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Cost-effectiveness of longitudinal surveillance for *Piscirickettsia salmonis* using qPCR in Atlantic salmon farms (*Salmo salar*) in Chile

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Abstract

Costs of diagnostic testing including sample collection, sampling frequency and sample size are an important consideration in the evaluation of the economic feasibility of alternative surveillance strategies for detection of infectious diseases in aquatic animals. In Chile, *Piscirickettsia salmonis* is the primary reason for antibiotic treatments in farmed Atlantic salmon. In 2012, a surveillance and control programme for piscirickettsiosis was established with an overall goal of reducing antibiotic use. The present study estimated the cost-effectiveness of different sampling frequencies and sample sizes to achieve at least 95% confidence of early detection of *P. salmonis* at the netpen and farm levels using a validated qPCR test. We developed a stochastic model that incorporated variability in test accuracy, within-pen prevalence and sampling costs. Our findings indicated that the current piscirickettsiosis surveillance programme based on risk-based sampling of five moribund or dead fish from 2 to 3 netpens is cost-effective and gives a high probability of detection of *P. salmonis* in Atlantic salmon farms in Chile at both the netpen and farm levels. Results from this study should incentivize salmon farmers to establish cost-effective strategies for early detection of *P. salmonis* infection and the application of this approach to other highly infectious diseases.

KEYWORDS

cost-effectiveness, population-level sensitivity, population-level specificity, qPCR, surveillance

1 | INTRODUCTION

Early detection of the bacterium *Piscirickettsia salmonis*, the aetiologic agent of piscirickettsiosis, also known as salmon rickettsial syndrome or salmonid rickettsial septicaemia (SRS), is an important goal of government- and industry-based surveillance in farmed Atlantic salmon in Chile (Sernapesca, 2019). Infection with *P. salmonis* causes annual losses of approximately 450 million USD (Camussetti et al., 2015), and is the primary reason for metaphylactic antibiotic use in farmed salmon (Cabello & Godfrey, 2019; Miranda et al., 2018). Regular surveillance testing usually starts one or two months after fish are stocked into marine netpens. Atlantic salmon typically becomes diseased with the *P. salmonis* within 1–6 months of seawater entry (Gaete-Carrasco et al., 2019; Jakob et al., 2014; Rees et al., 2014). This means that *P. salmonis* infection may establish early in the marine production cycle, negatively impacting the health and productive performance of survivor fish, and necessitating the use of antimicrobials (Smith & Mardones, 2020).

A production cycle of farmed Atlantic salmon lasts for about 14–18 months in Aysén and Los Lagos regions, whereas, in the Magallanes region, the production cycle is over 20 months. The prevalence of SRS in Los Lagos and Aysén regions is higher and might be explained by a combination of factors such as high farm density in the area/region, stocking density (i.e. 17 kg/m^3 for Atlantic salmon) and higher temperatures. In contrast, the low prevalence found in Magallanes could be due to the region's production and environmental conditions, such as the low number and/or geographical concentration of sea farms and low water temperature (Gaete-Carrasco et al., 2019). In Chile, regions where the water temperature is cooler (<10°C) may be at lower risk of disease (Rees et al., 2014) because of slower growth of *P. salmonis*.

Prevention and control of *P. salmonis* are challenging because vaccines and antibiotics treatments are reported to be of variable efficacy (Happold et al., 2020a, 2020b; Jakob et al., 2014; Maisey et al., 2017; Rozas-Serri & Enríquez, 2014; Rozas-Serri et al., 2019). Although the available vaccines have not prevented SRS in Chile, they have been shown to contribute to delayed onset of the first outbreak as well as reduce disease severity (Jakob et al., 2014; Rozas-Serri & Enríquez, 2014; Tobar et al., 2011). However, recent research has shown that there is limited benefit from the use of booster vaccinations against *P. salmonis* in the seawater phase of production (Happold et al., 2020b). The variable efficacy has been attributed to a combination of factors such as the pathogen's virulence and pathogenicity, the host's immune system and genetic resistance, as well as environmental factors and management practices (Rozas-Serri & Enríquez, 2014). In addition, several recent studies have shown that the gene expression associated with the fish immune response likely has an important effect on vaccine efficacy (Rozas-Serri et al., 2017, 2019; Rozas-Serri, Peña, Arriagada et al., 2018; Rozas-Serri, Peña, Maldonado 2018).

Although antibiotics are available to treat SRS, limited effectiveness of treatments has been reported in farmed salmonids (Price et al., 2016; Rozas-Serri & Enríquez, 2014). The main reason for poor treatment response is loss of appetite in *P. salmonis*-infected salmon which leads to insufficient consumption of medicated feed to reach therapeutic tissue concentrations of antibiotics (Price et al., 2018; Rozas-Serri & Enríquez, 2014); and factors such as antibiotic type, disease incidence in the population and time-to-treatment (Price et al., 2016; Rozas-Serri & Enríquez, 2014). Consequently, in order to achieve the successful SRS treatment and control, early detection of piscirickettsiosis is crucial.

In 2012, the Sanitary Programme of Control and Surveillance of SRS (PSVCSRS) was established by the National Fisheries Services or Sernapesca (Sernapesca, 2012). The objectives of the surveillance are to reduce the impact of SRS in Chile through early detection and timely and progressive implementation of measures to control early and advanced cases of SRS. The programme uses a risk-based approach that is focused on testing of recently dead and moribund fish (Oidtmann et al., 2013). Specimens are taken from a minimum of 15 fish (i.e. moribund or dead fish from 2 to 3 netpens) at each sampling and tested by real-time polymerase chain reaction (qPCR).

Diagnostic tests for infectious diseases in Atlantic salmon are mostly validated at the individual fish level rather than at an aggregate (population) level (*e.g.* netpen, farm site, company and management areas such as bays or regions established for disease control purposes). The latter are the epidemiological units to which interventions (*e.g.* mandatory movement restrictions, vaccination and antibiotic treatments) are applied. Exceptions where test validation is most relevant at the individual level include salmon broodstock (Laurin et al., 2019) and valuable ornamental fish species (*e.g.* koi carp) because intervention decisions are mostly done on a fish-by-fish basis.

