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MICROBIOLOGY

Seroprevalence of *Leptospira* spp. infection in sheep from northwest of Rio Grande do Sul, Brazil

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ABSTRACT: *Leptospirosis is a zoonosis that is widely distributed in tropical countries. This infection is also associated with reproductive losses in livestock, which has a significant economic impact. The objective of this study is to investigate the seroprevalence and risk factors associated with leptospiral infection in commercial sheep flocks from the northwestern mesoregion of Rio Grande do Sul. Serum samples were analyzed in 319 sheep using the Microscopic Agglutination Test (MAT). At the time of sampling, an individual and herd questionnaire was carried out to investigate the risk factors. On an individual level, positive reactions were observed in 5.6% (18/319) of the studied sera, with Sejroe being the most prevalent serogroup. Animals >3 years old were more likely to be seropositive than animals 1-3 years old (OR 14.4; 95% CI 1.9-110). The predominance of Sejroe serogroup in this manner draws attention to how subclinical infection is associated with economic losses. Therefore, the importance of maintaining measures for the prevention and control of leptospirosis among the sheep flocks of the studied region is reiterated.*
Key words: *Leptospira spp., sheep, MAT, seroprevalence.*

Soroprevalência da infecção por *Leptospira* spp. em ovinos do noroeste do Rio Grande do Sul, Brasil

RESUMO: *A leptospirose é uma zoonose amplamente distribuída em países tropicais. Essa infecção também está associada a perdas reprodutivas na pecuária, apresentando uma relevância econômica notável. O objetivo deste estudo foi investigar a soroprevalência e os fatores de risco associados à infecção por leptospirose em rebanhos comerciais de ovinos da mesorregião noroeste do Rio Grande do Sul. Amostras de soro de 319 ovelhas foram analisadas pelo Teste de Aglutinação Microscópica (MAT). No momento da amostragem foi realizado um questionário individual e de rebanho para investigar os fatores de risco. No nível individual, reações positivas foram observadas em 5.6% (18/319) dos soros estudados, sendo Sejroe o sorogrupo mais prevalente. Animals >3 years old were more likely to be seropositive than animals 1-3 years old (OR 14.4; 95% CI 1.9-110). A predominância do sorogrupo Sejroe chama a atenção para a infecção subclínica associada a perdas econômicas. Portanto, reitera-se a importância da manutenção de medidas de prevenção e controle da leptospirose entre os rebanhos ovinos da região estudada.*
Palavras-chave: *Leptospira spp., ovinos, MAT, soroprevalência.*

INTRODUCTION

Sheep farming is one of the most important activities in Rio Grande do Sul, offering great economic potential for the region. The state is the largest producer of sheep in Brazil (IBGE, 2014), with reproductive efficiency being the basis of profitability in sheep flocks (FTHENAKIS et al., 2015). However, there are several challenges for the maintenance of reproductive efficiency, with handling infectious diseases being key among them. Leptospirosis is a significant zoonotic bacterial disease (ALLAN et al., 2018; COSTA et al., 2015), which has been associated with reproductive failure and economic losses in sheep (RIDLER et al., 2015).

Leptospirosis causes subclinical infections in sheep such as infertility, an increased number of services per conception and extended

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delivery intervals, miscarriage, stillbirths, and weak lambs (MARTINS & LILENBAUM, 2014). The seroprevalence of anti-*Leptospira* antibodies in sheep flocks in Brazil varies from 3% in São Paulo (AMORIM et al., 2016) to 47.4% in Rio de Janeiro (MARTINS et al., 2012). Additionally, Sejroe is the serogroup most associated with this infection in small ruminants, causing many reproductive failures in our country (MARTINS et al., 2012; VALLÉE et al., 2018).

Leptospirosis is a neglected disease and few studies have been conducted in the state of Rio Grande do Sul, despite the great impact of the infection on the economy. In addition, there is a lack of information regarding the factors associated with such infection. Thus, this study aimed to investigate the seroprevalence and risk factors associated with leptospirosis infection in commercial sheep flocks of the northwestern mesoregion of Rio Grande do Sul.

