Severity Of Vaccine Induced Side Effects In Pre- And Post-Smolts Of Atlantic Salmon: Heritabilaties And Genetic Correlations

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Introduction

Vaccination is one of the most important tools to prevent outbreaks of a number of bacterial (furunculosis, vibriosis, cold water vibriosis, winter ulcer) and viral diseases (infectious pancreatic necrosis (IPN), pancreas disease (PD), infectious salmon anaemia (ISA)) in farmed Atlantic salmon *(Salmon salar)* (Gudding, Lillehaug and Evensen 1999; McLoughlin and Graham 2007). In comparison to water baser vaccines, oil adjuvant vaccines are needed to obtain lasting protection for some diseases, like furunculosis (Midtlyng, Reitan and Speilberg 1996), but an disadvantage can be reduced appetite and growth (Midtlyng and Lillehaug 1998; Sørum and Damsgård 2004; Berg, Rødseth, Tangerås *et al.* 2006). Also, oil adjuvated vaccines cause varying levels of intraperitoneal side effects (adhesions of intraperitoneal organs and melanin deposits) (Midtlyng, Reitan, Lillehaug *et al.* 1996;; Mutoloki, Berg, Rødseth, Tangerås *et al.* 2006;). The severity of these vaccine induced side effects varies but may in the severe cases lead to reduced fish welfare and downgrading of the fish quality (Midtlyng 1996; Poppe and Breck 1997). Previous studies have shown that time of vaccination, vaccine formulation, water temperature and fish size may affect the severity of the vaccine induced intra-abdominal lesions (Aucouturier, Dupuis and Ganne 2001; Berg, Rødseth, Tangerås *et al.* 2006; Berg, Rødseth and Hansen 2007). Genetics does also seem to play a role in the development of vaccine induced side effects as significant additive genetic variation in susceptibility to intra-abdominal lesions (h^2=0.18-0.19) at harvest size has been shown (Gjerde, Evensen, Bentsen *et al.* 2009). However, the data used in the latter study is more than 10 years old. Since then, vaccines have been substantially improved, which may have had an effect on the severity of the side effects of vaccination. Vaccine related side effects in Atlantic salmon are known to develop over time (Mutoloki, Alexandersen and Evensen 2004), and it would therefore be of interest to examine the severity of vaccine induced side effects caused by "modern" fish vaccines at different life stages of the fish. The objective of this study was to estimate the magnitude of the heritability of vaccine induced side effects in Atlantic salmon pre-smolts in fresh water and in post-smolts at 6 months after sea transfer, and of the genetic correlation coefficients between these observations.

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Material and methods

Fish and family material. The fish were the offspring of 85 sires and 150 dams (150 full-sib families) from the breeding material of the breeding company SalmoBreed AS. They were hatched in November 2006, and reared in separate tanks until a body size suitable for PIT-tagging (approximately 12g). The breeding population had been selected for growth, slaughter quality and resistance to furunculosis and ISA for two generations.

Vaccination. Each fish was vaccinated manually with a commercial 6-compontent vaccine (ALPHA JECT® 6-2, PHARMAQ AS) administered through the abdominal cavity in a dose of 0.1 ml. Pre-smolts were vaccinated in October 2007 and the post-smolts in March 2008. The average weight and temperature at vaccination was 44g (SD=14g) and 13°C for pre-smolts and 153g (SD=44g) and 7°C for post-smolts.

Rearing and sampling. Vaccine induced side effects were measured at two different life stages: pre-smolts (fresh water) and post-smolts sampled 6 months after sea transfer. To ensure sufficient measurable variation in vaccine induced side effects relatively short time after vaccination, the pre-smolts were kept at an elevated water temperature of 17°C for three months (November 2007 to February 2008), which have previously shown to be a stable laboratory model for inducing vaccine related side effects (PHARMAQ AS). The post-smolts were transferred to a net-cage in the sea in June 2008 and were scored for vaccine induced side effects 6 months later. Examined for vaccine side effects as described below. In total, 12-15 fish from each of the 150 families were sampled at each time point.

Scoring of vaccine induced side effects. Adhesions in the abdominal cavity were scored on a scale from 0-6 (0 = no adhesions, 6 = extremely severe adhesions: interval 0.5). Each fish was given an score in three different abdominal regions. Melanin deposits on internal organs and abdominal wall were scored on a scale from 0-3 (0 = no visible melanin, 3 = severe melanin spots: interval 1.0), and averages of these two are presented and used in the analysis in the present paper. For adhesions averages of the three regions were used. At each stage, the fish were examined and scored by 2-4 skilled persons from PHARMAQ.

