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Leptospirosis in Dairy Cattle from Southern Brazil - Risk Factors

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ABSTRACT

***Background*:** Cattle are susceptible to chronic leptospirosis infection, that results in reduced milk production and reproductive disorders such as abortions, stillbirths, fetal malformation, and mummified fetuses, causing significant economic losses. Commercially available vaccines against leptospirosis offer limited protection to cattle because they contain only the most prevalent serovars worldwide, even though they are not prevalent in the specific region. This study aimed to evaluate the prevalence of specific antibodies against *Leptospira* serogroups, reproductive disorders and the risk factors in dairy herds from different mesoregions of Rio Grande do Sul State, Southern Brazil.

***Materials, Methods & Results*:** An epidemiological survey was conducted, and serum samples from the bovine population representative of three mesoregions (MR1, MR2, and MR3) were studied; the samples were collected and tested for leptospirosis using the microscopic agglutination test (MAT) for 12 serogroups checking for the presence of agglutination. A total of 442 blood samples were collected from dairy cattle from November to December 2019 (MR1, 187; MR2, 88; and MR3, 167), including cows vaccinated with different commercial vaccines during the three months before sample collection (n = 295) and non-vaccinated against leptospirosis (n = 147). At the time of collection, an interview was conducted with the owners with questions about the health of the animals, management, habitat, feeding and reproduction. Chi-square tests univariate analysis with the SPSS® version 20.0 were performed to estimate the association of serogroup Djasiman seroreactivity with the occurrence of reproductive problems and related risk factors. The mean prevalence of antibodies against leptospires was 78.7% (MR1, 74.9 %; MR2, 84.1 %; and MR3, 80.2 %). Serogroup prevalence was different in each mesoregion evaluated and varied with vaccination status tested of the animals. The most prevalent serogroups in MR1 were Djasiman and Icterohaemorrhagiae. In MR2 and MR3, Djasiman was the most prevalent serogroup, regardless of vaccination status. Other prevalent serogroups in vaccinated animals were Icterohaemorrhagiae (MR1), Sejroe (MR2), Pomona, Sejroe, and Icterohaemorrhagiae (MR3). The other serovars tested had a prevalence of less or equal than 2%. The occurrence of reproductive problems with abortions and estrus repetition, was associated with reactivity to the serogroup Djasiman ($P > 0.05$). The results showed that the access of animals to flooded areas and the presence of rodents were significant risk factors, according to the literature.

***Discussion*:** The prevalence of antibodies against *Leptospira* in the studied areas was higher than expected, with a high prevalence of Djasiman serogroup. It is important to note that this serogroup is not present in current vaccine formulations. The presence of wetlands and rodents as risk factors in association with Djasiman is consistent with the scientific literature, since wild rodents are natural hosts, and swampy areas may allow *Leptospira* to survive for up to 180 days in the environment. Due to the presence and high prevalence found of the serogroup Djasiman in the experiment, new studies are being carried out to improve our knowledge about this serogroup and its possible inclusion in a commercial vaccine. Prophylaxis and control measures were recommended to rural producers in the analyzed properties.

Keywords: bovine leptospirosis, herds, zoonosis, antibodies, reproductive disorders, leptospires, Djasiman.

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INTRODUCTION

Leptospirosis is a zoonosis prevalent worldwide and is particularly common in tropical and subtropical regions [15]. Most bovines harbor a chronic infection characterized by reproductive disorders such as abortion, stillbirths, mummified fetuses, fetal and placental retention, death of calves within 72 h, infertility, and decreased milk production [4,23].

Leptospires are classified into more than 300 serovars, defined according to the outer membrane lipopolysaccharide (LPS) heterogeneity [20]. The most prevalent serovars in cattle herds worldwide are serovar Hardjo and serovar Pomona [4,12,19]. Milk is produced commercially in 93.6% of the municipalities of the Rio Grande do Sul State, making milk production a significant source of income [7]. In Brazilian livestock, approximately 30% cows suffer reproductive failure due to various causes [10]. Bovine leptospirosis is widespread in Rio Grande do Sul State, southern Brazil, with a prevalence of 38.75% [7].

