



A Serological Survey of Leptospirosis in Sheep in Chile

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ABSTRACT. In order to investigate exposure to *Leptospira* spp. in sheep in the Xth Region in the south of Chile, 629 ovine serum samples were collected from 11 convenience selected sheep farms, using within farm random sampling. The sera collected were examined for antibodies to the following serovars; *icterohaemorrhagiae*, *autumnalis*, *hardjo*, *pomona*, *ballum*, and *canicola*, using the Microscopic Agglutination Test. Ten out of eleven flocks had sheep which were seropositive for at least one *Leptospira* spp. Positive tests, the majority at low titres, were obtained from 36/629 (5.7%) of all the serum samples tested. The most frequently detected serovars were, in descending order: *icterohaemorrhagiae*, *autumnalis* and *hardjo*. The greatest proportion of positive samples came from sheep which were over 30 months old. Key words: Leptospirosis, Sheep, Serology.

RESUMEN. Se investigó serológicamente la leptospirosis ovina en la X Región del sur de Chile, para lo cual se le extrajo sangre 629 ovinos seleccionados al azar provenientes de 11 predios. Los sueros se examinaron mediante la prueba de aglutinación microscópica, determinando anticuerpos contra los siguientes serovares: *icterohaemorrhagiae*, *autumnalis*, *hardjo*, *pomona*, *ballum* y *canicola*. Se constató que 10 de los 11 ovejerías tenían ovejas positivas al menos frente a un serovar de leptospirosis. Treinta y seis (5,7%) sueros presentaron anticuerpos, la mayoría de los cuales reaccionaron a títulos bajos. Los anticuerpos más frecuentemente detectados en orden descendente lo hicieron en contra de los serovares *icterohaemorrhagiae*, *autumnalis* y *hardjo*. La proporción mayor de muestras positivas correspondió a a ovejas mayores de 30 meses de edad.

Palabras clave: Leptospirosis, Ovinos, Serología.

INTRODUCTION

Leptospirosis is an important zoonotic disease affecting most mammals, including all domestic livestock species, and it is widely recognised as a cause of considerable economic loss.^{11,32} Studies in southern Chile indicate that leptospirosis causes severe clinical disease and economic loss in cattle^{23,24} and pigs,²⁶ but little is known about leptospirosis in sheep. In other countries sheep are known to be infected with many serovars e.g. Australia,^{5,13} Canada,¹⁷ UK,²⁰ Ireland,⁹ and clinical disease has been recorded both experimentally²⁹ and on farms.^{7,22,27}

This study was undertaken to investigate the prevalence, strains and presence of clinical disease in sheep in the Xth Region of the south of Chile.

MATERIAL AND METHODS

Study population. Eleven flocks were convenience selected according to their management, distance from the University and willingness of the owners to participate in the study. There were four small (less than 50 sheep), three medium (51 to 300 sheep) and four large (more than 300 sheep) flocks. Blood was collected into plain vacutainers

from the jugular vein of all the ewes from the small flocks and from 25 randomly selected ewes from each of three different age categories (six to 18 months, >18 to 30 months and over 30 months) from the medium and large flocks.

Antibody serology. The serum was removed from each blood sample and stored at -20 °C. Agglutinins against *Leptospira* serovars *autumnalis*, *ballum*, *canicola*, *hardjo*, *icterohaemorrhagiae* and *pomona* were detected by a microscopic agglutination test (MAT) using doubling dilution from 1:100 to 1:1600. Seropositive samples were those with at least 50% agglutination at 1:100 final serum dilution.¹⁷

Statistical analysis. The association between seropositivity and size of flock and age of ewes was tested using a chi square test⁸. Alpha was set at 0.05 for a two-tailed null hypothesis.

RESULTS

Ten out of 11 flocks had sheep which were seropositive to at least one strain of leptospire (Table 1). The distribution of serovars varied between the flocks. Three flocks had antibodies for 3 or more serovars, two flocks had anti-



Table 1. MAT results according to the flock size and age of the sheep.

Flocks		Age in months**							
Size	Positive*	6-18		>18-30		>30		All Ages	
		Number	%	Number	%	Number	%	Number	%
Small	3/4	0/20	0	0/12	0	10/112	8.9	10/144	6.9
Medium	3/3	0/53	0	2/40	0	12/101	1.9	14/194	7.1
Large	4/4	2/98	2	5/74	6.8	5/119	4.2	12/291	4.1
Total	10/11	2/171	1.7	7/126	5.6	27/332	8.1	36/629	5.7

* Flocks affected/flocks tested

** Number of sheep positive/number of sheep tested

Table 2. Number of samples positive for each serovar, as determined by MAT

Serovar/Titres*	100	200	400	800	>1,600	Total	Percentage (%)
Icterohaemorrhagiae	7	6	1	0	0	14	38.9
Autumnalis	2	2	1	0	2	7	19.4
Hardjo	2	2	1	0	0	5	13.9
Ballum	1	1	0	1	1	4	11.1
Pomona	2	1	0	0	0	3	8.3
Canicola	0	1	0	0	0	1	2.8
Coagglutination c-b	1	0	0	0	0	1	2.8
p-i	1	0	0	0	0	1	2.8
Total	16	13	3	1	3	36	
Percentage	44.5	36.1	8.3	2.8	8.3		100

Reciprocal of the dilution. c-b, canicola-ballum; p-i, pomona-icterohaemorrhagiae.

bodies for 2 serovars and four flocks had positive samples for 1 serovar. One flock had coagglutination between serovars and one flock had no positive animals (Table 2).