MATERIALS AND METHODS

A cross-sectional study has been conducted using 319 serum samples from sheep collected between August to October of 2016. Samples were from 14 flocks located in 10 municipalities of the northwest mesoregion of Rio Grande do Sul (Figure 1), chosen by convenience but respecting the representative sample size distribution. To be included in this study, flocks should have at least 20 animals, being at least one adult male and one young male (1 year) and the remaining females. Serum samples were assayed by a microscopic agglutination test (MAT). The total population of sheep in the studied region was 327,828 according to the Brazilian Institute of Geography and Statistics (IBGE, 2014). Thus, the minimum representative number (n) of samples required for this study was 280 assessed considering a 22.8% of expected prevalence (RIZZO et al., 2014), 5% error and 95% CI using the software EpiInfo 7.1.5 (CDC, 2002). Blood samples were collected by venipuncture of the jugular vein in 10 mL tubes (Vacutainer®) without anticoagulant, then conditioned in an isothermal container at 5 ºC, centrifugated at 1,000 xg for 10 min and sera were conditioned in microtubes at -20 °C.

The MAT was performed as recommended by the OIE (2008). Samples were tested for a panel of ten live antigens of *Leptospira* serovars (serogroups), Hardjoprajitno (Sejroe), Icterohaemorrhagiae and Copenhageni (Icterohaemorrhagiae), Autumnalis (Autumnalis), Djasiman (Djasiman), Australis (Australis), Pomona (Pomona), Hebdomadis (Hebdomadis), Grippotyphosa (Grippotyphosa) and Canicola (Canicola). This panel of strains has been structured based on previous seroprevalence information (HERRMANN et al., 2004). In addition, in order to improve the sensitivity, a local isolate of *Leptospira noguchii* (serogroup Autumnalis) is

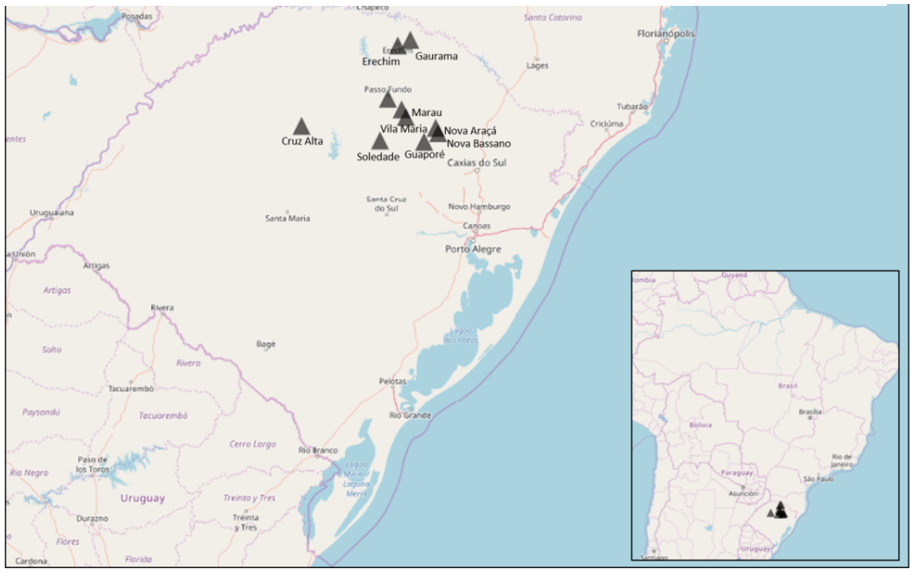

Figure 1 - Distribution of the sampled municipalities of the northwestern mesoregion of Rio Grande do Sul.

included in the panel. Strains were grown in liquid EMJH during 7 days at 28–30 ºC, evaluated under the darkfield microscopy for the absence of auto-agglutination and contamination.

Serological samples were initially analyzed at a dilution of 1: 100, and those with agglutination level equal to or greater than 50% were then diluted again in a geometric ratio of two (1: 200, 1: 400 and 1: 800). Serological titration was represented to correspond with the highest dilution that presented a positive result. Animals were considered positive when titers ≥100 (OIE, 2014). In the occurrence of sero-reactivity for more than one serovar, the most frequent strain in the flock was considered the infective one.

At the same time, an epidemiological questionnaire, including questions regarding individual (gender, age and breed) and flock-level characteristics (type and system of production, consortium with other animals, water source, type of sheepfold, veterinary care, presence of fetal malformation and abortion in the last 12 months, presence of dogs and rodents on the farm) is used to identify the risk factors related to seroreactivity against *Leptospira* spp.

The independent variables or explanatory variables (obtained in the epidemiological questionnaire) were analyzed on the explanation of the biological phenomenon (a result of serological analysis). Chi-square test was used to evaluate the association between serology results and explanatory variables. All analyses were conducted under a confidence level of 95%. Statistical analyses were performed in SPSS version 8.0 (SPSS, 1998). SPSS. 1998. Statistical Package for the Social Science. SPSS Inc., Chicago, IL.

