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# Impact of vaccination and selective breeding on the transmission of Infectious salmon anemia virus

Margo E. Chase-Topping [a,*], Chris Pooley [a,b], Hooman K. Moghadam [c], Borghild Hillestad [c], Marie Lillehammer [d], Lene Sveen [d], Andrea Doeschl-Wilson [a]

[a] Roslin Institute and Royal (Dick) School of Veterinary Studies, Easter Bush, Midlothian, UK
[b] Biomathematics and Statistics Scotland (BioSS), Edinburgh, UK
[c] Benchmark Genetics Norway AS, Sandviksboder 3A, N-5035 Bergen, Norway
[d] Nofima, N-1433 Aas, Norway

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ABSTRACT

A transmission trial was carried out using 420 Atlantic salmon (*Salmo salar* L., parr stage) to simultaneously test the impact of vaccination and selective breeding on the transmission of Infectious salmon anemia virus (ISAV). Genetic difference in disease resistance was based on mortality differences across 15 families (defined by a genomic breeding value (GEBV)) and organised into three groups (low GEBV (LBV), mid GEBV (MBV) and high GEBV (HBV)). Three different shedder groups were infected with ISAV before being placed in tanks (T1 – T18) with naive contact fish ($n = 15$), one representative from each of the 15 families. The shedder groups included LBV vaccinated ($LBV_v$), LBV not vaccinated ($LBV_{nv}$) and HBV not vaccinated ($HBV_{nv}$). The trial was run with two consecutive sets of nine tanks so the infectiousness of the shedder fish could be tested at different stages in their infection process (early (3–9 days post-infection (dpi) versus late (9–15 dpi)). Infection and mortality data of the contact fish were analysed using a Generalised Linear Mixed Model (GLMM) and a Bayesian epidemiological model. Neither vaccination nor genetic resistance prevented transmission, but both lowered the probability of infection in contact fish. Though not statistically significant, the effect of genetic resistance was larger ($LBV_{NV}$ vs $HBV_{NV}$: odds ratio: 8.35 (0.75–93.36)) than vaccination ($LBV_{NV}$ vs $LBV_V$: odds ratio: 4.52 (0.43–46.99)). There was no difference in the susceptibility of fish with different resistance breeding values, however, significant differences were found in their endurance to ISAV infection, with LBV fish dying 14 days earlier than HBV fish. Mortality as a resistance phenotype in breeding programmes appears to simultaneously improve survival of ISAV infected fish and reduce ISA transmission. However, it would be beneficial to evaluate mucus viral load (MVL) as an additional phenotype to more effectively reduce ISA transmission.

## 1. Introduction

Viral infections are one of the major challenges facing marine-farmed Atlantic salmon (*Salmo salar* L.) often leading to disease outbreaks and substantial economic losses. One of the most significant of all diseases is Infectious salmon anemia (ISA). The causative agent of ISA is the Infectious salmon anemia virus (ISAV), an enveloped single stranded RNA virus in the Orthomyxoviridae family (Thorud and Djupvik, 1988), the same family as influenza viruses (Falk et al., 1997; Krossøy et al., 1999). ISA is a notifiable disease, characterized by severe anemia and hemorrhagic lesions (Falk et al., 1995). The disease is listed as notifiable by the EU and the World Organization for Animal Health (OIE, 2016).

ISA was first detected in Norway in 1984 (Thorud and Djupvik, 1988) and has since been reported to occur globally where Atlantic salmon aquaculture is prevalent (Canada, Mullins et al., 1998; Scotland, Rodger et al., 1998; the Faroe Islands, Lyngøy, 2003; USA, Bouchard et al., 2001 and Chile, Godoy et al., 2008). ISA outbreaks typically occur during on-growth in sea, but ISA incidences have been detected in freshwater smolt production (Lyngstad et al., 2008). Outbreaks develop slowly, but the majority of fish in an infected population may succumb

* Corresponding author.
*E-mail addresses:* margo.chase@ed.ac.uk (M.E. Chase-Topping), chris.pooley@bioss.ac.uk (C. Pooley), hooman.moghadam@bmkgenetics.com (H.K. Moghadam), borghild.hillestad@bmkgenetics.com (B. Hillestad), marie.lillehammer@nofima.no (M. Lillehammer), lene.sveen@nofima.no (L. Sveen), andrea.wilson@roslin.ed.ac.uk (A. Doeschl-Wilson).

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during the production cycle resulting in mortalities as high as 90% (Jørgensen et al., 2008; Rimstad et al., 2011; Qviller et al., 2020). Outbreaks in Chile (2007–2009) (Mardones et al., 2009; Mardones et al., 2011) and Faeroe Islands (2000–2005) (Christiansen et al., 2011) resulted in the destruction of the entire farming industry in the affected region. In Scotland an outbreak in 1998–1999 (Anon, 2000; Stagg, 2003) was eradicated at a cost then of over £20 M (Hastings et al., 1999). In Norway, the peak of outbreaks was in the early 1990's when 80 outbreaks were recorded. Currently Norway experiences 0–7 outbreaks annually (Lyngstad et al., 2018). Due to its status as a listed disease, outbreaks of ISA call for mandatory disease control measure including the establishment of a disease control zone with surveillance of fish populations, culling of infected cages or entire farm populations, and ensuring a period of coordinated fallowing of the entire zone after depopulation (Anon, 2017; (Office International des Epizooties Manual of Diagnostic Tests for Aquatic Animals, 2016)). Effective ISA control thus needs to reduce the transmission of ISAV infection.

The disease can spread to other salmon sea farms through passive transmission in water or with contaminated equipment, boat traffic or movement of fish (Vågsholm et al., 1994; Jarp and Karlsen, 1997; Murray et al., 2002; Gustafson et al., 2007; Mardones et al., 2009; Aldrin et al., 2011; Mardones et al., 2014). The most important predictor of further spead of infection, excluding viral strain, is seaway distance between salmon sea farms (Aldrin et al., 2011; Qviller et al., 2020). Furthermore, the level of sea lice infection is thought to play a role (Oelckers et al., 2014) and there is also evidence of vertical transmission of ISAV from mother to offspring through ovarian fluid and eggs (Marshall et al., 2014). Atlantic salmon is the only susceptible species known to develop clinical disease, but ISA virus can replicate in rainbow trout (*Oncorhynchus mykiss*) and sea trout (*Salmo trutta L.*).

Biosecurity, vaccination and selective breeding are the main control measures currently being considered. Vaccination plays a crucial role in large scale commercial fish farming (Mutoloki et al., 2015) as one of the best methods to increase survival and profitability. However, depending on the vaccine and the method of delivery, fish are often either stressed and suffer various side effects or have reduced immunity. Furthermore, vaccinated fish can still carry the virus, and can therefore become asymptomatic carriers spreading the disease to other fish. Moreover, vaccination of fish can result in positive results in ISAV detection tests, which can exclude facilities from ISA free compartment status, preventing export of fish from these facilities (OIE, 2016). For these reasons, there are restrictions on the use of ISA vaccines, which are not allowed in some countries, therefore other strategies are necessary to reduce the transmission and limit the impact of ISAV.

