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# Within-farm spread of infectious salmon anemia virus (ISAV) in Atlantic salmon *Salmo salar* farms in Chile

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ABSTRACT: Spread of infectious salmon anemia virus (ISAV) at the cage level was quantified using a subset of data from 23 Atlantic salmon *Salmo salar* farms located in southern Chile. Data collected from official surveillance activities were systematically organized to obtain detailed information on infectious salmon anemia (ISA) outbreaks. Descriptive statistics for outbreak duration, proportion of infected fish, and time to secondary infection were calculated to quantify the magnitude of ISAV incursions. Linear and multiple failure time (MFT) regression models were used to determine factors associated with the cage-level reproduction number ($R_c$) and hazard rate (HR) for recurrent events, respectively. In addition, the Knox test was used to assess if cage-to-cage transmissions were clustered in space and time. Findings suggest that within farms, ISA outbreaks, on average, lasted 30 wk (median = 26 wk, 95% CI = 24 to 37 wk) and affected 57.3% (95% CI = 47.7 to 67.0%) of susceptible cages. The median time to secondarily diagnosed cages was 23 d. Occurrence of clinical ISAV outbreaks was significantly associated with increased $R_c$, whereas increased HR was significantly associated with clinical outbreaks and with a large number of fish. Spatio-temporal analysis failed to identify clustering of cage cases, suggesting that within-farm ISAV spread is independent of the spatial location of the cages. Results presented here will help to better understand ISAV transmission, to improve the design of surveillance programs in Chile and other regions in which salmon are intensively farmed, and to examine the economic impact of ISAV and related management strategies on various cost and demand shifting factors.

KEY WORDS: Infectious salmon anemia virus · ISAV · Within-farm transmission · Salmon farming · Chile



## INTRODUCTION

On June 2007, infectious salmon anemia (ISA), a highly infectious viral disease, was confirmed in southern Chile and resulted in the single largest ISA epidemic the world had ever experienced. Since then, ISA has had a profound negative economic impact on the Chilean salmon industry through job losses and decreased revenue. Subsequent outbreaks were spatially and temporally aggregated

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during the first year of the epidemic (Mardones et al. 2009). In addition, the difficulty of controlling outbreaks was demonstrated particularly in densely populated areas, due to a substantial fraction of super-shedding farms (Mardones et al. 2011). At the time of the epidemic, the official authority for fish health (National Fisheries Service, Sernapesca) along with the Chilean Salmon and Trout Growers Association (private representative body, SalmonChile) established husbandry measures aimed at controlling ISA, including early detection and depopulation (on a cage-by-cage basis), establishment of control zones, increased biosecurity, and control and treatment of processing wastes. Although such measures may have helped to reduce the number of reported infected farms, ISA virus (ISAV) continued to spread and persisted throughout southern Chile for almost 3 yr, in spite of the depleted number of susceptible farms (Mardones et al. 2009, 2011).

Introduction of ISAV into a salmon holding may have different sources and may affect a variable proportion of fish in different cages (McClure et al. 2004). In general, when fish in one or more cages are initially infected, ISAV spreads among susceptible cages directly through seawater (Løvdal & Enger 2002) via the excretion or secretion of infected fish mucus, blood, tissues, or feces (Thorud & Djupvik 1988, Nylund et al. 1994, Totland et al. 1996, Rimstad et al. 1999). Epidemic duration and cumulative mortality can be highly variable, with durations ranging from <1 mo to several months and cumulative mortalities exceeding 95% (Hammell & Dohoo 2005a, Rimstad et al. 2011). Few studies have evaluated factors associated with the spatial and temporal patterns of ISAV spread within a farm. However, a study of 3 ISA-outbreak farms in Maine, USA, found no significant association between cage adjacency and disease timing (Gustafson et al. 2007). This suggests that manifestations of ISA, subsequent to ISAV exposure, are affected by certain local conditions. A better understanding of the dynamics of aquatic infectious disease outbreaks within a farm, and particularly ISAV, is fundamental to salmon farm management. Such knowledge would allow a better estimate of the severity of an incursion, e.g. duration and the total number of infected cages and fish, which is needed to identify efficient control strategies that will diminish the consequences of disease in terms of costs and animal welfare.

The aims of the present study were to (1) describe and quantify the magnitude of an ISAV incursion in a typical Atlantic salmon *Salmo salar* farm of Chile, (2) identify factors associated with cage-to-cage transmission and incidence rates, and (3) assess spatio-temporal cage-to-cage transmission. Results presented here will help to understand the intra-farm dynamics of ISAV infection in cultured salmon and to parameterize transmission and spread models for disease prevention and control.

## MATERIALS AND METHODS

### Data source

Data were collected from Sernapesca's official surveillance program for the detection of highly infectious diseases or reported during the ISA epidemic (ISAV-specific active surveillance, Sernapesca 2008) that affected southern Chile from June 2007 through December 2009. At the time of the epidemic, data were provided mostly by public and private diagnostic laboratories, sanitary and production records from many salmon farms and companies during their internal monitoring activities, and epidemiological questionnaires carried out by veterinarians. This information was systematically compiled and organized to generate a unique dataset that contained specific farm-level information on ISA outbreaks. A unique identification number (ID), which was the reference code used for most laboratories to identify sampled fish, was assigned to each salmon farm. Surveillance information included data on date of sampling, number of fish, and production unit (cage or tank) sampled as well as the diagnostic test used and the test result (positive or negative). In addition, industry representatives willing to participate in the project provided historical information from past outbreaks.

