

# Estimation of the probability of freedom from infectious salmon anemia virus in farmed coho salmon in Chile using scenario tree modeling

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## Abstract

The infectious salmon anemia virus is a notifiable disease for the OIE member countries, including Chile. This disease primarily affects farmed Atlantic salmon causing important socioeconomic impact in regions where this species is intensively raised. However, the role, if any, of the coho salmon in the epidemiology of this disease has not been elucidated yet. Using a scenario tree modeling approach this study assessed the sensitivity of the surveillance program carried out between September 2007 and December 2014 and the probability of freedom from ISAV infection in the Chilean population of farmed coho salmon. Over this period a total of 4,059 surveys were conducted, for a total of 86,382 pools that were tested from 164 fresh water (FW) and 299 salt water (SW) farms. All the samples were negative for ISAV by real-time reverse-transcription-polymerase chain reaction, the test recommended by the OIE. The overall sensitivity of the surveillance varied in FW and SW, with a median estimate by quarter of 0.83 (min = 0.61, max = 0.99) and 0.94 (min = 0.61, max = 1), respectively. Combining both components the probability of freedom from disease estimated per quarter was within a range between 0.91-1. The overall probability of being free of resulted in a median estimate of 0.95. Results support the hypothesis that the Chilean farmed coho salmon population is free from ISAV infection despite this agent being prevalent in the farmed Atlantic salmon population in Chile.

**Keywords:** *Freedom from animal disease, infectious salmon anemia, ISAV, coho salmon, Scenario tree modeling*

## Introduction

Infectious salmon anemia (ISA) is a highly contagious disease caused by the ISA virus (ISAV) characterised by severe anemia and high mortality mainly in farmed Atlantic salmon (*Salmo salar* L.) (1). ISA, a World Animal Health Organisation (OIE) notifiable disease, was first reported in Norway in 1984 (2) and since then, the disease has spread into several regions where Atlantic salmon farming activities are carried out, including Canada, Chile, United States, Faroe Islands, and Scotland (3-7). ISAV has a narrow host range and only one species, Atlantic salmon, is considered the principal host of pathogenic infections, manifesting uniquely clinical disease with high mortality in farmed fish. In Chile, the epidemiology of ISAV

is still controversial. In 1997, a new disease condition was reported and limited exclusively to farmed coho salmon. This disease was named infectious hemolytic anemia (IHAS) with a marked spatial and temporal pattern (8-9). In 1999, ISAV was isolated from diseased coho salmon that manifested a different pathology from typical ISA, and thus was considered rare and related with the North American genogroup (10). Subsequent experiments failed to reproduce the disease, suggesting that the virus would not be the unique etiological agent associated with this disorder (8). Since 2001, the Chilean fish health authority (Sernapesca) has been coordinating surveillance activities to detect ISAV. Up to date, the IHAS is prevalent in the country; however, it has never been associated with any official positive ISAV-test result. The emergence of classical ISAV into the country was reported in 2007, when a Norwegian ISAV variant was detected for the first time affecting farmed Atlantic salmon from a marine site in southern Chile (11). The epidemic resulted in the largest ISA worldwide epidemic ever occurred (12) generating a sanitary crisis in the industry with devastating economic impacts. Despite the magnitude of the epidemic in Atlantic salmon, ISAV was never reported to have been detected or affect the farmed coho population. However, due to the early controversial studies regarding the role that coho salmon could play in the spread of the infection, surveillance activities were also carried out on this population. The aim of this study was to quantify the sensitivity and probability of freedom from ISAV in the population of farmed coho salmon in Chile taking into account the RT-qPCR results from the surveys carried out in Chile between September 2007 and December 2014. The approach used in this study may benefit other areas where coho salmon is produced and routinely sampled. The results presented are relevant to evaluate the need for sustaining surveillance activities for the disease in this species, and contribute to increase the efficiency in the use of resources for disease surveillance in farmed salmon in Chile and other regions.

## Materials and methods

A stochastic scenario tree model (13) was used to estimate the sensitivity (*Se*) of the surveillance system (SS) carried out by Sernapesca during the last seven years in different production stages and substantiate the freedom from ISAV in the farmed coho salmon population of Chile. Both estimates,

i.e. Se and probability of freedom, were assessed by a quarter period (four months). Here the proposed model builds up information from quarter to quarter, taking into account the confidence gained from consecutive testing and the probability of introduction of disease into this subpopulation over time.