Selective breeding for increased host disease resistance has proven a viable solution to control infectious diseases in aquaculture (Yáñez et al., 2014; Houston, 2017). Compared to other species, aquaculture breeding benefits from large family sizes and the ability to routinely collect disease or mortality data from large-scale sib-disease challenge experiments. A recent publication from Canada (Holborne et al., 2020) found family level mortality in an ISAV sib-challenge experiment ranged from 42% to 100%. Mortality is the main phenotype for disease resistance in fish, but there is emerging evidence that directly targeting survival via breeding or vaccination without considering the epidemiological effects does not reduce disease transmission and thus disease prevalence at the population level (Anche et al., 2014; Anacleto et al., 2019; Bitsouni et al., 2019). In Norway, the breeding company Benchmark Genetics Norway, formerly SalmoBreed, has had an active selection program against ISA since 2001 that produces fish with enhanced chances of survival when exposed to ISAV. However, it is unclear if selection based on the survival performance will provide an ideal phenotype for effective breeding to reduce the prevalence of this disease.

Recent studies point to three key epidemiological host traits affecting infectious disease prevalence and population mortality rates: susceptibility (an individual's propensity of becoming infected when exposed to infectious material), infectivity (the ability of an individual, once infected, to transmit infection) and endurance (the propensity of an individual, once infected, to survive the infection) (Doeschl-Wilson et al., 2018; Anacleto et al., 2019; Saura et al., 2019). Heritable genetic variation in susceptibility has long been the target of many genetic selection schemes in livestock and plants (Heringstad et al., 2000; Kover and Schaal, 2002; Bishop et al., 2010; Houston et al., 2010; Banos et al., 2017). However, emerging evidence suggests that endurance and infectivity may also be partly controlled by host genetics (Geenen et al., 2004; Ødegård et al., 2011; Raszek et al., 2016; Anacleto et al., 2019; Saura et al., 2019). Recent theoretical modelling studies have shown that although genetic selection on susceptibility reduces disease risk and prevalence, the additional gain from selection on infectivity can substantially accelerate disease eradication and reduce more efficiently the risk of new outbreaks (Anche et al., 2014; Tsairidou et al., 2018). In contrast, improving individuals' endurance may accidentally increase disease risk and prevalence if individuals with greater endurance are more infectious or infectious for longer (Gopinath et al., 2014; Bitsouni et al., 2019). Interventions aiming to reduce disease prevalence and impact may target improvement in any one of these traits. However, to date, very little is known about how vaccination or selective breeding alone or in combination affect these underlying epidemiological traits and ISAV transmission. In this study, a small scale ISAV transmission experiment was carried out to simultaneously examine the effects of vaccination and genetic resistance, defined by host survival, on the transmission of ISAV in Atlantic Salmon.

## 2. Methods

### 2.1. Experimental design

The experimental design is summarised in Fig. 1. The experiment comprised altogether 420 Atlantic salmon from 15 families (28 fish per family) that differed widely in their estimated genomic breeding values (GEBV) for resistance to ISA (five families with low GEBV (LBV), medium GEBV (MBV) and high GEBV (HBV), respectively; see Supplementary Material A for more detailed information). Fish were hatched and startfed in family tanks ($n = 15$ families) at the Benchmark Genetics Norway breeding station in Lønningdal, Norway. At the parr stage, the fish where ID marked by inserting a passive integrated transponder (PIT) tag into their abdominal cavity, DNA sampled and weighed. The fish were then transported to VESO Vikan (Namsos, Norway) testing facility and acclimated for approximately one week in fresh water (at ~12° temperature, flow system with 5–6 mg $O_2$/l, ad libitum feeding) and were then separated into shedders ($n = 150$) and naive contact fish ($n = 270$). Shedder fish comprised ten arbitrarily chosen fish from each of the 15 families (n = 150 fish); four fish per family ($n = 60$ fish) were vaccinated (AJ micro 7 ILA, delivered by Pharmaq AS) against ISAV (0.05 ml per fish) and then placed into a different tank while the remaining fish were not vaccinated ($n = 90$). Following vaccination, the fish were left for six weeks for stimulation of their immune response and then all 150 shedder fish were injected (intra peritoneal, 0.1 ml per fish) with ISAV (Glasvaer 080411 strain, 2 passage, grown in ASK-cells, estimated titre $10^6$/ml, delivered from the Norwegian Veterinary Institute (NVI) in Oslo). The ISAV strain originates from the first field outbreak of ISA in 1984.

Shedder fish were selected for the trial based on their GEBVs (high GEBV (HBV) and low GEBV (LBV)) in combination with whether or not they were vaccinated (see Supplementary Material A). There were three shedder groups used in the challenge: Low GEBV vaccinated ($LBV_v$); low GEBV not vaccinated ($LBV_{nv}$); and high GEBV not vaccinated ($HBV_{nv}$).

At three days post-infection (dpi) two shedder fish from the same shedder group were placed in pairs in nine new tanks (T1 - T9), each containing 15 contact fish (one random fish per family, total $n = 135$ fish). The amount of shedder fish per tank was decided based on cohabitation trials where the goal was to achieve 50% mortality by the end of trial. After six days (9 dpi), the shedders were transferred to nine

