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SHORT COMMUNICATION

Serological evidence of *Coxiella burnetii* in sheep herds from Lonquimay valley in the Chilean Andes

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ABSTRACT. *Coxiella burnetii* is the causative agent of Q Fever, a worldwide zoonotic disease that causes acute flu-like illness and chronic manifestations in humans in the form of endocarditis, hepatitis, and other symptoms. Domestic ruminants are the most important reservoirs of the bacteria, transmitting infections to humans during the calving/lambing season through direct contact with contaminated fetal tissues or inhalation of dust particles. The aim of this study was to provide serological evidence and estimate the individual true seroprevalence of *C. burnetii* exposure in sheep herds in Lonquimay, and to characterize farmers' knowledge of coxiellosis. A disease freedom survey was conducted in 30 sheep herds selected from the Indigenous Territorial Development Program database (PDTI). A total of 260 blood samples were tested using ELISA for *C. burnetii* antibody detection. Disease freedom and true animal-level prevalence were estimated, and a questionnaire was administered during farm visits to characterize farmers' zoonotic knowledge. A positive result was found in 3% (1) of the sampled herds, and the true animal prevalence (mTALP) was higher than previous unpublished estimations (mTALP 4.2%, 95% PPI 1.6%–9%). The estimated probability of the study sheep population not being free of *C. burnetii* was 34%. A lack of knowledge regarding *C. burnetii* or the consequences of Q Fever was detected, along with risky behaviors that could facilitate pathogen transmission. This study revealed evidence of exposure to *C. burnetii*, with low individual and herd-level prevalence. Initiatives to improve zoonotic knowledge among farmers need to be implemented in the short term.

Keywords: Q Fever; coxiellosis; sheep; prevalence; zoonoses; Chile.

INTRODUCTION

Coxiella burnetii is the etiologic agent of Q Fever, a zoonotic disease that usually manifests as asymptomatic cases or acute flu-like symptoms. A chronic form of the infection has been documented, presenting endocarditis, hepatitis, and lung fibrosis (Straily *et al.*, 2017). Transmission to humans occurs mainly through direct contact with birth fluids and tissues from domestic ruminants (main reservoirs), consumption of raw milk or derived products, or inhalation of contaminated dust particles (Angelakis & Raoult, 2010). In animals, infection is mostly asymptomatic; however, reproductive disorders, such as abortion and low birth weight, may be observed (Palmer *et al.*, 1983).

Q fever outbreaks have been reported in several countries, including Australia, the USA, Spain, France, Germany, Great Britain, and Switzerland (Bellini *et al.*, 2014; Biggs *et al.*, 2016; van Woerden *et al.*, 2004). The largest reported outbreak occurred in The Netherlands between 2007-2010, associated with goat and sheep farming, with over 4,000 confirmed cases (Dijkstra *et al.*, 2012; Schneeberger *et al.*, 2014).

In Chile, the first reported outbreak occurred in 1998 when members of the Agricultural and Livestock Service (SAG) were infected through contact with imported sheep. This led to the declaration of Q Fever as an occupational disease, and its inclusion in the list of notifiable diseases (Ministerio de Agricultura, 2000). Throughout the following years, no data about *C. burnetii* presence in the country was published. In 2017, an outbreak of atypical pneumonia occurred in persons related to dairy farms in southern Chile (Ministerio de Salud, 2017), 20% of 357 suspected Q Fever cases were subsequently confirmed (Tapia *et al.*, 2020). In response, research has been performed to update the disease status and provide insights into the infection risk (Cornejo *et al.*, 2019; Hernández-Agudelo *et al.*, 2023; Tapia *et al.*, 2021; Weitzel *et al.*, 2016). However, research has focused on dairy herds, leaving the situation for other ruminants unexplored. Nevertheless, SAG reported a seroprevalence of 0.041% in sheep herds near the zone in which the 2017 outbreak occurred; however, these data have not been published (Rosenfeld, 2022).