#### *Surveillance program*

The surveillance program is a two-stage systematic random sampling of all farmed salmon at both fresh and marine sites that have operated. Sampling is conducted in at least two visits at each farm per year, with a minimum interval of four months. Reports from surveillance activities include a farm-level unique identification code, location of the farm, type of farm, i.e. fresh water (FW) or salt water (SW), number of farmed coho salmon, sampling date, number of pools, and testing results. The targeted population included all farms that raised coho salmon based on reports from individual producers to Sernapesca. The number of farms varied monthly because of stocking, re-stocking, and harvesting practices within the salmon industry. Coho salmon farms were categorised into two production types, FW and SW farms.

#### *Laboratory testing and case definition*

In FW farms a minimum of 60 fish are collected during each visit. The selection of samples prioritises the collection of fish that have died recently or are moribund. If the FW water farm raises breeders, 15 fish are additionally taken in one of the two visits. The surveillance carried out on SW farms is similar to FW farms. In each visit, 30 fish are collected, and randomly selected from at least three cages. If moribund fish or fresh mortalities are available, they are preferred. A SW farm that raises broodstock has a different sampling scheme, in which 60 fish are collected in addition to 15 broodstock in one of the visits. Tissue pools from 1 to 3 fish of kidney, heart, and gills were submitted to Chilean official laboratories for diagnostic testing using RT-qPCR and confirmatory sequencing. All laboratories employed TaqMan probes (14) for the RT-qPCR. The test's sensitivity was estimated from results of a ring test carried out by Sernapesca (data not shown) and modeled using a Pert distribution (0.9, 0.95, 0.99). Here, a farm that raised coho salmon was considered infected if the sequential testing resulted positive, including the follow-up investigation required to confirm infection. On this basis, the specificity of the surveillance was considered 1.

#### *The stochastic scenario tree model*

The model included all the steps of the surveillance that need to be considered to detect the ISAV infection in the coho salmon in the event of being infected. The model described the processes from the starting points (in the event of infected population) to the outcome (infection detected or not detected). Here, we performed a quantitative analysis of historical surveillance data to capture the Se of surveillance and test the freedom from ISAV hypothesis for the population of farmed coho salmon in Chile. Two SS components (SSC) were assessed: FW and SW farms, which represent two distinct production stages. The unit of analysis was the

pooled sample taken from a surveyed coho salmon farm. Each sample was tested and the results were reported from authorised laboratories to Sernapesca. The design prevalence ( $P^*$ ) at farm level was set at 0.2% and the ISAV prevalence of farmed coho salmon farm ( $P_U^*$ ) was assumed at 15%.

#### *Surveillance system sensitivity (SSSe)*

The SSSe indicates the probability of detecting the ISAV infection through the surveillance activities carried out given the fish were infected at the design prevalence. Between September 2007 and December 2014 the SSSe of farmed coho salmon was estimated per quarter for FW and SW. First, the model computed the Se for each sampled farm considering  $P_U^*$ , the number of pools sampled from each salmon farm, the number of individual fish by pool, and sensitivity of RT-qPCR by pool. The SSSeq for FW and SW was computed using a hypergeometric distribution that included the minimum expected ISAV prevalence among farms, the number of farms sampled by quarter, and the total number of coho salmon farms that comprised the investigated component.

#### *Probability of freedom from ISAV infection*

The model computed the probability that the farmed coho salmon population was free from ISAV throughout the study period.

Moreover, between consecutive samplings there could exist a risk of incursion of ISAV. Finally, we combined the sensitivities from each component to obtain the overall surveillance sensitivity, assuming that these two components were independent (15), and subsequently, the overall probability of freedom from ISAV was obtained. Finally, the probability of being free from ISAV during all the period was calculated as the area under the curve (AUC) for each component and combining both components.

#### *Software*

The descriptive analyses, models and plots of this work were performed using the R software (16) and specific statistical packages.

