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Risk factors for outbreaks of infectious salmon anemia in farmed Atlantic salmon, *Salmo salar*

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Abstract

Infectious salmon anemia (ISA) is a viral disease occurring in farmed Atlantic salmon (*Salmo salar*) that is characterized by lethargy, anorexia, anemia and death. To control the disease in New Brunswick, Canada, 7.5 million fish from outbreak cages have been destroyed since 1997. Despite changes made by farmers, 2002 was the worst year ever for ISA losses in the region.

We evaluated the associations between potential risk factors and ISA outbreaks in the Atlantic-salmon sites in New Brunswick. This was a multilevel study in which the site-level design was a retrospective cohort study while the cage-level design was a modified case-cohort study. The questionnaire was divided into site-level questions, cage-level questions and hatchery information.

The important factors identified by this study can be categorized as environmental, farmer controlled or industry controlled according to the capacity to change or eliminate them. Environmental risk factors such as increasing the depth of the net (if nets were $\leq$9 m, odds ratio (OR) = 3.34) and decreasing the depth of water underneath the net (if depth of water underneath the net $>$3 m, OR = 3.34) are for the most part dictated by site location. Wild pollock (*Pollachius virens*) in the cage reflects the number of wild pollock that live in the site location. If there were $\geq$1000 pollock in the cage, the odds of disease in the cage increased 4.43-fold. Risk factors that are under farm control include increasing the number of times that the salmon are treated for sea lice (OR = 3.31 if lice treatments are $\leq$2 times), transferring small smolts into seawater (OR = 2.40 if smolts weighed $>$99 g) and improving on the adaptation of smolts to seawater to reduce post-transfer mortalities (OR = 4.52 if there was at least one cage with post-transfer mortalities $>$5%). The industry-controlled factors need to be addressed by the industry as a whole. Organizing boat travel to minimize the time

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and frequency of boats travelling to or by sites currently is being reviewed. This will be extremely important because the OR = 9.43 if processing boats travel within 1 km of the site and the OR = 4.03 if the site has dry feed delivered by the feed company. Because the hazard ratio increased stepwise from 1 if the nearest neighbor with ISA was ≥5 km up to 5.5 if the nearest site with ISA was within 0.5 km, increasing the distance between sites might be necessary for effective control.

Keywords: Risk factor; Infectious salmon anemia; Atlantic salmon; Outbreak

1. Introduction

Infectious salmon anemia (ISA) is a disease caused by the infectious salmon anemia virus (ISAV). The disease occurs in farmed Atlantic salmon (*Salmo salar*) and is characterized by lethargy, anorexia, anemia and death (Thorud and Djupvik, 1988). The first case of ISA in the Bay of Fundy (New Brunswick) Atlantic-salmon sites occurred in 1996 (O'Halloran et al., 1999). The ISA virus continued to cause disease sporadically through the Bay of Fundy farms, reaching a peak with the 1999 year-class (fish that entered seawater in 1999) that had 24 out of the 86 sites with clinical disease. Since the 1999 year-class, the incidence of clinically diseased sites dropped to 9 affected sites in the 2000 year-class and 15 affected sites in the 2001 year-class (McGeachy and Moore, 2003). Unfortunately, there was a resurgence of disease in the 2002 year-class. This year-class of fish has experienced greater depopulation events associated with ISA than any previous year-class during the first half of the growing cycle (up to early 2003).

The economic impact from this disease has been very damaging to the industry (financial losses to the farmers US$ 4.8–5.5 M per year). To control the disease, 7.5 million fish from outbreak cages have been destroyed since 1997 (Moore, 2003). Current detection-and-depopulation measures applied to fish from outbreak cages do not appear to be a solution to ISA in the New Brunswick sites.

Other control measures have been adopted to reduce the amount of disease. Many of these control measures have been suggested as a result of laboratory studies. Reduction of sea lice (*Lepeophtheirus salmonis*) on the skin of the salmon has been recommended because sea lice in laboratory experiments were mechanical transmitters of the ISA virus (Nylund et al., 1993). The ISA virus can be spread horizontally through blood and tissues of infected fish; thus, frequent removal of dead fish from a cage has been recommended (Totland et al., 1996). The virus remains infective after 20 h in seawater, providing evidence that proper hygiene and disinfection should be maintained at all sites (Nylund et al., 1994).

Epidemiologic studies also have identified important control methods. In Scotland, there was increased risk of ISA at the site if well-boats (boats with interior compartments for holding live fish) visited a site to move live fish between sites or to the processing plant (Murray et al., 2002). Two case–control studies in Norway used the site as the unit of concern (Vagsholm et al., 1994; Jarp and Karlsen, 1997). Increased risk of ISA was associated with the site's proximity to another site with ISA and to a salmonid slaughterhouse that did not disinfect its wastewater, the purchase of smolt (young fish adapted for transfer into seawater) from more than one hatchery, transportation of the smolt over a long distance and removal of dead fish less than daily in the summer.

The risk-factor study from New Brunswick, Canada in 1997 (Hammell and Dohoo, in press) differed from the Norwegian studies because it was multilevel; it investigated both factors associated with the site becoming an ISA problem site (>50% of the cages at the site were ISA positive) and factors specifically associated with a cage becoming ISA positive. Cage-level factors for increased risk were higher initial number of smolt stocked in a cage, medium fish density (2.5–5.0 fish/m^3) within a cage, higher cumulative mortality for the cage in the first year in seawater, weight sampling the fish in the second year in seawater and fewer treatments for sea lice. Site-level factors were harder to analyze due to the small number of sites, but in a Cox proportional-hazards model, feed delivered by the feed company (as opposed to being picked up at the factory) increased the hazard of a site becoming positive. Having only one year-class of fish on the site and increasing months of feeding moist feed reduced the hazard of a site becoming an ISA problem site.