Lonquimay is a district located in the Araucanía Region in southern Chile, the country's third most important livestock region. Lonquimay has the second highest sheep population in the region, primarily destined for extensive fattening (Rojas *et al.*, 2022). Situated in a high-alti-

tude valley. To the east and southeast, it is contained by the Andes Mountain range and border with Argentina (Figure 1). The territory experiences harsh, cold winters that limit agricultural activities, which are mostly for basic subsistence. The temperature in winter can drop to -20°C, making the area nearly inaccessible, whereas in summer, can reach 30°C (Santibañez *et al.*, 2017). An important characteristic is the use of state-owned high-elevation pastures for transhumance from December to May, which allows farmers to store the forage and use it to feed animals between May and December, when direct grazing is hindered by snow (Catrileo & Alvarado, 2009).

Due to the predominantly subsistence agriculture and significant poverty is expected that farmers' knowledge about zoonotic diseases is limited. Given this context, increasing the awareness of zoonotic and infectious diseases is crucial. The aim of this study was to provide serological evidence and estimate individual true seroprevalence of *Coxiella burnetii* exposure in sheep herds in Lonquimay and to characterize farmers' knowledge of coxiellosis.

MATERIAL AND METHODS

Study population, design, and sample size estimation

The study population consisted of sheep herds from Lonquimay commune. This contains nearly 1300 sheep herds (Rojas *et al.*, 2022), most of which use an extensive, self-sub-

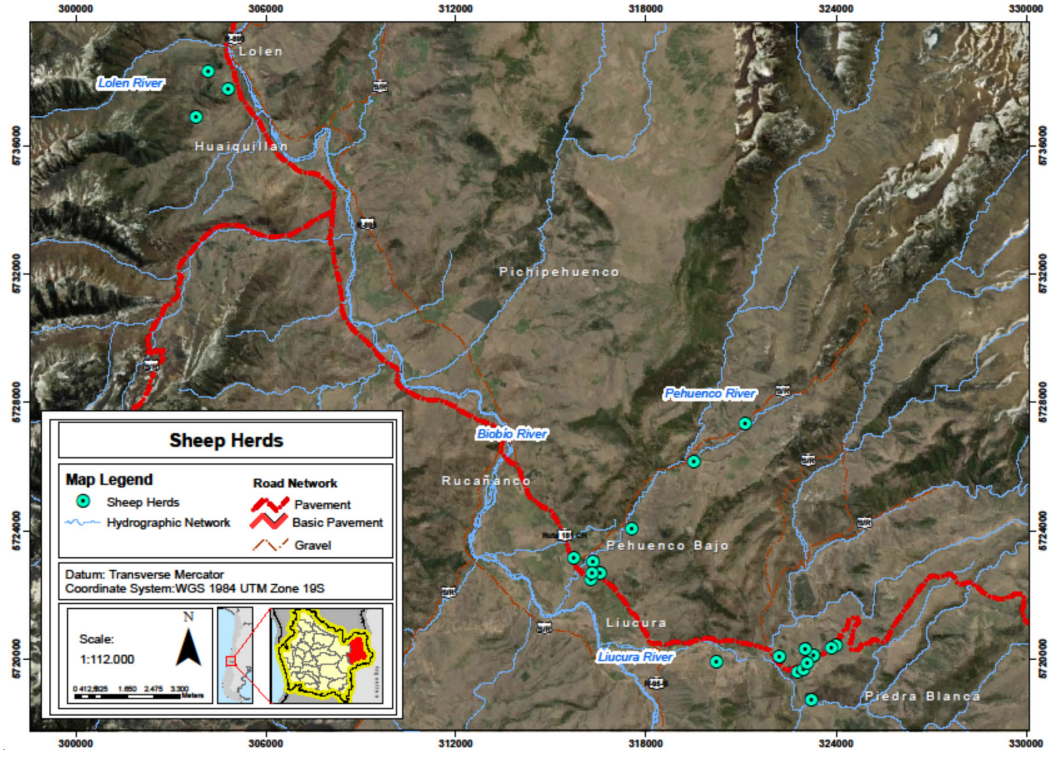

Figure 1.
Spatial distribution of sheep herds sampled to obtain serological evidence of *Coxiella burnetii* in Lonquimay.

sistence fattening system; the lambs are sold mainly through non-established commercial channels.

