used, a live attenuated viral vaccine and a formalin-killed bacterial vaccine. Our results are coupled with histological observations of the ontogeny of the trout olfactory organ from hatch to 1050 DD. Altogether, the present study provides novel insights into fish nasal immunity and contributes towards the implementation of nasal vaccines in rainbow trout aquaculture facilities.

2. Materials and methods

2.1. Fish

Three hundred specific-pathogen-free (spf) rainbow trout of three different ages (1050° days (DD) (group A); 450 DD (Group B); and 360 DD (group C)), or 70, 30 and 24 days post-hatch (dph), were obtained from Clear Springs Foods Inc. (Buhl, Idaho). The mean weight of fish in group A was 4.69 g; the mean weight of fish in group B was 2.9 g whereas in group C was 2.37 g. Fish were maintained in 378 L tanks that received single-pass spf spring water at a constant temperature of 14.5 °C and a dissolved oxygen content of 9.2 ppm. Fish were fed twice daily a commercial rainbow trout diet (Clear Springs Foods, Inc.).

2.2. Vaccination trials and challenge experiments

Vaccination experiments were conducted using two different vaccine models: an IHN live attenuated viral vaccine (LaPatra et al., 2004) and an ERM bacterin (Nelson et al., 2015). One hundred fish from each age group were vaccinated with each vaccine or saline using a 10 or 25 μl volume dependent on the size of the fish. Duplicate 25-fish groups from each treatment were challenged with either IHNV or Yersinia ruckeri at 28 (400 DD) days post-immunization (dpi) and followed for an additional 28 days as previously described (Tacchi et al., 2014). Kidney, spleen and liver were collected from rainbow trout that died prior to challenge. Tissues were weighed and homogenized. The presence of IHNV in homogenized samples was measured as explained elsewhere (LaPatra et al., 1989).

2.3. Histology

Whole rainbow trout (N = 5) were sampled every day from hatching (15 DD) to 600 DD. The heads from these series of samples were dissected. Additionally, olfactory organs from adult individuals (N = 4) were also collected. All samples were fixed in 10% neutral buffered formalin overnight and embedded in paraffin following standard histological procedures. Five μm-thick sections were stained with hematoxylin-eosin and observed under a Zeiss Axioscope microscope coupled with a digital camera using the AxioVision software.

2.4. Statistical analysis

To estimate the expected mortality profile of vaccination trials and challenge experiments in practice, Kaplan–Meier survival curves with pointwise 95% confidence bands were computed and plotted. To test for differences in the proportion of mortality by treatment and age for each pathogen in both the vaccination trials and challenge experiments, the 28-day survival and mortality frequencies were used in Fisher's exact test of conditional independence for two-dimensional contingency tables as implemented in R version 3.1.2 stats package (R Core Team, 2014) fisher.test() function. Familywise error rate was controlled by the most conservative Bonferroni correction by multiplying all p-values by the total number of hypothesis tests performed (16 tests). All test results are presented in Table 1.

All p-values are shown in Table 1.

3. Results

3.1. Vaccination-related mortalities prior to challenge

The Kaplan–Meier survival profiles shown in Fig. 1a indicate increased and earlier mortality only for younger fish vaccinated with live attenuated IHN vaccine; total mortalities are shown in Fig. 1b. No mortalities associated with I.N delivery of ERM vaccine or saline were observed in any of the age groups that were vaccinated (p-value = 1). Vaccination with the live attenuated IHN vaccine resulted in mortalities of 4%, 19% and 40% in groups A, B and C, respectively (Fig. 1b). Furthermore, all age group pairs were significantly different (A vs B p-value = 0.0227, A vs C p-value = 4.62 E^{-9}, and B vs C p-value = 0.0287). This means that early life stages of rainbow trout are more susceptible to residual virulence present in the IHNV vaccine than older life stages tested in this study (p-value = 9.87 E^{-9}). Some rainbow trout that died as a result of nasal IHNV delivery showed typical gross signs of IHN although no attempts to characterize the histopathology of moribund fish were performed. IHNV levels were titrated in kidney, spleen and liver homogenates of 43% (27/63) of the fish that died. IHNV was detected in 70% (19/27) of the animals tested. The mean titers were 6.0 × 10^3 plaque forming units (pfu)/g for group A; 1.4 × 10^3 pfu/g for group B and 4.9 × 10^5 pfu/g for group C.

Table 1
p-Values obtained from the statistical analyses performed in the present. Study. "Side" indicates mortalities recorded prior to challenge to the live pathogen.

Description	p-Value
Age_ERM_Side	1
Age_IHNV_Side	6.15E-10
Age_Control_Side	0.775538147
TreatmentPairs_C_ERM_A_B	1
TreatmentPairs_C_ERM_A_C	1
TreatmentPairs_C_ERM_B_C	1
TreatmentPairs_C_IHNV_A_B	0.001415719
TreatmentPairs_C_IHNV_A_C	2.89E-10
TreatmentPairs_C_IHNV_B_C	0.001796509
TreatmentPairs_C_Control_A_B	0.497487437
TreatmentPairs_C_Control_A_C	1
TreatmentPairs_C_Control_B_C	1
TreatmentPairs_A_ERM_Control_Vaccinated	1.41E-09
TreatmentPairs_A_IHNV_Control_Vaccinated	1.48E-16
TreatmentPairs_B_ERM_Control_Vaccinated	8.21E-12
TreatmentPairs_B_IHNV_Control_Vaccinated	2.53E-18
TreatmentPairs_C_ERM_Control_Vaccinated	5.17E-09
TreatmentPairs_C_IHNV_Control_Vaccinated	1.94E-13
Age_ERM_Control	1
Age_ERM_Vaccinated	0.549087619
Age_IHNV_Control	0.302214595
Age_IHNV_Vaccinated	0.772112235