The integration of ISA research, vaccine technology and established farming principles has led to some major changes in salmon farming in New Brunswick. Virtually all sites have only one year-class of fish on a site and many areas have only one year-class of fish in a Bay Management Area (BMA). This practice was established to minimize contact between older fish and young fish to reduce the risk of infection transmission. Another change that has been implemented is proper disinfection of the wastewater released by the processing plants. A third change that has been made is the treatment to remove sea lice. Laborious and stressful bath treatments were performed until the introduction of an oral ectoparasiticide – emamectin benzoate (SLICE^{TM}, Schering-Plough Animal Health, Quebec) – which is incorporated into the feed (thus removing many of the stresses associated with bath treatments). Since 1999, inactivated ISA-virus vaccines have been available (although they have not been evaluated in large-scale clinical trials).

Despite the changes, 2002 was the worst year for clinical ISA disease in the New Brunswick sites resulting in the highest level of cage depopulation as a control measure (Moore, 2003). Our current study evaluated risk factors given the current situation in the Atlantic salmon sites in New Brunswick. Our objective was to identify host, environment and management risk factors associated with a site or a cage experiencing an ISA outbreak.

2. Materials and methods

2.1. Selection of sites and cages

2.1.1. Site selection

The New Brunswick Department of Agriculture, Fisheries and Aquaculture (NBDAFA) provided a list of all farms in New Brunswick. Between June 2002 and September 2002, all farm owners that had stocked fish in the years 2000 and 2001 were asked to participate in the study. Upon agreeing, the owner was asked if the farm was ISA positive (case farm) or ISA negative (control farm). ISA-positive farms were defined as having had at least one cage of the 2000 or 2001 year-class of fish that had been removed because it had been diagnosed with clinical ISA prior to the start of the interview process (summer 2002). ISA-negative farms had no cages diagnosed with clinical ISA during this time period.

2.1.2. Cage selection

Once the owner agreed to participate, either the owner or the site manager from the case farm was asked which of the cages on the farm had been removed due to ISA. At the time of this study in New Brunswick, moribund fish from every farm were tested for ISA every 6 weeks. ISA tests that were available included indirect fluorescent-antibody test (IFAT) (Falk et al., 1998), reverse transcription-polymerase chain reaction test (RT-PCR) (Mjaaland et al., 1997) and virus isolation on cell culture (VI) (Dannevig et al., 1995; Bouchard et al., 1999). The sensitivities and specificities (respectively) have been estimated to be 0.79 and 0.96 for IFAT, 0.93 and 0.98 for RT-PCR (McClure et al., 2005) and $\geq$0.84 and $\geq$0.90 for VI (Nerette et al., 2005). Although the sensitivities and specificities of these tests were unknown at the time, a cage was considered ISA positive (case cage) if at least two fish were positive on two different tests, there were clinical signs consistent with ISA, and mortality rates were elevated (usually $\geq$0.05% per day). This strict requirement was designed to minimize the chance of misdiagnosing a negative cage. In a few farms in which the positive testing occurred in cages with very-young fish or where there already had been cages removed due to high ISA mortalities, cages also were considered ISA positive (case cage) even if they did not meet the requirement of $\geq$0.05% mortalities per day.

If a case farm had only one case cage, two control cages were selected randomly by drawing cage numbers out of a hat. If a case farm had more than one case cage, then two case cages and four control cages were selected randomly by drawing numbers out of a hat. For the control farms, three control cages were selected randomly in the same way.

2.2. Questionnaire

A questionnaire was developed for administration by personal interview of the site manager (or of the owner if the site manager was not available). The questionnaire was divided into site-level questions and cage-level questions. Sections for each level were further divided into information subsections such as area and site, health, feed, equipment and personnel, mortality removal, smolt history, holdovers, predators, weight sampling, wild fish, net care, sea lice and harvest. Important predictors derived from the questionnaire, which had some association with ISA risk are listed in Table 1. The complete questionnaire is available from the senior author on request.

For control farms, information was collected covering the period from the time the fish were transferred to seawater until the time the cage was harvested or until the time of the interview if the site/cage had not been harvested. For the case farms, information was requested for a case cage and two control cages from the time the fish entered seawater until the case cage was diagnosed with ISA.

The interviews were conducted from July 2002 through December 2002. By this time, all of the 2000 year-class fish had been harvested and harvest of the 2001 year-class fish had started. No data were collected on 2002 year-class fish on any of these farms. Prior to the interviews, the questionnaire was tested with two farm managers and final amendments to the questionnaire were made.

Table 1
Definitions of selected predictors of infections salmon anemia and their description

Predictor name	Group	Description
ProcBoatDist	Site	If processing boats travel within 1 km from the site
ProcBoatNum	Site	Number of processing boats that travel past the site
CageMortNum	Site	If $\geq$1 cages had >5% mortalities within 30 days post seawater transfer
CageMortPer	Site	Percent of total fish that died within 30 days post seawater transfer
ISANeigDist	Site	Categorical data, how close the nearest neighbor with ISA is to the site
ISANeighClose	Site	If the nearest neighbor with ISA is within 0.5 km of the site
DryFeedDel	Site	If the site had dry feed delivered by the feed company
DiveAug	Site	If the site had mortalities removed more than once a week in August
CageVol	Cage	Continous variable representing the volume of the cage in m^3
CageType	Cage	Polar circle or square metal cages
CageSize	Cage	Circumference of the circle cage or the length of a side of the square cage
CageDeep	Cage	Depth of the net in meters
LiceTx	Cage	If cage was treated for sea lice <3 times with any product
SliceTx	Cage	If cage was treated for sea lice <3 times with SLICE$^{\text{TM}}$
MeterUnder	Cage	If the cage had >3 m of water underneath the net at low tide
CageDepth	Cage	If the net was <10 m in depth
Pollock	Cage	If the farmer perceived that there were at least 1000 pollock in the cage
SmoltWeight	Hatchery	If the smolt weighed $\geq$99 g at the time of seawater entry

2.3. Data collection

Interviewer training to reduce inter-observer variation took place by mock interviews in a group setting with discussion of questioning techniques and recording the appropriate answer. Questionnaire interviews took between 45 and 90 min depending on the site and were carried out by the team of trained graduate students and veterinary students. At the completion of the interview, farmers were asked to provide farm records including cage mortality data, medication usage and disease diagnoses and site maps. If there were any questions that the interviewee could not answer, then farm records were consulted. Questions referring to distances between farms were cross-checked using an official Bay of Fundy marine-aquaculture site map provided by the NBDAFA.