A disease freedom survey was conducted between August 2022-August 2023. The required sample size was calculated using a one-stage sampling method for disease freedom assessment. Considering a population size of 1300 herds, expected herd-level prevalence of 10%, and 95% confidence level, a sample size of 29 herds was required. On every farm visited, 8-10 adult sheep were tested, depending on herd size. In herds smaller than 8 animals, all adult sheep were tested. Herds were selected from the Indigenous Territorial Development Program (PDTI) of the Agricultural Development National Institute (INDAP), based on their willingness to participate.

Sample collection and laboratory analysis

Five mL blood samples were collected by puncturing the jugular vein using additive-free vacutainers. The samples were kept cold until reception at the Veterinary Diagnostic Laboratory of the Universidad Católica de Temuco, Chile. Serum was obtained by centrifugation, and then frozen and stored until processing. A commercial ELISA test IDEXX Q

Fever Ab Kit (Maine, USA) was used to detect the presence of *C. burnetii* antibodies (IgG) following the manufacturer's instructions. This kit uses purified antigens from phases I and II of *C. burnetii*.

The ratio between the optical density (OD) of the samples (S) and the OD of the positive control (P) was calculated to obtain the ELISA results. The cut-off values for the S/P ratio were obtained from the test insert and interpreted as follows: samples with S/P% ≥ 40% were positive, samples with S/P% ≥ 30% and ≤ 40% were suspect, and samples with S/P% < 30% were negative. Suspect samples were considered to be positive.

Farmers' knowledge of Coxiellosis

Knowledge about *C. burnetii* infection was characterized through a survey (Table 1). The questionnaire included questions related to knowledge about coxiellosis, Q Fever, introduction and dissemination risk behaviors, biosecurity, and health events in humans, probably related to the suspicion of Q Fever.

Statistical Analysis

Sample size estimation was performed using the epi.ssdetect function from the R package "EpiR" (Stevenson *et al*., 2015). A Bayesian approach was used to assess i) the mean true animal-level prevalence (mTALP) corresponding to the distribution of the true prevalence in an average infected farm, and ii) an overall true animal-level prevalence (oTALP) corresponding to a fitted beta distribution across all infected herds. The model was constructed as follows:

$$
\begin{aligned}
&y[i] \sim binomial(Ap[i], n[i]),\\
&Ap[i] = Tp[i] \times Z[i] \times Se + (1 - Tp[i] \times Z[i]) \times (1 - Sp),\\
&Z[i] \sim bernoulli(tau),\\
&Tp[i] \sim beta(\alpha, \beta),\\
&ATp[i] = Tp[i] \times Z[i],\\
&Se \sim beta(\alpha, \beta),\\
&Sp \sim beta(\alpha, \beta),\\
&tau \sim beta(\alpha, \beta)
\end{aligned}
$$

where *y[i]* is the number of positive animals in each infected herd, *Ap[i]* is the apparent prevalence in each herd, and *n[i]* is the sample taken in every farm. *Tp[i]* is the true prevalence for a truly infected herd. To obtain the actual true prevalence, a mixture of a point probability mass at zero and continuous beta distribution was used to model the probability of animal prevalence being zero as *ATp[i]=Tp[i]×Z[i]* (Verdugo *et al*., 2018). The prior values of the sensitivity (Se) and specificity (Sp) parameters of the ELISA kit were sourced from literature (Lurier *et al*., 2021); Se was set as beta(53.43, 86.43) and Sp as beta(100, 3.02). The prior distribution values of true individual animal prevalence were derived from an unofficial estimation of *C. burnetii* seropositivity in Chile, which is the most likely value (Rosenfeld, 2022). The value reported for sheep herds in Brazil was adopted as the upper level (Guatteo *et al*., 2011) and set as a beta distribution (beta(1.07, 19.21)); the herd-level prevalence *(tau)* was set as beta(1.39, 4.53) (Guatteo *et al*., 2011). Analysis was conducted using OpenBugs 3.2.3 (Lunn *et al*., 2009) through the R2Openbugs R package (Sturtz *et al*., 2005). The assessment of model convergence involved simulating three parallel chains and visual evaluation of the trace and density plots. Gelman-Rubin diagnostics were also used. The model was simulated for 25,000 iterations, with 5,000 discarded as a burn-in period.

