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Bayesian estimation of diagnostic sensitivity and specificity of a qPCR and a bacteriological culture method for *Piscirickettsia salmonis* in farmed Atlantic salmon (*Salmo salar* L.) in Chile

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Abstract

Early detection of piscirickettsiosis is an important purpose of government- and industry-based surveillance for the disease in Atlantic salmon farms in Chile. Real-time qPCRs are currently used for surveillance because bacterial isolation is inadequately sensitive or rapid enough for routine use. Since no perfect tests exist, we used Bayesian latent class models to estimate diagnostic sensitivity (*DSe*) and specificity (*DSp*) of qPCR and culture using separate two-test, single-population models for three farms (n = 148, 151, 44). Informative priors were used for *DSp* (culture (beta(999,1); qPCR (beta(98,2)), and flat priors (beta 1,1) for *DSe* and prevalence. Models were run for liver and kidney tissues combined and separately, based on the presence of selected gross-pathological signs. Across all models, qPCR *DSe* was 5- to 30-fold greater than for culture. Combined-tissue qPCR median *DSe* was highest in Farm 3 (sampled during *P. salmonis* outbreak (*DSe* = 97.6%)) versus Farm 1 (*DSe* = 85.6%) or Farm 2 (*DSe* = 83.5%), both sampled before clinical disease. Median *DSe* of qPCR was similar for liver and kidney, but higher when gross-pathological signs were evident at necropsy. High *DSe* and *DSp* and rapid turnaround-time indicate that the qPCR is fit for surveillance programmes and diagnosis during an outbreak. Targeted testing of salmon with gross-pathological signs can enhance *DSe*.

KEYWORDS

Bayesian latent class model, *Piscirickettsia salmonis*, piscirickettsiosis, qPCR, sensitivity, specificity

1 | INTRODUCTION

Piscirickettsia salmonis is the aetiologic cause of salmonid rickettsial septicaemia (SRS, also known as piscirickettsiosis) and a Gram-negative intracellular organism that is phylogenetically more similar to *Legionella*, *Francisella* and *Coxiella* species than to Rickettsia. *Piscirickettsia salmonis* was the first Rickettsia-like organism isolated from fish and has been shown to experimentally infect salmonid hosts, and the organism has also been detected in non-salmonid fish. (Fryer, Lannan, Giovannoni, & Wood, 1992; Rozas & Enríquez, 2014). Clinical signs and gross lesions of SRS are not pathognomonic, and evidence in Chile indicates that the clinical presentation at a population level in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) is highly variable (Rozas & Enríquez, 2014). *Piscirickettsia salmonis* isolates show high degrees of similarity, although the EM-90 isolate is phylogenetically separated

from LF-89 (Mauel, Giovannoni, & Fryer, 1999). LF-89-like and EM-90-like field isolates have genomic differences that may induce different degrees of virulence (Bohle et al., 2014) as well as differences in pathogenesis (Rozas-Serri et al., 2017) and immunity (Rozas-Serri, Peña, Arriagada, Enríquez, & Maldonado, 2018; Rozas-Serri, Peña, & Maldonado, 2018). For the last 8 years, the national fisheries and aquaculture service in Chile have implemented a surveillance and control programme for piscirickettsiosis with goals of early detection of the organism in Atlantic salmon in marine sites (Sernapesca, 2012).

Historically, isolation of *P. salmonis* on artificial media was considered to be the reference standard, but its lack of sensitivity as well as a culture time of approximately 2 weeks (Makrinos & Bowden, 2016) precludes its use in current piscirickettsiosis surveillance programmes. *Piscirickettsia salmonis* was previously considered to be cultivable only in eukaryotic cell lines (Birkbeck, Griffen, Reid, Laidler, & Wadsworth, 2004; Fryer et al., 1992; Lannan & Fryer, 1991), but several reports show that the bacterium may be cultured on cysteine-enriched agar media, verifying the facultative intracellular nature of this pathogen (Mauel, 2008; Mikalsen, Skjaervik, Wiik-Nielsen, Wasmuth, & Colquhoun, 2008; Gómez, Henríquez, & Marshall, 2009; Yañez et al., 2012, 2013). However, culture of the organism and in vitro tests are necessary to assess antimicrobial susceptibility and inform choice of an appropriate antibiotic for the treatment of SRS.

qPCR tests have mostly replaced bacterial culture for population diagnosis of *P. salmonis* and are commonly used techniques for prevalence estimation, risk factor studies and risk assessment related to movement of farmed salmon between hatcheries and marine sites. Immunohistochemistry, immunofluorescence (IFAT) and an enzyme-linked immunosorbent assay (ELISA) are useful for confirmatory diagnosis (Rozas & Enríquez, 2014). To our knowledge, there has been no formal validation study which has estimated the diagnostic sensitivity (*DSe*) and specificity (*DSp*) of bacterial culture or qPCR. Moreover, one of the key knowledge gaps identified in current epidemiological research of piscirikettsiosis is the need to have *DSe* and *DSp* estimates, as well as predictive values, both to develop new and to improve current diagnostic tests (Mardones et al., 2018).