Hatchery information relevant for each site/cage (including hatchery type, water source, number of smolts stocked in a cage, average weight of the smolt at the time of seawater stocking, condition of smolts at stocking, vaccines administered and time since vaccination) were collected directly from the hatchery managers by telephone interview, followed by a faxed questionnaire.

Data were entered into the Epidata (Lauritsen et al., 2003) data-management computer program twice and then cross-compared to reduce data-entry errors. When possible, interview answers were cross-checked with farm records.

2.4. Statistical analysis

2.4.1. Predictor variables

Continuous variables were kept as continuous variables if the relationship between the log odds of the outcome and the variable was approximately linear. If the graph evaluating this relationship was not approximately linear, the variable was categorized or dichotomized using cut points that represented natural distinctions between groups. Some categorical variables had the number of different categories reduced by combining categories if there were only a few sites in the group and it made biological sense. If there were many related variables, a new variable was created to reduce the risk of multicollinearity (Dohoo et al., 1997). An example of this was the variable for cage volume (CageVol). This variable incorporated the variables for cage type (CageType), size (CageSize) and depth (CageDeep) by using the shape of the cage (CageType) and the circumference or length of a side (CageSize) to estimate the surface area of the cage. The surface area was multiplied by the depth (CageDeep) to calculate the cage volume (CageVol). Collinearity was checked between pairs of variables by correlation for continuous variables and chi-square for categorical data. Some variables were dropped from the analysis because they were highly correlated to another factor that was kept in the analysis. An example of this was the variable for distance from the site to the processing boats travelling past the site (ProcBoatDist) and the variable for the number of processing boats that travel past the site (ProcBoatNum). The closer the site is to the processing plant, the closer the processing boats will travel by the site and the higher the number of processing boats that travel past the site in order to get to the processing plant. The variable for the number of processing boats (ProcBoatNum) was dropped from further analysis.

2.4.2. Logistic regression

2.4.2.1. Unconditional associations. Independent variables were first evaluated for unconditional associations with the outcome (case/control) using a χ^2-test for categorical data and a *t*-test for continuous data. All variables with $P < 0.20$ were tested in a multivariable logistic-regression analysis.

2.4.2.2. Model building using site level predictors. Site-level factors were analyzed using a dataset with the dependent variable ‘‘whether or not the site was a case farm.’’ Initially, all unconditionally associated significant ($P < 0.20$) variables were fit and the least-significant variable was removed one-at-a-time until only significant variables (Wald-test $P \leq 0.05$) were left in the model. The variable CageMortNum had many missing values, and a similar backward-elimination process was used to evaluate its significance in the subset of records for which it had been recorded.

Variables were evaluated for confounding by examining for a ‘‘clinically important’’ change in the magnitude of the coefficient of the variables in the model fit with and without the confounder (Hosmer and Lemeshow, 2000). Two-way interactions were assessed for any biologically reasonable interaction terms by adding the terms into the model and evaluating their significance by the Wald statistic.

The fit of all of the logisitic-regression models was assessed by the Hosmer–Lemeshow goodness-of-fit test. Pearson residuals and standardized Pearson residuals were inspected. The covariate patterns with the extreme values of the diagnostic parameters: leverage, delta-beta, delta-chi-square and the delta deviance were examined for their influence on the model.

2.4.2.3. Model building using cage- and hatchery-level predictors. Cage- and hatchery-level factors were analyzed with the dependent variable ‘‘whether or not the cage was a case cage." In most cases, case cages were matched to two control cages from the same site. Initially, a conditional logisitic-regression modelling was attempted for the evaluation of cage and hatchery factors to account for matching of case and control cages. However, use of the conditional logistic regression produced unstable estimates. A random-effects logistic-regression model to account for variation between sites was not considered because for most sites, there was a fixed number of case cages and control cages and thus no variation in risk across all sites. Thus, an ordinary logistic-regression model was used, even though the estimates from an ordinary logistic regression analysis can be biased towards the null when applied to matched data.

Cage-level factors and hatchery factors were evaluated in a manner similar to site-level factors. The significance of variables that had many missing values (e.g. the number of wild Pollock (*Pollachius virens*) in the cage (Pollock) and the variable for the time between vaccination and seawater entry (DegreeDays)) was evaluated in the subset of cage records for which it had been recorded.

2.4.2.4. Model building using a site, cage and hatchery predictors simultaneously. To identify the risk factors with the strongest associations from the site, cage and hatchery datasets, the data from site, cage and hatchery were merged to make a combined dataset. Unconditional associations and multivariable logistic regression analysis were performed in a manner similar to that used for the site-level factors on this combined dataset using the cage's ISA status as the outcome.