Risk factors such as tropical and subtropical climate, regions with high temperatures, well-distributed rainfall, and the presence of rodents and wetlands help the survival of *Leptospira* [10,11,15]. The diagnosis of bovine leptospirosis in a specific region and identifying the prevalent serovar/serogroup are essential for the epidemiology, prevention, and prophylaxis of the disease [18,19]. This study aimed to evaluate the prevalence of specific antibodies against *Leptospira* and identify the prevalent serovars and the risk factors in dairy herds from different mesoregions of Rio Grande do Sul State, Southern Brazil.

MATERIALS AND METHODS

Study area

Random sampling was performed to select herds and animals in 4 mesoregions of Rio Grande do Sul State (southeast, southwest, northeast, and northwest mesoregions). The target population composed of dairy herds belonging to local milk cooperatives and a producer's association.

Samples

A representative sampling method was used and determined using the EpiTools® (Epitools Epidemiological Calculators - Software)[1]. The sampling parameters were: 50% expected prevalence for leptospirosis, 10% sampling error, and 95% significance level. A total of 442 blood samples from lactating cows were collected in June 2016 (Table 1): 187 samples were collected from the Northeast and Northwest mesoregions (MR1), covering 26 municipalities in the Northwest region, 22 in the Northeast region, and one in the metropolitan area of Porto Alegre; 88 samples (MR2) were collected from 18 herds of 17 municipalities in the Northwest region, 167 samples and in the southeast (22 herds) and southwest (one herd only) mesoregions (MR3). Vaccinated with commercial bacteria up to 3 months before the collection data (n = 295) and non-vaccinated animals (n = 147) were considered in this study. The areas where the samples were collected are shown in Figure 1.

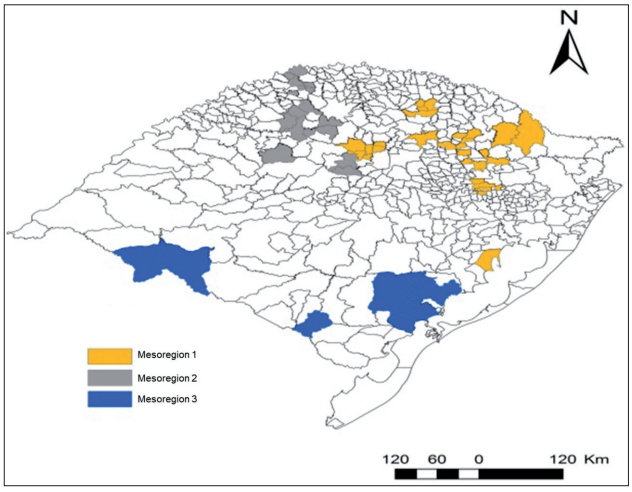

Figure 1. Cities with dairy cattle serum samples evaluated in Rio Grande do Sul State, Southern Brazil.

Epidemiological interview

The method used in this study consisted of collecting animal serum samples and information regarding the characteristics and sanitary conditions of each farm through an epidemiological interview (Table 2).

Serology

Blood samples were centrifuged at 3,500 × *g* for 10 min to separate the serum, stored at -20°C. Laboratory investigations were performed at the Vaccinology Laboratory, University Federal de Pelotas (UFPel). To screen the reactive animals, the serum samples were submitted to the microscopic agglutination test (MAT) as previously described [2]. Each serum sample was diluted 1:50 in PBS for screening and titration. For the test, 12 serogroups, comprising 13 serovars, were selected, as shown in Table 3. The strains were maintained in modified Ellinghausen,

McCullough, Johnson and Harris (EMJH) liquid culture media supplemented with 10% albumin and incubated at 30°C. MAT analyses were performed using 96 well concave bottom plates. Agglutination was examined by dark-field microscopy using a 20 × objective to verify the presence or absence of agglutination. Results were considered positive when the agglutination was greater than or equal to 50% compared to the control sample. Positive samples were serially diluted 2-fold from 1:100 to 1:3200 to determine the final titer of agglutinating antibodies. The serovar with the highest MAT titer was considered the infective serovar.