The objective of the present study was to compare the *DSe* and *DSp* of a real-time qPCR protocol and a bacteriological culture method (on solid media) of liver and kidney specimens for *P. salmonis* detection, using Bayesian latent class modelling. There are many qPCR and culture tests in use in diagnostic laboratories providing services to the salmon industry in Chile, but this validation study was restricted to the accuracy of the qPCR and bacteriological culture tests used by the Pathovet laboratory which was participating in a prospective epidemiological study of *P. salmonis* incidence in Atlantic salmon farms. This study is reported based on guidelines for test accuracy studies in aquatic animals (Gardner et al., 2016) using Bayesian latent class models (Kostoulas et al., 2017).

2 | MATERIALS AND METHODS

2.1 | Source populations

For logistical and financial reasons, the study was restricted to two Atlantic salmon farms in the Región de los Lagos (41.92°S, 72.14°W) participating in an epidemiological study of SRS as implemented by research team members, and a third farm in the Región de los Lagos with a clinical outbreak of SRS in market-weight fish. The participating farms had a *P. salmonis* diagnosis in the previous production cycle, and, after a fallowing period of at least three months, all fish in the farms were vaccinated against *P. salmonis* (ALPHA JECT LiVac® SRS by Pharmaq) before stocking into seawater sites. This vaccine contains a live strain of *P. salmonis* that can be detected by qPCR (Karatas et al., 2008). Vaccination may induce qPCR-positive results for up to two days post-vaccination, but at one to two months later, all salmon will be negative (A. Peña, unpublished data).

2.2 | Sampling methods and specimen collection

Salmon were sampled prospectively for bacteriological culture and PCR from three marine sites: Farm 1 ($n = 148$) and Farm 2 ($n = 151$) with five collections per farm (approximately 30 fish per collection time per farm) between February 20 and April 10, 2019; and Farm 3 which had a single sampling of 44 moribund or recently dead fish for both bacteriological culture and PCR testing on 29 October 2018. None of the sampled fish had received antibiotics against *P. salmonis* infection. For qPCR testing of samples from Farms 1 and 2, the goal was to sample a total of 10 live fish (collected with a catch net: four fish from one pen and three fish each from two pens) as well as five freshly dead fish (i.e. two, two and one from the same pens as the live fish collections) as part of an epidemiological study of *P. salmonis*. The targeted sample sizes for both diagnostic methods were 150 each for Farms 1 and 2, which allowed at least 95% confidence that the expected *DSe* of qPCR (80%) and bacterial culture (20%) would differ (frequentist calculation). There was no predetermined sample size for Farm 3 as logistical considerations with shipping large salmon to the laboratory needed to be managed. Live fish were killed with an overdose of benzocaine 20% using 1ml/litre of water (AVMA, 2013).

A veterinarian examined organs and tissues of fish for evidence of first gross signs of SRS (i.e. petechial haemorrhages in liver and muscles). Samples of liver and anterior and posterior kidney were removed using sterile instruments from fish on-site and were shipped on ice in separate sterile containers within five hours of collection to the Pathovet laboratory in Puerto Montt for testing. Samples were processed within 24 hr of arrival at the laboratory, which has Nch-ISO-IEC-17025:Of2005 accreditation (LE 1,364 to LE 1,366) and a quality management system for qPCR testing; testing was done as part of routine diagnostic workflows. Bacteriological culture and qPCR testing were done independent of knowledge of the other test's results.

2.3 | Bacteriological culture

Culture was preferentially done from lesioned areas in liver and kidney, whenever possible, using heat-sterilized loops that were dipped in ethanol. Agar plates with Austral-TSHem medium (Yañez et al., 2012) were used for isolation of *P. salmonis* and were incubated at 18°C for 21 to 28 days. To confirm that *P. salmonis* was the organism growing at the end of incubation, suspicious colonies were picked from the agar plate and suspended in 20 µl molecular biology grade water for qPCR analysis.