2.4.3. Survival analysis

The variables from the final site logistic-regression models were analyzed using a Cox proportional-hazard model to evaluate how the hazard of ISA at a negative site changed as newly diagnosed ISA-positive sites became progressively closer (in distance). The data were arranged such that it was possible to have multiple records per site. There was one record for the number of days from the time point that a site had a neighboring site with ISA in one distance category until the time point when there was a newly diagnosed site with ISA in a closer distance category. The start of the first time period at risk was defined as the time at which the smolts were transferred into the site. ISA-positive sites were classified as cases when they experienced an outbreak. Control sites were classified as censored at the time the fish were harvested, at the time of the interview if the fish were not harvested, or at 730 days (in most cases, maximum time for complete grow-out cycle) for control sites with missing harvest dates. The distance variable (ISANeigDist) was grouped into four categories appropriate for the location of farms within the Bay of Fundy (the closest site with ISA was <0.5 km ("neighboring site"), >0.5 km but ≤2 km ("in the same bay"), >2 km but ≤5 km ("next bay or in same large bay") and >5 km ("more than one bay away")). The failure event was set to whether or not the site was positive for ISA at the time that the disease came within a closer distance category. A backward-elimination process (removing the least-significant variable until all remaining variables were statistically significant with Wald-test $P > 0.05$) was used on unconditionally associated ($P < 0.20$) site risk factors to determine the final survival-analysis model. Potential confounders and interaction terms were evaluated in a similar manner as the logisitic-regression models.

The assumption of proportional hazards was evaluated using the test for proportional hazards based on the Schoenfeld residuals. Further examination of the proportional hazard for individual variables was assessed graphically looking for parallel lines for the log-cumulative hazard plot ($\log(-\log S(t))$ versus log time). In addition, time-dependent covariates were generated as interaction terms with individual variables. If the interaction terms were significant, then the proportional-hazard assumption had been violated.

Cox–Snell residuals were used to assess the overall fit of the model by graphing the cumulative hazard against the Cox–Snell residuals. (If the model fits well, then the line should be straight at a 45° angle.) Martingale and deviance residuals were used to identify any outliers by graphing the residuals against the days the sites were at risk.

All data analysis was performed using Stata (Version 7) software (College Station, TX).

3. Results

3.1. The data

Participation was 97.6% (83 of the 85 qualifying sites). There were nine sites that did not qualify because they did not stock fish in the years 2000 and 2001. Of the 83 participating sites, 27 were case sites and 56 were control sites. From the 27 case sites, there were data from 41 case cages and 79 control cages. There was data from 151 control cages from the 56 control sites. For seven sites, the cage data were not incorporated into the analysis due to many missing answers and the inability to verify given answers with site records.

3.1.1. Site-level data

There were 74 different site-level variables tested; 26 had unconditional associations ($P < 0.20$) with site being a case. Selected associations chosen by their importance in site-level models and their importance in past risk-factor studies are presented in Table 2. The best logistic-regression model using data from 82 sites and the separate model including the variable for the 65 sites with at least one cage with post transfer mortalities greater than 5% are in Table 3. Evaluation for interactions among the variables presented in the site risk-factor logistic-regression models was attempted, but discontinued due to the small number of records in the analysis.

3.1.2. Cage-level data

There were 37 different cage-level variables tested and 8 had unconditional associations between cage-level factors and the cage being a case (Table 4). One significant continuous variable was the variable for cage volume, CageVol. For this variable, there were 41 case cages and 226 control cages. The average volume for the case cages was 3230 m^3 and for the control cages was 3857 m^3. The odds for ISA in the cage were increased 1.18 times for every 1000 m^3 less volume in the cage. The 95% confidence interval for the OR was (0.98, 1.42) and the Wald's P for the logistic regression was 0.08. The two best logistic-regression models (one with data from 250 cages, and the separate model which included the variable

Table 2
Selected risk factors in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001 with unconditional associations ($P < 0.20$) between site-level factors and the site's ISA status

Variable	Level	Number of sites		P-value (χ^2)
		Case	Control	
Closest neighbor with ISA (ISA Neigh Close)	>0.5 km	16	51	0.001
	≤0.5 km	11	5	
Distance to processing boats travelling past site (ProcBoatDist)	>1 km	1	16	0.009
	≤1 km	26	40	
Dry feed is delivered by feed company (DryFeedDel)	No	12	44	0.001
	Yes	15	11	
Number of cages that had >5% mortalities during the first 30 days post seawater transfer (CageMortNum)	No cages	18	22	0.004
	≥1 cage	3	23	
Percent of total mortalities during the first 30 days post seawater transfer (CageMortPer)	≤5%	10	28	0.13
	>5%	10	12	
Fish are fed by hand (FeedHand)	No	13	40	0.04
	Yes	14	16	
Site allows visitors (Vistor)	No	6	21	0.16
	Yes	21	35	
Number of mortality dives per week in August (DiveAug)	≤1/week	12	46	0.001
	>1/week	15	10	
Number of mortality dives per week during times of high mortalities (DiveHighMort)	≤2/week	7	28	0.03
	>2/week	20	27	
Smolts transferred to seawater site by a ferry (SmoltFerry)	No	8	33	0.01
	Yes	19	23	
Has site been attacked by seals (SealAttack)	Yes	6	21	0.16
	No	21	35	
How often are fish weight sampled (WeightSample)	<1/month	9	30	0.08
	≥1/month	18	26	
Nets are cleaned in the water (CleanNets)	No	7	27	0.05
	Yes	20	29	

representing the cage containing at least 1000 pollock) are in Table 5. There were no significant interactions among the variables presented in the logistic-regression models for the cage risk factors.

3.1.3. Hatchery data

There were 14 different variables tested; five had unconditional associations ($P < 0.20$) with the cage being a case (Table 6). The best logistic-regression model ($P < 0.05$) consisted of only one risk factor (Table 7): weight of smolts at seawater transfer was <99 g (SmoltWeight). This model incorporated data from 233 cages.

