

Serosurveillance of pathogenic *Leptospira* in ruminants from a veterinary teaching hospital

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ABSTRACT. Leptospirosis can affect livestock health and production, potentially causing abortion, stillbirth, and infertility. An endemic presentation of infection in ruminants most likely occurs in southern Chile. A pilot study was conducted in a veterinary hospital in Puerto Montt to perform epidemiological surveillance to determine the seropositivity of pathogenic *Leptospira* in different ruminant species using a microscopic agglutination test (panel of eight serogroups) and to identify the most common serogroups and antibody titers in seropositive animals. Serum samples were collected from 43 ruminants (20 cattle, 18 sheep, 4 goats, and 1 alpaca). The overall seropositivity was 69.7% (95% confidence interval (CI) = 56.0–83.4), which is the highest rate reported so far in ruminants in the country. Elevated seropositivity was recorded in cattle (85.0%; 95% CI = 69.3%–100%), followed by sheep (66.7%; 95% CI = 45.0%–87.0%) and goats (25.0%; 95% CI = 0%–67.4%). The predominant serogroups were Tarassovi and Sejroe, followed by Autumnalis and Canicola, with different antibody titers according to the serogroup. It is recommended to consider the implementation of epidemiological surveillance for pathogenic *Leptospira* in different settings, such as other veterinary hospitals and farms, to elucidate the reproductive and economic consequences that the disease may cause in different ruminant species, and to apply preventive measures due to the zoonotic potential of the bacterium.

Keywords: Pathogenic *Leptospira*, Surveillance, Ruminants, Seropositivity, Serogroups, Antibody titers

INTRODUCTION

Leptospirosis is a zoonotic bacterial disease that affects the health and production of livestock worldwide (Mughini-Gras et al., 2014). In cattle, the clinical signs associated with leptospirosis are reproductive and may include abortion, stillbirth, and infertility. When the disease becomes acute and multi-systemic, usually affecting calves and lactating cows, calves present with fever, hemolytic anemia, hemoglobinuria, and jaundice (Mughini-Gras et al., 2014; Loureiro & Lilenbaum, 2020; Sykes et al., 2022). In sheep, acute leptospirosis is represented by anorexia, diarrhea, and “milk loss syndrome” (Ellis, 1994; Martins et al., 2014). In goats, the disease has an acute or chronic course, with clinical signs such as anemia, jaundice, and hemoglobinemia. There may also be afebrile and non-icteric animals (Monahan et al., 2009). In small ruminants, reproductive signs such as abortions, infertility, mortality, and birth of weak offspring are also frequent (Martins & Lilenbaum, 2014a).

The microscopic agglutination test (MAT) is the gold standard for serological diagnosis of leptospirosis due to its high specificity (Goris & Hartskeerl, 2014). This test provides information on *Leptospira* serogroups that cause seropositivity or infection, which is relevant from an epidemiological point of view because it allows us to determine the animal reservoirs from which serological reactivity originates and is a widely used method in research, especially if representative serovars/serogroups of the geographic area under study are included in the panel (Bourhy et al., 2013; Haji et

al., 2022). Identification of circulating serogroups is crucial for defining prevention measures for infection (Lilenbaum & Martins, 2014).

Animal disease surveillance involves the systematic collection of long-term data on a disease and determination of preventive measures (Kumar et al., 2021). *Leptospira* infection is likely endemic in many countries that lack surveillance or diagnostic facilities, particularly for animals (Cilia et al., 2021). Routinely collected public health surveillance data are often incomplete but serve as a useful resource for monitoring incidence and tracking progress during disease interventions (Benschop et al., 2021). A systematic review of spatial epidemiological approaches to inform leptospirosis surveillance and control found that 33 studies utilized mapping approaches to describe spatial heterogeneity in the incidence/prevalence and serological status of *Leptospira* infection among various reservoir animals, including companion animals, livestock, rodents, and wildlife. However, few studies have created prevalence maps at national or subnational levels (Dhewantara et al., 2018). This pilot study aimed to perform epidemiological surveillance and determine the seropositivity for pathogenic *Leptospira* in different ruminant species (cattle, sheep, and goats) in a veterinary hospital in southern Chile using MAT, as well as to identify the infecting serogroups and quantify the anti-*Leptospira* antibody titers.