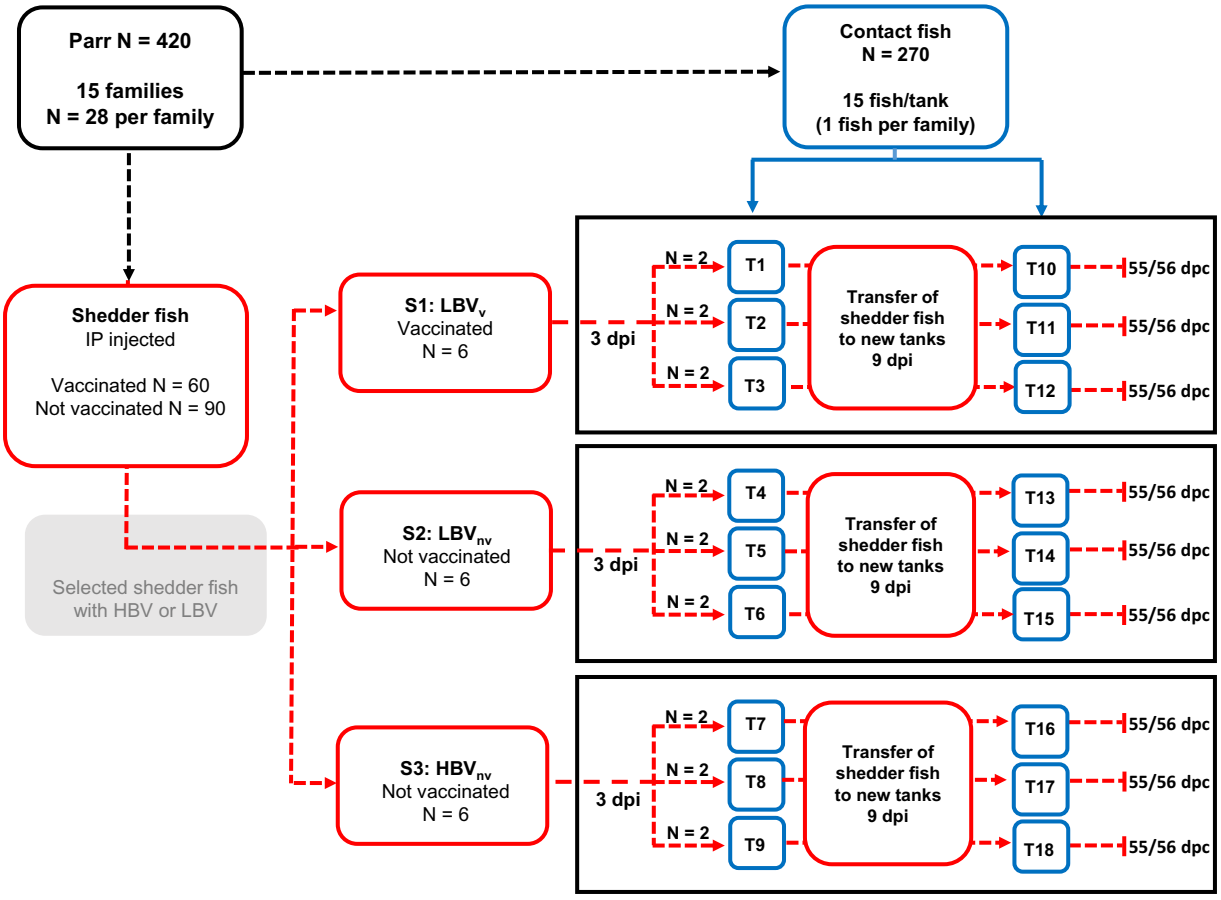


**Fig. 1.** Study design for transmission experiment. Atlantic salmon (*Salmo salar* L.) $n = 28$ from each of the 15 families ($n = 420$ total) were separated into fish that will be used as shedders ($n = 150$) and contact fish ($n = 270$). The shedder fish were separated, a portion ($n = 60$) were vaccinated, and the remainder ($n = 90$) were not vaccinated. All shedder fish were injected with ISAV six weeks after vaccination. Shedder fish from families with low breeding value (six vaccinated ($LBV_v$), six not vaccinated ($LBV_{nv}$)) and high breeding value (six not vaccinated ($HBV_{nv}$)) were selected for the transmission study. All contact fish were not vaccinated or infected. A total of 18 tanks (T) were set up (T1 - T18) into which 15 contact fish were placed (one from each family). Two shedder fish were placed in each of tanks T1 - T9 where they remained for six days. After six days the shedders were moved to tanks T10 - T19. T1 - T9 represent shedders early in their stage of infection (3–9 days post infection (dpi)) whereas tanks T10 - T18 represent shedders in a later stage of infection (9–15 dpi). After 15 dpi the shedder fish were removed. The contact fish remained in the tank until 55 (T1 - T9) or 56 (T10 - T18) days post contact (dpc).

new tanks (T10 - T18) with 15 new contact fish per tank (one random fish per family, total n = 135 fish). Six days after their second transfer (15 dpi), shedder fish were removed from the tanks. Hence there were two experimental replicates for each of the three shedder groups (Fig. 1), with contact fish exposed to different shedder groups at two different stages of infection: early, at 3–9 dpi (T1 - T9), and late, at 9–15 dpi (T10 - T18). Two shedder fish died on the day before the challenge was terminated in T13 and T15. According to the protocol the two fish were replaced with a shedder fish belonging to the same shedder group. Contact fish remained in the tanks until the challenge was terminated at 55 days post contact (dpc) for tanks T1 - T9 and 56 dpc for tanks T10 - T18.

### 2.2. Sampling and virus level determination

The mucus of shedder fish was sampled using swabs at three, nine and 15 dpi. All tanks were checked for moribund or dead fish twice a day from zero dpc to termination. At termination, tissue (tip of the heart) samples were collected from all remaining contact fish for quantitative reverse transcription PCR (qRT-PCR), on tubes containing RNALater. All qRT-PCR samples were stored on -20 °C until processing. Quantitative estimates of virus load in heart and mucus were obtained by qRT-PCR analyses performed by the Fish Vet Group Norway (http://fishvetgroup.no/en/). The cycle threshold value (Ct) was provided for both the ISAV and the reference gene, elongation factor 1-alpha (ef1a). For ISAV, a Ct value larger than 33 was considered as no ISAV detected in the sample.

### 2.3. Data management and statistical analyses

All data was stored in an Excel file with PIT tag as unique identifiers. The final file contained 265 contact fish and 20 shedder fish (five contact fish were lost to the study and two shedder fish were added as per protocol to the final days of T13 and T14). Univariable analysis was performed on the contact fish to look for patterns of infection (dead (i.e. found dead before the trial terminated), infected (i.e. survived to the end of the trial, virus detected in heart tissue) and uninfected (i.e. survived to the end of the trial, no virus detected in heart tissue) across the 15 different contact fish family groups (Supplementary Material A), shedder groups ($LBV_v$, $LBV_{nv}$, $HBV_{nv}$) and shedder stage of infection (early, late). Fisher's exact tests were performed to look for differences in the distributions of death and infection using StatXact version 11 (Cytel MA, USA).

Multivariable analysis was performed using the infection status (binary: infected (including dead) / not infected) of contact fish at the end of the trial as the response variable given that mortality was low. Data was analysed using generalised linear mixed models (GLMM, SAS Proc Glimmix). Family and tank were included as random effects. Contrasts

were developed to look at the effects of vaccination ($LBV_{nv}$ vs $LBV_v$) and genetic resistance ($LBV_{nv}$ vs $HBV_{nv}$) of shedder fish on contact fish infection status. For the infected contact fish only, delta Ct values for virus load were analysed using a linear mixed model (SAS Proc Mixed) with family and tank as random effects.

Unless stated otherwise all statistical analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC). Statistical significance was set at $p < 0.05$.

### 2.4. Estimating the impact of host genetics and vaccination on ISAV transmission

To assess the impact of vaccination and genetic resistance on ISAV transmission, a compartmental epidemiological model was fitted to data from each contact group from each tank in the transmission experiment (Pooley et al., 2020). Individuals were considered to be in one of three states: susceptible to infection (S), infectious (I) or dead (R). Transitions between states were assumed to be Markovian (i.e. they occur with a certain probability per unit time, irrespective of the history of the individual). The infection status of individuals at the beginning of the challenge was assumed known, with shedder fish in the I state and contact fish in the S state.