The probability of absence of *C. burnetii* in sheep was estimated using the probability formula for surveys to substantiate freedom from disease proposed by Cameron and Baldock (1998), using Epitools-Epidemiologic Calculators (Sergeant, 2018) and making the following assumptions: animal-level prevalence, value obtained by model estimation ELISA Se 40% and Sp 99 % (Lurier *et al*., 2021); and error of 5 %.

Sensitivity analysis

For the sensitivity analysis, two sets of prior values were employed. In Set (A), the test performance was modified by fitting the beta distributions for both Se and Sp derived from the values provided by the test manufacturer. For Se, a confidence level greater than 95% and a mode of 99% were used, whereas for Sp, a confidence level greater than 95% and a mode of 97.3% were applied. In Set (B), the prior parameter for *tau* was represented by a diffuse beta distribution (1,1) (Table 2).

RESULTS AND DISCUSSION

Before the 2017 outbreak, information on *C. burnetii* status in animals and humans was scarce, and several studies have been conducted since then. Results from these studies have shown that *C. burnetii* is highly present in cattle (Hernández-Agudelo *et al*., 2023); however, no studies in sheep have been published to date. The present study found a lack of knowledge about general concepts related to zoonoses among sheep farmers (Table 1). Only 11%, 18%, and 14% of farmers, respectively, stated that they knew the meaning of "zoonotic disease", "coxiellosis" or "Q Fever", although 86% were aware that they could contract diseases from animals; 37% of farmers observed abortions in the last lambing season, only 7% declared having experienced flu-like symptoms in the same period, and 7% said that they did not seek medical help in response. None of the farmers stated that they had suffered from chronic diseases that could be related to chronic Q Fever.

Livestock farming in Lonquimay is characterized by small-scale, extensive practices oriented towards own consumption, with low levels of technification. Farmers in this region tend to be older and have lower education levels (Baez, 2005). These factors, combined with geographical isolation, infrequent veterinary medical attention, and difficult access to health services, may contribute to insufficient awareness of the health risks associated with animal production.

Table 1.
The outcomes of a questionnaire designed to evaluate zoonotic understanding and hazardous behaviors.

Question	% †
Do you raise animals of different species (cattle, sheep, goats)?	75
Have you heard about zoonotic diseases?	11
Have you heard about coxiellosis?	18
Have you heard the disease called Q Fever?	14
Do you know that you can get diseases from your farm animals?	86
Do you introduce animals from more than one origin, with unknown status, to your herd?	75
Do you implement a quarantine period before animal introduction?	10
Do you buy supplementary forage from other sheep herds?	64
Do you attend lambing?	39
Do you use personal protection elements when attending lambing?	57
Do you implement a disposal method for parturition membranes?	70
Do you carry out disinfection of animal housing?	75
Do you carry out peri-partum ewe isolation?	46
Last lambing season, did you observe abortion, stillbirth, or weak lamb birth?	36
Do you remember if you experienced flu-like symptoms in the last lambing season?	7
If you experienced flu-like symptoms, did you seek medical attention?	7
Do you suffer chronic illness compatible with suspicion of chronic Q fever?	0

†Percentages correspond to positive answers to the questions

With respect to biosecurity, the questionnaire revealed management practices that could increase the risk of introduction and dissemination in a herd; 75% of farmers introduced animals from sources with unknown status, and only 10% used quarantine before introduction. However, this primarily involves the introduction of male breeding animals, which results in a lower impact on transmission (Amin *et al.*, 2022).

Risk-related conducts for farmer security were also detected. For instance, 39% of farmers attend lambing and only 57% use protective equipment when attending lambing or other management practices. Nevertheless, 70% of farmers have a disposal method for parturition membranes, and 75% disinfect animal housing. In view of the lack of awareness observed in the survey, it was expected that risky behaviors would promote the transmission of *C. burnetii*. The 2017 outbreak highlighted the need to inform and educate farmers about the potential risks and consequences of *C. burnetii* infection.