### Case-definition and ISAV spread

A list of infected farms was readily available (Mardones et al. 2011). Of these farms, only farms that reported raising Atlantic salmon as a single species (monoculture) and excluding broodstock, that were located in seawater, and that provided a cage-layout of the farm were included in the present study. Specifically, farms were restricted to those that reported using the predominant technology (FAO 2007), i.e. a marine floating system of square-metal frame cages (in a grid arrangement) at the time of the epidemic.

Within-farm ISAV spread was estimated from sampling and testing data as part of the monitoring activ-

ities carried out in many laboratories in Chile. According to Sernapesca, immediately after detection of ISAV in a particular farm, all farms that were within 5 km (farms at high risk) of a case were intensively sampled as part of the active surveillance plan (Sernapesca 2008). Veterinarians visited all high-risk farms and collected 30 fish from ≥1 targeted cages and handled samples as follows: tissues (brain, kidney, liver, or spleen), from individual or a pool of 5 fish, were submitted to a laboratory from the laboratory network and tested using one or more of the official diagnostic tests (real time RT-PCR, immunofluorecent antibody test, or cell culture). Accordingly, a farm was suspected of being subclinically infected if it had positive results using official diagnostic tests. Clinical infection was confirmed if, in addition to a positive test result, fish on a farm had clinical signs and/or post-mortem features attributable to ISA. The index cage (IC) was identified as the first test-positive cage at each infected farm and was considered as the earliest detection for ISAV infection at the cage level. Sampling and testing were continued every 15 d if a farm had a positive test, or 30 d if not, until the fish were completely harvested. Recurrent or secondary ISAV-positive cages were recorded until there was no positive test result or until complete depopulation of the farm. The time from the earliest detection until depopulation was referred to here as the outbreak duration. Although no additional information was available on the magnitude of the outbreak at the cage level (e.g. affected proportion of fish or mortalities), it was possible to confirm the presence of mortalities or clinical signs attributable to ISA and, in some cases, the highly polymorphic region (HPR) pattern contained in the hemagglutinin-esterase (HE) gene of ISAV, which is believed to be important for the pathogenicity of ISAV (Rimstad et al. 2001, Aspehaug et al. 2005, Markussen et al. 2008), was provided. In addition to surveillance activities, results from internal monitoring carried out by private veterinarians were available and complemented the official data.

### Estimation of the cage-level reproduction number ($R_c$)

The Martingale method, which uses cumulative incidence data with removal of infective population over time, was used to derive the threshold parameter or $R_c$ (Becker 1989). In the present study, $R_c$ refers to the mean number of secondary infections that result from a single infected cage. This approach has been applied to derive the value of $R_c$ in single epidemics in finite communities from the sizes of outbreaks among households, assuming homogenous mixing and closed populations. In a given farm $f$ of size $s + i$, there are initially, at time $T = 0$, $i$ infected and $s$ susceptible cages. The $R_c$ value was then estimated for each farm $f$ as follows:

$$R_{cf} = s\hat{\theta} = s\left[\frac{1}{s} + \frac{1}{(s-1)} + \ldots + \frac{1}{S(T_N)+1)}\right] / R(T_N) \quad (1)$$

where $S(T_N)$ denotes the number of susceptibles at the time when the infection process of the epidemic ends ($T_N$), i.e. when its intensity process becomes zero and there is no positive test result, and $R(T_N)$ is the cumulative incidence of diagnosed infected cages when $S(T_N) > 0$ (i.e. sum of new cases plus $i$).

Finally, assuming uniform random mixing, the infection potential associated with ISAV for a farm $f$ ($\hat{\theta}$) was estimated. For example, if a farm with 15 susceptible cages had 1 infective cage and a cumulative incidence of 10 infected cages,

$$R_c = 15\hat{\theta} = 15\left[\frac{1}{15} + \frac{1}{(14)} + \ldots + \frac{1}{7}\right] / 10 \quad (2)$$

where $\hat{\theta} = 0.087$, and $R_c = 1.3$. Subsequently, $R_{cf}$ was computed for each farm $f$. Additional details about the method of inference, equations and derivations are described elsewhere (Becker 1989, Chap. 7).

### Estimation of the HR for cage recurrences

A multiple-failure time (MFT) model was constructed as a generalization of the Cox proportional hazards model in which the hazard function is continued beyond a farm's IC to second and subsequent ordered and identical outbreaks (Prentice et al. 1981). This approach, also known as a conditional risk set model, uses recurrence data to produce a proportional hazards model with time-dependent strata, in which the dependence between event times is handled by stratifying the prior number of infected cages. Here, recurrence refers to a cage infection occurring after the IC in a given farm. Considering recurrences are not mutually independent observations and that they are ordered events, a conditional model was constructed by estimating robust standard errors. Because it is possible that more than one cage or event was diagnosed at the same time, ties were handled by specifying Efron's method (Efron 1977). All statistical analyses and methodological specifications were implemented in the statistical software STATA v. 11 (StataCorp).