Statistical model. Estimates of heritabilities and genetic correlations for the studied traits were obtained using a multivariate animal model. For each trait *i*, the model had the following general characteristics.

$$\mathbf{y_i} = \mathbf{X_i}\boldsymbol{\beta}_\mathbf{i} + \mathbf{Z_i}\mathbf{a_i} + \mathbf{e_i}$$

where $\mathbf{y_i}$ is the vector of the observations for the trait *i*, $\boldsymbol{\beta}_\mathbf{i}$ is the associated vector of fixed effects (including person responsible for the examination, a combined tank/net-cage by sex effect and a fixed regression on age of the fish nested within the tank/net-cage and sex effect), $\mathbf{a_i}$ is the additive genetic effect of each individual for the trait, $\mathbf{e_i}$ is a vector of random residuals and $\mathbf{X_i}$ and $\mathbf{Z_i}$ are appropriate incidence matrices.

Initially, bivariate models with adhesions and melanin at each life stage were run separately to estimate heritabilaties and genetic correlations between the two traits. Further, adhesions and melanin at the two life stages were run in two separate bivariate models to estimate the genetic correlation between vaccine injuries at the different life stages. The residual

correlation was set to zero in these last two models because vaccine injuries were measured on different individuals at each life stage.

Results and discussion

The mean score for abdominal adhesions was, as expected, higher for pre-smolts kept at 17°C for three months than for post-smolts kept under conventional farming conditions (Table 1). Mean score for melanin deposits on the other hand was lower for pre-smolts than for post-smolts (Table 1). Adhesions are thought to be established relatively soon after vaccination, and the immune response and severity of adhesions is known to be temperature dependent (Berg, Rødseth and Hansen 2007), while melanin deposits takes longer to develop. This is supported by the results in the present study.

Table 1: Means and standard deviations for body weight (g), adhesions score and melanin score for pre-smolts and for post-smolts.

		Body weight		Adhesions		Melanin	
	N	Mean	SD	Mean	SD	Mean	SD
Pre-smolts	1630	234	58	2.06	0.49	0.95	0.44
Post-smolts	1627	1007	293	1.68	0.65	1.49	0.62

The heritability for the susceptibility to abdominal adhesions was intermediate for pre-smolts, but lower for post-smolts (Table 2). The heritability for the susceptibility to melanin deposition was also intermediate at both life stages. The genetic correlation between the two traits was high at the post-smolt stage, but only intermediate at the pre-smolt stage. The reason for this might be that melanin deposits were not fully developed and therefore less visible at the pres-smolt stage. The results confirm that the susceptibility to vaccine injuries may be reduced by selection.

Table 2: Estimates of heritabilities (h^2) and genetic correlations (r_g) for adhesions score and melanin score for pre-smolts and for post-smolts.

	$h^2 \pm$ see		$r_g \pm$ see
	Adhesions	Melanin	Adhesions-Melanin
Pre-smolts	0.31±0.05	0.26±0.05	0.52±0.11
Post-smolts	0.19±0.05	0.27±0.05	0.89±0.06

The genetic correlation between melanin deposits score in pre- and post-smolts was relatively high (0.84±0.08) while the genetic correlation between adhesions score at the two different life stages was intermediate (0.62±0.12). This indicates that adhesions in pre- and post-smolts might be regarded as partly different traits, possibly because of the vaccine injuries were developed in two different scenarios: Pre-smolt; 3 months post vaccination, temperature at 17°C, not smoltified and in fresh water. Post-smolt; 6 months post vaccination, temperature natural with average 12°C, smoltified and in seawater. In commercial smolt production, vaccination usually takes place shortly (2-3 months, at least 550 day degrees) before the transfer to seawater. A selection program to reduce the effects of

vaccine injuries during grow-out and at harvest is then likely to be less efficient if based on records from pre-smolt compared to records from the grow-out period. However, the optimal timing of recording during grow-out remains to be determined. This will be discussed in a full paper where similar data recorded after 12 months in sea water will be included. However, including an additional trait in the breeding goal will reduce the genetic gain for other traits. Alternative approaches for reducing vaccine injures should therefore be given high priority as improvement of vaccines and management factors like time of vaccination, temperature and fish size as suggested by Berg, Rødseth, Tangerås *et al.* (2006) and Berg, Rødseth and Hansen (2007).

Conclusion

The results of this study show that it is possible to reduce the susceptibility to vaccine induced side effects in Atlantic salmon through selective breeding. Vaccine induced side effects found in pre-smolts or post-smolts should be considered as partly different traits. If the goal is reduced effects of vaccine injuries during grow out and harvest, selection is likely too be more efficient if based on records from the grow-out phase rather than from pre-smolt. However, management and improvement of vaccines should be considered to be the major factors in order to reduce side effects caused by oil adjuvanted vaccines in Atlantic salmon.

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