Transmission dynamics in the epidemiological SIR model is determined by three host traits: the susceptibility ($g$) of individuals in state S (i.e. propensity to become infected), the infectivity ($f$) of individuals in state I (i.e. propensity to transmit infection, once infected) and the endurance $m$ of individuals in state I (i.e. propensity to survive infection).

In the standard SIR model the probability per unit time of a susceptible fish becoming infected, or the "force of infection", is given by a transmission parameter β (which combines the rate at which fish come into contact and the probability of disease transmission upon each contact) multiplied by the total number of infected fish (which changes as a function of time as the shedder fish spread the infection to the contact fish). Here, however, we need to account for the fact that (a) the contact fish do not all have the same susceptibility to the disease, (b) the infected fish can be divided into those which are infected shedders and those which are contact fish (and we must allow for uncertainty if and when they become infected), and (c) the infectivity of the shedder fish may not only depend not only their group ($LBV_v$, $LBV_{nv}$, $HBV_{nv}$), but also on their stage of infection (early, late). An appropriate model is as follows:

For $t \leq 6$ dpc the force of infection experienced by contact fish in tanks after the introduction of $n_{shed}$ infected shedder fish is given by:

$$\lambda_{ijkl}(t) = \beta e^{g_i}\left(n_{shed}\, e^{f_{jk}} + I_l(t)\right) \quad (1a)$$

and for $t > 6$ dpc (after removal of the shedder fish):

$$\lambda_{ijkl}(t) = \beta e^{g_i} I_l(t), \quad (1b)$$

where the contact fish type $i$ refers to the estimated family breeding value category (low, medium, high) for ISA resistance (based on mortality records), shedder type $j$ at stage $k$ in tank $l$, and:

$\beta$ = avg. transmission rate.
$g_i$ = susceptibility effect of contact fish resistance type i on transmission rate $\beta$.
$f_{jk}$ = infectivity effect of shedder fish type j at stage k on transmission rate $\beta$
$n_{shed}$ = number of shedder fish per tank ($n_{shed} = 2$)
$I_l(t)$ = number of infected and infectious contact fish in tank $l$ at time t (whose uncertainty is accounted for during analysis).

The exponential link functions in the model above imply that susceptibility $g_i$ and infectivity $f_j$ parameters are expressed as fractional deviations in an individual's susceptibility and infectivity, respectively, as compared to that of the population as a whole (e.g. $g_j = 0.1$ corresponds to individual $j$ being ≃10% more susceptible than the population average, which corresponds to a value of $g = 0$).

In this model the infectivity of the shedder fish was not further stratified by stage of infection (i.e. early versus late) as the model did not produce realistic posterior model parameter estimates, indicating that the data provided insufficient information to estimate all parameters simultaneously.

The endurance of infected fish, defined by the infection-induced mortality rate $m$, was inferred from the estimated time from infection to death (i.e. low value of $m$ means high endurance). The endurance of a contact fish affects the force of infection on its group members through the $I_l(t)$ term in Eqs. (1a) and (1b). Under the SIR model, greater endurance implies a greater number of infectious fish at any time. The above full model thus accounts for potential genetic differences in the susceptibility and endurance of contact fish to ISAV infection (represented by the factor $g_i$ and mortality rate $m_i$, respectively) as well as for potential genetic or vaccine mediated variation, and differences in the ability of shedder fish to transmit the virus (represented by the infectivity factor $f_{jk}$) to susceptible contact fish. In the epidemiological model contact fish families were stratified into three distinct resistance Family BV categories (see supplementary Material A, Table A.1) to assess whether genetic differences in resistance (mortality after exposure) also confer genetic difference in susceptibility or endurance to infection.

Model fitting was carried out using a Bayesian approach which generated posterior samples for the unobserved infection and mortality events $\xi$ (which provide posterior estimates for $I_l(t)$ in Eqs. (1a) and (1b) above), and for the model parameters $\theta = \{\beta, g_{F1}, g_{F2}, g_{F3}, f_{S1E}, f_{S1L}, f_{S2E}, f_{S2L}, f_{S3E}, f_{S3L}, m_{F1}, m_{F2}, m_{F3}\}$ where the subscript F1,F2,F3 in parameter $g$ and $m$ refers to the contact fish resistance category (F1 = LBV; F2 = MBV; F3 = HBV), and the subscript S1,S2,S3 in parameter $f$ refers to the three shedder groups (S1 = $LBV_v$; S2 = $LBV_{nv}$; S3 = $HBV_{nv}$) and E,L refers to the stage of infection of the shedders (E = early; L = late).

Data used for model fitting comprised the binary diagnostic test results from the heart samples of each contact individual (specifically, binary +ve/−ve diagnostic test results were generated with the cut-off being set by the detection limit of the qRT-PCR analyses). Application of Bayes' theorem to this data $y$ implies that the joint posterior is given by:

$$\pi(\theta, \xi|y) \propto \pi(y|\xi) L(\xi|\theta) \pi(\theta), \quad (2)$$

where the observation model $\pi(y|\xi)$ takes the values one or zero depending on whether the events $\xi$ are consistent with $y$ or not, and the latent process likelihood is given by O'Neill and Roberts (1999):

$$L(\xi|\theta) = \prod_{z=1}^{Z}\left[\prod_{e=1}^{E_z} r_e e^{-\Lambda_e \times (t_e - t_{e-1})}\right], \quad (3)$$

where $z$ goes over all contact groups ($Z$=18 tanks) and $e$ goes over all infection and mortality events within each contact group (up to a total of $E_z$ events). The quantity $r_e$ denotes the transition rate corresponding to event $e$ (e.g. infection at time $t$ of a contact fish of resistance category 1 exposed to shedder fish of type 2 at the early stage of infection in tank 1 is given by $\lambda_{F1S2E1}(t)$) and $\Lambda_e$ is the sum of all possible event rates immediately prior to time $t_e$. The prior $\pi(\theta)$ in Eq. (2) consists of largely uninformative uniform distributions between 0 and 3 for each of the model parameters.

Bayesian inference was performed using Monte Carlo Markov Chain (MCMC) with a large number of iterations to ensure accurate estimates were generated (with effective sample size exceeding 400 for each model parameter after an initial 20% burn-in period) from four randomly initialised chains (used to confirm global convergence of parameters). Details of this procedure along with MCMC diagnostics are given in Supplementary Material B.