### Association between epidemiological factors and $R_c$ and HR

Values for hypothesized explanatory variables and confounders (Table 1) for $R_c$ and HR were obtained from surveillance data and previous spatial estimations (Mardones et al. 2011). Associations between these factors and $R_c$ and HR, computed at each farm *f*, were estimated using linear regression and an MFT model, respectively. Factors potentially associated with the dependent variables were selected in a univariate analysis ($p \leq 0.2$) and then incorporated into a preliminary, candidate model. For the MFT model, the number of fish and biomass at the farm were evaluated as temporally varying effects. The best-fitting models were defined after using forward and backward stepwise variable selection as the one that included significantly ($p \leq 0.05$) associated variables and that minimized the value of Akaike's information criterion. For the linear model, evaluation of the major assumptions was based on graphical methods by representing standardized residuals with fitted values (evaluation of homoscedasticity) and the model's quantiles versus quantiles of the normal distribution (evaluation of normality) (Dohoo et al. 2009). For the MFT, the assumption of proportionality of risks was assessed using Schoenfeld's residuals analysis and test of proportional hazards assumptions for each significant variable retained in the final model (Grambsch & Therneau 1994).

### Spatio-temporal analysis of cage-to-cage ISAV spread

To assess if cage-to-cage transmission were clustered in space and time, the null hypothesis of a random spatio-temporal distribution of cases was tested using the Knox test (Knox 1964). This test has been applied to a number of infectious terrestrial animal diseases (Ward & Carpenter 2000), and in aquaculture, it has been previously used to assess time-space clustering of white sturgeon iridovirus outbreaks in hatcheries (Georgiadis et al. 2001). Briefly, the Knox test requires preliminary assumptions about the maximum incubation period (time from infection to appearance of clinical signs) and the maximum transmission distance at which viral transmission may occur between cages in the marine site. Here, it was assumed that infected fish from a single sea cage were able to shed and transmit ISAV to cages either adjacent or diagonal to the cage; according to the dimension of the standard marine salmon site included in this study, this distance had a hypotenuse of 43 m. The incubation period ranges from 13 and 31 d after experimental inoculation of the virus (Dannevig et al. 1994, Totland et al. 1996, Rimstad et al. 1999, Simko et al. 2000). Consequently, 43 m and 31 d were considered as the maximum (critical) spatial and temporal distances, respectively. The Knox routine was simulated to estimate confidence intervals around the calculated statistic (Levine 2006), using the CrimeStat software (Levine 2010).

Table 1. Description of group variables evaluated in 2 regression models using retrospective data of cage diagnoses from 23 Atlantic salmon *Salmo salar* farms in southern Chile that experienced infectious salmon anemiavirus (ISAV) outbreaks between August 2007 and October 2008

| Category | Variable | Type | Description |
|---|---|---|---|
| Spatio-temporal | Time | Continuous | Time (wk) from the beginning of the largest ISAV epidemic (June 2007) |
| | Nearest | Continuous | Time-varying seaway distance (km) to the nearest and concurrent ISAV-infected farm |
| | Radius | Continuous | Time-varying number of concurrent ISAV infectious farms evaluated within a radius of 5, 10, and 15 km |
| On-farm | Initial population | Continuous | Fish number stocked |
| | No. of fish | Continuous | Time-varying number of fish (×100 000) |
| | Smolt weight | Continuous | Average weight smolts stocked (g) |
| | No. at ISAV | Continuous | Fish number at the ISAV first diagnosis |
| | Biomass | Continuous | Biomass at the ISAV first diagnosis |
| | Fallowing | Dichotomous | Farm fallowed >1 mo between production cycles (reference) or not |
| | Supply | Continuous | Number of smolt suppliers (farms) |
| | Origin | Dichotomous | Fish were imported (reference) or were national strains |
| Outbreak | Outbreak | Dichotomous | Outbreak was based solely on positive test results (subclinical, reference) or on developed mortalities attributable to ISAV |
| | Early detection | Dichotomous | Index cage (IC) was 1 (early, reference) or >1 (delayed detection) |
| | Surveillance | Dichotomous | Diagnoses were carried out by official campaigns (reference) or based on company's submissions of samples to private laboratories |

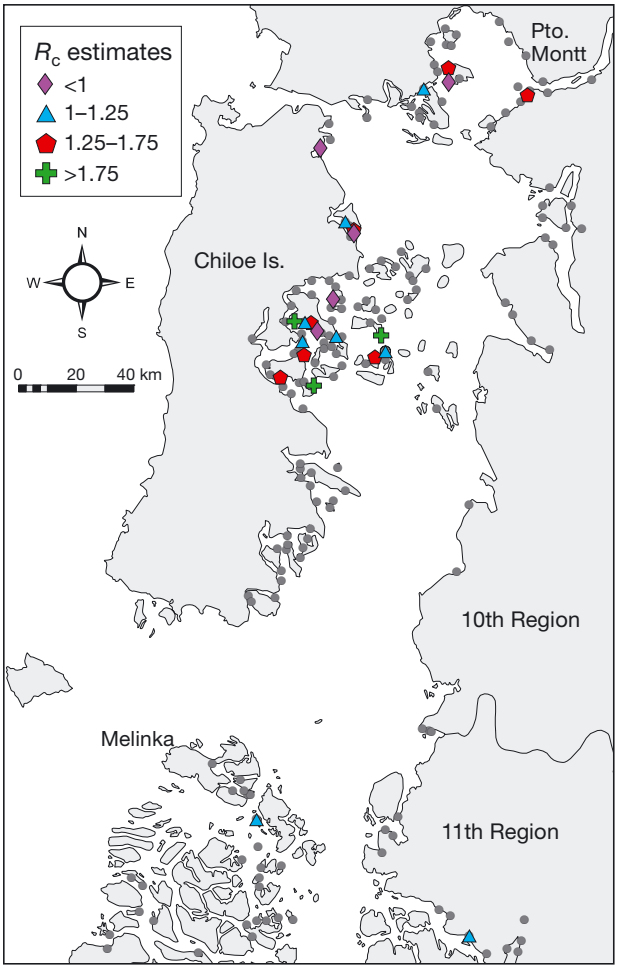


Fig. 1. Study area and cage-level reproduction numbers ($R_c$) for infectious salmon anemia virus (ISAV) estimated for 23 Atlantic salmon farms in southern Chile. Grey dots are the reported ISAV-infected farms from 2007 to 2009