Table 3
Logistic-regression models for SITE-LEVEL risk factors for ISA in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001

Variable	Level	Data from 82 sites			Data from 65 sites		
		Odds ratio	95% CI	P-value	Odds ratio	95% CI	P-value
Closest neighbor with ISA (ISANeighClose)	>0.5 km	1			1		
	≤0.5 km	3.58	1.04, 12.3	0.04	14.0	2.35, 83.4	0.004
Distance to processing boats travelling past site (ProcBoatDist)	>1 km	1					
	≤1 km	12.6	1.38, 114	0.03	–	–	–
Dry feed is delivered by feed company (DryFeedDel)	No	1					
	Yes	5.18	1.66, 16.1	0.005	–	–	–
Number of cages with post transfer mortalities greater than 5% (CageMortNum)	No cages				1		
	≥1 cage	–	–	–	38.0	4.04, 358	0.001
Number of mortality dives per week in August (DiveAug)	≤1/week				1		
	>1/week	–	–	–	11.9	2.01, 70.5	0.006
Overall P (final model)[a]				<0.0001			<0.0001
Degrees of freedom				78			61
Deviance				80.0			48.0

Data from 82 sites and data from 65 sites (includes the variable for a site that has at least one cage with post transfer mortalities greater than 5% in the first 30 days post seawater transfer).

[a] β_0 was -3.83 for the model with 82 sites and -5.00 for the model with 65 sites.

Table 4
Unconditional associations (with $P < 0.20$) between cage-level risk and the cage's ISA status factors in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001

Variable	Level	Number of cages		P-value (χ^2)
		Case	Control	
Depth of cage (CageDepth)	>9 m	6	69	0.04
	≤9 m	35	157	
Meters underneath net at low tide (MeterUnder)	≤3 m	10	88	0.06
	>3 m	31	131	
Number of lice treatments with SLICE™ (SliceTx)	>2 times	6	64	0.05
	≤2 times	35	152	
Number of pollock farmer perceived to be in the cage (Pollock)	<1000	28	158	0.005
	≥1000	10	17	
Fish had gill parasites (GillParasites)	Yes	1	24	0.10
	No	40	202	
Has the net been changed (NetChange)	No	27	164	0.10
	Yes	14	47	
At seawater entry, did the smolts come in a wide range of sizes (SmoltUngraded)	Yes	1	26	0.07
	No	38	181	

[a] P-value for t-test on a continuous predictor.

Table 5
Logistic-regression model for CAGE-LEVEL risk factors for ISA in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001

Variable	Level	Data from 250 cages			Data from 206 cages		
		Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Depth of cage (CageDepth)	>9 m	1			1		
	≤9 m	2.96	1.17, 7.52	0.02	3.51	1.16, 10.6	0.03
Meters underneath net at low tide (MeterUnder)	≤3 m	1			1		
	>3 m	2.26	1.03, 4.97	0.04	3.34	1.34, 8.36	0.01
Number of lice treatments with SLICE^{TM} (SliceTx)	>2 times	1					
	≤2 times	2.67	1.05, 6.81	0.04	–	–	–
Number of pollock farmer perceived to be in the cage (Pollock)	<1000				1		
	≥1000	–	–	–	4.40	1.59, 12.1	0.004
Overall *P* (final model)[a]				0.003			0.0001
Degrees of freedom				246			202
Deviance				209.2			176.4

Data from 250 cages and data from 206 cages (using the variable for the number of pollock farmer perceived to be in the cage).

[a] β_0 was −3.81 for the model with 250 cages and −3.57 for the model with 206 cages.

3.1.4. Combined data

All variables that had unconditional associations with χ^2 or *t*-test *P*-values <0.20 from the site-level, cage-level and hatchery data were combined. The best logistic-regression model used data from 260 cages and consisted of five risk factors (Table 8). The separate

Table 6
Unconditional associations (with $P < 0.20$) between hatchery risk factors and the cage's ISA status in farmed Atlantic salmon farms for New Brunswick during 2000 and 2001

Variable	Level	Number of cages (all cages)		*P*-value (χ^2)
		Case	Control	
Smolt weight (SmoltWeight)	<99 g	12	105	0.02
	≥99 g	25	91	
Vaccinated against ISA (VaxISA)	Yes	21	137	0.10
	No	19	70	
Immersion vaccinated against Aeromonas salmonicida (VaxIM)	No	21	137	0.18
	Yes	16	64	
Degree–days between intraperitoneal vaccination and seawater transfer (DegreeDays)	>700 degree–days	9	70	0.17
	≤700 degree–days	18	77	
Number of smolts transferred into a cage (SmoltNum)	>16000	15	97	0.16
	≤16000	24	94	

Table 7
Logistic-regression model for HATCHERY risk factors in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001

Variable	Level	Odds ratio	95% CI	*P*-value
Smolt weight (SmoltWeight)	<99 g	1		
	≥99 g	2.40	1.14, 5.06	0.02
Overall *P* (final model)[a]				0.02
Degrees of freedom				231
Deviance				198.3

Data from 233 cages.

[a] β_0 was −2.17 for the model.

model using the variables that had many missing records was fit, and the best logistic-regression model (Table 8) incorporated data from 199 cages. There were no significant interactions between the variables presented in the logistic-regression models for the combined risk factors.