## 3. Results

### 3.1. General epidemiology

Across the entire transmission experiment only 20% of the contact fish became infected ($n = 53/265$) and 4.5% died ($n = 12/265$). Fig. 2 and Supplementary Material A Table A.2 contain the results from all tanks (T1 - T18) with contact fish classified as dead, infected (excluding dead) and uninfected. There was considerable variability across tanks. Five of the nine tanks in the early stage of infection (T1 - T9) had infected contact fish despite non-detectable mucus viral load (MVL) in the shedder fish at the time of transfer. All shedder fish from the $LBV_{nv}$ group had detectable MVL at the time of transfer for the late stage of infection, whereas this was only the case for two and two shedder fish from $LBV_v$ and HBVnv group respectively. Seven tanks contained no dead or infected contact fish despite at least one shedder fish mucus sample testing positive. Infection was observed in all tanks with contact fish exposed to $LBV_{nv}$ shedders. However, four out of six tanks for which infection was seeded by the $HBV_{nv}$ shedder group contained no single infected or dead contact fish (3/3 and 1/3 associated with early and late infection stage, respectively). Similarly, three out of six tanks where the infection was seeded by the $LBV_v$ group did not result in any infected or dead contact fish, indicating that both genetic resistance and vaccination reduce ISAV transmission.

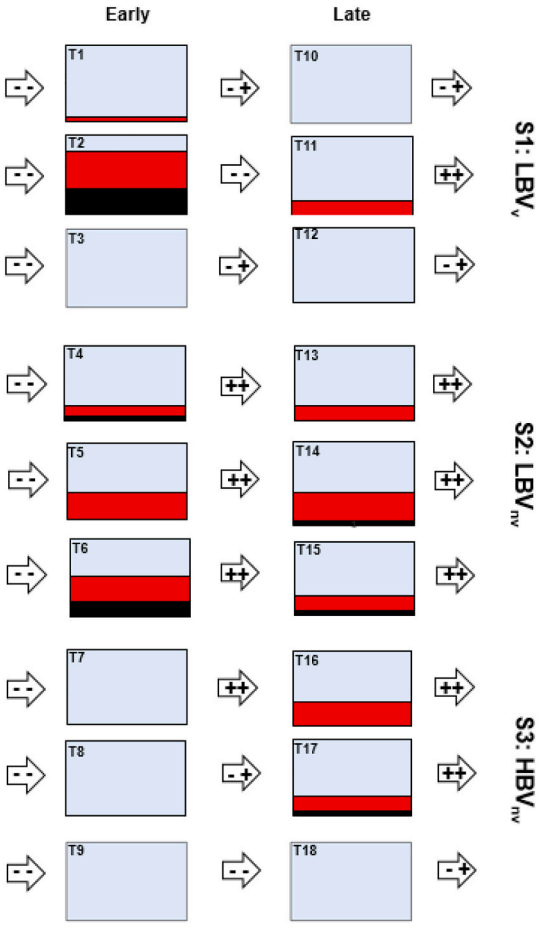


**Fig. 2.** Results from the challenge. Coloured boxes refer to the status of the contact fish (Dead, black; Infected (but not dead), red; uninfected, light blue). The width of the colour is in proportion to the number of animals in each category. Arrows refer to the results of the nasal swab test for mucus viral load (MVL) for shedder fish (two per tank) -, MVL negative; +, MVL positive. "Early" Stage of infection, T1 - T9 shedder fish 3–9 dpi; "Late" stage of infection, T10 - T18 shedder fish 9 - 15dpi. Shedder groups: low breeding value vaccinated ($LBV_v$), low breeding value not vaccinated ($LBV_{nv}$) and high breeding value not vaccinated ($HBV_{nv}$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.2. GLMM results: impact of genetic resistance and vaccination status of infected shedders on contact fish infection status and viral load

Fig. 3 shows the proportion of dead, infected and uninfected contact fish summarised by shedder group (A), stage of infection (B) and stage of infection within shedder group (C). There was a significant difference in the distribution of dead, infected and uninfected contact fish across shedder groups ($3 \times 3$ Fisher's exact test: 15.64 $p = 0.002$) (Fig. 3A). The distribution within the $LBV_{nv}$ group was statistically significant from $LBV_v$ and $HBV_{nv}$. Overall, there was no significant difference in the distribution of dead, infected and uninfected contact fish between the early and late stage of infection ($2 \times 3$ Fisher's exact test: 2.933, $p = 0.2468$) (Fig. 3B). However, within the shedder groups (Fig. 3C), stage differences in the contact fish distributions were found for $LBV_v$ ($p = 0.006$) and $HBV_{nv}$ ($p = 0.003$), but not $LBV_{nv}$ ($p = 0.647$).

Table 1 show the results from the GLMM analysis for the probability of transmission. Neither shedder group nor stage of infection was significant in the model. However, both vaccination and genetic resistance lowered the probability of infected fish to transmit the infection. Though not statistically significant, the effect of genetic resistance on contact fish probability of infection was larger ($LBV_{NV}$ vs $HBV_{NV}$: odds ratio: 8.35 (0.75–93.36); $p = 0.080$) than the effect of vaccination ($LBV_{NV}$ vs $LBV_V$: odds ratio: 4.52 (0.43–46.99), $p = 0.186$).

There was no significant difference in the viral load of infected contact fish in the heart as measured by delta Ct between either shedder group ($p = 0.2467$) or stage of infection ($p = 0.1933$), however, the fish that died generally had lower delta Ct values (hence higher viral load) than those fish that were infected but did not die (Fig. 4).

### 3.3. Epidemiological modelling results: impact of genetic resistance and vaccination status of infected shedders on ISAV transmission dynamics

Posterior distributions for shedder fish infectivity associated with different stages of infection strongly overlapped, hence a simpler model, assuming time constant shedder fish infectivity was fitted. The results of the epidemiological modelling are shown in Fig. 5 (A - D) and Table 2. Fig. 5A shows the posterior probability distributions for the infectivity (parameter *f*) of the different shedder groups (means and 95% credibility intervals for the model parameters are shown in Table 2). There was some overlap in the credible intervals for the infectivity effects of the shedder groups on ISAV transmission rate, with the trend supporting the results from the GLMM. $LBV_{nv}$ shedders generally were the most infectious (i.e. highest $f_{S2}$). Contact fish were less likely to become infected when exposed to $HBV_{nv}$ shedders ($f_{S3}$) and $LBV_V$ shedders compared to $LBV_{nv}$ shedders with the effects of shedder vaccination on transmission generally less pronounced than the genetic effects (Fig. 5A, Table 2). Fig. 5B shows the posterior estimates for contact fish susceptibility. The strong overlap in the credible intervals for susceptibility indicates that fish that differ in their genetic resistance to ISAV did not differ in their susceptibility to ISAV infection. Fig. 5C shows the posterior estimates for mortality rates (endurance) of infected contact fish different resistant types. Although the credible intervals were overlapping, there were pronounced differences in the endurance of contact fish to infection with the trend matching the GEBV of the family, i.e. genetically more resistant fish had also greater endurance. The results thus indicate that the observed differences in mortality across family groups (Supplementary Material A Fig. A.2) resulted from genetic differences in endurance rather than the susceptibility of fish to ISAV infection.