## RESULTS

### Within-farm spread

Only 38 of the 251 infected Atlantic salmon farms provided information useful to trace back and describe ISAV spread within farms. Of these 38 farms, 15 were excluded from further analysis because they raised >1 species and/or included broodstock (n = 6), were located in estuarine waters (n = 5), or reported inconsistent data (n = 4). Ultimately, data from a subset of 23 Atlantic salmon farms were traced back to describe ISAV spread within farms. Most farms (21) were located in the 10th region (Fig. 1), and all reported ISAV infections that occurred between August 2007 and October 2008 (Table 2). Of all farms, 18 (78.3%) experienced mortalities or the presence of moribund fish with clinical signs attributable to ISAV, i.e. clinical outbreaks. Additional demographic and epidemiologic features of each farm are shown in Table 2. Selected statistical indicators that describe the within-farm spread are shown in Table 3. Each infectious cage during an epidemic was found to infect ($R_c$) between 0.5 and 2.1 other cages (mean = 1.24, median = 1.2; SD = 0.404) (Fig. 1, Table 2).

### Regression and space-time analyses

Results from the final fitted models are presented in Tables 4 & 5. After the model-building process, only the category for outbreak type (subclinical versus clinical) was retained in the final model, where clinical outbreaks were significantly associated with increased $R_c$ (p = 0.042, $R^2$ = 0.18, 95% CI = 0.02–0.79) (Table 4).

In the MFT model, the numbers of fish as well as outbreak type were retained in the final model. Unfortunately, ISAV diagnoses after the fifth recurrence were not well captured in the model, resulting in a lack of power to detect additional differences. The global HR in the MFT model associated with clinical outbreaks was 3.19 (p = 0.022), and an overall Mantel-Haenzel estimate of the hazard ratio, controlling for time, of 2.8 (p = 0.015) was computed. The model also predicted a 1.17-fold greater HR for recurrences with every 100 000 increment in the number of fish at the farm (p = 0.028). The interaction term between these 2 significant variables was only tested in the MFT model and was not statistically significant (p = 0.24). The test using the scaled Schoenfeld residual showed no evidence of violating the proportional hazard assumption (p ≥ 0.69) (Table 5). The variable ISAV strain was not evaluated due to considerable missing values (43% of data, Table 2).

The Knox test failed to identify an overall time-space clustering of cases (p = 0.38).

## DISCUSSION

Results from the present study indicate that following an ISAV incursion, the virus was spread to more than half of the cages in standard marine Atlantic salmon farms, ranging from 16 to 89% cumulative cage incidence of infection. Clinical outbreak status and number of fish at the farm were significant predictors of the magnitude of within-farm ISAV spread. The pattern of ISAV spread described here is similar to previous investigations regarding the distribution

Table 2. Demographics and epidemiological features of 23 Atlantic salmon *Salmo salar* farms in southern Chile that experienced infectious salmon anemia virus (ISAV) outbreaks between August 2007 and October 2008. Outbreak duration was time from first infection date (diagnosis) of the index cage to final depopulation date. Outbreak type was classified as subclinical if it had positive results under official diagnostic tests (virus isolation, reverse transcriptase polymerase chain reaction or immunofluorescent antibody technique) in any cage but no fish with clinical signs. Clinical infection was confirmed if, in addition to a positive test result, a farm had clinical signs and/or post-mortem features attributable to ISA in at least 1 sea cage. $R_c$: cage-level reproductive number; HPR: highly polymorphic region group; nd: no data