The analogous measures of test accuracy at the population level are termed population sensitivity (*PopSe*) and population specificity (*PopSp*) which are functions of individual-level sensitivity (Se) and specificity (Sp), prevalence, sample size, correlation of test errors and cut-off number of positive test results to designate the population as positive or negative (Donald et al., 1994; Martin et al., 1992). Extrapolation of individual-level Se and Sp values to the population is predicated on having unbiased estimates of these parameters (Christensen & Gardner, 2000) and hence, it is important that values of Se and Sp represent the test performance for purposes of clinical diagnosis or surveillance testing. Similar to individual animal testing, *PopSe* and *PopSp* can be based on a single test or multiple tests where results are interpreted in series or parallel.

The objectives of the present study were to: (a) estimate the pen-level and farm-level sensitivities and specificity of qPCR testing for *P. salmonis* in Atlantic salmon for sample sizes between five and 30 fish, and (b) assess the cost-effectiveness of sampling frequency, sampling strategy effect (random versus risk-based sampling) and sample size to achieve 95% confidence of detecting *P. salmonis*. Findings from the study will be useful to the design of more cost-effective monitoring and surveillance strategies by integrating the value of the diagnostic tests and their costs which is a crucial research gap (Mardones et al., 2018) for a disease with enormous economic impact in the second-largest salmon farming country in the world.

2 | MATERIALS AND METHODS

2.1 | SRS surveillance in Chile

The programme uses a risk-based approach that is focused on testing of recently dead and moribund fish (Oidtmann et al., 2013). In freshwater farms, regular surveillance sampling is performed no sooner than 30 days (d) prior to the transfer of fish to sea farms. In seawater farms, the first sampling is carried out after a period of 30 days has elapsed following completed sea transfer, which usually lasts up to three months starting from the first date fish are stocked at sea. Subsequent sampling is performed every two months until the end of the production cycle. The exception is salmon farms located in the southernmost farming region of Magallanes (52.37°S, 70.99°W), where sampling is performed every four months because *P. salmonis* is rarely detected because the low water temperatures in such latitudes which limit survival of the bacterium (Gaete-Carrasco et al., 2019).

The surveillance programme also features a second component based on risk-based surveillance in which veterinarians should notify authorities and send samples to laboratory for analysis when farm show daily mortalities >0.05% for 5 days or 2% in up to 5 days, excluding sea lice and environmental mortalities. However, for the purposes of this study, we only evaluate the active surveillance component (regular surveillance).

Specimens such as brain, liver and muscle (when lesions are present) are taken from a minimum of 15 moribund or dead fish, from 2 to 3 netpens, at each sampling and tested by qPCR. The qPCR uses specific primers and probe reported by Karatas et al. (2008) to amplify the *P. salmonis* 16S rRNA gene. The qPCR testing was performed at the Pathovet laboratory, as described in Laurin et al. (2020), and cycle threshold (*Ct*) values lower than 33.01 were classified as positive. Laurin et al. (2020) estimated the qPCR Se and Sp in individual fish to be 85% and 97%, respectively, in early clinical and outbreak scenarios, using Bayesian latent class models.

2.2 | Model overview

We used a three-level hierarchical model (fish, netpen and farm with subscripts of *i*, *j* and *k*, respectively) to reflect the population structure of Atlantic salmon farmed in marine sites in southern Chile. Netpens typically contain approximately 50,000 fish and farms usually have 12 to 30 netpens per site often arranged in two or three groups of separate modules (Epivet, 2018). Recently, the salmon industry started to switch to larger netpens (40 × 40 m instead of 30 × 30 m) containing 90,000 fish. Maximum farming densities are set by authorities prior to stocking and depend on several factors (*e.g.* total number of stocked fish, expected growth and health performance of the prior cycles). The model was designed to estimate the pen-level and farm-level sensitivities and specificities of qPCR testing of salmon in sea netpens for *P. salmonis* for different sample size and prevalence scenarios.

The model was written in an Excel spreadsheet (Microsoft Corporation, Redmond,WA) using the risk-analysis add-in software @Risk (Version 7.5, Palisade Corporation, Newfield, NY). Monte Carlo simulations with 10,000 iterations were run for each scenario. Uncertainty was incorporated by randomly drawing input parameters from specified probability distributions. The list of variables and distributions used in the model is given in Table 1 and described in more detail in the following sections. Model outputs were pen-level sensitivity (*PenSe*) and specificity (*PenSp*); and farm-level sensitivity (*FarmSe*) and specificity (*FarmSp*).

2.3 | Pen-level sensitivity (*PenSe*) and specificity (*PenSp*)

First, we calculated *PenSe* and *PenSp* which are the probabilities of detecting *P. salmonis* in a netpen, if present, at or above a specified true

TABLE 1 Input values and distributions used to calculate the diagnostic sensitivity and specificity of qPCR, and sampling and laboratory costs of testing for *P. salmonis* at netpen and farm levels

Input	Value/distribution	Description	Source of data
Se_{qPCR}	Pert(0.6, 0.84, 0.99)	Sensitivity of qPCR test at fish level	Laurin et al., 2020
Sp_{qPCR}	Pert(0.98, 0.99, 1)	Specificity of qPCR test at fish level	Laurin et al., 2020
P_j	RiskUniform(0.02, 0.1)	Very low true within-pen prevalence scenario	Consensus of co-authors
	RiskUniform(0.11,0.3)	Low true within-pen prevalence scenario	
	RiskUniform(0.31,0.5)	Moderate true within-pen prevalence scenario	
	RiskUniform(0.51,0.7)	High true within-pen prevalence scenario	
N_{ij}	5,10, 15, 20, 25, 30	Number of fish tested from the j^{th} netpen	
N_{jk}	2 or 3	Number of netpens tested from the k^{th} farm	
$Sampling_{Gas}$	Pert(13.1, 74.2, 74.2)	Gas consumption and road tolls	Sampling costs from actual project
$Sampling_{Labour}$	Pert(126.2, 308.7, 342.2)	Labour (veterinarian and technical vet)	
$Laboratory_{Mat}$	Pert(7, 7.3, 11.6)	qPCR sample materials	
$Laboratory_{Tube}$	Pert(3.31, 3.7, 4.13)	Sampling tube, 70% ethanol (5 per fish)	
$Laboratory_{qPCR}$	Pert(11.7, 17.5, 22.8)	Cost of qPCR per sampling tube	

prevalence (P_j) and not detecting *P. salmonis* if absent from a netpen, respectively. A netpen was considered infected if at least one test-positive fish was detected but cut-off values of two or more positive fish were also explored. Since the proportion of the group of fish tested is small relative to the population size in a netpen, we used the binomial formula to estimate *PenSe* and *PenSp* (Christensen & Gardner, 2000; Martin et al., 1992). For an infected netpen ($P_j > 0$), the formula for a cut-off (threshold) value of ≥1 positive to class the j^{th} netpen as positive is.

