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Effect of sampling time and surveillance strategy on the time to onset and magnitude of piscirickettsiosis (Piscirikettsia salmonis) outbreaks in Chilean farmed Atlantic salmon

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ABSTRACT

Aquaculture is currently the primary source of fish for human consumption. However, the sustainability of this industry has been under scrutiny. In Chile, the main concern is the use of antibiotics to control piscirickettsiosis, and farmers have identified timely detection as a critical issue. Using data provided by the Fisheries and Aquaculture authority (Sernapesca), we assessed whether the sampling strategy or the time of the first detection of *Piscirickettsia salmonis* would affect the time to onset or the magnitude of an outbreak. We modeled time to onset using an Aalen additive-hazards survival model, and found that on average, shorter times between diagnosis and treatment were associated with earlier onset of disease. Piscirickettsiosis also occurred earlier when fish received a sea lice bath treatment at any point before the outbreak, and when farms submitted the samples, but these effects waned past 30 to 35 weeks into the production cycle. To assess the impact of these predictors on the proportion of fish that die during an outbreak, the magnitude of an outbreak, we used a mixed-effects linear model, controlling for the level of mortality when the treatment started. We found that mortality due to piscirickettsiosis was higher when the diagnosis was made closer to treatment; however, this was only observed when samples were obtained during an active surveillance activity. Our results showed that disease occurs later, and fewer fish die when the time between the detection of the agent and the occurrence of an outbreak is longer, especially when samples are collected during active surveillance activities. This suggests that active surveillance may be more likely to lead to early detection, and give the farmers time to implement adequate control measures. Despite the limitations of this study, the results suggest that the current surveillance program is effective in delaying and reducing the impact of piscirickettsiosis outbreaks, and that farmers may benefit from increasing their own surveillance efforts.

1. Introduction

Aquaculture has surpassed fishing as the main source of fish for human consumption (Food and Agriculture Organization of the United Nations, 2018). Considering the projected growth in aquaculture to meet the demands of the increasing human population, aquaculture industries need to develop solutions to production challenges that ensure sustainability. Globally, Atlantic salmon (*Salmo salar* L.) is one of the most intensely farmed and highly valued fish, with the majority of the world's production taking place in Norway and Chile. Currently, a major threat to finfish aquaculture sustainability in the latter country is the dependence on antibiotics to control piscirickettsiosis (Rozas and Enríquez, 2014), also known as salmonid rickettsial syndrome (SRS), a salmonid bacterial disease caused by *Piscirickettsia salmonis* (Fryer et al., 1992).

Antimicrobial use in Chilean aquaculture is highly regulated. Only registered products are allowed and must be prescribed by a veterinarian; all treatments must be reported to the authority, and treatment

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failure must be documented and reported; and, preventative use is prohibited, and isolation of the agent and susceptibility testing must be attempted (Sernapesca, 2015). Despite these regulations, between 2013 and 2016, an average 580 g of antibiotic per harvested ton were used annually in Chilean aquaculture (Miranda et al., 2018), more than 90% of this to treat SRS (Sernapesca, 2018). However, treatments are often considered unsuccessful and a subsequent antibiotic treatment might be needed to control an outbreak. Reasons for treatment failure are not fully understood, but antimicrobial resistance (Cartes et al., 2017; Contreras-Lynch et al., 2017; Henríquez et al., 2016; Henríquez et al., 2015; Saavedra et al., 2017; Sandoval et al., 2016), inadequate concentrations (Price et al., 2018), and delayed treatment start time (Price et al., 2016) have been identified as possible underlying factors.