| Farm ID | No. cages | Initial population (×100 000 000) | Smolt weight (g) | First infection date | Outbreak duration (wk) | Outbreak type | No. (%) of cages detected | $R_c$ | ISAV strain |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 1.5 | 101 | Aug 2007 | 14 | Clinical | 7 (88) | 1.6 | HPR7b |
| 2 | 12 | 1.16 | 104 | Aug 2007 | 23 | Clinical | 8 (67) | 1.3 | HPR7b |
| 3 | 18 | 1.47 | 69 | Aug 2007 | 52 | Subclinical | 5 (28) | 0.9 | nd |
| 4 | 22 | 1.22 | 106 | Aug 2007 | 43 | Clinical | 12 (55) | 1.1 | HPR7b |
| 5 | 18 | 1.88 | 113 | Aug 2007 | 15 | Clinical | 16 (89) | 2.1 | HPR7b |
| 6 | 16 | 1.01 | 167 | Aug 2007 | 29 | Clinical | 8 (50) | 1.1 | HPR7b |
| 7 | 20 | 2.2 | 133 | Sep 2007 | 47 | Clinical | 16 (80) | 1.6 | HPR7b |
| 8 | 26 | 0.85 | 137 | Sep 2007 | 30 | Subclinical | 12 (46) | 1.2 | HPR7b |
| 9 | 16 | 1.1 | 130 | Oct 2007 | 11 | Clinical | 14 (88) | 1.8 | HPR7b |
| 10 | 22 | 1.08 | 113 | Nov 2007 | 64 | Clinical | 7 (32) | 0.8 | HPR2 |
| 11 | 16 | 0.93 | 116 | Dec 2007 | 58 | Clinical | 10 (63) | 1.3 | HPR2 |
| 12 | 18 | 1.17 | 119 | Jan 2008 | 26 | Clinical | 14 (78) | 1.6 | HPR7b |
| 13 | 15 | 1.2 | 109 | Jan 2008 | 13 | Subclinical | 3 (20) | 0.7 | HPR7b |
| 14 | 14 | 0.91 | 125 | Jan 2008 | 43 | Clinical | 8 (57) | 1.2 | nd |
| 15 | 18 | 1.13 | 121 | Jan 2008 | 51 | Subclinical | 3 (17) | 0.7 | nd |
| 16 | 28 | 1.91 | 69 | Jan 2008 | 37 | Clinical | 6 (21) | 0.5 | nd |
| 17 | 17 | 1.19 | 118 | Apr 2008 | 17 | Clinical | 14 (82) | 1.8 | HPR7b |
| 18 | 20 | 1.32 | 105 | May 2008 | 27 | Clinical | 16 (80) | 1.7 | nd |
| 19 | 23 | 1.38 | 92 | Jul 2008 | 21 | Clinical | 8 (35) | 1.0 | nd |
| 20 | 24 | 1.27 | 114 | Aug 2008 | 12 | Clinical | 13 (54) | 1.1 | nd |
| 21 | 12 | 1.56 | 189 | Sep 2008 | 23 | Subclinical | 10 (83) | 1.2 | nd |
| 22 | 19 | 1.07 | 107 | Sep 2008 | 20 | Clinical | 10 (53) | 1.0 | nd |
| 23 | 22 | 1.09 | 140 | Oct 2008 | 24 | Clinical | 12 (55) | 1.3 | nd |

Table 3. Epidemiological indicators for the within-farm spread of infectious salmon anemia virus (ISAV) from 23 Atlantic salmon *Salmo salar* farms in southern Chile that experienced outbreaks between August 2007 and October 2008

| Indicator | Mean | Median | Min.–max. |
|---|---|---|---|
| Number of index cages | 1.4 | 1 | – |
| Outbreak duration (wk) | 30.4 | 26 | 11–64 |
| Percentage cages infected | 57.4 | 55 | 17–89 |
| Time (d) to 2nd infected cage | 51.3 | 23 | 3–186 |

Table 4. Results of a linear regression model fitted for the threshold parameter $R_c$ (cage-level reproduction number) estimated from 23 Atlantic salmon *Salmo salar* farms in southern Chile that experienced infectious salmon anemia virus (ISAV) outbreaks between August 2007 and October 2008. CI: confidence interval

| Variable | Level | Coefficient | Lower 95% CI | Upper 95% CI | p |
|---|---|---|---|---|---|
| ISAV outbreak | Subclinical vs. clinical | 0.41 | 0.02 | 0.79 | 0.042 |
| Intercept | | 0.98 | 0.59 | 1.27 | <0.001 |

of the virus within 3 Atlantic salmon grow-out farms in Maine, USA (Gustafson et al. 2007). Interestingly, in both Chile and the USA, incident cages were not spatio-temporally aggregated within farms, and limited associations with certain factors were associated with the outbreak. Here, ~9% (23 of 251) of the reportedly affected farms during the 2007 to 2010 epidemic (Mardones et al. 2009, 2011) were investigated further, providing important findings for disease planning control.

Table 5. Results of an multiple-failure time (MFT) regression model fitted for the cage-level recurrences of infectious salmon anemia virus (ISAV) estimated from 23 Atlantic salmon *Salmo salar* farms in southern Chile that experienced ISAV outbreaks between August 2007 and October 2008. HR: hazard rate; CI: confidence interval

| Variable | Level | HR | Lower 95% CI | Upper 95% CI | p |
|---|---|---|---|---|---|
| No. of fish (×100 000) | | 1.17 | 1.02 | 1.35 | 0.028 |
| ISAV outbreak | Subclinical vs. clinical | 3.19 | 1.18 | 8.6 | 0.022 |

Within-farm spread was larger for farms with clinical cases of the disease compared to those in which no clinical cases were identified. This is certainly expected, given that clinically infected fish likely shed larger amounts of virus into the environment compared to subclinically infected fish. During subclinical outbreaks, clinical and macroscopic changes may be restricted solely to anemia and some minor circulatory disturbances, which can be overlooked easily if no diagnostic investigations are undertaken (Rimstad et al. 2011). In many cases, increased mortality and clinical outbreaks can be related to stressful events, such as fish handling and delousing (Hammell & Dohoo 2005b, McClure et al. 2005, Rimstad et al. 2011). Moreover, it has been shown that fish can clear the infection 25 d after exposure, where some infected fish may remain apparently healthy for long periods, becoming subclinically infected, and harbor ISAV for weeks and months, likely with shedding rates and virus loads significantly smaller than those of clinically infected fish (Mikalsen et al. 2001, Rimstad & Mjaaland 2002). In all cases, the categorization of disease outbreaks into clinical versus subclinical is merely a simplification of a complex dynamic that was not fully evaluated here, and it was assumed that both farm and authorities' veterinarians agreed in this category according to the definition stated in the sanitary program (Sernapesca 2008).