$$PenSe_j = 1 - (1 - AP_j)^{N_{ij}} \quad (1)$$

where AP_j is the apparent (test) prevalence, and N_{ij} is the number of fish tested from the j^{th} netpen, where j is either 2 or 3. For each netpen, AP_j is a linear function of fish-level sensitivity (Se_{qPCR}) and specificity (Sp_{qPCR}) and true within-pen prevalence (P_j) as shown in Equation 2. Diagnostic test accuracy is assumed to be constant across netpens.

$$AP_j = (1 - Sp_{qPCR}) + (Se_{qPCR} + Sp_{qPCR} - 1) \times P_j \quad (2)$$

For a non-infected netpen ($P_j = 0$), the probability that a single fish will test negative for *P. salmonis* is Sp_{qPCR}. Assuming that individual-level specificity is a known and constant value for all fish in the netpen, the probability that all fish in a sample of size N_{ij} will test negative by qPCR is $Sp_{qPCR}^{N_{ij}}$. Note that when Sp = 1, *PenSp* also equals 1 and equation 1 simplifies to $PenSe_j = 1 - (1 - Se_{qPCR} \times P_j)^{N_{ij}}$. *PenSe* and *PenSp* were calculated (*i.e.* consensus of co-authors) assuming four categories of true within-pen prevalence: very low ($2\% < P_j \leq 10\%$), low ($10\% < P_j \leq 30\%$), moderate ($30\% < P_j \leq 50\%$) and high ($50\% < P_j \leq 70\%$; Table 1).

2.4 | Farm-level sensitivity (*FarmSe*) and specificity (*FarmSp*)

During surveillance sampling, there are situations where netpen identification is not recorded for sampled fish. In this case, *FarmSe* and *FarmSp* for the *k*th farm are calculated as in section 2.2 with the modification that N_{ij} is the total number of fish sampled across all netpens, and for infected netpens, P_j represents an average prevalence that does not account for clustering of infection among netpens. For example, an average prevalence of *P. salmonis* across 3 netpens might be obtained from 3 netpens each with prevalence of 50% or with prevalence that averages out at 50% (*e.g.* 10%, 50% and 70%). In practice, within-pen prevalence will differ especially if netpens were infected at different times and within-pen transmission rates are not constant.

When netpen identification is recorded for each sampled fish, $FarmSe_k$ is the product of the false-negative proportions (1- $PenSe_j$) for the sampled netpens ($j = 2$ or 3).

$$FarmSe_k = 1 - \prod (1 - PenSe_j) \quad (3)$$

and $FarmSp_k$ is the product of the respective *PenSp* for the sampled non-infected netpens.

2.5 | Likelihood ratios

In the context of a population test, we calculated the likelihood ratios to consider the magnitude and certainty of estimated *PenSe* and *PenSp*. The likelihood ratio (LR) for a positive pen-test result (*PenLR+*) reflects how much more likely infected netpens are to test positive compared with non-infected netpens (Caraguel et al., 2011). The likelihood ratio for a negative pen-test result (*PenLR–*) reflects how much less likely infected netpens are to test negative compared with non-infected netpens. An advantage of use of LR is that they can be interpreted regardless of the value of herd-level prevalence. *PenLR +* ranges from 1 to infinity, and the *PenLR–* ranges from 0 to 1. The higher (lower) the *PenLR* of a positive (negative) pen-test result, the more useful that result is as an indicator of true pen-level infection status.

$$PenLR+ = PenSe/(1 - PenSp) \quad (4)$$

$$PenLR- = (1 - PenSe)/PenSp \quad (5)$$

2.6 | Cost-effectiveness analysis

Cost-effectiveness analysis was done to identify the lowest cost sampling strategy (combination of sampling frequency, sample size and random versus risk-based sampling) to achieve at least 95% confidence of detecting *P. salmonis* at a farm level. The cost-effectiveness value (CEV) for each sampling strategy was defined as the ratio of total costs (C) to probability of detection (PD), *that is* CEV = C/PD.

Total costs included laboratory (median price of qPCR per fish including materials and sampling tubes), and transportation and labour costs for each sampling events (Table 1). All costs were calculated in 2020 Chilean pesos (CLP) and converted to 2020 US dollars (USD, conversion rate 1 USD = 803 CLP).

Two conditions were considered for the PD, the probability of detecting *P. salmonis* in a farm (*FarmSe*) and the temporal sensitivity (*TempSe*), which is the probability of detecting an agent in a given time period (Thurmond, 2003). Suppose there are two different scenarios, sampling fish at either 4-week or at 8-week intervals. If we assume that a marine netpen typically becomes diseased with *P. salmonis* within at least four weeks of seawater entry, the *TempSe* applying the 4-week sampling interval is 100%, whereas if the 8-week interval is used, the *TempSe* decreases to 50%. Hence, the shorter the interval between sampling events, the greater the probability to cover the minimum time for the development of the disease and, consequently, detecting early disease transition states (Thurmond, 2003).

The time interval used in the model was one week to allow flexibility in the frequency of sampling. We also assumed that surveillance would start one month after fish were put to sea and a minimum production cycle duration of 72 weeks. We used probability theory to combine *FarmSe*TempSe* into a single PD value for the *k*th farm.

$$PD_k = 1 - \prod (1 - FarmSe_k * TempSe) \quad (6)$$

Different scenarios were simulated based on a combination of sampling method (random versus risk-based sampling), sample size and sampling frequency (Figure 1).