Critical to avoiding treatment delay is the timely detection of *P. salmonis* infection. Salmon farmers have identified timely diagnosis coupled with necropsy training as key strategies to control SRS (Estévez et al., 2019). In Chile, government authorities have implemented a control and surveillance program for this disease (Sernapesca, 2012), and a key objective of the surveillance and control program is to achieve early detection in order to initiate timely control measures (Rozas and Enríquez, 2014), ultimately leading to decreased piscirickettsiosis outbreak occurrence and severity in Chilean salmon farms. The program relies on active surveillance, and all farms are required to submit a sample of at least 15 individuals to an accredited lab to test the fish for the presence of *P. salmonis* 30 days before and after stocking, and every two months thereafter. The program does not preclude farmers from carrying out additional testing, and they frequently submit samples when they suspect the presence of the pathogen or want to confirm a clinical diagnosis. Although the program was established in 2012, the effectiveness of this sampling scheme and any associated strategies to reduce the impact of piscirickettsiosis have not been fully assessed, particularly regarding the timing of several other factors (e.g., diagnostic testing, diagnostic interpretation, antibiotic treatment(s), and mortality). Therefore, our objective was to assess whether the sampling strategy (active surveillance vs. farm-submitted samples) and the time of first *P. salmonis* detection within a farm site would affect either the time to disease onset (using first antibiotic treatment as a proxy) or the magnitude of the outbreak as measured by cumulative mortality attributable to SRS.

2. Material and methods

2.1. Data sources

Three sources of data were available for our study: a weekly farm-level record of all mortality classified by cause of death, a record of antibiotic and antiparasitic treatments delivered by cage and/or farm, and a farm-level record of all the samples submitted for *P. salmonis* diagnostics. All of the information was provided by the Chilean Fisheries and Aquaculture Authority known as Sernapesca (Servicio Nacional de Pesca y Acuicultura).

2.2. Data management

We used year, calendar week, and the farm identification code to merge all of the information into one master dataset, which also included geographical production regions (Los Lagos and Aysén regions). We chose production cycles within a farm as our study unit and collapsed the data to a single observation per production cycle. In the absence of a standardized case definition for a piscirickettsiosis outbreak, we used antibiotic treatment against *P. salmonis* as a proxy for identifying an outbreak within a production cycle. For each production cycle, we then recorded the week of the production cycle in which the farm received antibiotic treatment for the first time. We also recorded the number of weeks before the outbreak that the first detection of *P. salmonis* was made (either by a polymerase chain reaction (PCR) or immunofluorescent antibody (IFAT) test), and whether the samples were submitted by the farm voluntarily or in the context of an active surveillance activity. Considering bath treatment intensity was recently associated with increased piscirickettsiosis outbreak severity (Meyer et al., 2019), we also recorded the production cycle week when the first bath treatment for sea lice was administered and the number of bath treatments between the first *P. salmonis* detection and the first antibiotic treatment. Additionally, we calculated the average weekly mortality percentage due to SRS in the three-week period preceding the treatment, as well as the percentage of accumulated mortality due to SRS in the four weeks that followed the start of antibiotic treatment.

2.3. Statistical methods

We assessed trends in our data using summaries and descriptive statistics. To explore the impact of different factors on the time to treatment onset, we used the Kaplan-Meier estimator to visualize differences in survival time for our categorical predictors (i.e., sampling strategy, bath before treatment, and year). To model the time to piscirickettsiosis onset, we used an Aalen additive-hazards survival model (Aalen, 1989). We chose this model because it can deal with time-varying covariate effects and because these effects are estimated on an absolute rather than on a relative scale, allowing us to relax the assumption of proportional hazards.

To assess the effect of our predictors on the magnitude of the outbreak, we used a linear model of the log-transformed percent of the cumulative mortality in the four weeks after the start of antibiotic treatment. We selected predictors for our final models based on plausible biological relationships, including interactions between our predictors, and a stepwise forward selection and backward elimination strategy. We compared the models' fit using the Akaike Information Criterion (AIC) and chose the model with the smallest AIC. Models with a difference in AIC of more than 2 points were considered significantly different. We graphically checked the assumptions of the model using residual plots. Statistical significance for both regression analyses was set at $P < .05$.