RESULTS

A total of 5.6% (18/319), 95% CI (3.4-8.8), animals were reactive by MAT. The most prevalent serogroups were Sejroe 33.3% (6/18), Djasiman 27.7% (5/18) and Grippotyphosa 22.2% (4/18). Titers of 100 were the most commonly observed (72.2%) followed by 200 (22.2%) and 400 (5.5%). Animals >3 years old were more likely to be seropositive than animals 1-3 years old (OR 14.4; 95% CI 1.9-110) (Table 1). The seroactivity of the animals in the herds is presented in table 2.

DISCUSSION

Seroreactivity against *Leptospira interrogans* was observed in 5.6% of the samples analyzed. For more than a decade, HERMANN et al. (2004) observed a prevalence higher than that observed in our study, describing 34.26% of reactive animals in southeast and southwest mesoregions of the same state. This study was carried out in another region and in a different research period (January to March), which makes it difficult to compare. This period is characterized by higher temperatures when compared to our study, conducted between August and October, which had a minimum temperature of 10ºC and a maximum of 21ºC in August (INMET, 2016). Leptospires has an optimum growth temperature of 28–30ºC (ADLER & DE LA PEÑA MOCTEZUMA, 2010) and temperatures below 24ºC hinder bacterial proliferation. So, we have hazarded that the low prevalence verified in our study can be related to a climate differences, region and a possible better preventive management currently employed in sheep herds.

In other regions of Brazil, the general average is 20–30% (CARVALHO et al., 2014; RIZZO et al., 2014). Overall, sero-reactivity is low in this study and similar results were reported in the central-western region of the state of São Paulo, where 3% of the sheep were seropositive for *Leptospira* spp. (AMORIM et al., 2016) and in the northeast of Brazil, Piauí and Pernambuco State, where 4.6% of the animals were positive (DOS SANTOS et al., 2017). Recently, a low seroprevalence for leptospirosis was reported in cattle in the western part of the state of Santa Catarina, a region near the northwest of Rio Grande do Sul (FÁVERO et al., 2017).

Conversely, higher prevalence rates of *Leptospira* spp. were observed in Rio de Janeiro, where leptospirosis was described as the main infectious problem, representing 47.4% of the sheep sampled and the researchers concluded that this may contribute to the decrease in the productivity of small ruminants (MARTINS et al., 2012). Variation in results indicated that herd management conditions and, animal density, as well as the climatic conditions reported in southeastern Brazil, contribute to the spread of the disease.

High seropositivity to *Leptospira* has also been often associated with exposure to environmental risk factors, such as rainfall and flooding. According to CORREIA et al. (2017), an increase in the prevalence of *Leptospira* spp. infections in cattle is related to an enhancement in precipitation rates; however, the infections associated with adapted serogroups were not affected by environmental conditions. In this study, although the collections were performed during times of greater precipitation in Rio Grande do Sul, the temperature was low so does not favor the survival of the bacterium, meaning they did not happen with a high prevalence of leptospirosis or acute clinical cases

Table 1 - Chi-squared test of risk factors associated with the presence of anti-*Leptospira* antibodies in sheep flocks of northwestern of Rio Grande do Sul.

Variables	Categories	Serology				*P
		Negative		Positive		
		n	%	n	%	
Gender	Male	57	96.6	2	3.4	0.534
	Female	244	93.8	16	6.2	
Age	1 a 3	138	99.3	1	0.7	<0.001
	> 3	163	90.6	17	9.4	
Breed	(Dorper, Lacaune, Poll Dorset, Suffolk, Mixed Breed)	110	94.0	7	6.0	0.841
	Texel	191	94.6	11	5.4	
Production objective	Meat	117	94.4	7	5.6	0.469
	Mixed	47	97.9	1	2.1	
	Genetics	137	93.2	10	6.8	
Production system	Intensive	24	100.0	0	0.0	0.213
	Semi-intensive	277	93.9	18	6.1	
Consortium with other animals	Yes	141	95.3	7	4.7	0.511
	No	160	93.6	11	6.4	
Source of water	Weir	50	92.6	4	7.4	0.326
	Cistern	20	100.0	0	0.0	
	Artesian well	121	92.4	10	7.6	
	Mixed	110	96.5	4	3.5	
Type of sheepfold	Suspended	122	91.7	11	8.3	0.158
	Ground floor	155	95.7	7	4.3	
	Absent	24	100.0	0	0.0	
Veterinary care	Yes	281	94.0	18	6.0	0.615
	No	20	100.0	0	0.0	
Presence of dogs	Yes	282	94.3	17	5.7	1.000
	No	19	95.0	1	5.0	
Presence of rodents	Yes	280	94.0	18	6.0	0.619
	No	21	100.0	0	0.0	
Fetal malformation	Yes	39	97.5	1	2.5	0.482
	No	213	93.0	16	7.0	
Abortion in the last 12 months	Yes	161	95.8	7	4.2	0.228
	No	140	92.7	11	7.3	