2.4 | qPCR

The qPCR evaluated in the present study used specific primers and probe reported by Karatas et al. (2008) to amplify the *P. salmonis* 16S rRNA gene. The assay had been previously validated analytically to the end of stage 1 of the World Organisation for Animal Health validation pathway (OIE, 2019) with no evidence of cross reactions with other freshwater and marine bacterial agents (*Renibacterium salmoninarum*, *Vibrio ordalli*, *Yersinia ruckerii*, *Flavobacterium psychrophylum* and *F. columnare*, *Tenacibaculum maritimum* and *T. dicentrarchi)* as well as viruses (infectious salmon anaemia, infectious pancreatic necrosis and piscine orthoreovirus) which are known to infect Atlantic salmon in Chile. The qPCR detects the two main strains (LF-89 and EM-90) circulating in salmonids in Chile and has an estimated limit of detection of 100 copies of plasmid containing the qPCR target (16S rRNA gene) sequence (Karatas et el., 2008) as well as good repeatability of duplicate samples. Testing by three operators in the Pathovet laboratory yielded coefficients of variation (CV) between 0.08% and 0.29% for repeatability.

For the diagnostic validation (stage 2 of OIE pathway), DNA was extracted from kidney and liver samples using the EZNA Tissue DNA kit (Omega Bio-Tek) according to the manufacturer's instructions. The qPCR assays were carried out using the StepOne Plus Real-Time qPCR System. The qPCR was performed using a total volume of 15 µl for each sample, containing 2X KAPA PROBE FAST qPCR Master Mix Universal, 300 nM of each primer, 200 nM of probe, 0.3 µl of 50X ROX High and 2 µl DNA of each sample. The qPCRs were carried out in the StepOne Plus Real-Time qPCR System (Thermo Fischer Scientific) using the following parameters: 95°C for 3 min for initial denaturation, 95°C for 1 s and 60°C for 20 s for 40 cycles. A positive control (*P. salmonis* DNA), a negative control without DNA and negative extraction control were also included in every run. The qPCR for confirming colonies of *P. salmonis* used the same conditions except for 2 µl of suspended bacteria as template. In the case of tissues, all samples were run in single wells (as for surveillance), but in the case of the colony qPCR, they were run in duplicate. Cycle threshold (Ct) values were recorded (run to a maximum of 40 Ct), and a Ct of < 33.01 was considered positive, and negative if otherwise.

The cut-off Ct of 33.01 was determined based on results of 1:5 serial dilutions of a *P. salmonis* positive DNA sample including two dilutions after the qPCR displayed negative results. Ten technical replicates were run for each dilution. The initial cut-off was the first endpoint dilution showing 100% positive Ct values. The initial endpoint dilution was then subjected to 1:2 serial dilutions followed by qPCR. The cut-off Ct was calculated as the average Ct of the 10 replicates at the endpoint dilution which showed 100% positive Ct results.

2.5 | Statistical analysis

Two-way tables were created to compare test results overall, and then by tissue and whether gross signs of SRS were identified or not at necropsy. For qPCR Ct values, boxplots were created based on the presence or absence of gross pathologic signs and by site. Stata/SE 15.0 (College Station, Texas) was used for descriptive analyses.

For evaluation of diagnostic accuracy estimates (*DSe*, *DSp*) and true prevalence, Bayesian latent class models (BLCM) were used to obtain posterior median estimates of these parameters with their 95% probability intervals (PI). The latent (unknown true) state being modelled is infection with *P. salmonis*. Counts of the joint test results from the two-way tables were used as data inputs for BLCM, which were run in OpenBugs 3.2.3 (Lunn, Spiegelhalter, Thomas, & Best, 2009). Separate conditionally independent BLCM were created for the three populations (farms) and two tests (*P. salmonis* culture and qPCR), following the BLCM code described by Branscum, Gardner, and Johnson (2005). Although both tests detect organisms (cultivable bacteria and genomic material in live/dead bacteria, respectively), they have different limits of detection; therefore, the assumption of conditional independence is reasonable (Assis, de Oliveira, Gardner, Figueiredo, & Leal, 2017). This was verified by comparing results of conditional independence and conditional dependence models. The former is a special case of the dependence model in which the sensitivity covariance (correlation) of bacteriological culture and qPCR is zero. OpenBUGS code is listed in the Appendix A.