All analyses were carried out using survival package (Therneau and Grambsch, 2010) in R 3.5.1 (R Core Team, 2018). All figures were generated using the ggplot2 (Wickham, 2016) and ggfortify (Tang et al., 2016) packages.

3. Results

In total, we successfully merged information from 489 Atlantic salmon complete production cycles in the “Los Lagos” and “Aysén” regions of Chile between January 2012 and June 2016. Of these, 393 cycles received antibiotic treatments reported as piscirickettsiosis treatments. We identified 179 cycles that had missing ($n = 83$) or unreliable ($n = 36$) information about sampling (e.g. first sampling occurred later than required by law, therefore we could not tell if it was the first sampling for sure) or there was no record of sampling prior to treatment ($n = 60$); these cycles were not included in the analyses. For our linear model, we excluded an additional 61 cycles because mortality attributed to piscirickettsiosis was not recorded prior to or during the treatment period. Therefore, our final dataset had 214 production cycles matching our selection criteria for the survival model and 153 cycles matching our selection criteria for the linear model.

The first antibiotic treatment occurred between six and 56 weeks after stocking, with a median time from stocking to treatment onset of 20 weeks. Only four of the production cycles that met our selection criteria were not treated during the study period. The median time from detection to treatment was three weeks, with a range of 0 to 64 weeks; however, more than 80% of the outbreaks occurred within 12 weeks of first *P. salmonis* diagnosis. The median number of sampling events it took to prove the presence of the agent was 2, and 80% of the production cycles needed 4 or less sampling events to have a positive result.

The piscirickettsiosis-specific mortality rates in the three-week period that preceded treatment ranged from 0 to 0.3% per week, but 82% of the production cycles had levels below 0.01%. Cumulative mortality during the four-week period after treatment began ranged from 0.02 to 5.7%, but 79% of the cycles accumulated mortalities of less than 0.2%.

Using survival curves based on the Kaplan-Meier estimator, we observed longer times to a piscirickettsiosis outbreak for production cycles where there were four or fewer weeks between the detection of *P. salmonis* to the onset of antibiotic treatment (the weeks-since-detection parameter was dichotomized at four weeks for the exploratory analysis), where active surveillance occurred, and where farms did not report an antiparasitic bath treatment before the outbreak. We only found a small difference between the "Los Lagos" and "Aysén" regions, but a larger difference between the outbreaks that occurred before 2016 and the ones that occurred in 2016 was observed. However, only the statistically significant parameters, namely weeks-since-detection ($P < .001$), sampling strategy ($P < .001$), bath treatment before outbreak ($P < .001$), and year 2016 ($P = .003$), were retained in our final model. Our Aalen additive hazards survival model showed an overall increase in the hazard of piscirickettsiosis as the production cycle progressed. In this model (Table 1, Fig. 1), longer times between detection and treatment (outbreak) of piscirickettsiosis were associated with lower hazard of piscirickettsiosis (i.e., in these farms outbreaks occurred later in the production cycle than in comparable farms with shorter intervals between diagnosis and treatment). Another factor that decreased the hazard of an outbreak was the sampling strategy. Outbreaks that occurred after detection from an active surveillance event had lower hazard than outbreaks where the sampled fish were submitted voluntarily by the farm. However, this effect was no longer significant after approximately 30 weeks into the production cycle. Farms that received an antiparasitic bath treatment before the outbreak had significantly higher hazard than farms that did not, but the effect faded after approximately 35 weeks into the production cycle. Finally, in our model, we controlled for a higher hazard of piscirickettsiosis observed in 2016.

In our linear model, the most parsimonious model included only the predictor for the average mortality in the three-week period before antibiotic treatment started ($P < .001$). However, we chose to also include the interaction between weeks-since-detection and sampling strategy ($P = .021$) because it allowed us to more fully assess our predictors of interest (Table 2).