Statistical analysis

Statistical analysis to verify the association of clinical signs and risk factors was performed using Pearson's chi-square univariate analysis with the SPSS® (Version 20.0)[2] considering $P \leq 0.05$ as significant. The analysis was conducted at the herd level. The chi-square test was used to evaluate the association between positivity for the most prevalent serovar/serogroup and reproductive problems. The strength of association (or risk) was calculated using the odds ratio (OR) and its 95% confidence interval (CI).

Table 1. Population data of cattle and animal herds sampled for the study of leptospirosis prevalence, divided by mesoregions of Rio Grande do Sul state, Southern Brazil.

Mesoregions	Herds		Animals*	
	Population	Sampled	Population	Sampled
Northeast/Northwest (MR1)[1]	3,070	47	33,234	187
Northwest (MR2)[2]	53	17	658	88
Southeast/Southwest (MR3)[3]	193	21	1930	167

*Cows older than 24 months; [1]Farms associated to Cooperative 1; [2]Farms associated to a Producers Association; [3]Farms associated to Cooperative 3.

Table 2. General presentation of the epidemiological questionnaire applied to owners on dairy farms in Rio Grande do Sul State, Southern Brazil.

Characteristics	Dependent variables
Farm	Production System (Confinement, semi-confinement, extensive and semi-extensive)
	Technical Assistance (Agricultural Technician, Veterinary or Agronomist)
	Animal feed (Silage, concentrate or native pasture)
	Age and category of animals (calves, heifers, dairy cows and dried cows)
Reproductive Performance	Type of Reproduction used (natural breeding (NB), artificial insemination (AI) or (AI + NB).
	Reproductive problems (miscarriages, stillbirths, placental retention, malformations)
	Diagnosis of reproductive diseases (IBR, BVDV, neosporosis, brucellosis or leptospirosis)
Biosecurity	Presence of other animals (rodents, goats, sheep, pigs,dogs, cats or birds)
	Presence of wetlands and animal access
	Animal disposal (age, reproductive failure, poor production or disease)
	Animal replacement (purchase or own herd)
	Vaccination against leptospirosis
	Synantropic rodents control

Table 3. List of *Leptospira* serogroups used in MAT.

Genomic species	Serogroup	Serovar	Strain
L. interrogans	Autumnalis	Autumnalis	Akiyami A
	Icterohaemorrhagiae	Copenhageni	M20
	Canicola	Canicola	Hond Utrecht IV
	Djasiman	Djasiman	Djasiman
	Hebdomadis	Hebdomadis	Hebdomadis
	Sejroe	Hardjo	Hardjoprajitno
	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
	Pomona	Pomona	Pomona
L. kirshneri	Grippotyphosa	Grippotyphosa	Moskva V
	Javanica	Javanica	Veldratbatavia 46
L. borgpetersenii	Tarassovi	Tarassovi	Prelepelitsin
	Sejroe	Hardjo	Hardjobovis
L. noguchii	Panama	Panama	CZ 214 K
L. santarosai	Shermani	Shermani	1342 K

RESULTS

Of the 442 dairy cow samples, 348/442 (78.7%) presented titers equal to or greater than 100. The prevalence was different among the 3 mesoregions analyzed. The prevalence of MR1, MR2, and MR3 were 74.9% (140/187), 84.1% (74/88), and 80.1% (134/167), respectively. The prevalence of agglutinating antibodies against *Leptospira* was different in animals recently vaccinated (< 3 months) against leptospirosis (81.4%, 240/295) compared to the non-vaccinated ones (73.5%, 108/147).

The prevalent serogroups were different in each mesoregion evaluated, varying in vaccinated (V) and non-vaccinated (NV) animals. In MR1, the predominant serogroups were Icterohaemorrhagiae (35.1%) and Djasiman (22.8%) in vaccinated animals and Djasiman (53.8%), followed by Icterohaemorrhagiae (19.2%) in non-vaccinated animals (Figure 2A). The other serogroups evaluated showed a prevalence of less than 10%. In MR2, the predominant serogroups were Djasiman (28.6%) and Sejroe (14.3%) in vaccinated and Djasiman (36%), followed by Canicola and Icterohaemorrhagiae (12% each) in non-vaccinated animals (Figure 2B). In MR3, the predominant serogroups were Djasiman (52.0%), followed by Sejroe, Pomona, and Icterohaemorrhagiae (5.2% each) in vaccinated Djasiman (68.4%), and Icterohaemorrhagiae (8.8%) in non-vaccinated animals (Figure 2C).