MATERIALS AND METHODS

Study design and sample collection

This was a cross-sectional, descriptive, and quantitative observational study (Hernández *et al.*, 2011). This study was conducted on ruminant patients who attended the Veterinary Clinical Hospital, Universidad San Sebastián, Puerto Montt, Los Lagos Region, Chile. Cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*), and alpacas (*Vicugna pacus*) were the animal species analyzed. Each owner of the animal was asked to sign an informed consent form, in which authorization to participate in the study was expressed. Although vaccines against leptospirosis are used in certain species in Chile, the animals sampled had no vaccination history against the bacteria, which was determined by anamnesis.

All animals admitted to the medical care area for large animals of the Veterinary Clinical Hospital between December 2022 and June 2023 were included in the study. Serum samples stored in a serum bank belonging to the hospital's clinical laboratory that were collected during the study period were also used. A convenience sampling method was applied (Thursfield, 1990). The inclusion criteria for admission into the study were that the animal was a ruminant and that it received medical attention at the aforementioned health center. A total of 43 animals were sampled: 20 cattle, 18 sheep, four goats, and one alpaca. Approximately 1 ml of blood was collected from the jugular vein of each animal, which was distributed in a tube without additives, kept between 4°C and 6°C for 24 h, and centrifuged. The serum was stored at -20°C until analysis.

Microscopic agglutination test (MAT)

MAT was performed according to a standard methodology (Faine, 1994; WHO-ILS, 2003). A panel of eight pathogenic *Leptospira* serogroups/serovars of frequent presentation in domestic animals in Chile were used: *Leptospira interrogans* serovar Hardjo (serogroup Sejroe), Pomona (serogroup Pomona), Canicola (serogroup Canicola), Icterohaemorrhagiae (serogroup Icterohaemorrhagiae), Autumnalis (serogroup Autumnalis), and Bratislava (serogroup Australis); *Leptospira borgpetersenii* serovar Tarassovi (serogroup Tarassovi); and *Leptospira kirschneri* serovar Grippotyphosa (serogroup Grippotyphosa). The samples were subjected to screening and determination of antibody titers at 1:100, 1:200, 1:400, 1:800, and 1:1600, with the highest positive sample being recorded (Faine, 1994; WHO-ILS, 2003). The same antibody titers for different serogroups were classified as coagglutination (Silva & Riedemann, 2007).

Data Analysis

The overall seropositivity by animal species was calculated using the number of individuals that tested positive for MAT divided by the the number of individuals sampled (Dohoo *et al.*, 2003). The 95% confidence intervals (95% CI) were also calculated (Noordhuizen *et al.*, 1997). The detected serogroups and antibody titers for ruminants were deter-

mined. The data were then grouped by animal species, and the distribution with absolute and relative frequencies was expressed as descriptive statistics.

RESULTS AND DISCUSSION

Anti-*Leptospira* antibodies were detected in 30 of 43 samples, corresponding to an overall seropositivity rate of 69.8% (95% CI = 56.0–83.4%). The highest seropositivity was recorded in cattle (85.0%; 95% CI = 69.3–100%), followed by sheep (66.7%; 95% CI = 32.2–87.0%) and goats (25.0%; 95% CI = 0–67.4%). The alpaca sample tested negative. None of the sampled or seropositive animals exhibited clinical signs suggestive of leptospirosis. In Chile, serological studies on leptospirosis in ruminants date back several decades. In cattle, Zamora *et al.* (1975; 1991) reported seroprevalences of 59.1% and 44.9%, respectively, using MAT. More recent research has determined that herd prevalence ranges from 42.2% to 75.0% (Salgado *et al.*, 2014; Montes & Monti, 2021). In sheep and goats, only two studies have estimated anti-*Leptospira* seroprevalence in Southern Chile. One estimated a prevalence of 7.4%, and reported an antibody prevalence of 24.8% using MAT in goats (Zamora *et al.* 1975), and another conducted in the Los Lagos Region, which estimated a seroprevalence of 5.7% (Zamora *et al.*, 1999).

The Sejroe serogroup presented 15 reactive samples, nine of which belonged to cattle with antibody titers of 1:400 (44.4%), 1:200, 1:800 (22.2% each), and 1:1600 (11.1%). Five sheep samples tested positive, with antibody titers of 1:400, 1:800 (40.0% each), and 1:1600 (20.0%). In goats, one sample (100%) contained antibodies at a titer of 1:400 for this serogroup (Table 1). In the study by Montes and Monti (2021) on cattle in Chile, the most frequently detected serovars in unvaccinated animals were Hardjo, Tarassovi, and Pomona. A systematic review found that the most reported circulating serogroup in European cattle is Sejroe (Sohm *et al.*, 2023). Similar results have been reported for cattle, sheep, and goats in Iran and Malaysia (Rahman *et al.*, 2020; Haji *et al.*, 2022).