Average mortality during the three-week period before antibiotic treatment started was positively associated with mortality accumulated in the four-week period after treatment started. That is, the higher the average mortality before treatment, the larger the mortality accumulated during and after treatment. The interaction term in our model suggests that the number of weeks-since-detection was only negatively associated with the magnitude of the outbreak when the detection was in samples obtained during an active surveillance event (Fig. 2).

Table 1
Slope, coefficient, standard (Std.) error, and *P*-value results of the Aalen additive-hazards survival model for the time to piscirickettsiosis onset.

	Slope	Coefficient	Std. Error	P-value
Intercept	0.066	0.001	0.001	
Weeks since detection	−0.003	−0.001	4.8×10^{-5}	< 0.001
Sampling strategy: farm-submitted	(reference)			
Sampling strategy: active surveillance	−0.041	−0.004	0.001	< 0.001
Bath before treatment: No	(reference)			
Bath before treatment: Yes	0.045	0.005	0.001	< 0.001
Year: before 2016	(reference)			
Year: 2016	0.056	0.007	0.001	0.003

4. Discussion

One means of avoiding piscirickettsiosis treatment delay or failure is timely detection, as supported by the piscirickettsiosis surveillance program in Chile. For our study, weekly farm-level mortality records, cage and/or farm records of antibiotic treatments, and farm-level piscirickettsiosis sampling records were provided by Sernapesca. Our objective was to evaluate these data for any effect patterns of the sampling strategy or the time of first pathogen detection, with respect to the time to disease onset or the magnitude of the farm's outbreak. Utilization of this information can benefit the timing of management, sampling, and diagnostic strategies at the site level to avoid or decrease the magnitude of piscirickettsiosis outbreaks.

We found that time since detection and sampling strategy have a significant influence on both the timing of the outbreak and its magnitude. Our results suggest that longer times between the detection of the pathogen and the outbreak of the disease (via time of first antibiotic treatment) are associated with outbreaks that occur relatively later in the production cycle. This pattern may arise from detecting the agent at different stages of the onset of disease. Early detections, represented by longer times between diagnosis and disease, may result in lower hazard because less individuals are compromised and the agent might be propagating at a slower rate. Furthermore, in the presence of a positive diagnostic test result for *P. salmonis*, farm administrators usually react proactively by implementing control measures, such as the use of functional diets, increasing the frequency of mortality removal, and/or avoiding stressful activities for the fish (e.g., delousing baths) further delaying the onset of disease.

An alternative explanation may be that our model is identifying outbreaks caused by low-pathogenicity *P. salmonis* strains. A difference in pathogenicity between strains has been previously described by Saavedra et al. (Saavedra et al., 2017). More specifically, the presence of EM90-like strains has been associated with less severe outbreaks than LF89-like strains in affected Atlantic salmon populations. Unfortunately, the retrospective nature of our study design and the lack of strain identification in the available data limited our ability to differentiate between these competing hypotheses.

In contrast to challenge experiments where higher mortality was associated with higher lice counts (Figueroa et al., 2017; Lhorente et al., 2014) and to observational studies where higher sea lice burden and higher bath treatment rates were associated with higher SRS mortality risk (Meyer et al., 2019), we were not able to detect significant differences in magnitude of the outbreak between farms with and without a history of bath treatments. Using our survival model, however, we found that farms that had a delousing bath treatment at any point before starting antibiotic treatment had an increased hazard of having an outbreak. This effect might represent the stress associated with the burden of lice, the handling needed to deliver antiparasitic bath treatments, or a combination of both. It is not clear why the effect tends to fade as the production cycle advances, but it might be partially explained by the increasing base hazard of piscirickettsiosis that may diminish the role of stress in the occurrence of an outbreak. Differences in exposure between lab-induced and natural infection, unit of study, and observation periods might explain the differences between the findings of this study and previous studies.

