Control of key diseases of Atlantic salmon, Coho and trout in the Chilean aquaculture industry: design of a prospective cohort study

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PROGRAMA PARA LA GESTIÓN SANITARIA EN LA ACUICULTURA

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INTRODUCTION

The prospective study is intended to assess the effectiveness of several interventions currently being applied for the control of key diseases of Atlantic salmon, Coho salmon and rainbow trout in the Chilean aquaculture industry.

The primary exposures of interest are:

- treatment with an alternative pesticide (Lufenuron) to control Caligus spp.
- vaccination with a new vaccine to control SRS
- genetic characteristics expected to confer resistance to SRS and/or Caligus spp.

The key outcomes of interest are:

- the occurrence of Salmon Rickettsial Septicaemia (SRS, Piscirickettsiosis)
- infestation with Caligus rogercresseyi.

These exposures and outcomes are described in more detail below. The prospective cohort study will be conducted across a number of farms and barrios over a period of 24 months.

OVERVIEW

A cohort study is a type of observational study in which study participants are selected and classified according to their exposure status; for example, whether or not they have been vaccinated to control SRS. Having determined the prevalence (preferably absence) of the outcome(s) of interest at the start of the study, a cohort study then compares the incidence of the outcome(s) of interest in the groups (as defined by the exposure status) during the specified follow-up period.

It is intended that several discrete cohort studies (each focussing on a specific exposure/intervention) will be designed and implemented within one overarching study. A general outline of the cohort study approach is provided in the following sections.

SELECTION OF THE GROUPS (COHORTS) TO BE STUDIED

Broad description of the population from which cohorts will be selected

Although species is not one of the primary exposure variables, it is anticipated that the study will include all three species of salmonid fish that are farmed commercially in Chile:

- Salmo salar (Atlantic salmon)
- Oncorhynchus kisutch (Coho or Pacific salmon)
- Oncorhynchus mykiss (rainbow trout)

The target population (i.e. the population to which results of the study will be extrapolated) is assumed to include all salmonid fish farms in Regions X, XI and XII. Cohorts will be selected from farms that agree to participate in the study and that are required to submit the required data for regulatory purposes. It is anticipated that the population from which cohorts will be selected will include the majority of commercial salmonid fish farms located in Regions X, XI and XII.

The geographical extent of the study, selection of barrios and the identification of farms eligible for inclusion in the study will be determined in consultation with government and industry stakeholders.

Unit of concern

The unit of concern relates to the level at which exposures and outcomes are measured, and the level that we are interested in from a disease control perspective.

The sea-cage is likely to be the appropriate unit of concern if:

- all fish within a sea-cage have the same exposure status
- treatments and other management factors are applied at the cage level (with some variability among cages within a farm)
- sea-cages are uniquely identified and data is consistently collected at the sea-cage level.

If sea-cage is the unit of concern, then we would need to consider the effects of clustering at the farm level, and account for farm-level effects (and barrio-level effects) in the analysis.

However, it is possible that the farm could be the appropriate unit of concern if:

- exposure status is the same for all cages within a farm
- treatments and other management factors are applied at the farm level
- data is only available at farm level.

It will be useful to discuss this issue during refinement of the study design.

Timing of the study with respect to production cycles and calendar

The timing of the study will coincide with the production cycle of participating farms. It is assumed that participants will enter the study when smolts are transferred to sea-cages. The exact timing of the study this will be described after consultation with stakeholders and consideration of any practical and logistical matters.

Inclusion and exclusion criteria

It may be necessary to define specific criteria for inclusion and/or exclusion from the study. Ideally, exposed and unexposed groups will be as similar as possible with the exception of the exposure of interest. Consideration should be given to the possibility of matching to increase

the likelihood of similarity between cohorts (with respect to major confounding variables) and improve the efficiency of the study.

Sample size and sampling method

The approach to selection of study participants will be influenced by statistical considerations and practical considerations such as the availability of resources. There are two main scenarios:

1. No requirement (or opportunity) to select a sample. If all the necessary data is being collected from all (or a large proportion) of farms in the target population—irrespective of this study—then there is no need to select a sample. In this case, we effectively have a census of participating farms, and we can try to determine if these are representative of the broader target population of interest.

2. Necessary to select a sample from a broader population. If additional data collection is required for this study—and additional costs prevent the data from being collected on all farms—then we will need to select a sample from the target population. For example, we might use cluster sampling to select farms, and include all cages on selected farms [with cluster sampling, all cages within selected farms would be included].

Calculations about sample size and power should be done during refinement of the study design. In the first scenario (no sampling and no control over the number of study participants), these calculations can be used to estimate the achieved power of the study. In the second scenario, these calculations can inform the number of participants required, given a desired level of confidence in the estimates obtained from the study and power to detect real difference between the exposed and unexposed groups.

In either case, these calculations require that we:

- clearly define how outcomes will be measured

- identify the appropriate way of comparing the exposure groups (for example, comparison of means, proportions, survival times, etc.)
- decide on the desired level of confidence (i.e. the certainty that any difference is real ant not due to chance)
- decide on desired power of the study to detect a real difference between groups, if a difference of a defined magnitude exists
- estimate the measure of the outcome for both treated and untreated groups and statistical variability around this; e.g. proportion and standard error.