3 | RESULTS

3.1 | Pen-level sensitivity (*PenSe*) and specificity (*PenSp*)

The *PenSe* at different true within-pen prevalence (P_j) of infection and number of fish tested (5, 10, 15, 20, 25 and 30) are presented in Table 2. *PenSp* is unaffected by true within-pen prevalence and depended only on the number of fish sampled in non-infected pens and the pen-level cut-off value (usually 1) to designate the netpen as positive. As the number of fish tested increased, *PenSe* increased and *PenSp* decreased. In contrast, as the netpen cut-off value increased from one to two, there was a corresponding increase in *PenSp* with a decrease in *PenSe*. In an infected netpen, *PenSe* increased substantially if P_j was very low (≤10%) or low (≤30%). Calculations are based on the assumption that 2% is the minimum detectable prevalence because this value is used as the threshold for surveillance to certify freedom from OIE-listed pathogens. In a high within-pen prevalence scenario, if a sample of 5 fish is taken, these data show, for instance, that with a cut-off number of positive fish set to 1 (to classify the netpen as positive), the median *PenSe* was 97% (95% PI: 92.6–99.2) compared to 82% (95% PI: 65.3–94.0) if the cut-off was 2.

Table 3 shows the number of samples, cut-off values, cost and cost-effectiveness values at different within-pen prevalence of infections, necessary to obtain *PenSe* of at least 90%, 95% or 99%, and *PenSp* of at least 90% for the detection of *P. salmonis*. The cost per individual fish tested using qPCR was USD $29 (median value estimated from the stochastic model).

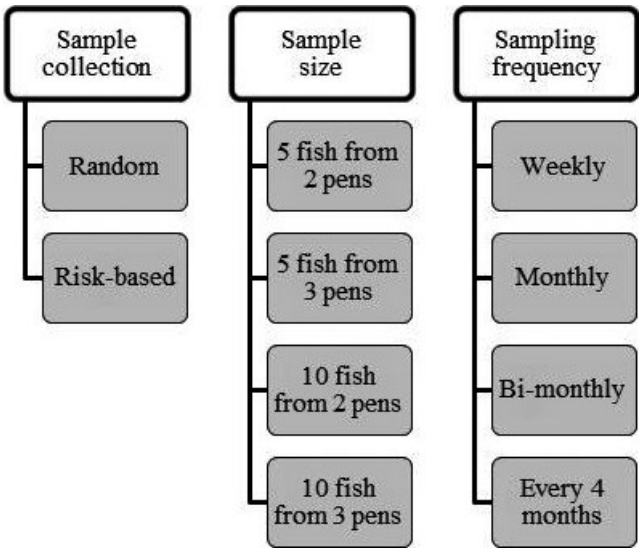

FIGURE 1 Sampling scenarios to compare the cost-effectiveness of alternative surveillance strategies

For example, if we assume the within-pen prevalence of *P. salmonis* is low (10%<P_j ≤ 30%), the median cost of testing to achieve a *PenSe* of at least 95% (Table 3) is $724 ($n$ = 25 fish per netpen). However, the minimum cost of testing is only $145 ($n$ = 5 fish) if moribund fish were sampled (risk-based sampling), assuming the prevalence is at least 50% in these fish. At very low within-pen prevalence (2%<P_j ≤ 10%), it is unlikely to be financially feasible to detect *P. salmonis* because the required sample size is too large (n = 102). The CEV is strongly influenced by within-pen prevalence, especially at very low prevalence (*i.e.* <10%) when it can be 20-fold higher than in high prevalence (>50%) scenarios. Therefore, sampling of moribund fish and/or fish with clinical signs is much more cost-efficient for *P. salmonis* detection than random sampling of apparently healthy fish. Use of risk-based sampling increased *PenSe* without decreasing *PenSp*. Figure 2 shows a scenario where a confirmatory test with perfect specificity (Sp = 1) is used to screening test-positive samples to obtain *PenSp* of 100%.

Positive (*PenLR+*) and negative likelihood ratios (*PenLR−*) varied with within-pen prevalence and the cut-off number of positive fish to designate the netpen as positive (Table 4). *PenLR* + was calculated as an indication of the strength of positive results. For example, for a sampling of 5 fish at moderate within-pen prevalence, a positive result is 18 times more likely to come from a netpen infected with *P. salmonis* than from a netpen without the infection. For the same sample size, the *PenLR−* means that a negative result is approximately 0.1 times as likely (*i.e.* 10 times less likely) to come from an infected netpen, than a netpen without the infection. The best approach should have the highest *PenLR* + and the lowest *PenLR−*. Thus, *PenLR* + and *PenLR−* at high within-pen prevalence indicate a great ability to discriminate between farms infected and not infected with *P. salmonis*, minimizing misclassification of pen-infection status. An advantage of use of LRs is that they can be interpreted regardless of the value of pen-level prevalence.

3.2 | Farm-level sensitivity (*FarmSe*) and specificity (*FarmSp*)

FarmSe and *FarmSp* at different combination of within-pen prevalence, number of fish sampled and number of netpens selected are presented in Table 5. *FarmSp* was unaffected by within-pen prevalence but varied from 90% to 86% depending whether 2 or 3 netpens were selected, respectively. At moderate and high prevalence, any of the sampling strategies (a combination of number of netpens within-farm and fish within-pen) was very likely to achieve a *FarmSe* of at least 95%.

Overall, sampling 5 fish from 2 netpens or 3 netpens (official sampling scheme) was more cost-effective than other sampling schemes (Table 5). At moderate and high prevalence, the median *FarmSe* reached 98% and 100%, respectively. However, sampling 5 fish from 2 netpens had better *FarmSp* (90%). Sampling 15 fish from each of 3 netpens was the least cost-effective for all prevalence scenarios, despite achieving a high *FarmSe*, even at very low within-pen prevalence.