A few samples showed cross-reaction (CR) with more than 1 serovar; MR1 was 16.4% (23/140), MR2 27.0% (20/74), and MR3 18.7% (25/134). At least 25% of the samples that presented CR in vaccinated animals showed a reaction with serogroups contained in traditional vaccines against bovine leptospirosis; however, at least 10% of the samples reacted with the serogroup Djasiman. In non-vaccinated animals, CR was observed in approximately 30 samples. This CR was predominantly between Djasiman and another serogroup.

Chi-square statistical analysis showed that 86% ($P = 0.035$) of animals that reacted to serogroup Djasiman presented reproductive problems. The occurrence of miscarriages at the end of pregnancy ($P = 0.017$), presence of birth problems ($P = 0.005$), stillbirths ($P = 0.017$), and malformations ($P = 0.011$) were the most statistically significant problems, as shown in Table 4.

In the analysis of risk factors (Table 5), the diagnosis of leptospirosis in the herd was not significant ($P = 0.085$), as well as vaccination ($P = 0.072$). The presence of wetlands and access to animals were significant ($P = 0.012$ and $P = 0.019$, respectively). The presence of rodents in the property ($P = 0.004$) and the accomplishment of its control ($P = 0.025$) were statistically significant.

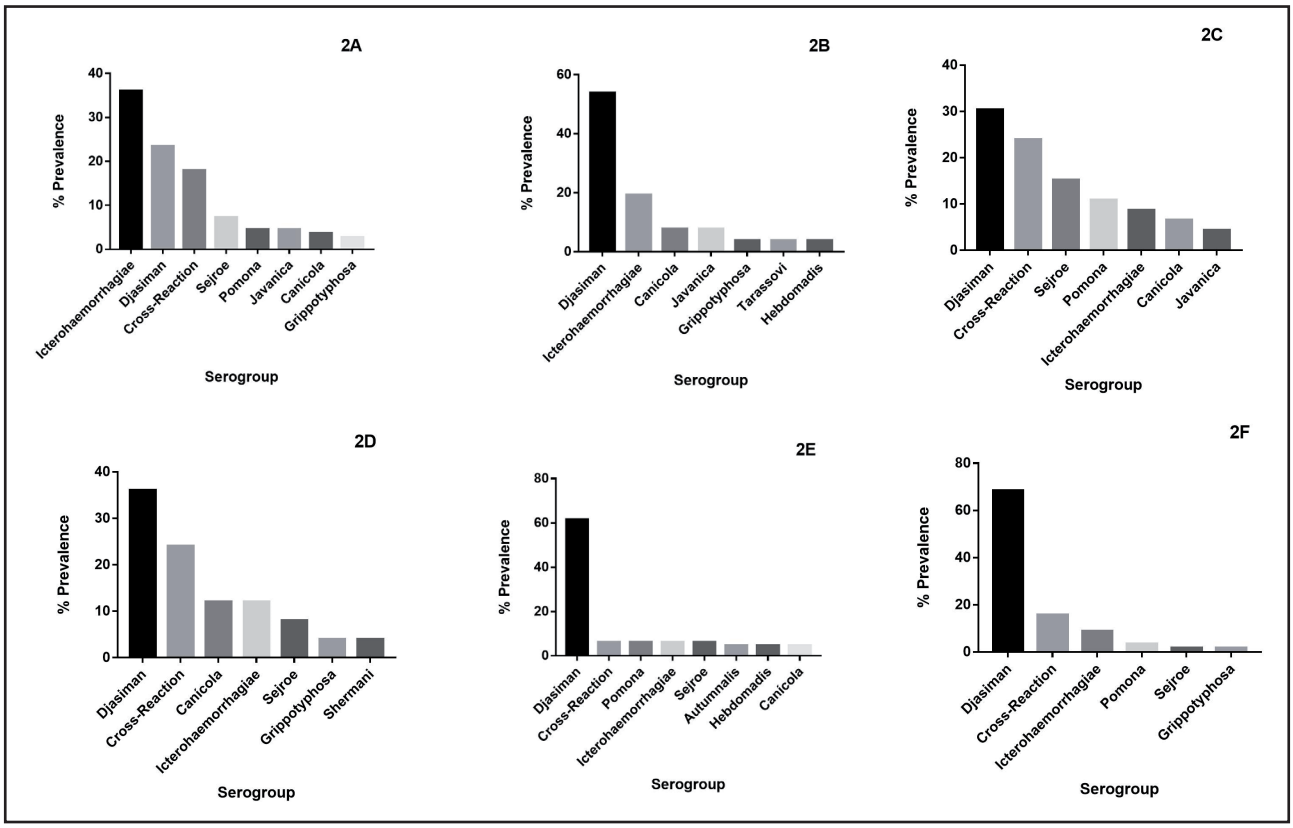

Figure 2. Serogroup's prevalence in Mesoregion 1 (A), 2 (B) and 3 (C), vaccinated and unvaccinated animals.

Table 4. Results statistical analysis Chi-square independent variable Serovar Djasiman and dependent variables reproductive and delivery problems.

Variables		Djasiman serogroup		*P* Value	OR	CI 95%
		Yes	No			
Reproductive problems	Yes	43(89.6%)	19 (70.4%)	*P* = 0.0035	3.62	1.04-12.52
	No	5 (10.4%)	8 (29.6%)			
Late-gestation abortion	Yes	20 (41.7%)	4 (14.8%)	*P* = 0.017	4.11	1.22-13.73
	No	28 (58.3%)	23 (85.2%)			
Return to estrus	Yes	38 (79.2%)	18 (66.7%)	*P* = 0.232	1.90	0.65-5.48
	No	10 (20.8%)	9 (33.3%)			