For each farm, nine separate models were run: three per tissue type (liver and kidney combined, liver alone and kidney alone) and for each of the three subpopulations created from the gross observations at necropsy (signs present, signs absent and ignoring that information). All models were run with three chains, with dispersed starting values and 100,000 iterations (the first 10,000 discarded as burn-in) of the Gibbs sampler for each chain. In addition to *DSe* and *DSp* and prevalence, functions of these parameters (*e.g.* $DSe_1 - DSe_2$) can be calculated at each iteration. The STEP function in OpenBUGS then assigns a 1 if $DSe_1 - DSe_2$ is positive and a 0 if the difference is negative. The proportion of 1's across all iterations can be interpreted as the probability (P) that a test has a higher *DSe* than another test, where $P = 1$ indicates certainty and $P = .5$ indicates no difference in *DSe*. Visual evaluation of model convergence was assessed based on history and Gelman–Rubin (brg) plots. Autocorrelation plots were also examined to determine whether thinning of iterates was needed.

2.6 | Prior distributions for the BLCM

For single-population BLCM, there are five parameters (*i.e.* two *DSe*, two *DSp* and prevalence) to be estimated and three degrees of freedom. Hence, to make the model identifiable (have a unique combination of parameters), prior information must be placed on two parameters. We used a beta (999,1) prior for *DSp* of bacteriological culture to allow for rare false positives caused by cross-contamination or mislabelling of tissues (one error in 1,000 analyses of non-infected specimens) as used for *Salmonella* spp. culture in pigs (Mainar-Jaime, Atashparvar, & Chirino-Trejo, 2008). For the *DSp* of qPCR, we used a beta 98,2 prior based on expert opinion of a co-author (A. Peña), who believed that in 100 non-infected samples processed, there would be a maximum of 2 false positives with a most likely value of between one false-positive sample in 100 (beta 99,1) and one false-positive sample in 1,000 (beta 999,1). The priors were based on her experience testing samples from marine sites not known to be infected with *P. salmonis* based on a combination of diagnostic criteria, and negative qPCR results in non-infected fish prior to stocking into marine net-pens where exposure to *P. salmonis* occurs.

We used the conservative value of beta (98,2) as the *DSp* for all initial model runs and then did sensitivity analyses with the other beta priors (99,1 and 999,1) to assess how influential the *DSp* prior was on posterior inferences. We also down-weighted the beta (98,2) prior fourfold to a beta (24.5, 0.5) to allow for more spread (median = 99%; 95% probability interval of 90.2 to 99.99%). Flat (beta 1,1) priors were used for the two *DSe* and for prevalence in each model.

3 | RESULTS

Early gross signs of piscirickettsiosis (*i.e.* petechial haemorrhages in liver or muscle) were evident at necropsy in 101 (68.2%), 70 (46.3%) and 20 (45.5%) fish from Farms 1, 2 and 3, respectively. Descriptive data for participating farms are in Table 1. PCR detected SRS in both farms at the first sampling, culture first detected SRS at the second sampling which was seven and six days later for Farms 1 and 2, respectively.

3.1 | Descriptive analyses

The percentages of positive test results (positive on qPCR or bacteriological culture on Austral-TSHem agar) for Farms 1, 2 and 3 were 55.4%, 17.9% and 97.7%, respectively. All farms had at least three culture-positive results, but overall, there were 11-fold fewer culture positives ($n = 14$) compared to qPCR-positive results ($n = 152$). Of the 14 culture-positive kidney and liver samples, qPCR Ct values ranged from 15.67 to 30.08 with a median of 23.92. There were 138 samples that were qPCR positive when culture was negative, but no qPCR-negative results when culture was positive, regardless of tissue type. The joint results of both bacteriological culture and qPCR of kidney and liver (positive–positive, positive–negative, negative–positive and negative–negative) and by gross-pathological signs (present or absent) and across farm sites are shown in Table 2A, and those for kidney tissue alone are in Table 2B. Liver results are presented in Appendix S1.

In Figure 1, we present Ct values from qPCR testing of different tissue types, across sites and by the presence or absence of gross signs of piscirickettsiosis. We included as a reference line, the Ct cut-off from the laboratory (33.01 Ct) for qPCR. However, in some cases, there were a number of samples with Ct values in the high 30s that might be considered positive if the Ct cut-off was increased. Furthermore, there were almost all positive results (with lower Ct levels, which would equate to higher load of the bacterial analyte) for Farm 3, but the opposite for Farm 2.