diagnosed in the sampled sheep. In this study a limitation was the use of a convenience sample, as formal random sampling was not possible. Thus, data analysis should ideally consider the cluster sampling design.

The importance of the knowledge of the associated risk factors is emphasized, which allows for a more appropriate view of the leptospirosis in the locality, with better defined control perspectives. In sheep production systems the presence of dogs, rodents and wild animals, as well as animal consortiums have been considered important factors in the epidemiology of leptospirosis (CAMPOS et al., 2017). Dogs and rodents were present in all farms, which made it impossible to assess the influence of their presence inside the farms and might prove to be a bias in this study. Animals at three or more years old proved to be a significant observed variable, which agreed with other studies (DOS SANTOS et al., 2017) and is related to the longer exposure to bacteria.

No statistical significance was observed for the abortion in sheep in the studied herds. The questionnaire answered by the producers may contain a bias regarding reproductive data, fetal malformation, and abortion in the last 12 months, since the tabulation of this data on the property is often absent or inadequate, making it difficult to report the period in which the changes occurred. According to CHOI & PAK, (2004), failure to identify a common starting time for exposure

Table 2 - Sero-prevalence of *Leptospira* spp in sheep from northwestern Rio Grande do Sul State, Brazil.

Municipality	Flock ID	N	Seroreactive (%)	Range of titres	Predominant serogroup
Passo Fundo	1	30	0	-	-
Vila Maria	2	30	4 (13.3)	100-400	Sejroe
Marau	3	21	0	-	-
Nova Araça	4	20	1 (5)	800	Sejroe
Nova Bassano	5	20	1 (5)	100	Grippotyphosa
Soledade	6	24	0	-	-
Guaporé	7		0	-	-
Erechim	8	23	2 (8.7)	100	Grippotyphosa
Erechim	9	20	3 (15)	100	Grippotyphosa
Gaurama	10	20	5 (40)	100-200	Sejroe
Erechim	11	20	1 (5)	100	Grippotyphosa
Cruz Alta	12	27	1 (3.7)	100	Icterohaemorrhagiae
Passo Fundo	13	20	0	-	-
Soledade	14	21	0	-	-
Total		319	18 (5.6)	100-800	Sejroe

or illness may lead to bias. Although, the outbreaks of leptospirosis have attracted more attention, the silent, endemic and chronic disease is important because it causes constant economic damage (MARTINS & LILENBAUM, 2014). Among these losses, estrus repetition was strongly associated with leptospirosis and the serogroup Sejroe was predominant in (LIBONATI et al., 2018). This might be related to the absence of a significant level of abortion in our study. Despite the presence of the serogroup Sejroe, reproductive failure was not considered a factor associated with leptospirosis in those flocks. In order to recognize subclinical leptospirosis, it is necessary to monitor the management and to look more critically at reproductive failures in the herds.

CONCLUSION

In this study, the seroprevalence for leptospirosis in sheep was low and animals >3 years old were more likely to be seropositive than animals 1-3 years old. Predominance of Sejroe serogroup draws attention to the subclinical infection associated with economic losses. Therefore, the importance of the maintenance of measures for prevention and control of leptospirosis among the sheep flocks of the studied region is reiterated.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The Animal Ethics Committee of the Federal Fluminense University approved this study under the protocol 832 and the Animal Ethics Committee of Universidade de Passo Fundo, RS, Brazil under the protocol number 035.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest. The authors are responsible for the content and writing of the paper.

AUTHORS' CONTRIBUTIONS

Angélica Consalter and Eraldo Lourenso Zanella conceived, designed and performed experiments. Angélica Consalter, Gabriel Mendes de Souza Martins and Hugo Libonati de Araujo carried out the lab analyses. Ana Maria Reis Ferreira and Walter Lilenbaum supervised and coordinated the animal experiments and provided clinical data. Guilherme Nunes de Souza performed statistical analyses of experimental data. All authors critically revised the manuscript and approved of the final version.

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