TABLE 2 Comparisons of pen-level sensitivity (*PenSe*) and specificity (*PenSp*) among different sampling scenarios (sample sizes and true within-pen prevalence)

	True within-pen prevalence of infection				
Sample size	Very low [0.02–0.1]	Low [>0.1–0.3]	Moderate [>0.3–0.5]	High [>0.5–0.7]	*PenSp*
Cut-off = 1					
5	26 (12.6–39.7)	62 (41.4–79.2)	88 (78.0–94.3)	**97 (92.6–99.2)**	95 (92.2–98.1)
10	46 (23.7–63.7)	85 (65.7–95.6)	**98 (95.2–99.7)**	**100 (99.5–100)**	90 (85.0–96.3)
15	60 (33.5–78.1)	94 (80.1–99.1)	**100 (98.9–100)**	**100 (99.9–100)**	86 (78.3–94.5)
20	71 (41.2–86.8)	**98 (88.3–99.8)**	**100 (99.7–100)**	**100 (99.9–100)**	82 (72.1–92.7)
25	78 (48.7–92.2)	**99 (93.0–99.9)**	**100 (99.9–100)**	**100 (99.9–100)**	78 (66.5–91.0)
30	84 (55.1–95.1)	**100 (95.9–100)**	**100 (99.9–100)**	**100 (99.9–100)**	74 (61.2–89.2)
Cut-off = 2					
5	3 (1.0–7.6)	22 (8.3–41.0)	58 (37.1–74.4)	82 (65.3–94.0)	100 (99.7–100)
10	12 (2.7–25.0)	57 (26.8–79.8)	91 (75.8–97.7)	**99 (95.0–100)**	100 (99.0–100)
15	22 (5.9–42.9)	78 (45.2–94.1)	**98 (91.9–99.8)**	**100 (99.4–100)**	99 (97.4–100)
20	34 (9.7–58.5)	90 (61.4–98.4)	**100 (97.7–100)**	**100 (99.9–100)**	98 (96.0–100)
25	44 (14.0–71.0)	**95 (73.6–99.6)**	**100 (99.3–100)**	**100 (99.9–100)**	97 (93.3–99.8)
30	54 (19.0–79.5)	**98 (81.9–99.9)**	**100 (99.8–100)**	**100 (99.9–100)**	96 (90.7–99.6)

Note.: A cut-off of 1 or 2 positive individual tests was used to classify the population (netpen) as infected or not with *P. salmonis*. 95% prediction intervals for *PenSe* and *PenSp* are in parentheses. Sampling scenarios that reached a minimum *PenSe* of 95% are shown in bold font.

TABLE 3 Minimum number of samples (*N*), netpen cut-off (*c*), median cost of testing ($) and the cost-effectiveness values (CEVs) at different within-pen prevalence of infection, necessary to obtain a minimum pen-level sensitivity (*PenSe*) of at least 90%, 95% or 99%, and a minimum pen-level specificity (*PenSp*) of 90% for the detection of *P. salmonis*

	True within-pen prevalence of infection											
	Very low [0.02–0.1]			Low [>0.1–0.3]			Moderate [>0.3–0.5]			High [>0.5–0.7]		
PenSe and *PenSp*	*N* (c)	$	CEV[a]	*N* (c)	$	CEV	*N* (c)	$	CEV	*N* (c)	$	CEV
90%	87 (3)	2,523	28.0 (22–62)	12 (1)	348	3.9 (3–5)	6 (1)	174	1.9 (1–2)	4 (1)	116	1.3 (1–2)
95%	102 (3)	2,958	31.1 (25–60)	25 (2)	725	7.6 (6–9)	7 (1)	203	2.1 (2–3)	5(1)	145	1.5 (1–2)
99%	157 (4)	4,553	46.0 (38–60)	33 (2)	957	9.7 (8–12)	11 (1)	319	3.2 (3–4)	6 (1)	174	1.8 (1–2)

[a]95% prediction intervals for CEV are in parentheses.

Table 6 shows *FarmSe*, *FarmSp* and CEV results for the *k*th farm if we consider that *P. salmonis* infection does not cluster at the farm level, and netpen identification is not recorded for sampled fish. Sampling 10 fish from the *k*th farm achieved a *FarmSe* of at least 96% and a CEV of 3. Similarly, risk-based sampling of 5 fish from 2 netpens had a CEV of 2.9 but higher *FarmSe* (Table 5), especially if the selection of netpens is also risk-based (*e.g.* netpens having higher sea lice counts).

3.3 | Sampling frequency

Sampling 5 fish from 2 or 3 netpens, at moderate or high within-pen prevalence (risk-based sampling), was shown in section 3.2 to be the most cost-efficient sampling strategy to achieve a *FarmSe* of at least 95%. Considering that the key objective of the surveillance and control programme is to achieve early detection in order to initiate timely control measures, we explored different time frames for SRS development. To calculate the *TempSe*, we also explored alternative time frames for when a marine netpen typically becomes diseased with *P. salmonis* after seawater entry. Bi-weekly and monthly sampling achieved a median *TempSe* of 100% regardless of the disease time frame. However, *TempSe* decreased to 50% (25%) if sampling was carried out every 2 months (every 4 months) and if we assumed that a marine netpen typically becomes diseased with *P. salmonis* within four weeks of seawater entry. Sampling carried every two months (every four months) achieved median *TempSe* over 95% only in scenarios where a marine netpen became diseased with *P. salmonis* after 2 months (4 months) after seawater entry.

Figure 3 shows the cost-effectiveness values (CEVs) to achieve at least 95% probability of detection (PD) of *P. salmonis* associated with different sampling strategies and time frames for SRS development