Table 8
Logistic-regression model for COMBINED (SITE, CAGE AND HATCHERY) risk factors for ISA in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001

Variable	Level	Data from 260 cages			Data from 199 cages		
		Odds ratio	95% CI	*P*-value	Odds ratio	95% CI	*P*-value
Closest neighbor with ISA (ISANeighClose)	>0.5 km	1					
	≤0.5 km	2.26	1.11, 5.21	0.03	–	–	–
Distance to processing boats travelling past site (ProcBoatDist)	>1 km	1					
	≤1 km	9.43	1.18, 75.4	0.03	–	–	–
Dry feed is delivered by feed company (DryFeedDel)	No	1			1		
	Yes	4.03	1.85, 8.76	<0.001	2.68	1.13, 6.37	0.03
Number of cages with post transfer mortalities greater than 5% (CageMortNum)	No cages				1		
	≥1 cage	–	–	–	4.52	1.27, 16.1	0.02
Depth of cage (CageDepth)	>9 m	1	1.20, 9.30	0.02	1		
	≤9 m	3.34			3.28	1.03, 10.5	0.04
Number of lice treatments with any product (LiceTx)	>2 times	1					
	≤2 times	3.31	1.38, 7.92	0.007	–	–	–
Smolt weight (SmoltWeight)	<99 g				1		
	≥99 g	–	–	–	2.95	1.23, 7.06	0.02
Overall *P* (final model)[a]				<0.0001			<0.0001
Degrees of freedom				254			194
Deviance				193.0			143.3

Data from 260 cages and data from 199 cages (using the variables for the number of pollock farmer perceived to be in the cage, number of cages with post transfer mortalities greater than 5%, and Smolt weight).

[a] β_0 was −6.53 for the model with 260 cages and −4.98 for the model with 199 cages.

Table 9
Survival analysis model for modified site risk factors for the time to ISA disease in farmed Atlantic salmon stocked in New Brunswick during 2000 and 2001

Variable	Level	Hazard ratio	95% CI	*P*-value
Nearest neighbor with ISA categorized (ISANeigDist)	≥5 km	1		
	≥2 km but <5 km	1.17	0.34, 4.07	0.81
	≥0.5 km but <2 km	2.01	0.63, 6.46	0.24
	<0.5 km	5.50	2.03, 15.0	0.001
Distance to processing boats travelling past site (ProcBoatDist)	>1 km	1		
	≤1 km	7.47	1.00, 55.8	0.05
Dry feed is delivered by feed company (DryFeedDel)	no	1		
	yes	2.66	1.20, 88	0.02

Data from 83 sites.

3.1.5. Testing the fit of all logistic-regression models

In the Hosmer–Lemeshow goodness-of-fit testing, all final models with more than one predictor had $P > 0.05$. The Pearson residuals and standardized Pearson residuals were inspected showed no obvious outliers. The leverage and the extremes of the delta-beta, delta-chi-square and the delta deviance were examined, and no influential covariate patterns were found.

3.2. Survival analysis of site data

There were 82 sites used in the data analysis, and the model identified three significant risk factors (ISANeigDist, ProcBoatDist and CageDepth) (Table 9).

The proportional-hazard assumption was confirmed by all methods described in the methods sections. The plot of cumulative hazard against the Cox–Snell residuals yielded a reasonably straight line (indicating that the model fit well). Graphs of the Martingale and deviance residuals against the time at risk identified one outlier. This site was positive for all of the risk factors in the model, but it was not a case site. Rerunning the model with this outlier removed only slightly changed the coefficients of the model, but did not affect their significance.

4. Discussion

4.1. Significant risk factors

4.1.1. Site risk factors

Laboratory experiments have shown that the virus can survive in seawater (Nylund et al., 1994; Loevdal and Enger, 2002) as well as be spread to naive Atlantic salmon by blood, infected tissues and feces (Nylund et al., 1994; Totland et al., 1996). A site that has a neighboring site with ISA within 500 m (ISANeigClose) has increased odds of ISA disease. This might have been due to the spread of the virus through the water.

Feed boats (DryFeedDel) might carry ISA virus from site to site. Similar to this study, the previous study in New Brunswick did find an increase risk associated with feed delivery (Hammell and Dohoo, in press), but the one study of well-boats in Scotland did not find an association between feed delivery and ISA disease (Murray et al., 2002). However, that study did find an association between boats carrying fish from site to site and increased risk. It was not possible to measure the boat traffic at particular sites in our study but if processing boats travelled within 1 km of the site (ProcBoatDist) then the site had higher odds for disease. Currently, members of the NBDAFA are reviewing boat traffic in the area of the sites and are making a plan to reduce the risk of ISA viral spread by boats.

Similarly to our CageMortNum, Hammell and Dohoo (in press) found an increased risk of disease for cages with high cumulative mortality during the salmons' first year in seawater (perhaps an indicator for general fish health).

4.1.2. Cage risk factors

In the summer, the surface water is often warm (>14 °C) and this reduces the available oxygen in the water for the fish (increasing the stress level and, in severe cases, limiting the number of times the fish are offered feed). Deeper water is usually cooler, allowing for better oxygen-carrying capacity. There are other possible explanations for the increased odds when cages are not <10 m (CageDepth). Deep nets could be an indicator for plastic (PVC) circular cages since they are usually larger and can carry a deeper net than smaller steel square cages or for an increased cage volume and thus more fish initially stocked. However, CageType and FishNum were not highly correlated with CageDepth and neither they nor CageVol were retained in the final model.

Having >3 m between the bottom of the net and the ocean floor at low tide (MeterUnder) increased the odds of having disease in that cage. Perhaps greater water depth allows for more distortion of the shape of the net enclosure increasing fish stress or, in severe cases, the fish may have decreased room to maintain swimming patterns. Another possible reason for this increased odds is that cages that have more depth underneath the cages were in deeper water and thus experienced higher currents. If currents are high, fish might get pushed against the nets, causing skin and fin damage allowing ISAV to enter.

Sea lice transmit ISAV (Nylund et al., 1993). The risk of ISA is less when sea lice are more frequently controlled (Hammell and Dohoo, in press). Sea lice remain an ongoing management issue and treatment infrequency with SLICE^{TM} remains a cage risk factor.