If sampling occurs in this study, sample sizes should be adjusted (increased) to account for clustering (at the level of farm and barrio), confounding and interaction.

These statistical considerations and non-statistical considerations should be discussed during refinement of the study design.

DEFINITION OF THE PRIMARY EXPOSURES AND THEIR MEASUREMENT

At the beginning of the study, selected units will be classified as 'exposed' or 'unexposed' to the interventions of interest. In an observational study, the people conducting the study will not seek to influence how and where the interventions ('exposures') are applied, but simply observe and compare the groups with respect to specified outcomes.

Some exposures (such as genotype) are fixed, while the exposure status for other factors (such as lufenuron treatment) may change during the study period. If exposure status changes, it will be important to record exposure status over time to calculate time-at-risk and enable rate-based analysis. Classification of exposure status should consider durations of effect (of treatment and vaccination).

In some situations, it may be possible to have degrees of exposure; when this occurs, the demonstration of a 'dose-response' trend can strengthen the conclusions.

In addition, some farms may be treated with combined treatments (exposures, e.g. genetics and lufenuron) and this will also be included as a unique treatment, or in other words considered statistically as an interaction.

1. Vaccination with a novel live vaccine for control of SRS

In consultation with stakeholders, this exposure will be described in more detail to include vaccine type, vaccination protocol and how vaccination status (and potentially immunological status) will be determined.

We will also need to define the ‘unexposed’ group (or groups); for example, there may be an ‘unvaccinated’ group and an ‘other vaccines’ group.

2. The use of lufenuron to control Caligus

In consultation with stakeholders, this exposure will be described in more detail to include active ingredient and formulation, dose, route and frequency of administration. It will also be necessary to determine how exposure status is determined (intention-to-treat or per protocol) and the expected duration of therapeutic effect after treatment.

We will also need to define the ‘unexposed’ group (or groups); for example, there may be a ‘no treatment’ group and an ‘other treatments’ group.

It will also be useful to estimate the proportion of farms in the study population that are expected to use lufenuron. If a large proportion of farms use lufenuron, then there may be insufficient farms in the ‘unexposed’ groups to allow meaningful comparison.

3. Genotypic characteristics expected to confer resistance to SRS and/or Caligus

In consultation with stakeholders, the nature of the genetic characteristics and how they are determined will be described in more detail. For example, the ‘exposed’ group may be defined by quantitative trait loci (QTL) mapping or other molecular markers.

MEASUREMENT OF POTENTIAL CONFOUNDING VARIABLES AND SECONDARY EXPOSURES

In these studies, we seek to obtain unbiased measures of association between the primary exposures and the outcomes of interest. However, as observational studies take place in a 'real world' environment, it is possible that other factors ('confounding variables') may influence the development of these outcomes. These variables include management practices, and environmental variables such as water temperature, salinity and turbidity. In consultation with stakeholders, we will identify other variables that should be measured and dealt with in the design (for example, by matching or exclusion) or analysis. In an observational study, it may be difficult to control for confounding in the design, but if the appropriate data are collected then confounding can be addressed in the analysis.

We will also need to consider whether interactions may occur if a selected farm applies more than one of the interventions of interest: for example, in the SRS cohort study, treatment for Caligus spp. could be measured as a secondary exposure to determine if there is an interaction between these exposures on the incidence of SRS.

The following variables are derived from existing databases and/or relevant literature on SRS and Caligus. They are not intended to define specific data requirements, but to illustrate the sort of data that might be collected during the study. Further consultation is required to determine which variables should be included in the study.

Inherent (fixed) exposures

- species

Hatchery system exposures

- water flow: type and flow rate
- growth rate
- use of prophylactic treatments
- food type/source

Note: alternatively, we might simply record hatchery as 'Hatchery 1, Hatchery 2, etc.

Sea-cage exposures

Host factors

- age
- time in sea water (month of the production cycle, or degree days)
- average weight of smolt at entry to sea cage
- average fish weight (although this might be correlated with time in sea water)

Management

- company
- farm size
- timing (e.g. calendar week) of smolt entry
- stocking density (kg per cubic metre)
- food type/source
- variability of fish size within cage
- use of prophylactic treatments (e.g. treatment status in whatever past time period is appropriate with respect to protective period)
- previous fallowing period

- level of biosecurity
- level of coordination at the barrio level
- netting construction/materials and level of biofouling
- cage depth

Environment

- water temperature
- turbidity/transparency
- salinity
- current speed
- sea floor contaminants/pollutants

Spatial factors

- location (site, barrio, macrozone, region)
- sea-distance from 'neighbour' farms
- density of active farms within a certain radius

DEFINITION OF THE OUTCOMES AND THEIR MEASUREMENT

Outcomes will be measured regularly throughout the production cycle (or, in the case of the lufenuron study, the period for which the exposure is expected to remain; for example, 150 days after administration).