## 4. Discussion

Biosecurity, vaccination and recently selective breeding for disease

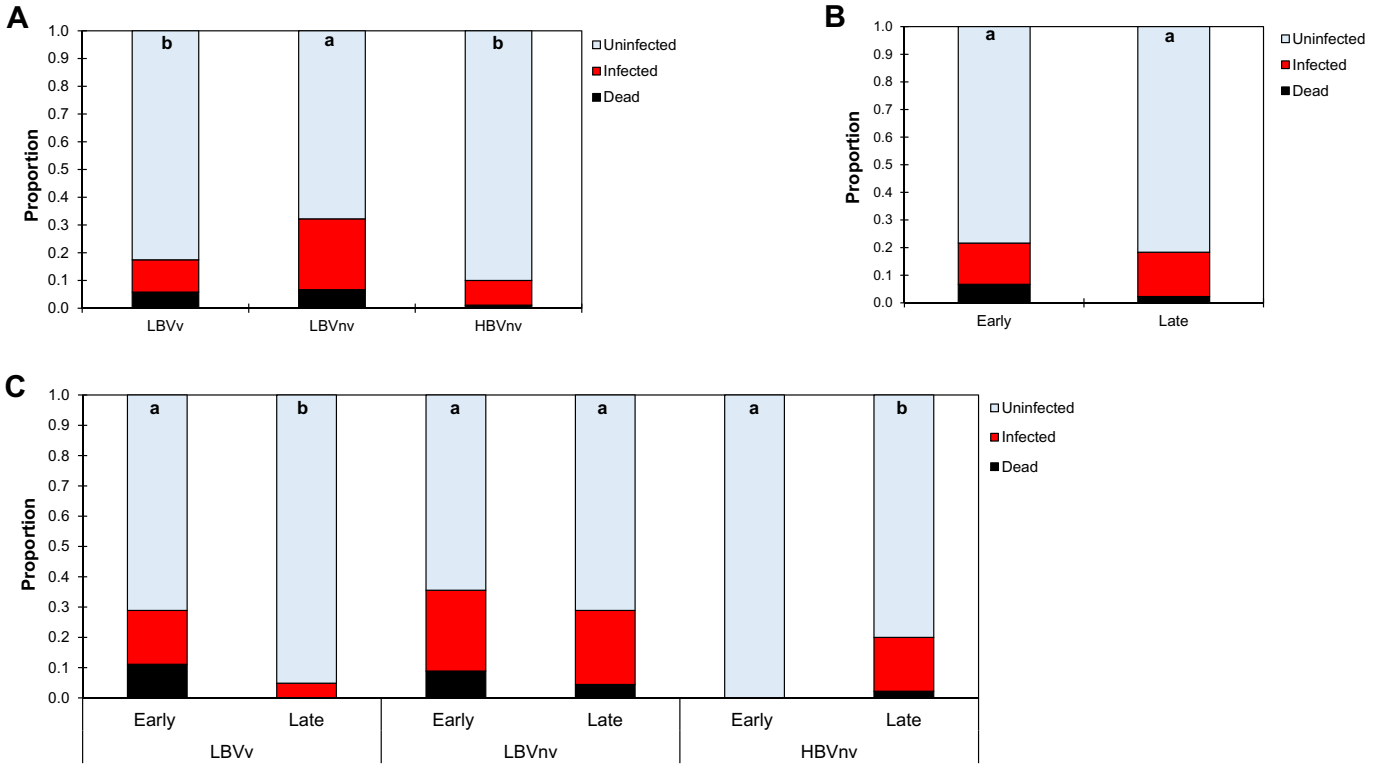


**Fig. 3.** Infection status of contact fish. Univariate analysis of the infection status (Dead, black; Infected (but not dead), red; uninfected, light blue) of the contact fish across all tanks by A. shedder group ($LBV_v$, $LBV_{nv}$, $HBV_{nv}$); B. Stage of infection (Early, Late); C. Stage of infection (within shedder group). The same small letter indicates no significant difference based on Fisher's exact test. $P < 0.05$ was considered statistically significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**
Generalised linear mixed model (GLMM) adjusted least-square means for the proportion of contact fish positive in A. the different shedder groups: low breeding value vaccinated ($LBV_v$), low breeding value not vaccinated ($LBV_{nv}$) and high breeding value not vaccinated ($HBV_{nv}$) and B. the different stage of infection: early (3–9 days post infection (dpi)) vs late (9–15 dpi).

| Variable | Level | Estimate | se | p | Mean | 95% CI |
|---|---|---|---|---|---|---|
| Intercept | | −2.8099 | 0.9286 | 0.0095 | | |
| Shedder group | $HBV_{nv}$ | −2.1223 | 1.1183 | 0.0800 | 5.28 | 0.88–25.88 |
| | $LBV_v$ | −1.5075 | 1.0736 | 0.1859 | 9.35 | 1.79–36.87 |
| | $LBV_{nv}$ | – | – | – | 31.78 | 8.72–69.43 |
| Stage of infection | Early | −0.1529 | 0.9146 | 0.8699 | 11.40 | 2.99–34.93 |
| | Late | – | – | – | 13.04 | 3.67–37.12 |

resistance are the main tools for controlling ISAV in Atlantic Salmon. However, both host genetics and current vaccines only provide partial protection raising concerns about their effectiveness in the field. In particular, very little is known about their impact on ISAV transmission and hence their effect on ISA prevalence upon which control strategies are built. This is partly because routinely carried out disease cohabitation experiments only capture genetic or vaccine effects on mortality of susceptible fish exposed to the virus, but not on how these impact transmission. In particular, current cohabitation trials provide little information on whether fish with greater genetic resistance or vaccinated fish are less likely to transmit infection when infected. In this study, the transmission experiment coupled with an epidemiological model provided first-time insights into these effects on the traits underlying disease transmission namely susceptibility, infectivity and endurance.

In this study, both genetic resistance and vaccination reduced the infectivity of the shedders by lowering the probability of the infected fish to transmit the infection to naive contact fish. Though not statistically significant as the confidence / credible intervals overlapped considerably, the interesting trend in the GLMM and Bayesian epidemiological models for the host genetic effects in this study to be larger than the effects of vaccination should be further investigated in future studies. Due to cost restrictions, the effect of vaccination, however, was only tested for the shedder fish with low genetic resistance, where vaccination effects were expected to be largest. It would be interesting to find out how genetic resistance and vaccination interact, and in particular whether vaccinated fish with high genetic resistance still transmit the virus.