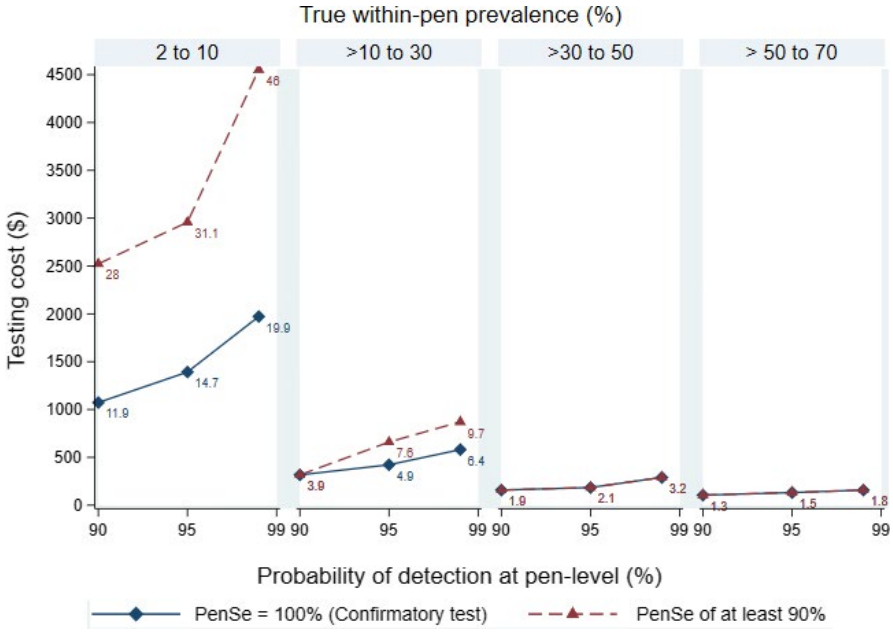

FIGURE 2 Cost-effectiveness values (CEVs), at different true within-pen prevalence of infection, necessary to obtain a minimum pen-level sensitivity (PenSe) of at least 90%, 95% or 99%.*Note*. The blue diamond represents the scenario where a confirmatory test with perfect specificity (Sp = 1) is used to screening test-positive samples to obtain*PenSp*of 100%. The red triangle represents a minimum pen-level specificity (*PenSp*) of at least 90% (red triangle) when no confirmatory test is used

TABLE 4 Descriptive statistics of expected numbers of positive test results predicted by the model and likelihood ratio for a positive netpen test (*PenLR+*) and for a negative netpen test (*PenLR−*) given several levels of true within-pen prevalence of infection with *P. salmonis*, sample size (N_{ij}) and different cut-off points

	Number of positive test results					Cut-off = 1		Cut-off = 2	
Inputs/outputs	Min	5th	50th	95th	Max	*PenLR+*	*PenLR−*	*PenLR+*	*PenLR−*
$N_{ij} = 5$									
Very low	0	0	0	0	0	5 (2–18)	0.8 (0.6–0.9)	32 (6–341)	1.0 (0.9–1.0)
Low	0	0	1	1	1	13 (6–43)	0.4 (0.2–0.6)	226 (51–2,568)	0.8 (0.6–0.9)
Moderate	1	2	2	3	3	18 (10–60)	0.1 (0.1–0.3)	574 (180–6,460)	0.4 (0.3–0.6)
High	1	2	3	3	4	20 (12–67)	0 (0–0.1)	843 (282–9,551)	0.2 (0.1–0.3)
$N_{ij} = 10$									
Very low	0	0	0	1	1	5 (2–15)	0.6 (0.4–0.8)	27 (5–277)	0.9 (0.8–1.0)
Low	0	1	1	2	3	9 (5–29)	0.2 (0.1–0.4)	131 (36–1,443)	0.4 (0.2–0.7)
Moderate	2	3	4	5	5	10 (6–34)	0 (0–0.1)	213 (74–2,332)	0.1 (0.0–0.3)
High	3	4	5	6	7	10 (6–35)	0	232 (83–2,571)	0
$N_{ij} = 15$									
Very low	0	0	0	1	1	4 (2–13)	0.5 (0.3–0.8)	23 (5–231)	0.8 (0.6–0.9)
Low	1	1	2	4	4	7 (4–22)	0.1 (0–0.2)	81 (26–823)	0.2 (0.1–0.5)
Moderate	3	4	5	7	8	7 (4–23)	0	102 (37–1,111)	0 (0–0.1)
High	5	6	8	9	11	7 (4–23)	0	104 (38–1,132)	0

Note: 95% prediction intervals for likelihood ratios are in parentheses.

(1, 2, 3 and over 4 months). If the time frame for SRS development is one or two months, targeted sampling of 5 fish from 2 or 3 netpens every 2 months would be the most cost-efficient strategy. If SRS time frame is more than three months, target sampling of 5 fish from 2 or 3 netpens every 4 month would be the most cost-efficient strategies. Consequently, risk-based sampling would be more cost-efficient to detect *P. salmonis* early in the agent's transmission.

Overall, bi-monthly risk-based sampling was the most cost-effective sampling strategy to detect *P. salmonis* early in the agent's transmission (first or second month after a period of 30 days has elapsed after sea transfer is completed).

On the other hand, the median PD of at least one infected cage in a farm over the production cycle was over 95% for all risk-based sampling strategies except if sampling was performed every four months (Table 7). However for random sampling, as sampling became more sporadic, PD was highly influenced by disease time frame. As expected, cumulative probability of detection decreased when the sampling frequency was more sporadic as did the cost-effectiveness value.

TABLE 5 Comparisons of farm-level sensitivity (*FarmSe*) and the cost-effectiveness values (CEVs) among different sampling scenarios for *P. salmonis* detection

Sampling			Within-pen prevalence							
			Very Low		Low		Moderate		High	
N_{ij}	N_{jk}	$	*FarmSe*	CEV	*FarmSe*	CEV	*FarmSe*	CEV	*FarmSe*	CEV
5	2	290	46 (24–63)	6 (4–13)	85 (68–95)	4 (3–5)	**98 (95–100)**	3 (2–4)	**100**	3 (2–4)
5	3	435	60 (36–77)	7 (5–14)	94 (82–99)	5 (4–6)	**100 (99–100)**	4 (3–5)	**100**	4 (3–5)
10	2	580	71 (41–87)	8 (6–14)	**98 (90–100)**	6 (5–7)	**100**	6 (5–7)	**100**	6 (5–7)
10	3	870	84 (59–95)	10 (8–16)	**100 (97–100)**	9 (7–10)	**100**	9 (7–11)	**100**	9 (7–11)
15	2	870	84 (56–95)	10 (8–16)	**100 (97–100)**	9 (7–11)	**100**	9 (7–11)	**100**	9 (7–11)
15	3	1,305	94 (74–99)	14 (11–20)	**100 (99–100)**	13 (11–15)	**100**	13 (11–16)	**100**	13 (11–16)

Note: "Very Low" prevalence (2% < P_j ≤ 10%); "Low" prevalence (10%< P_j ≤ 30%); "Moderate" prevalence (<30% P_j ≤ 50%); "High" prevalence (50%< i ≤ 70%); "N_{ij}" is the number of fish tested from the *j*th netpen; and "N_{jk}" is the number of netpens tested from the *k*th farm. Sampling scenarios that reached a minimum *FarmSe* of 95% are shown in bold font.

