

# Agglutinins to *Leptospira* spp. in equines slaughtered in the southern region of Brazil

## Aglutininas anti- *Leptospira* spp. em equídeos da região sul do Brasil abatidos em matadouro-frigorífico

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### Abstract

The objective of this study was to investigate the presence of anti-*Leptospira* spp. antibodies in serum samples of horses slaughtered in an abattoir, under the Brazilian federal food inspection service, in the southern region of Brazil. We tested 767 blood samples from adult horses slaughtered from April to May, 2013. The animals came from 45 municipalities in the states of Rio Grande do Sul, Santa Catarina, and Paraná. For the diagnosis we used the microscopic agglutination test (MAT). The results showed that 687 horses reacted to at least one of the 24 serovars of *Leptospira* spp., with titer equal to or greater than 100, representing 89.57% (95% CI: 87.41%–91.73%). The most likely serovars were Patoc (9.91%), Butembo (9.13%), Australis (7.82%), and Bratislava (5.87%). There was no significant difference ( $p = 0.2795$ ) in the number of positive animals by state. The proportion of MAT-positive males and females differed significantly ( $p = 5.4444 \times 10^{-5}$ ) since 85.26% (95% CI: 82.82%–88.70%) of the males and 94.44 (95% CI: 92.07%–96.81%) of the females were reactive. The results of this study demonstrate a high rate exposure to several serovars of *Leptospira* in slaughtered horses. For the protection of animal, public, and occupational health, we suggest attention to infections in this host in order to reduce the risk of leptospirosis.

**Key words:** Horses. Immunoglobulins. Leptospirosis. Microscopic agglutination. Slaughterhouse.

### Resumo

Objetivou-se investigar a presença de imunoglobulinas anti-*Leptospira* spp. em amostras de soros sanguíneos de equídeos abatidos em matadouro-frigorífico, sob Serviço de Inspeção Federal, localizado na região Sul do Brasil. Utilizaram-se 767 amostras de sangue de equídeos adultos, abatidos no período de abril a maio de 2013. Os animais foram provenientes de 45 municípios dos Estados do Rio Grande do Sul, Santa Catarina e Paraná. Para o diagnóstico foi utilizado o teste de soroprecipitação microscópica (SAM). Foram observados 687 equídeos sororreagentes a pelo menos uma das 24 sorovariedades utilizadas de *Leptospira* spp. com título igual ou superior a 100, representando 89,57% (IC 95%: 87,41%–91,73%). As sorovariedades mais prováveis foram Patoc (9,91%), Butembo (9,13%), Australis (7,82%), Bratislava (5,87%). Não houve diferença significativa ( $p = 0,2795$ ) entre as proporções de reagentes a

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*Leptospira* spp. nos estados. Já em relação à proporção de machos e fêmeas reagentes houve diferença significativa ( $p = 5,4444 \times 10^{-5}$ ), sendo 85,26% (IC 95%: 82,82% – 88,70%) de machos reagentes e 94,44% (IC 95%: 92,07% – 96,81%) de fêmeas reagentes. Os resultados obtidos no presente estudo demonstram uma exposição elevada a diversas sorovarietades de *Leptospira* nos equídeos abatidos no matadouro-frigorífico. No âmbito da saúde animal, pública e ocupacional, sugere-se a atenção a essa doença, visando diminuir o risco de infecção.

**Palavras-chave:** Abatedouro. Equinos. Imunoglobulinas. Leptospirose. Soroaglutinação microscópica.

## Introduction

Brazil is the eighth largest exporter of horsemeat. In 2011, a total of 168,000 animals were slaughtered, resulting in a production of 21,800 tons of meat (FAO, 2013). Belgium, the Netherlands, Italy, Japan, and France are the main importers of Brazilian horsemeat, which is also consumed in the United States (MAPA, 2013).

The equidae herds in Brazil comprise approximately 5,508,546 horses, 1,269,198 mules, and 974,532 donkeys. The Northeast region has the biggest equine herd (2,831,610), followed by the Southeast (1,618,045), Midwest (1,318,450), North (1,009,481), and South (974,690). Regarding states, the largest number of horses is located in Minas Gerais (787,129), representing 14.3% of the total herd of the country, then Bahia (555,905), with 10.1% of the total, and Rio Grande do Sul (472,778), with 8.6% of the total (IBGE, 2011a).