In earlier cohabitation trials, involving the same fish families as in this study, the mortality rate of the vaccinated contact fish was very low (< 2%) but there was considerable variation in the infection rate (36.2% - 81.4%) (unpublished data). The variability in the tanks containing the $LBV_v$ shedder group was high in this study with results ranging from no infected contact fish to the highest contact fish infection and mortality rate observed in this study. The majority of the infected contact fish were those exposed to the $LBV_v$ shedders during the early stage of infection. This may suggest that vaccination, unlike genetic resistance, does not reduce infectivity early on, although this could not be verified by the epidemiological model. Alternatively, the observed differences may represent natural variation in vaccination uptake, and that the chosen shedder fish had poor vaccine uptake. Variability in responsiveness to vaccination has been observed in other studies (Chase-Topping et al., 2020), were 31% of pigs in the study were vaccine non-responders and were shown to have characteristics more associated with unvaccinated animals. Heterogeneous vaccine responsiveness highlights the potential merit of assessing the combined effects of both host genetics and vaccination for effective ISA control.

The results of the GLMM indicated that infection rates in tanks are not only controlled by the shedder type, but also depend on the genetic resistance of the contact fish. In line with common practice, variation in resistance in this study was based on estimated breeding values at a

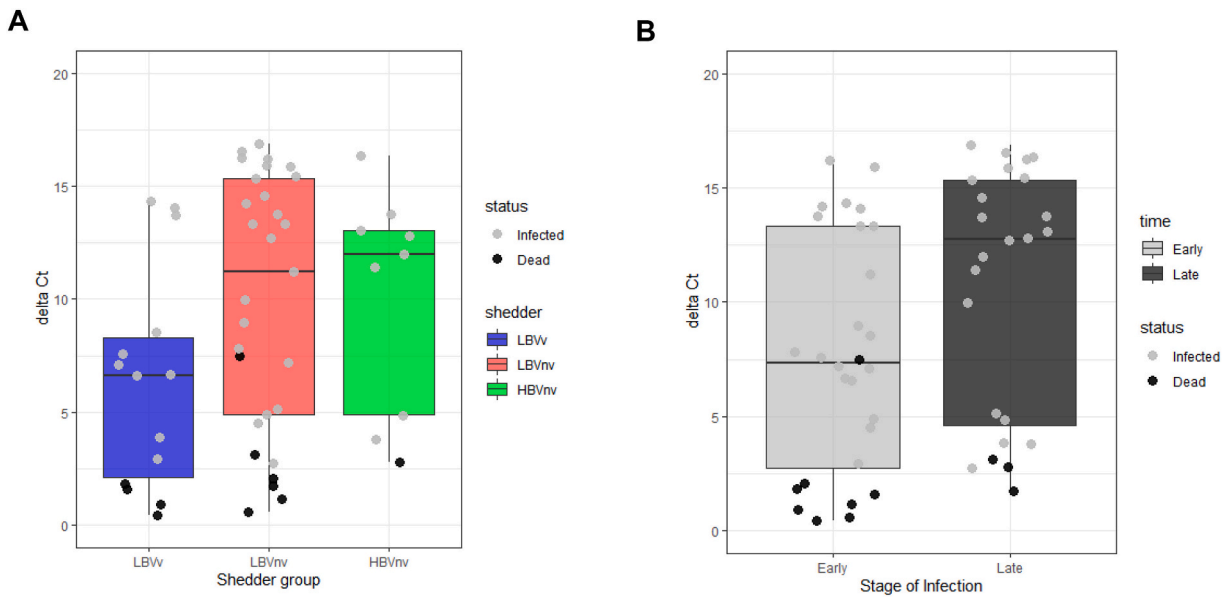


**Fig. 4.** Delta Ct values representing viral loads of infected contact fish. A. shedder group ($LBV_v$, $LBV_{nv}$, $HBV_{nv}$); B. Stage of infection (Early, Late). Individual points show whether a contact was dead (black) or infected (grey) at termination.

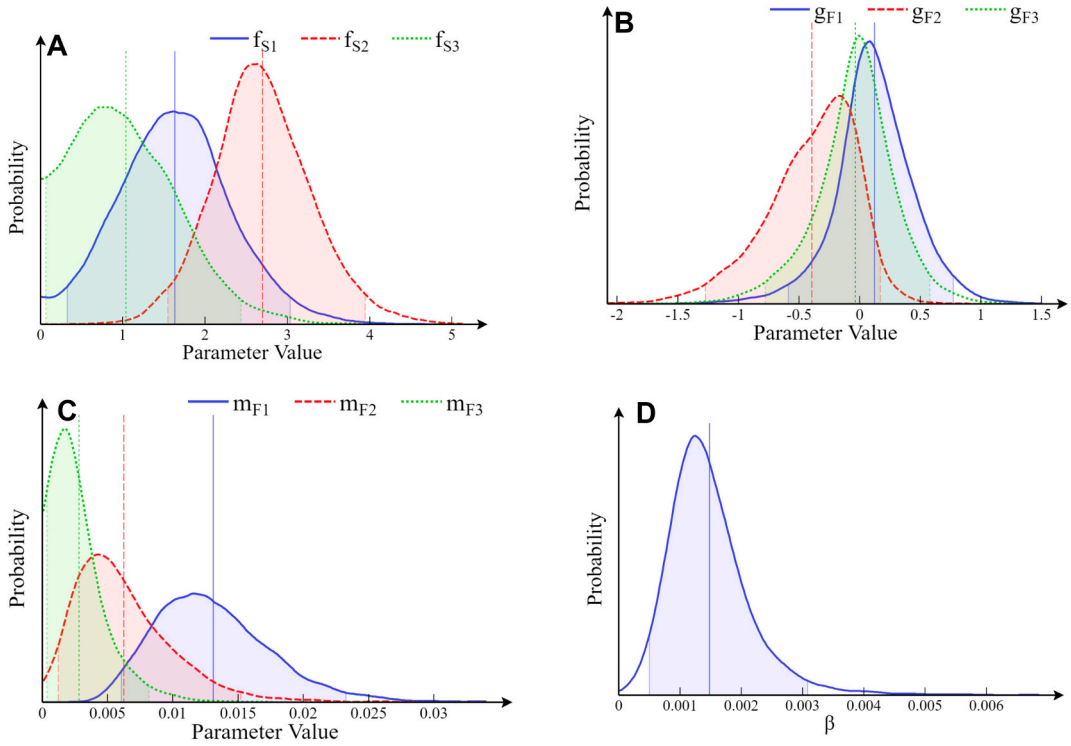


**Fig. 5.** Posterior distributions from the Bayesian epidemiological model. A. the infectivity (parameter *f*) for the different shedder groups (Blue, S1: shedder group $LBV_v$ ($f_{S1}$); Red, S2: shedder group $LBV_{nv}$ ($f_{S2}$); Green, S3: shedder group $HBV_{nv}$ ($f_{S3}$)); B. the endurance/mortality (parameter m) for the contact fish from different resistance types (Blue, Family's with low breeding value (F1); Red, Family's with mid breeding value (F2); green, Family's with high breeding value (F3)); C. the susceptibility (parameter g) of contact fish from different resistance types (Blue, Family's with low breeding value (F1); Red, Family's with mid breeding value (F2); green, Family's with high breeding value (F3)); D. Average transmission rate of shedder fish with average infectivity to contact fish (parameter β). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

family level related to mortality in response to ISAV challenge. We found that contact fish from different family BV groups differed in their endurance but not in their susceptibility to infection. This was supported by both the GLMM and the fitted epidemiological model. These findings may have potential important implications for ISA control. ISA is a notifiable disease and culling is required once the virus is detected. Individuals with greater endurance to infection will live longer when infected and therefore, may have more chances of infecting others (Saura et al., 2019). An alternative explanation is that contact fish in the more resistant family groups were infected but were able to clear the infection, thus reducing their risk of infecting others. Unfortunately there is no data available to distinguish between the two alternative