The epidemiology of leptospirosis is closely related to the presence of maintenance and incidental hosts in certain geographic areas (Cilia *et al.*, 2021). Cattle serve as maintenance hosts for the Hardjo serovar (Ellis, 1994). Although rats excrete higher concentrations of leptospire, cattle produce significantly more urine, leading to greater environmental contamination, with shedding lasting approximately 28–40 weeks (Leonard *et al.*, 1992). Transmission of the infection within cattle as well as among cattle, sheep, and goats could explain the seropositivity detected in this study, particularly because of the shared grazing locations and the coexistence of different ruminant species on the farms of origin. Infection with serovar Hardjo in cattle may occur without clinical signs, but can also lead to decreased milk production, abortions, and reproductive issues (Grooms, 2006). However, the role of apparently healthy animals in the maintenance and transmission of leptospirosis can be better assessed by

determining the predominant serovars associated with seropositivity and infection (Sriji *et al.*, 2022). Monti *et al.* (2023) studied the dynamics of pathogenic *Leptospira* elimination in naturally infected cattle across six farms with a history of leptospirosis in the Los Ríos and Los Lagos regions of Chile. They observed patterns of urine elimination lasting between 79 and 259 days, with bacterial loads ranging from 3×10^4 to 4.4×10^4 bacteria/ml, where many of the positive animals were asymptomatic.

The serogroup with the second highest seropositivity in this study was Tarassovi, with 10 reactive samples, six of which were from cattle, exhibiting antibody titers of 1:200, 1:400, and 1:1600 (33.3% each). Four sheep samples had antibody titers of 1:400 (75.0%) and 1:1600 (25.0%) (Table 1). A recent study at the local Wildlife Rehabilitation Center in Puerto Montt, (Los Lagos Region, Chile), detected seropositivity for the Tarassovi serogroup in wild mammals and birds (Balcázar *et al.*, 2024), which is consistent with another report in horses from the Los Lagos Region (Moreno *et al.*, 2024). These findings suggest that serological reactions indicative of an immune response to the Tarassovi serogroup are common in southern Chile. Cilia *et al.* (2020; 2021) pointed out pigs and wild boars as maintenance hosts for this serovar, while Zamora *et al.* (1999) associated it with wild animals, such as the Culpeo and gray foxes (*Lycalopex culpaeus* and *Lycalopex griseus*, respectively). Human

encroachment on wildlife habitats has been linked to potential conflicts that can facilitate the emergence of diseases in livestock, wild species, and humans (Jiménez-Ruiz *et al.*, 2024). According to Muyulema *et al.* (2024), the interaction between domestic and wild animals in the epidemiology of leptospirosis is evident, and spillover could occur; thus, avoiding contact between cattle and pigs is a practical preventive measure. Although the pathogenicity of the Tarassovi serovar in animals remains unclear, it has been associated with aseptic meningitis in humans in a case series study in Sri Lanka (Bandara *et al.*, 2021).

Another serogroup with reactive samples was Autumnalis, with one positive sample from cattle and two from sheep exhibiting antibody titers of 1:100 (100%), 1:100 (50.0%), and 1:200 (50.0%), respectively. For the Canicola serogroup, only one sample tested positive in cattle, with titers of 1:400 (100%), and one sample was reactive at a titer of 1:800 (100%) in sheep (Table 1). Serological reactions to the Autumnalis serogroup have been reported to originate in species such as mice (*Mus musculus*), which are its maintenance hosts (Greene *et al.*, 2008). These animals typically do not exhibit clinical signs but harbor leptospires in their kidneys, which becomes a significant source of infection (Adler & de la Peña Moctezuma, 2010). The contact between ruminants and rodents may be associated with the serological reactions detected. Moinet *et al.* (2023) indicate that

Table 1.

Number and frequency (%) of samples positive for serogroups with serological reactions and antibody titers for pathogenic *Leptospira* according to animal species. Veterinary Clinical Hospital, Universidad San Sebastián. December 2022 to June 2023. Puerto Montt, Los Lagos Region, Chile.