TABLE 6 Comparisons of farm-level sensitivity (*FarmSe*) and specificity (*FarmSp*), and the cost-effectiveness values (CEV) among different sampling scenarios for *P. salmonis* detection

N	Cost of testing	*FarmSe*	CEV	*FarmSp*
Netpen cut-off = 1				
5	145	80 (70.1–86.8)	2 (1.5–2.2)	**95 (91.8–98.5)**
10	290	**96 (91.0–98.3)**	3 (2.6–3.5)	90 (84.2–97.1)
15	435	**99 (97.4–99.8)**	4 (3.8–5.0)	86 (77.2–95.7)
20	580	**100 (99.2–100)**	6 (5.0–6.7)	82 (70.9–94.3)
25	725	**100 (99.8–100)**	7 (6.2–8.3)	78 (65.0–92.9)
30	870	**100 (99.9–100)**	9 (7.5–10.0)	74 (59.7–91.6)
Netpen cut-off = 2				
5	145	42 (29.3–53.9)	3 (2.6–5.0)	**100 (99.7–100)**
10	290	81 (66.6–89.7)	4 (3.0–4.5)	**100 (98.8–100)**
15	435	**95 (86.6–98.1)**	5 (3.9–5.4)	**99 (97.4–100)**
20	580	**99 (94.9–99.7)**	6 (5.1–6.8)	**98 (95.5–99.8)**
25	725	**100 (98.2–100)**	7 (6.3–8.4)	**97 (93.2–99.8)**
30	870	**100 (99.3–100)**	9 (7.5–10.0)	**96 (90.7–99.6)**

Note: "*N*" is the total number of fish tested from the *k*th farm. Sampling scenarios that reached a minimum *FarmSe* of 95% and/or *FarmSp* of 95% are shown in bold font.

It is noteworthy that, if confirmatory testing of qPCR-positive fish is not carried out, *FarmSp* would be strongly influenced by the sampling frequency, decreasing substantially over the months, resulting in a high number of false-positive results.

4 | DISCUSSION

Findings from the present study indicated that risk-based (targeted) sampling of Atlantic salmon in sea netpens using qPCR increased *PenSe* and *FarmSe* and decreased costs associated with misclassification of population status. Furthermore, sampling of recently dead and moribund fish, especially in subclinically infected populations, is much more cost-effective for *P. salmonis* detection than random sampling of apparently healthy fish. The median *FarmSe* varied from 46% (at very low true within-pen prevalence) to 100% (at moderate to high true within-pen prevalence) if 10–15 fish were sampled per farm. Thus, focusing on risk-based sampling increased *PenSe* (*FarmSe*) without decreasing *PenSp* (*FarmSp*) and without testing any more fish. Laurin et al. (2020) showed that preferential testing of fish with signs of petechial haemorrhages in internal organs can further enhance qPCR sensitivity for *P. salmonis* at netpen and farm levels. Risk-based sampling is a useful tool when detection of disease is the primary objective and there is prior knowledge of disease clustering by factors such as clinical signs (Oidtmann et al., 2013).

The current Chilean surveillance programme for piscirickettsiosis is based on a targeted sampling of a minimum of 15 fish (five recently dead and/or moribund fish from three netpens) at each sampling event (Sernapesca, 2012). Assuming the prevalence is at least 50% in these fish, the median *PenSe* was 97% and *FarmSe* was

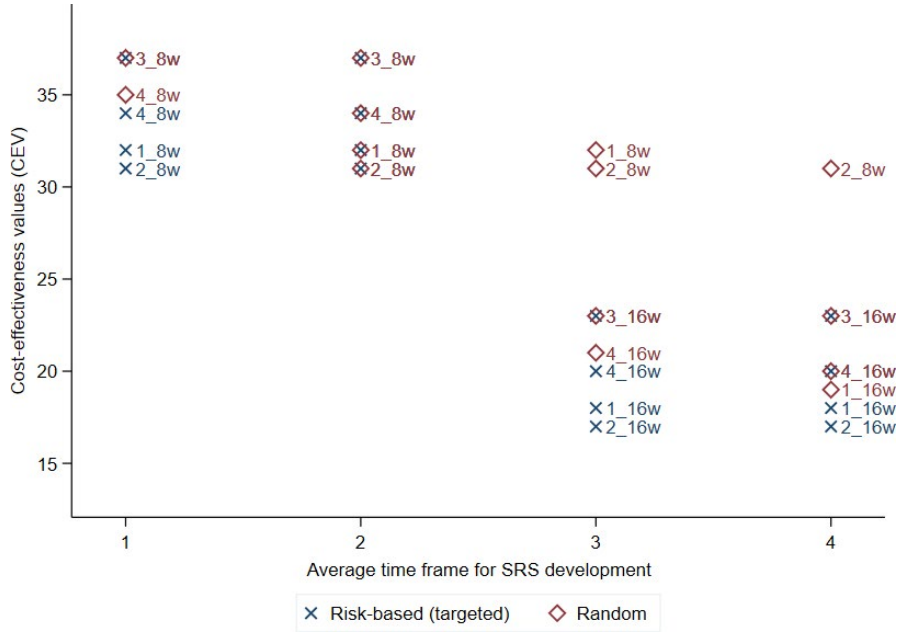

FIGURE 3 Cost-effectiveness values (CEVs) to achieve at least 95% probability of detection (PD) of P. salmonis associated with different sampling strategies and time frames for SRS development (1, 2, 3 and over 4 months).*Note*. Sampling strategies were a combination of sample size and sampling frequency. Sample size included th following: "1" = 5 fish from 3 netpens; "2" = 5 fish from 2 netpens; "3" = 10 fish from 3 netpens; and "4" = 10 fish from 2 netpens. Sampling frequency included the following: "4w" = monthly; "8w" = bi-monthly; and "16w" = every two months. For random sampling, the$P_j$was considered very low (≤10%), while for risk-based sampling, the$P_j$was considered high (≥50%)