During refinement of the study design, the outcomes of the literature review and consultation with stakeholders will be considered to determine:

- the specific outcomes which will be measured throughout the study period
- clear diagnostic criteria on which to ascertain the outcome status
- the means and frequency at which measurements will be made.

The following sections list some of the outcome measures that might be considered.

Disease and infestation outcomes

- Degree of Caligus infestation (continuous or ordered categorical variable)
- Piscirickettsia infection (‘outbreak) status (binary)
- Weekly mortality attributed to SRS
- Infection with other pathogens (incidence, by primary cause)

Production outcomes

- Average fish weight (although this is expected to be correlated with time in sea water)

Consultation with stakeholders will be important to assess the feasibility and cost of monitoring of exposure status and measuring outcomes throughout the study period.

ANALYSIS

Statistical analyses appropriate for a cohort study will be conducted to allow comparison of the exposed group with the unexposed group. Initial description of the data and exploration of crude associations will occur. This will be followed by an appropriate method such as generalised linear regression modelling (with an appropriate link function such as Poisson or logistic) or survival analysis, with the most appropriate method being determined by the study design (e.g. open or closed design). These models will allow the development of an adjusted measures of association in the exposed and unexposed groups, taking into account the possible confounding effects of other exposure variables and interactions.

Although the primary exposures are the focus of this study, the substantial amount of data that is expected to be available through the integrated data management system will support a range of secondary analyses. However, the possibility of making a Type 1 error (e.g. concluding that there is a difference between groups, when in fact there is no difference) increases if multiple analyses are conducted. Alternatively, information theoretic approaches may be employed which do not raise the Type 1 error rate.

Information about management practices will be required to determine whether the study population should be considered closed (no additions and no/few losses to follow-up, in which case a risk-based analysis is appropriate) or open (individuals may be lost from the study, or exposure status may change, in which case a rate-based approach is appropriate). If it is possible that a rate-based analysis is appropriate, then data should be collected to determine time-at-risk for each cage (or farm).

Risk or rates will be estimated to allow comparison of the exposed group with the unexposed group. If the study population is considered to be open and a rate-based analysis is appropriate, the assumption that the rate is constant over time may be suspect and survival analysis or Poison regression might be more appropriate.

INTERPRETATION

Results of the analysis will be interpreted in light of hypotheses formulated at the beginning of the study, possible biases and residual confounding, and the influence that these may have on the quantitative results.

PRACTICAL CONSIDERATIONS AND PROJECT PLANNING

Timing of the study

The timing of the study will have to coincide with key events in the salmonid fish production cycle. It would be useful to talk with industry experts during refinement of the study design to develop a work plan that is feasible with respect to the production cycle, management practices and opportunities for data collection.

Economic considerations and resources

Early in the design and planning phase, it will be important to describe the roles and responsibilities of the agencies and people involved in the study, and ensure that human resources are adequate. If it is necessary to collect additional data specifically for the project, it will also be necessary to estimate the associated costs and ensure that sufficient budget is available.

Principles and philosophical approach to the study

This substantial, multi-year study provides an important opportunity for collaboration and knowledge sharing between Sernapesca, representatives of the aquaculture industry, producers (farmers) and Ausvet consultants.

Stakeholder engagement

This study will be conducted to address important concerns of aquaculture industries and government agencies in Chile. If it is to be effective, it will be important to communicate well during refining of the design and during implementation. It would be useful to identify the target audiences for the outputs of this study and discuss ways to invite design inputs and disseminate results.

PROS AND CONS OF A PROSPECTIVE COHORT STUDY

There are several **advantages** to a (prospective) cohort study in this situation:

- A cohort study has the potential to provide unbiased, temporal associations between the exposures and outcomes of interest
- A cohort study will yield absolute (rather than relative) estimates of disease incidence in exposed and unexposed populations
- A range of exposures and outcomes may be measured in the context of a broader study, although if many variables are measured then some statistical associations may occur by chance

- In a prospective cohort study, exposure status is determined before outcomes are measured which avoids some forms of bias
- In reality, it may not be possible to control the allocation of the interventions (as would occur in a controlled trial), in which case an observational study is the preferred approach. Cohort studies can be conducted in situations where the controlled allocation of interventions is not possible.
- Determination of the way in which exposures and outcomes will be measured occurs before the data is collected, so there is a lesser risk of misclassification.

However, there are some potential **limitations and disadvantages:**

- If farms are the appropriate unit at which to ascertain exposures and outcomes, then a large number of farms are likely to be required
- Without having control of the exposures (vaccination protocols and treatments), there may be insufficient contrast between groups, making it difficult to detect meaningful effects
- The measurement of exposures and outcomes will depend on available methods and capacity of people and institutions involved in the study
- In all observational studies, control of confounding is a considerable challenge, although this can be addressed to some extent in the design and analysis
- The efficiency of the study will be poor if outcomes are rare (i.e. if there is low incidence of the outcomes of interest during the study period).
- One study cannot constitute proof of a causal relationship.


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