One cage risk factor with many missing observations was whether the farmer perceived there were at least 1000 pollock in the cage (Pollock). Wild pollock are commonly found in Atlantic salmon cages in New Brunswick. Small pollock can swim through the net (tempted by the presence of salmon feed) and become residents of the cage because they grow too large to swim out. They also get into the cages during net changes. Other wild fish such as sea trout (Devold et al., 2000), brown trout (Nylund et al., 1995) and herring (Nylund et al., 2002) are carriers of ISAV, and have infected naïve salmon in laboratory trials. There has been no proof that pollock can carry ISAV or infect salmon (Snow et al., 2002; McClure et al., 2004), but their presence in the cage might be stressful to the fish and increases the stocking density. This factor might be an indicator of the presence of other wild fish that could be carriers of ISAV.

4.1.3. Hatchery risk factors

The size of the smolt (SmoltWeight) perhaps indicates better adaptation for seawater by the smaller smolts. Alternatively, larger smolts are more likely to have problems such as conformational deformities due to the rapid growth. This factor also could be an indicator for the type of hatchery from where the smolt came. Recirculation hatcheries filter, and then reuse, the water that the fish live in. Usually, this water is heated and is warmer than water used in flow-through hatcheries that do not reuse the water. Because the water is warmer, the smolt will grow faster and be larger at the time of seawater transfer. However, the variable for hatchery type (HatchType) was measured and was neither significant nor a confounder for SmoltWeight.

4.1.4. Combined risk factors

The combined data did not reveal any new risk factors, except that the number of lice treatments with any product (LiceTx) fit in the model better than the similar variable SliceTx. ProcBoatDist and DryFeedDel had the largest ORs, which emphasizes the importance of regulating boat traffic near the sites.

4.1.5. Survival analysis

In some cases, the ISA at the neighbor site occurred after the outbreak at the study site. In addition, it is likely that the risk of disease changes at a negative site over time as the nearest neighbor with ISA becomes closer. This change in risk is not accounted for in the logistic-regression model.

4.1.6. Unmeasured risk factors

There is always the chance that there are other important risk factors for ISA, for which we did not have records. It is also possible that the risk factors that were significant in this study were actually indicators for other unmeasured factors. An example of this is the variable for MeterUnder. This variable measured whether the meters under the net at low tide were >3, but it could be an indicator for, e.g. the velocity of the current.

4.2. Minimizing bias

Selection bias for the site was minimized by selecting almost the entire population.

Information bias can occur if the site manager knows the purpose of the survey and answers questions according to what he believes to be the desired response. Recall can be introduced if the interviewee is more likely to remember an answer because the site has had ISA. Unfortunately, this type of bias is differential, in that it would be an issue only for information from ISA positive sites or cages. We tried to avoid any leading questions and all surveyors were trained not to direct the site manager's responses unintentionally (interviewer bias).

4.3. Causality

4.3.1. Time sequence

All factors except for one (DiveAug) precede the outcome of disease because they are constant events (such as the shape of the cage) or they were asked for the period up until the cage became diseased. Clinical ISA is most common during the late spring until fall. Many of the case sites were experiencing ISA outbreaks during August. Thus, frequent mortality dives during the month of August should be minimized as a cause of increased risk of ISA because it is possible that the site already had disease and dive frequency was increased as a control method.

4.3.2. *Consistency and reproducibility*

Many of the significant risk factors were identified in previous laboratory and epidemiological studies. Laboratory studies have shown that the virus can spread through seawater (Nylund et al., 1994; Loevdal and Enger, 2002) and be transferred from fish to fish by sea lice (Nylund et al., 1993, 1994) (relavent for ISANeigDist, LiceTx and SliceTx). Other epidemiologic studies have shown the importance of boat traffic (Murray et al., 2002; Hammell and Dohoo, in press), proximity to a site with ISA (Vagsholm et al., 1994; Jarp and Karlsen, 1997), lice treatment (Hammell and Dohoo, in press) and healthy young fish (Hammell and Dohoo, in press) (relevant for ProcBoatDist, DryFeedDel, ISANeigDist, LiceTx, SliceTx and CageMortNum). Newly identified risk factors such as CageDepth, MeterUnder, SmoltWeight and Pollock should be tested either in the laboratory or with other epidemiologic studies to test for reproducibility.

4.3.3. *Dose–response relationship*

As the nearest neighbor with ISA progressed from site to site drawing closer to the site in question, the risk of ISA also increased as (Table 9).

4.3.4. *Coherence with existing knowledge*

Only DiveAug that was identified in our study in contradiction to a previous ISA risk-factor study (Jarp and Karlsen, 1997) or any laboratory studies. This is less of a concern because this variable was discounted because of time sequence.

4.4. *Statistical analyses*

Because the case cages were matched to control cages, estimates for individual predictors would have been more appropriate if the conditional logistic-regression model could have been used for cage, hatchery and combined datasets. Unfortunately, because cage and hatchery predictors often did not vary within a site, estimates for predictors were unstable and ordinary logistic regression modeling was used, resulting in conservative estimates (Dohoo et al., 2003).

For the site-level factors, there were 74 variables and only 83 records. By removing variables that were not significant on unconditional associations, variables that had many missing records and combining a group of variables into a new index variable, the number of important risk factors available for analysis was decreased considerably. Still, it was not possible to assess interaction in the site level data because of the small number of records.

5. Conclusions

Environmentally controlled risk factors (such as the depth of the net, depth of water underneath the net and presence of wild pollock) mostly are dictated by site location.

Factors that are under site control and easier to change include improving lice control, transferring healthy smolt into seawater (possibly avoiding large smolt) and improving on the adaptation of smolt to seawater to reduce post transfer mortalities. Industry-controlled factors need to be addressed by the government officials and the industry representatives. Organizing boat travel to minimize the time and frequency of boats travelling to or by sites is currently being reviewed. Increasing the distance between sites to increase the distance to the nearest neighbor with ISA may be necessary if control of the disease is ever to be realized.