An alternative explanation is that mortality caused by ISAV may trigger husbandry and control practices that, if improperly planned and conducted, may unfortunately promote ISAV spread within the farm. During a clinical outbreak, removal of dead fish from affected cages (and sometime from the entire farm) is increased to prevent disease spread. In addition, the farmer is required by the authorities to depopulate or harvest the affected cage within 3 wk of detection to eliminate the main source of infection (Sernapesca 2008). Such procedures would involve more intense handling of dead and/or moribund fish and consequently may favor discharge of contaminated tissues or materials, particularly contaminated blood and carcasses. In fact, blood has been considered as the most infective tissue from experimental trials carried out in Atlantic salmon (Totland et al. 1996) where a large viral shedding event occurs before death (Gregory et al. 2009). Husbandry procedures during the grow-out stage, such as sharing equipment and personnel within a multiple-site company and feeding practices, modified the risk of experiencing mortalities caused by ISA at the cage level in New Brunswick, Canada (Hammell & Dohoo 2005b).

A third possibility is that clinical outbreaks are related to highly virulent strains of ISAV, increasing spread between cages due to its higher infectivity and/or pathogenicity (Johnson et al. 2008, Ritchie et al. 2009). Unfortunately, molecular information for each outbreak was only partially available, as indicated in Table 2, and no further analysis was carried out; however, it seems that the magnitude of an outbreak is at most minimally associated with the ISAV molecular type for a number of reasons. First, it has been reported that ~80% of ISAV isolates sequenced during the Chilean epidemic corresponded to the same segment 6 HPR group, which is referred to as HPR 7b (Kibenge et al. 2009), and in the present study, 11 out of the 13 (85%) reported this variant (Table 2). Second, most outbreaks were confined to the same geographic region (Fig. 1), and according to recent findings in Norway (Lyngstad et al. 2008, 2011), there is a high degree of genetic similarity between ISAV isolates from aggregated ISA sites. Third, previous analyses comparing cage-level ISAV mortality rates of different ISAV strains showed no significant differences after controlling for confounding factors related to exposure time and location of the site (Johnson et al. 2008).

The number of fish and the likelihood for additional recurrences was additionally identified as potential predictors for ISAV spread. This can be explained by the fact that with more fish, additional productive units (cages) are needed at the farm, increasing the chances of ISAV spread. The effect was identified as a time-varying covariate in the MFT model approach but not identified by the linear approach that looked at the cumulative cage incidence. This density-dependency process is in line with the expectation that as more fish become infected, the probability of spread to other cages also increases. It has been also reported that the likelihood of developing clinical outbreaks increases with fish population size (Hammell & Dohoo 2005b). However, a certain influence from simultaneously infected neighboring farms or infection pressure was expected, but neither analysis resulted in statistically significant associations to neighboring infected farms. It is well known that horizontal transmission through short seaway distances is a major transmission pathway for ISAV between sites (Mardones et al. 2009, Aldrin et al. 2011), but the magnitude and spread within-farm once fish have become infected is, to some extent, independent of the concurrent ISAV status and distance to the nearest infected farms, and likewise, is independent of the effect of having a concurrent large number of infected farms

within variable radii (5, 10, or 15 km). While managing ISA based on its farm-level impact might be reasonable, given that most subsequently infected cages result from within-farm transmission, the farm remains a source of infection to neighboring farms, and so ISA management must also consider potential transmission to other farms and therefore must also be area-based. Alternatively, it is possible that the large number of reportedly infected farms, 65 and 50% of salmon farms located in the 10th and 11th regions, respectively, of Chile (Mardones et al. 2011), has masked the effect of distances and infection pressure. Most farms were located on the mid-east coast of Chiloe Island (Fig. 1), where space-time clustering was previously reported (Mardones et al. 2009) as well as the presence of 'super-spreaders' (Mardones et al. 2011). Although effects of nearby infected farms were not demonstrated here, it is necessary to clarify this important aspect with additional research.

One of the conclusions of a similar study conducted in Maine (Gustafson et al. 2007) was that aggressive removal of cages would be the most beneficial strategy when attempting to control ISAV. This is also a conclusion here, with a clear distinction between clinical vs. subclinical outbreak. Whenever clinical signs and/or mortalities are observed in a farm, it is probably important to allocate extra time and resources to reduce the impact of ISAV given that a farmer would expect at least 3-fold greater risk of developing a new infection in another cage or more. This scenario will be worse if more fish are at the farm.