3.2 | Comparison of test accuracy

In Table 3, we show the BLCM results (median and 95% PI) for *DSe* and *DSp* of bacteriological culture and qPCR and true prevalence

TABLE 1 Production, mortality and environmental data for the three farms included in the test validation study of qPCR and bacteriological culture for piscirickettsiosis in Atlantic salmon in Chile

Variable	Farm 1	Farm 2	Farm 3
Total fish on farm	1,030,377	2,285,200	879,960
Number of cages on farm	12	20	28
Production week when SRS first detected by qPCR	13	30	14
Production week when SRS first detected (onset of 1st mortality)	21	30	14
Weekly mortality (%) when SRS was first detected	0.77	1.12	1.06
Stocking density (kg per cubic metre) when SRS first detected	5.5	12	1.31
Weight of fish at first treatment for SRS (kg)	1.13	1.95	1.2
Water temperature (^{O}C) at 5 m depth	11	12	14
Salinity (parts per thousand)	32	29	28

TABLE 2 Two-way tables of bacteriological culture and qPCR test results for kidney and liver in combination, assuming a positive on either tissue is positive (a) and for kidney tissue alone (b), across sites and whether gross signs of piscirickettsiosis were visible at post-mortem

			All fish		Gross signs + (191 fish)		Gross signs – (152 fish)	
			qPCR		qPCR		qPCR	
			+	–	+	–	+	–
(a) Kidney and liver tissues combined								
Farm 1 (148 fish)	Culture	+	**3**	0	**3**	0	**0**	0
		–	79	66	64	34	15	32
Farm 2 (151 fish)	Culture	+	**3**	0	**3**	0	**0**	0
		–	24	124	14	53	10	71
Farm 3 (44 fish)	Culture	+	**8**	0	**4**	0	**4**	0
		-	35	1	16	0	19	1
(b). Kidney tissue only								
Farm 1 (148 fish)	Culture	+	**1**	0	**1**	0	**0**	0
		–	71	76	56	44	15	32
Farm 2 (151 fish)	Culture	+	**2**	0	**2**	0	**0**	0
		–	18	131	13	55	5	76
Farm 3 (44 fish)	Culture	+	**4**	0	**0**	0	**4**	0
		-	39	1	20	0	19	1

Note: + (positive), – (negative).

The bold values are to highlight the values when both tests are positive for easier readability.

for the three farms. All models were based on farm-specific results of the joint test results. History and quantile plots and brg traces showed no evidence of lack of convergence of chains. Model estimates were similar for the conditional dependence and independence models, and probability intervals for the sensitivity covariance included zero. Hence, we reported all results assuming conditional independence of the two tests. Within-farm true prevalence based on results of kidney and liver samples interpreted in parallel was 63.8% (Farm 1), 19.8% (Farm 2) and 97.3% (Farm 3).

Overall, the *DSe* of qPCR was substantially higher than bacteriological culture, ranging between fivefold and 30-fold greater depending on model and 100% probability that qPCR was more sensitive than culture. For Farms 1 and 2, testing of both kidney and liver samples together increased the median *DSe* by about 5 to 8% compared to the use of kidney or liver samples alone. The wide and overlapping PI for these comparisons shows that the increase was not different from zero. The increase was not evident in Farm 3 which was tested during a clinical outbreak. Similarly, testing of lesioned tissues (kidney alone or combined kidney and liver) increased the median *DSe* by 16 to 30% compared with testing of fish without gross lesions (Table 3), with wide and overlapping PI. Model inferences for liver specimens are in Table S2.

The posterior distributions for *DSp* for culture and qPCR mostly reflected the prior distributions. Sensitivity analysis of a change in the qPCR *DSp* prior from beta (98,2) to beta (99,1) and (999,1) resulted in minimal changes (<1%) in the *DSe* and prevalence values. Similarly, the down-weighted prior (beta 24.5,0.5) resulted in wider PI but very similar medians to the original prior. Effects of changes in *DSp* of qPCR on posterior inferences are presented in Table S3.

The use of a two-test, two-population model (Branscum et al., 2005) for estimation of the *DSe* and *DSp* in Farms 1 and 2 (surveillance samples) yielded slightly higher *DSe* median estimates for qPCR (e.g. combined kidney and liver results of 89.3% versus 85.6% for Farm 1 and 83.5% in the farm-specific models) when beta (98,2) or beta (24.5,0.5) was used for the *DSp* prior of qPCR. The median *DSe* of bacteriological culture was 5.4% for combined kidney and liver results in the two-test, two-population model compared with farm-specific values of 3.7% and 11.6%. The conclusion that *DSe* of qPCR was superior to *DSe* of bacteriological culture was made with 100% certainty (based on results of the OpenBUGS STEP function) in all sensitivity analyses.