Serogroups*	Antibody titers	Cattle		Sheep		Goats	
		Number	%	Number	%	Number	%
Sejroe	1:200	2	22.2	0	0	0	0
	1:400	4	44.4	2	40	1	100
	1:800	2	22.2	2	40	0	0
	1:1600	1	11.2	1	20	0	0
	Total	9	100	5	100	1	100
Autumnalis	1:100	1	100	1	50	0	0
	1:200	0	0	1	50	0	0
	Total	1	100	2	100	0	0
Tarassovi	1:200	2	33.3	0	0	0	0
	1:400	2	33.3	3	75	0	0
	1:800	2	33.3	1	25	0	0
	Total	6	100	4	100	0	0
Canicola	1:400	1	100	0	0	0	0
	1:800	0	0	1	100	0	0
	Total	1	100	1	100	0	0

* Only serogroups with positive results are shown.

farm surroundings often provide ideal habitats for mice; thus, careful land management is necessary for effective pest control. Mulyema *et al.* (2024) added that longitudinal epidemiological studies could help determine whether the density of infected rodents reflects the risk of spillover to other species.

An epidemiological study conducted on domestic cats (*Felis catus*) in southern Chile also determined seropositivity for Autumnalis (Azócar-Aedo *et al.*, 2014a), similar to a study on sheep (Zamora *et al.*, 1999). This serogroup has been detected in bovine studies in Iran (Shafiqhi *et al.*, 2010), sheep and goats in Brazil (Martins *et al.*, 2014a; Barbante *et al.*, 2014; Moraes *et al.*, 2024), and among goats in Poland (Czopowicz *et al.*, 2011). Regarding the Canicola serogroup, domestic dogs are its maintenance hosts (Greene *et al.*, 2008), and it has been frequently diagnosed in bovines in Ecuador (Burgos *et al.*, 2019) and Egypt (Selim *et al.*, 2024), as well as in sheep and goats in India (Balamurugan *et al.*, 2021). The prevalence reported across studies varied. This may be explained by the climatic conditions and landscape differences in these countries, which influence the presence of leptospires in the environment (Azócar-Aedo & Monti, 2022), as does the use of different diagnostic tests (direct or indirect methods), sample sizes, characteristics of the sampled animals, sampling designs, and cut-off values for categorizing an animal as “positive” for the bacteria (Azevedo *et al.*, 2005).

An antibody titer of 1:100 for *Leptospira* is considered a positive serological reaction (OIE, 2021), indicating exposure to bacteria (Faine, 1994). Antibody titers of 1:400 or higher in endemic areas and 1:100 or higher in non-endemic areas were classified as clinical cases of infection if the animal presented with compatible clinical signs (WHO, 2010). In humans, these levels of serological reactivity are associated with probable active infections (WHO-ILS, 2003). Molecular tests based on DNA amplification enable the detection of *Leptospira* spp. during the acute phase of the disease, with antibodies below the detection limits of most serological tests (Ahmed *et al.*, 2014; Denipitiya *et al.*, 2016). PCR assays offer high sensitivity by detecting specific nucleotide sequences of the leptospiral genome in blood, urine, or tissue samples, confirming the infection, whereas their presence in kidney tissue or urine indicates either infection or colonization, making them an alternative for accurate diagnosis in places where specialized laboratories exist (Mancel *et al.*, 1999). Therefore, the use of molecular tests is recommended in future studies.

In this study, a proportion of ruminants from all three species exhibited antibody titers equal to or greater than 1:400 for serovars Hardjo, Tarassovi, and Autumnalis. In Chile, vaccines are available for cattle that include various serovars, including Hardjo, Pomona, Canicola, Icterohaemorrhagiae, and Grippotyphosa (Zoetis, 2024). Vaccination can significantly reduce the occurrence of clinical signs of leptospirosis in herds (e.g., abortions) (Lilenbaum & Martins, 2014). Adaptive immunity in leptospirosis is serotype-spe-

cific, and the protection conferred by vaccination is directed toward homologous serovars of the vaccine without cross-immunity (Murray *et al.*, 2013). Although protection is limited, vaccines represent a viable preventive measure for livestock producers, and circulating serovars or serogroups in the geographic area must be considered (Bautista *et al.*, 2014). The productivity of cattle and small ruminant breeding can be improved if adequate sanitary conditions are maintained, and if *Leptospira* reservoir animals are identified, this could potentially control the infection and reduce the economic risks associated with reproduction (Martins & Lilenbaum, 2014b).