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References

Bouchard, D., Keleher, W., Opitz, H.M., Blake, S., Edwards, K.C., Nicholson, B.L., 1999. Isolation of Infectious salmon anemia virus (ISAV) from Atlantic Salmon in New Brunswick, Canada. Dis. Aquat. Organ. 35, 131–137.

Dannevig, B.H., Falk, K., Namork, E., 1995. Isolation of the causal virus of infectious salmon anaemia (ISA) in a long-term cell line from Atlantic salmon head kidney. J. Gen. Virol. 76, 1353–1359.

Devold, M., Krossoy, B., Aspehaug, V., Nylund, A., 2000. Use of RT-PCR for diagnosis of infectious salmon anaemia virus (ISAV) in carrier sea trout Salmo trutta after experimental infection. Dis. Aquat. Organ. 40, 9–18.

Dohoo, I.R., Ducrot, C., Fourichon, C., Donald, A., Hurnik, D., 1997. An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Prev. Vet. Med. 29, 221–239.

Dohoo, I.R., Martin, S.W., Stryhn, H., 2003. Veterinary Epidemiologic Research. AVC Inc., Charlottetown, Prince Edward Island, Canada.

Falk, K., Namork, E., Dannevig, B.H., 1998. Characterization and applications of a monoclonal antibody against infectious salmon anaemia virus. Dis. Aquat. Organ. 34, 77–85.

Hammell, K.L., Dohoo, I.R. Risk factors associated with mortalities attributed to infectious salmon anemia virus in New Brunswick, Canada. J. Fish Diseases, in press.

Hosmer, D.W., Lemeshow, S., 2000. Applied Logisitic Regression. John Wiley & Sons Inc., New York.

Jarp, J., Karlsen, E., 1997. Infectious salmon anaemia (ISA) risk factors in sea-cultured Atlantic salmon *Salmo salar*. Dis. Aquat. Organ. 28, 79–86.

Lauritsen, J.M., Bruus, M., Myatt, M.A., 2003. EpiData (2.1b), an extended tool for validated entry and documentation of data. The EpiData Association, Odense Denmark, 2001–2003. http://www.epidata.dk.

Loevdal, T., Enger, O., 2002. Detection of infectious salmon anemia virus in sea water by nested RT-PCR. Dis. Aquat. Organ. 49, 123–128.

McClure, C.A., Hammell, K.L., Dohoo, I.R., Gagné, N., 2004. Lack of evidence of infectious salmon anemia virus in pollock *Pollachius virens* cohabitating with infected farmed Atlantic salmon *Salmo salar*. Dis. Aquat Organ. 61, 149–152.

McClure, C.A., Hammell, K.L., Stryhn, H., Dohoo, I.R., Hawkins, L.J., 2005. Application of surveillance data in evaluation of diagnostic tests for infectious salmon anemia. Dis. Aquat. Organ. 63, 119–127.

McGeachy, S.M., Moore, M.J., 2003. Infectious salmon anemia in New Brunswick: an historical perspective and update on control and management practices (1997–2002). In: Miller, O., Cipriano, R.C. (Eds.), International response to infectious salmon anemia: prevention, control, and eradication, Washington, DC. US Department of Agriculture, pp. 145–153.

Mjaaland, S., Rimstad, E., Falk, K., Dannevig, B.H., 1997. Genomic characterization of the virus causing infectious salmon anemia in Atlantic salmon (*Salmo salar* L.): an orthomyxo-like virus in a Teleost. J. Virol. 71, 7681–7686.

Moore, M., 2003. ISA in New Brunswick: putting it in perspective. In: Proceedings of the New Brunswick ISA Research Workshop, March 31–April 1, 2003, St. Andrews, New Brunswick.

Murray, A.G., Smith, R.J., Stagg, R.M., 2002. Shipping and the spread of infectious salmon anemia in Scottish aquaculture. Emerg. Infect. Dis. 8, 1–5.

Nerette, P., Dohoo, I., Hammell, L., 2005. Estimation of specificity and sensitivity of three diagnostic tests for infectious salmon anaemia virus in the absence of a gold standard. J. Fish. Dis. 28, 89–99.

Nylund, A., Alexandersen, S., Rolland, J.B., Jakobsen, P., 1995. Infectious salmon anemia virus (ISAV) in brown trout. J. Aquat. Anim. Health 7, 236–240.

Nylund, A., Devold, M., Mullins, J., Plarre, H., 2002. Herring (*Clupea harengus*): a host for infectious salmon anemia virus (ISAV). Bull. Eur. Assoc. Fish Pathol. 22, 311–318.

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

Nylund, A., Wallace, C., Hovland, T., Boxshall, G.A., Defaye, D., 1993. The possible role of Lepeophtheirus salmonis (Kroyer) in the transmission of infectious salmon anaemia. In: Boxshall, G.A., Defaye, D. (Eds.), Pathogens of wild and farmed fish: sea lice, Ellis Harwood Lmt., West Sussex, UK, pp. 367–373.

O'Halloran, J.L.F., L'-Aventure, J.P., Groman, D.B., Reid, A.M., 1999. Infectious salmon anemia in Atlantic salmon. Can. Vet. J. 40, 351–352.

Snow, M., Raynard, R., Bruno, D.W., Van Nieuwstadt, A.P., Olesen, N.J., Loevold, T., Wallace, C., 2002. Investigation into the susceptibility of saithe *Pollachius virens* to infectious salmon anaemia virus (ISAV) and their potential role as a vector for viral transmission. Dis. Aquat. Organ. 50, 13–18.

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

Totland, G.K., Hjeltnes, B.K., Flood, P.R., 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. Organ. 26, 25–31.

Vagsholm, I., Djupvik, H.O., Willumsen, F.V., Tveit, A.M., Tangen, K., 1994. Infectious salmon anaemia (ISA) epidemiology in Norway. Prev. Vet. Med. 19, 277–290.