4 | DISCUSSION

The purpose of the present study was to use Bayesian LCM to estimate the *DSe* and *DSp* of qPCR for purposes of surveillance and diagnosis of piscirickettsiosis during an outbreak compared with bacteriological culture on solid media, an imperfect reference test with low *DSe* but perfect *DSp*. Other tests such as immunofluorescence (IFAT) could have been chosen as comparator tests, but we selected bacteriological culture because it has a *DSp* of 100% and is thus suitable for confirmatory diagnosis. In addition, antimicrobial sensitivity testing of *P. salmonis* isolates can be done using plate colonies, and phylogenetic or genomic characterization is facilitated with entire organisms.

All study farms had at least three culture-positive samples for *P. salmonis*, with a higher percentage (18.2%) in the outbreak farm

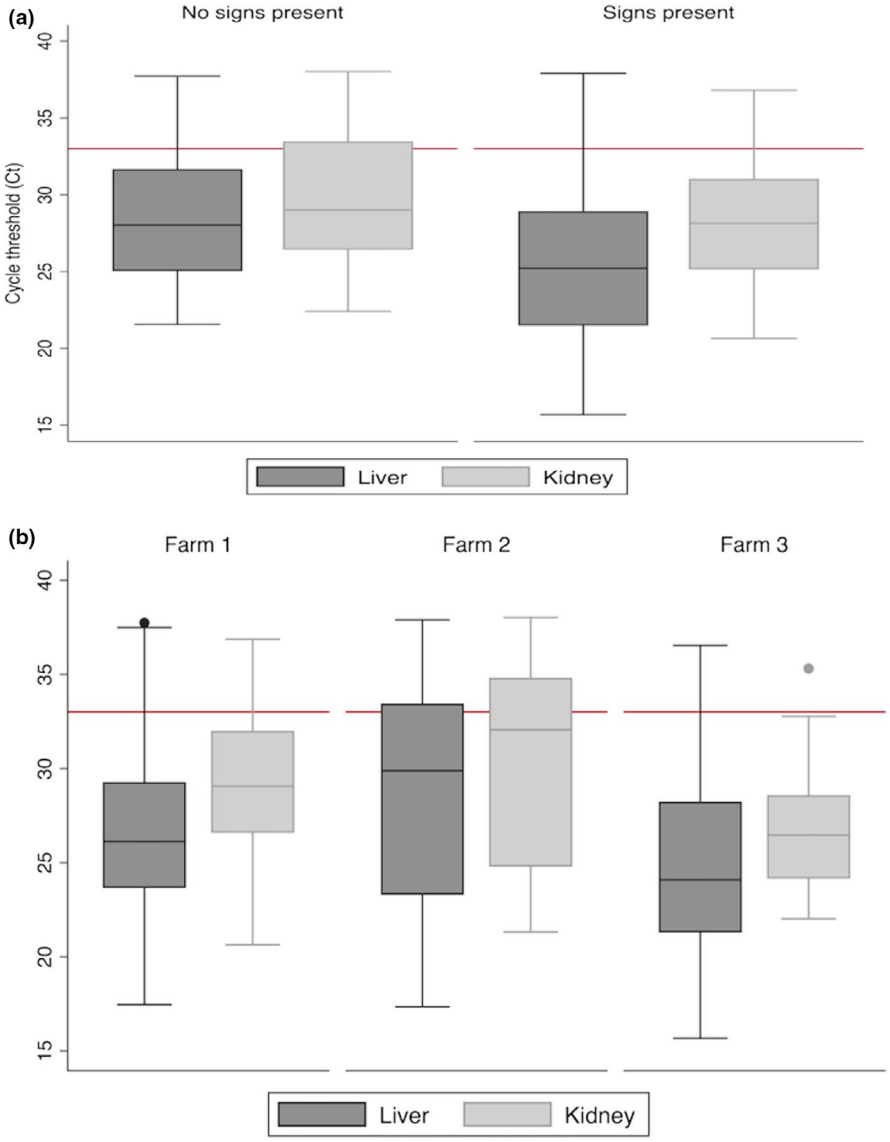

FIGURE 1 Boxplot of Ct values for qPCR by tissue type and presence or absence of gross signs of salmonid rickettsial septicaemia at post-mortem (a) and across sites (b). The y-axis reference line is the laboratory's Ct cut-off (33.01) [Colour figure can be viewed at wileyonlinelibrary.com]

than in the other two farms (2.0%). However, for Farms 1 and 2, there were no culture positives until the second sampling, approximately one week later than the first sampling when qPCR was already detecting SRS in these farms. Data from Farms 1 and 2 reflected surveillance scenarios, but Farm 3 was experiencing a clinical outbreak. Accordingly, the data for Farm 3 were analysed separately from Farms 1 and 2, as *DSe* for both culture and qPCR was expected to be higher on Farm 3 than on the other two farms. A critical assumption of constant *DSe* across populations would have been breached if a two-test, three-population model had been used, as evidenced by higher *DSe* in Farm 3 (Table 3).