Mid-trimester abortion	Yes	19 (39.6%)	6 (22.2%)	*P* = 0.126	2.29	0.78-6.72
	No	29 (60.4%)	21 (77.8%)			
Placental retention	Yes	2 (4.2%)	0 (0.0%)	*P* = 0.282	1.04	0.98-1.10
	No	46 (95.8%)	27 (100.0%)			
Delivery problems	Yes	21 (43.8%)	3 (11.5%)	*P* = 0.005	5.96	1.57-22.57
	No	27 (56.2%)	23 (88.5%)			
Stillbirths	Yes	9 (19.1%)	0 (0.0%)	*P* = 0.017	1.24	1.07-1.42
	No	38 (80.9%)	26(100.0%)			
Delivery of weak calves	Yes	9 (19.1%)	3 (11.5%)	*P* = 0.401	1.82	0.44-7.40
	No	38 (80.9%)	23 (88.5%)			
Bad fetal formations	Yes	10 (21.3%)	0 (0.0%)	*P* = 0.011	1.27	1.09-1.47
	No	37 (78.7%)	26 (100%)			

Table 5. Results statistical analysis Chi-square independent variable Serovar Djasiman and risk factors as dependent variables.

Variables		Djasiman Serogroup		P valeu	OR	CI 95%
		Yes	No			
Leptospirosis diagnosis	Yes	27 (35.1%)	50 (64.9%)	$P = 0.085$	0.57	0.29-1.08
	No	38 (48.7%)	40 (51.3%)			
Leptospirosis vaccination	Yes	93 (31.2%)	205 (68.8%)	$P = 0.072$	0.69	0.45-1.03
	No	60 (39.7%)	91 (60.3%)			
Synantropic rodents	Yes	105 (39.8%)	159 (60.2%)	$P = 0.004$	1.84	1.21-2.78
	No	47 (26.4%)	131 (73.6%)			
Wetlands	Yes	91 (39.9%)	137 (60.1%)	$P = 0.012$	1.67	1.11-2.48
	No	61 (28.5%)	153 (71.5%)			
Cattle access to Wetlands	Yes	79 (40.3%)	117 (59.7%)	$P = 0.019$	1.60	1.07-2.37
	No	73 (29.7%)	173 (70.3%)			
Rodent Control	Yes	147 (35.9%)	262 (64.1%)	$P = 0.025$	2.92	1.09-7.76
	No	5 (16.1%)	26 (83.9%)			

DISCUSSION

The prevalence of specific antibodies against *Leptospira* in dairy cattle in the mesoregions studied was higher than expected, indicating the exposure of non-vaccinated animals to leptospires and vaccine-induced seroconversion. Moreover, seroreactivity against serogroup Djasiman was the most prevalent in non-vaccinated animals in MR1 and vaccinated and unvaccinated from mesoregions 2 and 3, indicating the presence of a wild reservoir. Previous studies have suggested that wild rodents are carriers of this serogroup in the studied area [6,8].