As in all observational studies, there were limitations in the way our data were collected, and certain details about field and laboratory information, including virus and environmental characteristics, were unavailable to the researchers here. Recording the exact time of an outbreak was not feasible, as infection may not be known until a full examination of all fish for a given cage is made. However, there is no reason to believe that this imprecise estimate would have occurred differentially among farms since monitoring activities were standard for all farms. In this study, restricted sampling was adopted despite of the reduction of sample size, which resulted in only 9% of infected farms being used for statistical modeling. Although statistical models identified few predictors, suggesting that variations in the patterns of ISA outbreaks (introduction and subsequent spread) may depend upon extraneous factors not accounted for here, it is important to consider this paucity of significance cautiously since low power may limit additional findings. However, the novelty of the present approach to estimate the $R_c$ is likely the simplest one, which can be improved greatly by incorporating the population structure under study, for example, the level of crowdedness at the cage level, the contact structure, and/or certain interventions, e.g. vaccination, which could modify the pattern of spread (Pellis et al. 2009). Knowing the $R_c$ can provide important insights for the design of more efficient control and surveillance programs for ISAV. First, by applying the concept of herd immunity (Fine 1993), it is possible to estimate the proportion of the population that would have to be successfully vaccinated to avoid an outbreak. This proportion can be estimated as $1 - 1/R_0$, where $R_0$ is the basic case reproduction number. Assuming that $R_c$ is a good approximation of $R_0$, it can be expected that, on average, $R_c = 1.24$; therefore, vaccinating at least 19% $(1 - 1/1.24)$ of the population at risk against ISAV would prevent an outbreak, provided the vaccine was 100% effective. However, unknown control measures were likely established during each outbreak in this study, and consequently, this estimate may underestimate the true infection potential of ISAV, and also that achieving perfect immunization in field conditions is difficult. As with other viral fish vaccines, commercially available vaccines against ISA are based upon inactivated whole virus antigens and have questionable field effectiveness, as reflected by outbreaks reported in vaccinated fish farms (Gomez-Casado et al. 2011). There are additional formulations that take into account the different vaccine efficacies (Keeling & Rohani 2008).

Secondly, understanding the between- and within-farm transmission dynamics for ISAV is crucial to understanding the relative importance of intervention strategies at these 2 levels. Previously, the reproduction number at the farm level varied substantially between the epidemic times and geographical characteristics, requiring interventions to be more aggressive at the beginning of the epidemic (Mardones et al. 2011).

In conclusion, the present study quantified important epidemiological aspects of the within-farm spread of ISAV. Because the farm is often the fundamental unit at which control policies are targeted, this study will help to prevent and mitigate the impact of an ISAV infection and will be useful for farmers and veterinarians to quantify the impact of such infection in fully susceptible populations. Future studies should highlight the need for regular monitoring of mortalities to identify clinical outbreaks as early as possible, followed by the rapid removal of infected fish.

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## LITERATURE CITED

Aldrin M, Lyngstad TM, Kristoffersen AB, Storvik B, Borgan Ø, Jansen PA (2011) Modelling the spread of infectious salmon anaemia (ISA) among salmon farms based on seaway distances between farms and genetic relationships between infectious salmon anaemia virus isolates. J R Soc Interface 8:1346–1356

Aspehaug V, Mikalsen AB, Snow M, Biering E, Villoing S (2005) Characterization of the infectious salmon anemia virus fusion protein. J Virol 79:12544–12553

Becker NG (1989) Analysis of infectious disease data. Chapman & Hall, London

Dannevig BH, Falk K, Skjerve E (1994) Infectivity of internal tissues of Atlantic salmon, *Salmo salar* L, experimentally infected with the etiologic agent of infectious salmon anemia (ISA). J Fish Dis 17:613–622

Dohoo IR, Martin SW, Stryhn H (2009) Veterinary epidemiologic research. AVC, Charlottetown

Efron B (1977) The efficiency of Coxs likelihood function for censored data. J Am Stat Assoc 72:557–565

FAO (2007) Cage aquaculture – regional reviews and global overview. In: Halwart M, Soto D, Arthur JR (eds) FAO Fisheries technical paper, Book 498. Food and Agriculture Organization of the United Nations, Rome

Fine PE (1993) Herd immunity: history, theory, practice. Epidemiol Rev 15:265–302

Georgiadis MP, Hedrick RP, Carpenter TE, Gardner IA (2001) Factors influencing transmission, onset and severity of outbreaks due to white sturgeon iridovirus in a commercial hatchery. Aquaculture 194:21–35

Gomez-Casado E, Estepa A, Coll JM (2011) A comparative review on European-farmed finfish RNA viruses and their vaccines. Vaccine 29:2657–2671

Grambsch PM, Therneau TM (1994) Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 81:515–526

Gregory A, Munro LA, Snow M, Urquhart KL, Murray AG, Raynard RS (2009) An experimental investigation on aspects of infectious salmon anaemia virus (ISAV) infection dynamics in seawater Atlantic salmon, *Salmo salar* L. J Fish Dis 32:481–489

Gustafson L, Ellis S, Robinson T, Marenghi F and others (2007) Spatial and non-spatial risk factors associated with cage-level distribution of infectious salmon anaemia at three Atlantic salmon, *Salmo salar* L., farms in Maine, USA. J Fish Dis 30:101–109

Hammell KL, Dohoo IR (2005a) Mortality patterns in infectious salmon anaemia virus outbreaks in New Brunswick, Canada. J Fish Dis 28:639–650

Hammell KL, Dohoo IR (2005b) Risk factors associated with mortalities attributed to infectious salmon anaemia virus in New Brunswick, Canada. J Fish Dis 28:651–661

Johnson A, Binette SL, Cook-Versloot M, Beattie MJ and others (2008) Association between ISAV mortalities and ISAV molecular type in the Bay of Fundy, Canada. Can Tech Rep Fish Aquat Sci 2782

Keeling MJ, Rohani P (2008) Modeling infectious diseases in human and animals. Princeton University Press, Princeton, NJ

Kibenge FS, Godoy MG, Wang Y, Kibenge MJ and others (2009) Infectious salmon anaemia virus (ISAV) isolated from the ISA disease outbreaks in Chile diverged from ISAV isolates from Norway around 1996 and was disseminated around 2005, based on surface glycoprotein gene sequences. Virol J 6:88