#### *Results*

From September 2007 until December 2014, a total of 4,336 surveys were registered and were included in the analyses. The population of FW farms under surveillance ranged quarterly between 74 and 102 (median = 87). The median number of FW farms surveyed per quarter were 32 (min = 18, max = 58), covering approximately 38% of the FW farms at a given q, with an average number of coho salmon per farm of 3.8 million (95% CI = 1.83, 5.8). The quarterly number of SW farms ranged from 86 to 167 (median = 109) with an average of 0.73 million of coho salmon per farm (95% CI = 0.42, 1.04). The median proportion of SW farms surveyed per quarter was 50%. Over all the period the surveillance efforts covered a total of 164 FW and 299 SW farms, totalising 86,382 tested pools that resulted all negatives to RT-qPCR. The estimates of SSSeq,FW were more variable than the sensitivity at SW, and generally were higher in winters. In contrast, SSSeq,SW

were relatively stable over all the study period, with a drop by the end of 2011. The median values estimated for the SS<sub>Se</sub> were 0.83 (min = 0.61, max = 0.99) and 0.94 (min = 0.61, max = 1) for FW and SW, respectively. The SS<sub>Seq,Total</sub> varied over time within a range between 0.85 and 0.99 and a median of 0.99. These outcomes indicated that the SW surveillance had the highest contribution to the total surveillance sensitivity.

The probability of freedom by quarter combining both components was estimated as a median of 0.99 within a range between 0.96 and 1. The probability of being free over the entire period expressed as AUC was 0.95 (Figure 1).

## Discussion

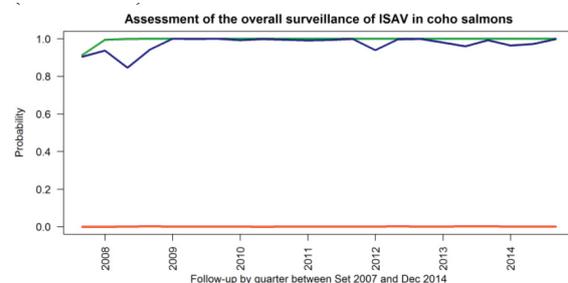
Results indicated that the probability of freedom from ISAV in the Chilean farmed coho population is high (>0.95). Despite ISAV being prevalent in farmed Atlantic salmon in Chile, the large amount of tested samples over time without any positive results strongly support the freedom from ISAV hypothesis in the Chilean coho salmon. After the publication of Kibenge *et al.* (10) that detected ISAV using RT-qPCR from coho salmon tissue coming from Chile, Sernapesca established an active surveillance program following the OIE guidelines for highly infectious animal diseases including ISAV. Since then, no sample has ever yielded a positive test result for ISAV in coho salmon.

A better knowledge on the species' susceptibility for these pathogens is critical since these species can play a major role in the epidemiology of the disease. Susceptibility in aquatic species is determined if the infection can be demonstrated by natural occurrence of the disease in the species, or by experimental exposure of the species to the disease agent through a pathway that mimics a natural route of infection. If there is a species that is non-susceptible, there is no reason to plan surveillance activities for them. On the other hand, knowledge on the non-susceptibility of the species may provide another sanitary perspective of this subpopulation and their natural resistance to a number of disease conditions, which can be thought as an advantage to control certain salmon diseases, at least in SW. For example, researchers found that the least susceptible species to sea lice infestation was the coho salmon (17). Sea lice is one of the most serious threats to the sustainability of the global salmon industry and has been previously associated with ISAV infection in Chile (18). Thus, coho salmon farms may serve as an epidemiological "firebreak", if they were to be spatially located between Atlantic salmon farms, i.e. increasing distance between susceptible sites and thus preventing spread of these diseases from infected to susceptible sites. Removing the disease transmission risk of such contacts could reduce the size of potential epidemics by creating "firebreaks". In this context, farmed coho salmon can replace other susceptible species to design "fire breaks", to mitigate potential ISAV and sea lice outbreaks.

Another important aspect that is worth to review is the associated costs that have been invested for ISAV surveillance of a non-susceptible species. Surveillance costs include, among others, those related to fish sampling (more expensive

in brood-stocks and cheaper in FW fish), materials used to sample and man labor costs, transportation and in-house laboratory operations, specimen processing, and handling for viral load testing. Assuming that individual pool sample costs are estimated to be around 21.5 USD and that during the study period a total of 86,382 samples were analysed, which is equivalent to US 1.8 million or roughly 260,000 USD per year. Results from a scenario tree model support the claim that the Chilean farmed coho salmon population is likely be free from ISAV and are in line with previous research that demonstrates that coho salmon is relatively resistant to ISAV. Funds used to sustain ISAV sampling in coho salmon may be more efficient to support the sustainability of the salmon industry if used, instead, to control other diseases known to represent a treat for the industry.

**Figure 1.** Probabilities of freedom (green), sensitivity (blue) and probabilities of incursion of ISAV (red) estimated for the overall surveillance system for the coho salmon population between September 2007 and December 2014 (AUC = 0.95).



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