The high prevalence of reactivity to serogroup Djasiman in all mesoregions studied, regardless of the vaccination status, raised suspicion regarding the identity of the strain used as antigen in MAT. In order to confirm the identity, we performed variable-number tandem repeat (VNTR) analysis [13], which confirmed the identity (result not shown). This study is the first to report a high prevalence of Djasiman serogroup in dairy cattle. Our findings are unusual, since the serogroup Sejroe (mostly, Hardjobovis strain) has been identified as the most prevalent in cattle worldwide as the largest cause of abortions in cattle [9,17]. This finding offers new insights into leptospirosis epidemiology in the study area.

The association of reproductive problems with the Djasiman serogroup was consistent with the symptoms of chronic leptospirosis infection manifested in dairy herds. Abortion caused by leptospirosis infection usually occurs in outbreaks and can occur at any time during pregnancy; however, abortions caused by serogroup Djasiman occur mainly from the sixth month of pregnancy onwards [6,19]. In this study, the observation of calving problems in herds, stillbirths, and malformations is one of the main causes of reproductive failure in cattle [21].

The presence of wetlands and the access of animals to them were statistically significant. These results validate those of previous studies that reported that the presence of synanthropic rodents and wetlands favors the occurrence of the disease and is an important risk factor. Infection in cattle by adapted serovars, such as Hardjo, is independent of region or precipitation, whereas incidental infections are dependent on environmental factors and are more important in tropical and subtropical climate countries [4,15].

Commercial leptospirosis vaccines available in Brazil for the immunization of bovines are polyvalent and contain prevalent serovars, including Pomona, Icterohaemorrahagiae, Hardjo, Canicola, Wolffi, Grippothyphosa, Bratislava, and Tarassovi [14,22]. Several leptospirosis vaccines sold commercially in Brazil are produced abroad, imported, and only packaged in Brazil [16]. Even those produced in the country use reference *Leptospira* strains, which are antigenically distinct from those in the field and thus are incapable of promoting effective protection against disease, infection, or establishment of the renal carrier state when the animals are exposed to local strains [3,5,22]. This

study identified a high prevalence of specific antibodies against serovar Djasiman in dairy herds in the study area, indicating the need to develop new vaccine formulations that include local strains of serogroup Djasiman for prophylaxis and to control bovine leptospirosis in the study area.

Both humoral and cellular immunity elicited by bacterins against leptospires are limited to serovars and, in some cases, serogroups included in the vaccine composition [1]. It is necessary to revise the criteria applied to the production of leptospirosis bacterins sold commercially in Brazil for the immunization of bovines [22]. Studies on *Leptospira* epidemiology should be supported, as they contribute to the development of vaccines more effectively, given that bacterins are the key to the reduction of chronic and reproductive problems related to *Leptospira* infection in cattle.

CONCLUSIONS

This study showed an uncommon high prevalence of the Djasiman serogroup in dairy cattle from the study area. The *Leptospira interrogans* serogroup Djasiman is considered an incidental infection. The most prevalent serogroups varied in each mesoregion studied; however, the combined prevalence was much higher than that reported in previous studies, reinforcing the need to adopt control and prevention measures. A commercial vaccine should include a Djasiman serogroup representative of its formulation to achieve effective control of leptospirosis in the area studied.

MANUFACTURERS

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***Ethical approval*.** This study was approved by Ethics Committee for Animal Experimentation (CEEA) of UFPel, under protocol number 7027-2015. The CEEA at UFPel is accredited by the Brazilian National Council for Animal Experimentation Control (CONCEA). All handling of animals in connection with sampling was performed, considering animal welfare, and following international and national guidelines.

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

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