The use of Bayesian LCM is the method of choice for estimation of *DSe* and *DSp* for infectious diseases in field samples because no single test is perfect. A LCM allows demonstration of the superior accuracy (*DSe*, *DSp* or both) of one test over another. Prior examples of its use for other bacterial infections in fish include *R. salmoninarum* in broodstock and market-weight salmon (Jaramillo, Gardner, Stryhn, Burnley, & Hammell, 2017; Laurin et al., 2019) and *Francisella noatunensis* subsp. *orientalis* in Nile tilapia (Assis et al., 2017). A critical component of a Bayesian analysis is the inclusion of prior information based on the knowledge of subject-matter experts. For this analysis, the most reasonable choice was to use prior distributions of *DSp* of culture and qPCR based on precedents established from prior studies and data sourced from non-infected Atlantic salmon populations in Chile. Posterior inferences changed minimally with changes in the qPCR prior indicating the robust nature of estimates from the Bayesian LCM.

The median *DSe* of qPCR and bacteriological culture was higher (97.6% and 19.4%, respectively) during the outbreak in Farm 3 than in the other two farms where surveillance samples were evaluated. The finding of superior *DSe* of qPCR compared with culture is in agreement with prior studies of *R. salmoninarum* in market-weight Atlantic salmon (Jaramillo et al., 2017) and francisellosis in Nile tilapia (Assis et al., 2017), and the present study provides evidence that sampling of lesioned tissue enhances *DSe* for surveillance purposes.

The main limitation of the study was the limited number of study sites, which impacted generalizability of the *DSe* estimates. The

TABLE 3 Bayesian latent class model results for true prevalence of *P. salmonis* for three farms and diagnostic sensitivity and specificity for two tests (bacteriological culture and qPCR). Results are median posterior estimates with 95% probability intervals in parenthesis

	Tissue	Gross signs	True prevalence	Diagnostic sensitivity		Diagnostic specificity	
				Culture	qPCR	Culture	qPCR
Farm 1	Liver and kidney	Both[a]	0.638 (0.499–0.947)	0.037 (0.011–0.090)	0.856 (0.570–0.994)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
		Present	0.750 (0.599–0.974)	0.047 (0.014–0.111)	0.873 (0.648–0.995)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
		Absent	0.475 (0.234–0.947)	0.028 (0.001–0.163)	0.647 (0.289–0.982)	0.999 (0.996–1.000)	0.982 (0.943–0.997)
	Kidney only	Both	0.613 (0.439–0.961)	0.017 (0.002–0.061)	0.778 (0.487–0.990)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
		Present	0.688 (0.505–0.974)	0.023 (0.003–0.079)	0.806 (0.546–0.991)	0.999 (0.996–1.00)	0.983 (0.944–0.997)
		Absent	0.475 (0.234–0.947)	0.028 (0.001–0.163)	0.647 (0.289–0.982)	0.999 (0.996–1.000)	0.982 (0.943–0.997)
Farm 2	Liver and kidney	Both	0.198 (0.111–0.425)	0.116 (0.031–0.288)	0.835 (0.384–0.993)	0.999 (0.997–1.000)	0.982 (0.940–0.997)
		Present	0.276 (0.152–0.567)	0.177 (0.049–0.409)	0.835 (0.393–0.993)	0.999 (0.996–1.000)	0.982 (0.942–0.997)
		Absent	0.193 (0.038–0.834)	0.038 (0.001–0.311)	0.538 (0.123–0.976)	0.999 (0.996–1.000)	0.981 (0.934–0.997)
	Kidney only	Both	0.148 (0.061–0.403)	0.112 (0.021–0.350)	0.784 (0.285–0.991)	0.999 (0.997–1.000)	0.981 (0.937–0.997)
		Present	0.257 (0.128–0.631)	0.136 (0.028–0.373)	0.786 (0.311–0.991)	0.999 (0.996–1.000)	0.982 (0.941–0.997)
		Absent	0.074 (0.003–0.641)	0.098 (0.002–0.808)	0.461 (0.045–0.971)	0.999 (0.997–1.000)	0.978 (0.940–0.997)
Farm 3	Liver and kidney	Both	0.973 (0.890–0.999)	0.194 (0.097–0.324)	0.976 (0.896–0.999)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
		Present	0.967 (0.836–0.999)	0.219 (0.083–0.419)	0.967 (0.838–0.999)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
		Absent	0.950 (0.811–0.998)	0.189 (0.070–0.369)	0.957 (0.820–0.998)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
	Kidney only	Both	0.973 (0.892–0.999)	0.104 (0.037–0.215)	0.975 (0.896–0.999)	0.999 (0.996–1.000)	0.983 (0.945–0.997)
		Present	0.967 (0.836–0.999)	0.033 (0.001–0.161)	0.967 (0.839–0.999)	0.999 (0.996–1.000)	0.983 (0.944–0.997)
		Absent	0.950 (0.811–0.998)	0.189 (0.070–0.369)	0.957 (0.820–0.998)	0.999 (0.944–1.000)	0.983 (0.944–0.997)