A total of 36 of the 629 samples taken were seropositive (Table 2). The majority of titres were 1:100 or 1:200 (Table 2). The serovar detected most frequently was *icterohaemorrhagiae* since it was found in 8 of the 11 flocks, and 38.9% of the samples. This was followed by *autumnalis* and *hardjo* (Table 2).

None of the sheep had evidence of clinical leptospirosis.

DISCUSSION

From this study we estimate that 5.7% of the sheep sampled had positive serology to *Leptospira* spp. The prevalence of positive sera, with titres of 1:100 or higher, is similar to the prevalence described previously in Chile of 7%.³⁴ Similar prevalences have been reported by some workers in England and Wales.¹⁴ However, higher preva-

lences have been described in Australia²¹ and Germany³⁰ and also in some parts of England.¹⁵ Slightly lower percentages of seropositive sheep have been described in Italy³¹ and Barbados.¹⁹ However different results have been reported in some South American countries, for example in Bolivia⁴ a seroprevalence of 14.3% was reported and in Brazil² a seroprevalence of 63.8% was reported.

The prevalence of seropositive samples would be lower if interpreted according to the criteria that considers titres of 1:100 as negative, 1:200 as suspicious and 1:400 as positive as was done by Witt et al.³³ While the latter criteria may be used meaningfully during an acute infection, the titres in this study of 1:100 are probably a consequence of a previous contact with the bacteria.

In this study no sheep showed clinical signs of the disease. The sheep have either been sick in the past or seroconverted without disease. Sheep in the Xth Region of Chile are not presented sick with leptospirosis, so it is possible that sheep less susceptible to clinical leptospirosis.³² This also has been suggested by authors in the UK^{11,14,20} and Spain¹⁸ although others¹⁶ recorded mortality rates in



sheep of up to 70%.

It has to be remembered that animals without clinical signs could be infected, Gordon¹³ reported that sheep with titres of 1:64 had leptospiuria. Other researchers considered as seropositive lambs with titres of 1:40²⁸, 1:48¹, 1:50²¹, and 1:100³³. In order to demonstrate an active infection serial test should be done.

From this study it can be concluded that *icterohaemorrhagiae* is the most important in the sheep and flocks investigated in this study. This is in agreement with previous reports³⁵ and it has been reported as one of most important causes of abortion in Spain.¹⁸ However, this serovar is of secondary importance in other countries.^{1,7,10,30}

In past serological surveys made in different species, including sheep, low titres of antibodies against serovar *autumnalis*, with a low prevalence 0.83% in cattle, 2.41% in horses and 2.81% in dogs, were generally found.³⁴ Consequently, it is drawn to attention that in this study 19.4% of the animals were seropositive to this serovar, even at the highest titres in two occasions; this is specially interesting considering that this serovar has not been isolated in domestic animals or wild rodents in Chile.²⁵

In other countries results are variable; Egan and Yearsley⁹ reported a 0.25% of seropositive sheep in Ireland; on the other hand, Shönberg et al³⁰ in Germany found that 14.4% of 3,000 sheep examined were seropositive to this serovar. Hathaway et al¹⁴ in England and Wales found that the highest percentage of seropositive sheep, reacted to serovar *autumnalis* (7.3%).

According to Hathaway et al¹⁴ serovar *autumnalis* usually presents crossreactions with other serovars. Therefore, considering that in Chile this serovar has not been isolated neither from sheep nor from other species of domestic or wild animals the authors are in agreement with Hathaway et al¹⁴ in that, until the serovar *autumnalis* has not been isolated, its importance as a source infection for sheep should be considered with caution.

Some authors have suggested that sheep are maintenance host of serovar *hardjo*^{5,6,13,14} but others are of the opinion that the sheep may be an accidental host⁹ and that cattle are the natural maintenance host.¹¹ Nevertheless, some Italian researchers,^{3,12} working with sheep infected either naturally or experimentally, point out that sheep can play an important role as a reservoir of this serovar. The apparently high seroprevalence of *hardjo* in this work (13.9%) could be associated to the fact that in some flocks sheep grazed together with cattle; in Chile *hardjo* is commonly found in cattle.³⁶ It is also common to find antibodies to this serovar in wild rodents in rural areas of Valdivia.³⁸

Blackemore et al¹ recorded a 3.8% of prevalence of *pomona* considering it lower than the prevalence recorded for cattle. The serovar *pomona* had low prevalence (8.3%) in this study. In the south of Chile *pomona* is now decreasing in importance in cattle having been the most prevalent leptospiral serovar.^{23,35,37} *Pomona* has been described as

one of the most important causes of abortion in Spain¹⁸. The effect of *pomona* in ovine fetuses has also been shown by experimental studies²⁹ and in flocks of lambs.^{7,29}

The low prevalence of *canicola* despite the frequent contact of sheep with dogs who are an important host in Chile,³⁴ may indicate that sheep are highly resistant to this serovar.

In this study the proportion of seropositive samples increased with the age of the sheep. This could indicate a greater opportunity for exposure to the infection. The size of the flock does not seem to influence the presence of antibodies.

This study suggests that the infection occurs at approximately 6% and that there are at least six serovars which sheep develop antibodies to. The importance of these serovars to the health of sheep or to spread of *Leptospira* serovars through the sheep causing disease in other domesticated species was beyond the remit of this study. Further work is needed to determine whether infection in sheep is associated with clinical disease, carrier status or of economical significance in southern Chile.

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