In our data, the outbreaks that followed an official active surveillance sampling event took, on average, longer to develop and had lower mortality than outbreaks that followed a farm-submitted voluntary sample event. Furthermore, longer times between detection and outbreak were associated with significantly lower mortality, but only when samples were obtained during active surveillance activities. This is not surprising if we consider that farms typically submit samples only when clinical signs are evident or once SRS-related mortality has already occurred. In contrast, detection of the pathogen during a surveillance event may occur at any point during the development of disease. If the agent is discovered in early stages of the disease, the preventative

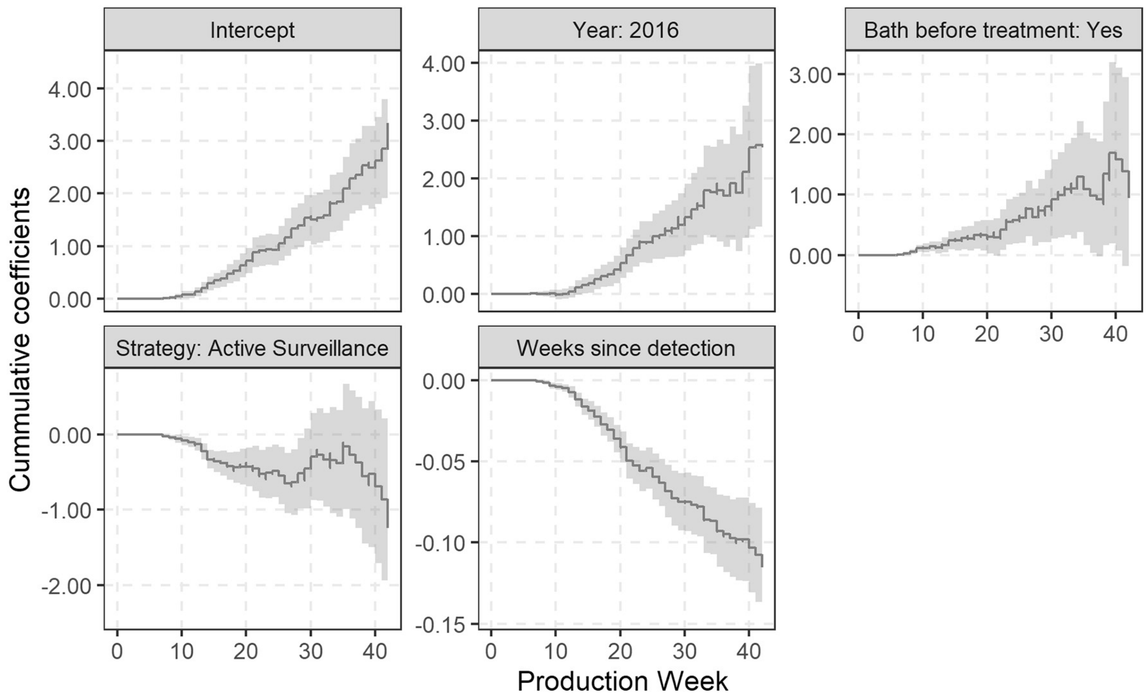

Fig. 1. Cumulative hazards with 95% pointwise confidence intervals based on the Aalen additive-hazards model for time to piscirickettsiosis onset.

Table 2
Coefficient, standard (Std.) error, 95% confidence intervals, and *P*-value results of the linear model for the magnitude of mortality in the four-week period after antibiotic treatment started.