**Table 2**
Parameters estimates from the epidemiological model obtained by Bayesian Inference. S1, Shedder group 1 (Low Breeding Value vaccinated ($LBV_v$)); S2, Shedder group 2 (Low breeding value not vaccinated ($LBV_{nv}$)); S3, Shedder group 3 (High Breeding Value not vaccinated ($HBV_{nv}$)); F1, LBV family group; F2, MBV family group; F3, HBV family group; LCI, lower 95% credible interval; UCI, Upper 95% credible interval; BV, breeding value.

| Variable* | Definition | Mean | LCI | UCI |
|---|---|---|---|---|
| $f_{S1}$ | Infectivity; shedder fish; $LBV_v$ | 1.63 | 0.327 | 3.03 |
| $f_{S2}$ | Infectivity; shedder fish; $LBV_{nv}$ | 2.70 | 1.55 | 3.94 |
| $f_{S3}$ | Infectivity; shedder fish; $HBV_{nv}$ | 1.04 | 0.0678 | 2.44 |
| β | Average transmission rate of shedder fish with average infectivity to contact fish | 0.00148 | 0.000501 | 0.00308 |
| $m_{F1}$ | Infection mortality rate; contact fish; LBV family group | 0.0131 | 0.00605 | 0.0233 |
| $m_{F2}$ | Infection mortality rate; contact fish; MBV family group | 0.00624 | 0.00123 | 0.0152 |
| $m_{F3}$ | Infection mortality rate; contact fish; HBV family group | 0.00281 | 0.000358 | 0.00817 |
| $g_{F1}$ | Susceptibility; low family breeding value (LBV) | 0.121 | −0.589 | 0.772 |
| $g_{F2}$ | Susceptibility; mid family breeding value (MBV) | −0.395 | −1.27 | 0.173 |
| $g_{F3}$ | Susceptibility; high family breeding value (HBV) | −0.0367 | −0.779 | 0.579 |

See methods 2.5, Estimating the impact of host genetics and vaccination on ISAV transmission for further details on the parameters.
* The rates for β and m are in units $[Day]^{-1}$ and all other quantities are dimensionless (they represent fractional deviations, as shown in Eq. (1)).

scenarios. In this study we assumed that fish become infected and die rather than recover.

Obtaining accurate infectivity estimates of individuals is not straightforward and usually requires carefully designed transmission experiment or field sampling protocols combined with sophisticated statistical inference models (Anacleto et al., 2019; Biemans et al., 2019; Pooley et al., 2020). A phenotypic trait closely correlated with infectivity that can be easily measured on individuals would be extremely useful. One such possible proxy trait would be a measurements of pathogen shedding (Tsairidou et al., 2018). Previous studies in other species have already demonstrated that vaccine-induced reduction in host infectivity is mediated by a reduction in virus load at shedding sites (Chase-Topping et al., 2020; Bailey et al., 2020). In this study, most shedders (61%) had detectable MVL at the time of transfer (nine dpi) and at the end of the trial (83%). However, no virus was detected in the mucus of any of the infected shedder fish at three dpi, although they were clearly able to transmit the virus. In this study, the shedder fish for the tank (T2) with the highest infection and mortality among contact fish had non-detectable MVL at 3 dpi as well as the time of transfer (nine dpi) suggesting perhaps MVL is not a reliable indicator for infectivity. However, more detailed evaluations of MVL as potential infectivity phenotype are needed to avoid preliminary false conclusions.

### 4.1. Study limitations

The overall infection and mortality rates in this experiment were surprisingly low. A recent transmission study in Canada recorded a cumulative mortality of 83% with mortalities ranging from 74% to 97% across 18 tanks (Holborne et al., 2020). The low mortality observed in this study may be due to the low initial force of infection resulting from only two infected shedders per tank. A higher mortality was expected given that the shedder to contact fish ratio was the same as that used in previous large scale ISAV cohabitation trials including the same families. The low mortality in this study may reduce the estimates of the genetic and vaccination effects on disease transmission.

The logistics of performing this investigation meant that the trial was small and hence the power to show highly significant statistical results was lacking. To extract as much information from the data as possible, whilst taking into account uncertainties in parameter estimates, an epidemiological model with a Bayesian inference framework was applied to separately examine the infectivity of the shedders and the susceptibility and endurance of the contact fish. Despite the complexity of the model (which contained 10 estimable parameters, which is already high compared to standard animal breeding applications that usually rely on much more data) and lack of power, the trend of the data was the same across both the GLMM and the fitted epidemiological model. Assumptions, however, were made regarding the infection status of the shedder fish. In particular, all the shedder fish in this study were assumed to be infected, despite non-detectable MVL for all shedder fish at the onset of the trial. It was also assumed that all infections were via contact with infected fish i.e. we assumed that infectious viral particles excreted by infected fish decayed rapidly in the tanks. The virus can be transmitted through water, however, experimental studies suggest the virus is quickly inactivated in sea water (Gustafson et al., 2014).

The results from contact and shedder fish together indicate that more genetically resistant fish (HBV) are equally susceptible as LBV fish, but have lower infectivity and greater endurance (i.e. live longer when infected). Future studies should investigate the genetic or vaccine-mediated relationship between infectivity and endurance, and their relative impact on disease spread in the population. Furthermore, the potential of using mucus virus load as a direct indicator / phenotype for individuals' infectivity warrants further examination as this would allow direct targeting of breeding and vaccination programmes towards reducing ISA transmission.

## 5. Conclusions

The results of this transmission study indicate that both vaccination and genetic selection reduce, but do not prevent ISA transmission. It would be very beneficial to assess the combined effects of genetic selection and vaccination on ISA transmission, and to evaluate mucus viral load as a potential in-vivo indicator for individuals' infectivity. Given that ISA is highly contagious, and the strict regimes for controlling ISAV spread through culling the entire population following confirmation of the virus infection, genetic selection based on estimated mortality breeding values or vaccination that primarily boosts survival and productivity may not be enough to control ISA.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.736365.

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