TABLE 7 Cumulative probability of detection (PD) and cost-effectiveness value (CEV) to detect *P. salmonis* over the production cycle, considering risk-based and random sampling and different sampling frequencies

	Bi-weekly		Monthly		Bi-monthly		Every four months	
Sampling	**PD**	**CEV**	**PD**	**CEV**	**PD**	**CEV**	**PD**	**CEV**
Risk-based	100	217–415	100	109–208	100	51–98	68–100	26–72
Random	100	217–415	100	109–208	88–100	63–101	39–99	28–90

over 99.9% for detection of *P. salmonis*, as shown by our results. The high probability of detection is consistent with a prior study that suggested that the current surveillance programme for SRS in Chile is effective (Price et al., 2020). In a scenario where recently dead and moribund fish are not available on the day of the official surveillance sampling, a larger sample size (at least 45 fish per farm) would be required to achieve a *FarmSe* of 95%, considering that the expected prevalence would be very low (≤10%). However, sampling healthy fish is not economically feasible to detect *P. salmonis*.

Targeted sampling of ten fish (five fish from two netpens) was shown to be sufficient to achieve a *FarmSe* of 99% and it is more cost-effective than sampling of three netpens. The costs of testing per farm could be reduced by approximately $145 ($29 × 5), if sampling is performed from two netpens instead of three. Moreover, larger sample sizes provided more chance for erroneous false-positive results when *Sp* was less than 1. However, if we assume clustering of *P. salmonis* infection by netpens, it is expected that sampling from three netpens would have a higher *FarmSe* and more power to detect an infected farm, by increasing the number of animals and netpens sampled.

The use of a confirmatory test with very high specificity to screen qPCR-positive fish should be considered, since the consequences of falsely diagnosing a netpen as infected with *P. salmonis* (*i.e.* likely to be given antimicrobial treatment) and the consequences of falsely diagnosing a cage as uninfected with *P. salmonis* (*i.e.* late intervention would increase the likelihood of treatment failure) are equally important. Addition of a second test interpreted in series reduces the likelihood of a false positive but is not considered here.

For tests measured on a continuous scale, the selection of a cut-off used to classify an individual result as positive or negative also affects *PenSe* and *FarmSe* and is usually based on analytical and epidemiological considerations (Caraguel et al., 2011). In our study, we assumed a fixed cut-off at fish level (Laurin et al., 2020), thus *PenSp* and *FarmSp* depended only on the number of fish sampled in non-infected netpens and the pen-level cut-off value to designate the netpen as positive. Clearly, maximal netpen and farm sensitivities will be obtained if the cut-off value was set at 1.

PenLR + and *PenLR*− at high within-pen prevalence (risk-based sampling) indicate a great ability to discriminate between netpens infected and not infected with *P. salmonis*, minimizing pen-infected

status misclassification, and hence enhancing *PenSe*. If the pen-level cut-off was set at 1, we found a better balance between positive results (*PenLR*+ = 20) and negative results (*PenLR*− = 0), whereas for pen-level cut-off set at 2, much higher confidence was given to positive results (*PenLR*+ = 843) than to negative results (*PenLR*− = 0.2). Use of the magnitude of the test results for interpretation can reduce the risk of a result being a false positive (Gardner & Greiner, 2006). Where the costs of a false-negative result are much greater than the costs of a false-positive result, a lower cut-off (i.e. 1 instead of 2) is required to achieve a higher sensitivity. Conversely, if the cost of a false positive is relatively high, alternatives cut-off should be considered as well as protocol for managing false-positives results (*e.g.* retesting procedures).

Sampling performed every two months (official frequency for Chile, except in farms located in the Magallanes region, where it is performed every four months) was identified as the most cost-effective approach for early detection of *P. salmonis* (lowest CEV), considering that *P. salmonis* infection is established early in the production cycle (Smith & Mardones, 2020). However, if *P. salmonis* transmission dynamics was less than 2 months, we could miss the time window for early detection. Therefore, switching to a monthly targeted sampling of 10 fish per farm would cost approximately 60% more than the official sampling, which is bi-monthly targeted sampling of at least 15 fish. That translates into a difference of $1,500 per farm at the end of 6 months after surveillance started, which might not be economically feasible. However, SRS transmission dynamics is an important factor that should be taken into consideration when making decision about the sampling frequency.

Early detection is a key objective of the piscirickettsiosis surveillance programme in Chile (Estévez et al., 2019; Rozas-Serri & Enríquez, 2014) and might encourage farmers to increase their own surveillance effort (Price et al., 2020). Additionally, a risk-based approach is recommended for the (target) selection of netpens to increase *FarmSe*. For example, it is expected a higher probability of detection of *P. salmonis* in netpens having a history of sea lice infestation (Price et al., 2020), cumulative mortality rate (Jakob et al., 2014), SRS-attributed mortality (Hillman et al., 2020) and fouling of cages (Estévez et al., 2019). If we had more knowledge of the population dynamics of *P. salmonis* transmission, it would have allowed us to better explore cost-effectiveness of different sampling frequencies. Despite this limitation, our results showed that the current piscirickettsiosis surveillance programme has a high probability of detection of *P. salmonis* in Atlantic salmon farms in Chile.

Although cost-effectiveness analysis has been extensively used in economic assessments in human health (Babo Martins & Rushton, 2014), helping to guide decision-makers in integrating evidence in the allocation of scarce resources (Rushton, 2017), there are few studies (Lyngstad et al., 2010; Nérette et al., 2008) on cost-effectiveness of surveillance programmes in farmed aquatic animals for disease detection. To our knowledge, the present study is the first in aquatic animals that includes a stochastic model to account for uncertainty in costs, diagnostic test accuracy and within-pen prevalence when assessing the cost-effectiveness of longitudinal surveillance of other endemic diseases.

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CONFLICT OF INTEREST

There were no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study and not presented in the figures and tables are available upon reasonable request.

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