Thirty (30) herds were tested, resulting in a total of 260 samples. At least one ELISA-positive animal was found in only one herd (3%) (CI95% = 0.36%-14%). In this herd, 5 animals tested positive (2% of all animals tested). The mTALP was estimated at 4.2% (95% PPI = 1.6-9%), while oTALP was 4.6% (95%PPI = 1.7-10%). *C. burnetii* presence was found to be higher in the population studied than has been reported in unofficial information from SAG, which, in the context of the 2017 Q Fever outbreak, reported a sheep seroprevalence of 0.041% from herds located near cases (Rosenfeld, 2022);

Table 2.
Posterior distribution Median and 95 % PPI for mTALP and oTALP, Se and Sp with two sets of prior values for sensitivity analysis (Set A: Prior sensitivity 99%, Prior specificity 97.3%, Set B: Prior for tau Beta (1,1) 0.041%, sensitivity and specificity are the same as default model.

Parameter	Posterior distribution (Median (95% PPI))		
	Default Model	Set A	Set B
mTALP	4.2 (1.6-9)	4 (1.6-10)	4 (1.6-8)
oTALP	4.6 (1.7-10)	4.3 (1.7-10)	4.1 (1.6-8.5)

however, this estimation is not fully comparable because no information is known about the study methodology used. Nevertheless, in South America, *C. burnetii* seroprevalence is highly variable, ranging from 0% to 66%, suggesting that the presence of *C. burnetii* depends vastly on the characteristics of the country and the specific zone (Epelboin *et al.*, 2023).

The low animal-level prevalence can be explained by the less likely introduction and transmission of *C. burnetii* within and between herds owing to the extensive productive system used, especially during the lambing season (Carrié *et al.*, 2019), and the restricted animal trade, which mostly involves males for breeding (Baez, 2005) (Table 1). The environmental conditions were also considered. The lambing

season begins at the end of winter (September) and lasts until late October, when pastures are still covered with snow. During this season, the animals are mainly fed with stored forage, reducing the probability of *C. burnetii* being acquired from the soil. Although bacterial survival under conditions of extreme cold has been reported, the cold and humidity due to snow reduce dust and dryness, decreasing airborne transmission (EFSA, 2010). Another important aspect is ineffective reproductive management, resulting in a longer breeding season with a low concentration of births despite the relatively small herd sizes, leading to a lower risk of spreading and environmental contamination.

It is likely that the seroprevalence of *C. burnetii* is underestimated. This can be attributed to the selection of ELISA for diagnosis because of its low sensitivity (40%) when used as a screening method. This low sensitivity is caused by false negatives in infected animals due to long exposure periods, infected animals that are still in their incubation period, and infected animals with slow or failed humoral response (Berri *et al*., 2001).

Disease freedom probability estimation showed that the likelihood of the studied population not being free of *C. burnetii* is 34%. This suggests that even when a small number of positive samples were found, it is unlikely that the population is free from the disease.

In the sensitivity analysis, mTALP and oTALP show no significant variation between analysis with Se and Sp set with the manufacturer's values (Set A), and analysis with the herd level prevalence set with a diffuse beta distribution (1,1) (Set B) (Table 2). Across all models, mTALP and oTALP remained very similar, with overlapping PPI intervals. Model convergence ensures that the estimation obtained can be safely utilized for drawing inferences (Dodds & Vicini, 2004).

CONCLUSIONS

This study revealed evidence of exposure to *C. burnetii* in sheep herds in Lonquimay, indicating a low individual and herd-level prevalence. The characteristics of the production system, geography, and environment of the study area are likely to provide a low-risk scenario for bacterial transmission. Future studies are needed to estimate the prevalence of *C. burnetii* active infection in at-risk animals and to investigate in greater depth the factors contributing to this low-risk transmission scenario. Initiatives to improve zoonotic knowledge among farmers need to be implemented in the short term.

DECLARATIONS

Competing interest statement

The authors declare that they have no competing interests.

Ethical statement

All procedures were approved by the Universidad Católica de Temuco Ethical Research Committee on 29 December 2023, reference number: CEIUCT1229001/23.

Author contributions

OAV: supervision, conceptualization, methodology, data curation, formal analysis, funding acquisition, writing-review & editing. SN: investigation, validation. CA: investigation, validation, writing-review & editing. RP: investigation, resources.

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