	Coefficient	Std. Error	95% Conf. Interval	P-value
Intercept	1.676	0.398	0.889 — 2.462	
Percent mortality before treatment (log transformed)	0.774	0.058	0.660 — 0.888	< 0.001
Weeks since detection	0.009	0.015	−0.021 — 0.039	
Sampling strategy: farm-submitted	(reference)			
Sampling strategy: active surveillance	0.257	0.274	−0.285 — 0.798	
Weeks since detection × sampling strategy	−0.067	0.029	−0.124 — -0.011	0.021

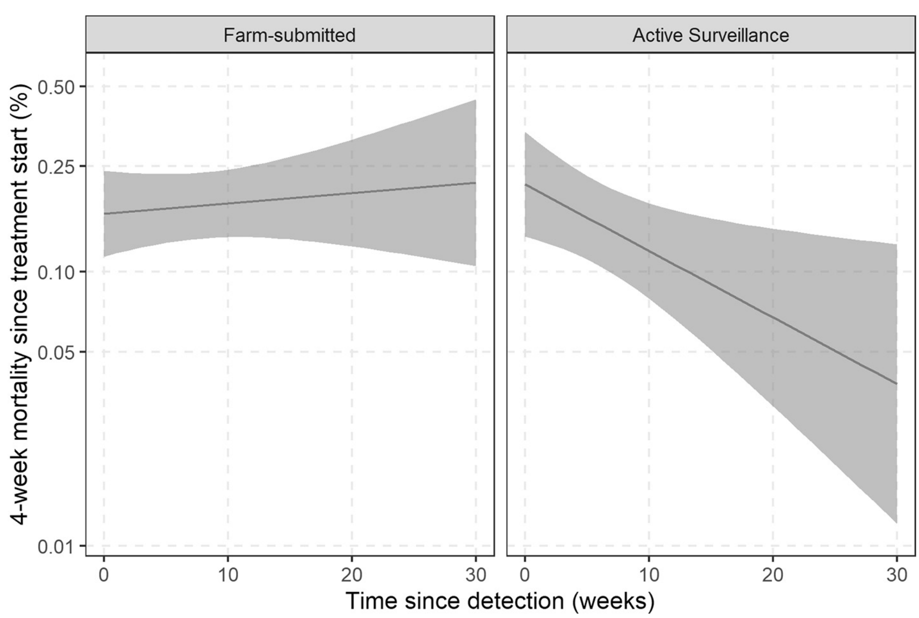

Fig. 2. Interaction plot of the effect of weeks since detection on the log-transformed* mortality accumulated during the four-week period after the treatment started by sampling strategy (farm-submitted (left) or active surveillance (right)).
* Labels were back-transformed to percent (%) for easier interpretation.

measures implemented by farmers may help protect a larger proportion of healthy individuals before the pathogen can establish in the population. Similarly, the effectiveness of antibiotic treatments have been shown to be dependent on how early in the disease process they are delivered (Price et al., 2016). This may help explain why we see lower mortality rates when samples are obtained from surveillance activities.

The use of farm-level instead of pen-level data may have limited our ability to more precisely identify the time to piscirickettsiosis onset and the magnitude of the outbreak. In general, however, when *P. salmonis* is detected in one or more cages, the farm is often assumed to be infected and all cages are treated with in-feed antibiotics. Another source of uncertainty is the use of treatment as a proxy for a disease outbreak. Having standardized outbreak identification criteria would have allowed us not only to determine the time to onset more precisely, but it would have also allowed us to assess the timeliness of treatment.

Despite the limitations in our study, our results suggest that the current piscirickettsiosis surveillance and control program implementation provides useful tools for the control and mitigation of the disease by making it possible to detect the agent at early stages of the disease. This might allow farmers to adapt their management practices to delay the onset and minimize the impact of SRS. We deem that an increase in the surveillance effort, or the adoption of a more preventative sampling behavior when farms submit samples, may further improve the overall results of the program. The knowledge obtained may help authorities and farmers elucidate critical aspects of the timing between agent detection and disease outbreak to adjust their sampling strategy and emphasize the importance of implementing management practices to help delay the onset and/or minimize the magnitude of piscirickettsiosis outbreaks.

Author statement

All authors contributed equally to this work.

Declaration of Competing Interest

The authors certify that they have no conflict of interest.

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