[a]Regardless of the presence or absence of gross-pathological signs.

three sites were owned by two different salmon-farming companies and thus represented some management variability. In addition, the sample size was insufficient in the two farms with surveillance samples to detect differences across subpopulations (gross lesions present or absent). For financial reasons, the study did not evaluate qPCR protocols used in other diagnostic laboratories in Chile or other methods of bacteriological culture. There was no testing of samples for other bacteria or viruses as the purpose of the study was validation of tests rather than assessing the possible role of co-infections or other infectious agents as a cause of mortalities.

The Karatas et al. (2008) qPCR for *P. salmonis* detection has been used by Pathovet for 6 years, and qPCR results are typically available within 24 hr for population-level diagnosis. Bacteriological culture may still be necessary on some farms as a confirmatory test, but the two-week time lag for results means that culture has minimal utility for making management decisions. The costs of qPCR and culture were approximately $US 20.25 each at the time of testing. Up to five samples (tissues or fish) can be cultured on a single Austral-TSHem agar plate, but suspect *P. salmonis* colonies need confirmation by other techniques, commonly by qPCR. In conclusion, the study findings indicate that the qPCR assay is fit for the purpose of presumptive diagnosis and surveillance for detection of *P. salmonis* cases in endemically infected regions of Chile. Compared with testing of only kidney samples,

testing of both kidney and liver is unlikely to be cost-effective as costs are doubled and the incremental improvement in *DSe* is small over testing of kidney alone.

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CONFLICT OF INTERESTS

There were no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX A

OpenBUGS code for Bayesian latent class model of qPCR (PCR) and bacteriological culture (Bac) accuracy for *P. salmonis* in three Atlantic salmon farms. For each farm, nine models (three tissues with three gross sign categories for each tissue) were run: liver and kidney combined, kidney only, and liver only, fish with gross signs, fish without gross signs and ignoring gross signs.

```
model
{
y [1:4] ~ dmulti(p[1:4], n)
p[1] <- pi*(SePCR*SeBac + CovSePCRBac) + (1-pi)*(1-SpPCR)*(1-SpBac)
p[2] <- pi*(SePCR*(1-SeBac) - CovSePCRBac) + (1-pi)*(1-SpPCR)*SpBac
p[3] <- pi*((1-SePCR)*SeBac - CovSePCRBac)+ (1-pi)*SpPCR*(1-SpBac)
p[4] <- pi*((1-SePCR)*(1-SeBac) + CovSePCRBac) + (1-pi)*SpPCR*SpBac
SePCR ~ dbeta(1,1)
SpPCR ~ dbeta(98,2) # sensitivity analyses done with 3 other priors
SeBac ~ dbeta(1,1)
SpBac ~ dbeta(999,1)
Sediff<-SePCR-SeBac
Spdiff<-SpPCR-SpBac
PSediff<-step(Sediff)
PSpdiff<-step(Spdiff)
pi ~ dbeta(1,1) # true prevalence in each farm population
CovSePCRBac<-0 # conditional independence model assuming sensitivity covariance is
zero#CovSePCRBac ~ dunif(LowerCovSePCRBac,UpperCovSePCRBac)
#LowerCovSePCRBac<- (SePCR-1)*(1-SeBac)
#UpperCovSePCRBac<- min(SePCR,SeBac) - SePCR*SeBac
}
list(n = 148, y = c(3,79,0,66)) #farm 1 - liver and kidney combined
list(n = 151, y = c(3,24,0,124)) #farm 2 - liver and kidney combined
list(n = 44, y = c(8,35,0,1)) #farm 3 - liver and kidney combined
```