To ensure the efficacy of current *Leptospira* vaccines in each animal species, such as bovines, it is essential to survey infected animals and characterize the recovered isolates to identify all species and serovars involved in the disease (Hamond *et al.*, 2022). The findings of this study indicate that further research with a larger sample size is necessary to determine the frequency of serovar presentation and their potential inclusion in future vaccines, as well as to ascertain the association between seropositivity and environmental factors, such as contact with rodents, pigs, or wild boars coexisting with ruminants.

To provide optimal patient care, appropriate efforts must be directed toward preventing disease transmission with measures based on results obtained through surveillance. Every facility is unique; thus, efforts should be tailored to the distinctive physical attributes and organizational limitations of individual practices (Burgess & Morley, 2015). Given that leptospirosis has a complex epidemiology involving a variety of domestic and wild animal species combined with environmental factors (Azócar-Aedo *et al.*, 2014b), some preventive measures that could be implemented include improved husbandry practices and providing information about the disease to animal producers to raise awareness.

The limitations of this study are the relatively small sample size and the fact that the animals sampled were patients from a veterinary hospital. However, the detection of anti-*Leptospira* antibodies in cattle, sheep, and goats confirms the presence of seropositivity and exposure to these bacteria. Therefore, attention must be paid to potential clinical cases. Southern Chile is the center of the country's livestock and milk production, and the implications for animal health and the economic significance of *Leptospira* infections require awareness. Therefore, epidemiological studies on leptospirosis in different ruminant species have become increasingly relevant. For future research, the use of molecular diagnostic tests to detect pathogenic *Leptospira* is recommended, such as PCR in urine samples or bacteriological cultures of kidney and/or liver tissue samples. In addition, the study should be expanded to include other animal species and larger sample sizes, considering the One Health approach in the design of these studies.

This study reports the detection of serologically reactive ruminants of the genus and species *Leptospira* at a veterinary hospital facility. The key takeaways are that epidemiological

surveillance for *Leptospira* infection should be implemented more frequently in various environments to identify seropositive animals and that biosecurity measures should be applied to hospital staff (and in other health centers) due to the zoonotic risk, along with preventive measures to minimize the potential for transmission among different animal species attending these facilities. All these actions will help reduce the perception of leptospirosis as a “neglected disease.” Despite the relatively small sample size, this study highlights that animals without clinical signs of the disease can exhibit high seropositivity to bacteria when visiting a health center for various reasons.

Regarding the zoonotic potential of *Leptospira*, humans can be infected through direct contact with blood, aborted fetuses, vaginal secretions, or birthing products from infected animals. Indirectly, infections can be caused by environmental contamination of urine (Montes & Monti, 2021). Epidemic outbreaks of leptospirosis have been reported in humans with occupational exposure to cattle in Australia (Brown *et al.*, 2022). A study conducted in Malaysia found a prevalence of anti-*Leptospira* antibodies in 75.0% of cattle farmers (Daud *et al.*, 2018). Therefore, it is crucial for people to implement preventive measures for leptospirosis, such as using personal protective equipment (including gloves, sleeves, and work clothing) and practicing strict hand hygiene after contact with potentially infected animals (Azócar-Aedo, 2023). Epidemiological surveillance of the disease in humans and animals at the local, regional, or national level is a viable option, although at present, it is extremely limited (Arunima *et al.*, 2024).

In conclusion, ruminant animals at a Veterinary Clinical Hospital in the Los Lagos Region, Puerto Montt, Chile, were exposed to pathogenic *Leptospira*, with an overall seropositivity of 69.7%, representing the highest prevalence reported to date in ruminant studies in the country. The most frequently diagnosed serogroup was Sejroe, followed by Tarassovi, Autumnalis, and Canicola. The predominant antibody titers were 1:400 and 1:800. It is recommended that epidemiological surveillance be implemented in various settings, such as other veterinary hospitals and farms, to establish preventive measures and conduct research using the One Health approach. This will elucidate the bacterial load and the economic, productive, and reproductive consequences that the agent may cause in different ruminant species.

Competing interests

The authors declare that they have no competing interests.

Ethical statement

This study was approved by the Institutional Committee for Ethics in the Use and Care of Animals of the Universidad San Sebastián, Chile (certification number: 015-22).

Author Contributions

Conceptualization, MU and LAA; methodology, MU and LAA; software, MU and LAA.; validation, MU, LAA, and MG.; formal analysis, MU

and LAA; data curation, MU, LAA, and MG.; writing-original draft preparation, LAA and MG; writing-review and editing, LAA and MG; and funding acquisition, LAA.

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