Knox EG (1964) The detection of space-time interactions. J R Stat Soc Ser C (Appl Stat) 13:25–29

Levine N (2006) Crime mapping and the CrimeStat program. Geogr Anal 38:41–56

Levine N (2010) *CrimeStat*: a spatial statistics program for the analysis of crime incident locations. Ned Levine & Associates, Houston, TX, and the National Institute of Justice, Washington, DC

Løvdal T, Enger O (2002) Detection of infectious salmon anemia virus in sea water by nested RT-PCR. Dis Aquat Org 49:123–128

Lyngstad TM, Jansen PA, Sindre H, Jonassen CM, Hjortaas MJ, Johnsen S, Brun E (2008) Epidemiological investigation of infectious salmon anaemia (ISA) outbreaks in Norway 2003–2005. Prev Vet Med 84:213–227

Lyngstad TM, Hjortaas MJ, Kristoffersen AB, Markussen T, Karlsen ET, Jonassen CM, Jansen PA (2011) Use of molecular epidemiology to trace transmission pathways for infectious salmon anaemia virus (ISAV) in Norwegian salmon farming. Epidemics 3:1–11

Mardones FO, Perez AM, Carpenter TE (2009) Epidemiologic investigation of the re-emergence of infectious salmon anemia virus in Chile. Dis Aquat Org 84:105–114

Mardones FO, Perez AM, Valdes-Donoso P, Carpenter TE (2011) Farm-level reproduction number during an epidemic of infectious salmon anemia virus in southern Chile in 2007–2009. Prev Vet Med 102:175–184

Markussen T, Jonassen CM, Numanovic S, Braaen S, Hjortaas M, Nilsen H, Mjaaland S (2008) Evolutionary mechanisms involved in the virulence of infectious salmon anaemia virus (ISAV), a piscine orthomyxovirus. Virology 374:515–527

McClure CA, Hammell KL, Dohoo IR, Nerette P, Hawkins LJ (2004) Assessment of infectious salmon anaemia virus prevalence for different groups of farmed Atlantic salmon, *Salmo salar* L., in New Brunswick. J Fish Dis 27: 375–383

McClure CA, Hammell KL, Dohoo IR (2005) Risk factors for outbreaks of infectious salmon anemia in farmed Atlantic salmon, *Salmo salar.* Prev Vet Med 72:263–280

Mikalsen AB, Teig A, Helleman AL, Mjaaland S, Rimstad E (2001) Detection of infectious salmon anaemia virus (ISAV) by RT-PCR after cohabitant exposure in Atlantic salmon *Salmo salar*. Dis Aquat Org 47:175–181

Nylund A, Hovland T, Hodneland K, Nilsen F, Lovik P (1994) Mechanisms for transmission of infectious salmon anaemia (ISA). Dis Aquat Org 19:95–100

Pellis L, Ferguson NM, Fraser C (2009) Threshold parameters for a model of epidemic spread among households and workplaces. J R Soc Interface 6:979–987

Prentice RL, Williams BJ, Peterson AV (1981) On the regression analysis of multivariate failure time data. Biometrika 68:373–379

Rimstad E, Mjaaland S (2002) Infectious salmon anaemia virus: an orthomyxovirus causing an emerging infection in Atlantic salmon. APMIS 110:273–282

Rimstad E, Falk K, Mikalsen AB, Teig A (1999) Time course tissue distribution of infectious salmon anaemia virus in experimentally infected Atlantic salmon *Salmo salar*. Dis Aquat Org 36:107–112

Rimstad E, Mjaaland S, Snow M, Mikalsen AB, Cunningham CO (2001) Characterization of the infectious salmon anemia virus genomic segment that encodes the putative hemagglutinin. J Virol 75:5352–5356

Rimstad E, Dale OB, Dannevig BH, Falk N (2011) Infectious salmon anaemia. In: Woo PTK, Bruno DW (eds) Fish diseases and disorders, Vol 3: Viral, bacterial and fungal infections. CABI Publishing, Cambridge, MA, p 189–194

Ritchie RJ, McDonald JT, Glebe B, Young-Lai W, Johnsen E, Gagné N (2009) Comparative virulence of infectious salmon anaemia virus isolates in Atlantic salmon, *Salmo salar* L. J Fish Dis 32:157–171

Sernapesca (2008) Programa sanitario especifico de vigilancia y control de la anemia infecciosa del salmon (PSEC-ISA, Res2638). www.sernapesca.cl/index.php?option=com_remository&Itemid=246&func=select&id=546 (accessed on 15 Nov 2010)

Simko E, Brown LL, MacKinnon AM, Byrne PJ, Ostland VE, Ferguson HW (2000) Experimental infection of Atlantic salmon, *Salmo salar* L., with infectious salmon anaemia virus: a histopathological study. J Fish Dis 23:27–32

Thorud KE, Djupvik HO (1988) Infectious anaemia in Atlantic salmon (*Salmo salar* L.). Bull Eur Assoc Fish Pathol 8:109–111

Totland GK, Hjeltnes BK, Flood PR (1996) Transmission of infectious salmon anaemia (ISA) through natural secretions and excretions from infected smolts of Atlantic salmon *Salmo salar* during their presymptomatic phase. Dis Aquat Org 26:25–31

Ward MP, Carpenter TE (2000) Analysis of time-space clustering in veterinary epidemiology. Prev Vet Med 43:225–237

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