Because of equine meat production in Brazil comes from unproductive animals and most of them are traction animals, there are concerns about the health of these animals, particularly about zoonotic infections such as leptospirosis, toxoplasmosis, brucellosis, and trichinosis.

Bacteria of the genus *Leptospira* are widespread in many countries, and these microorganisms can infect humans as well as many species of wild and domestic animals (BRASIL, 2005). In horses, infection with *Leptospira* spp. can manifest itself in acute or chronic form, with many animals presenting clinical signs such as recurrent uveitis, abortions, and other reproductive disorders. Moreover, this pathogen may also be transmitted to humans from equids (LINHARES et al., 2005). Leptospirosis

presents an economic and public health burden, and because it is a zoonosis, the bacterium can be transmitted by direct contact with infected animals (BRASIL, 2005).

Thus, the objective of this study was to investigate the presence of immunoglobulins anti-*Leptospira* spp. in serum samples of horses slaughtered in slaughtering houses from the south region of Brazil.

## Materials and Methods

### Samples

A total of 767 blood samples were collected from adult equidae (762 horses and 5 mules), males ( $n = 407$ ) and females ( $n = 360$ ) that were slaughtered in an establishment under the Federal Inspection Service located in the southwestern region of Rio Grande do Sul state from April to May, 2013. During this period, samples were collected from all animals for slaughter.

Animals slaughtered in the slaughterhouse during the sampling period animals came from 45 municipalities in the states of Rio Grande do Sul, Santa Catarina, and Paraná. In the state of Rio Grande do Sul, the slaughtered animals were from 37 municipalities, namely: Alegrete, Bagé, Caçapava do Sul, Cacequi, Canguçu, Capão Cipó, Cristal, Cruzeiro do Sul, Dom Pedrito, Encruzilhada do Sul, Itacurubi, Itaquí, Lavras do Sul, Maçambará, Pinheiro Machado, Piratini, Pelotas, Quaraí, Rosário do Sul, Santa Cruz do Sul, Santa Maria, Santa Vitória do Palmar, Santana da Boa Vista, Santana do Livramento, Santiago, Santo Ângelo, Santo Antônio das Missões, São Borja, São Francisco de Assis, São Gabriel, São Lourenço do

Sul, Taquari, Unistalda, Vacaria, and Vila Nova do Sul. The animals from the State of Santa Catarina were from municipalities of Bandeirante, Lages, Saltinho, and São Miguel do Oeste. In the State of Paraná, the animals were from the municipalities of Ampere, Nova Laranjeiras, Paulo Frontin, and São Mateus do Sul.

Blood samples were collected in disposable tubes at the time of bleeding on the slaughter line. After separating the serum, the samples were transferred to labeled tubes, and then stored at  $-20^{\circ}\text{C}$ . For detection of *Leptospira* antibodies, we used the microscopic agglutination test (MAT).

#### *Microscopic Agglutination Test (MAT)*

The *Leptospira* spp. antigens used in serological tests were obtained from bacteria subcultured weekly in liquid EMJH culture medium (Ellighausen, McCullough, Johnson and Harris), with 10% of the medium volume used to seed cultures that were maintained in a bacteriological incubator at  $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$  (OIE, 2008).

The serovars of leptospira employed were Andamana, Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Sentot, Grippytyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Javanica, Panama, Pomona, Pyrogenes, Serovar, Wolffi, Patoc, Shermani, and Tarassovi.

The blood serum samples were diluted in saline, at an initial dilution of 1/50. Aliquots (25  $\mu\text{L}$ ) of serum were placed in polystyrene plates with a flat bottom, with an equal quantity of antigens of the 24 serovars of *Leptospira* spp., resulting in a dilution of 1/100. The serum-antigen mixture was homogenized gently and incubated in an environmental incubator

at a temperature of  $28^{\circ}\text{C}$  for 40 to 120 minutes. Results were read by dark field microscopy with  $10\times$  objective, directly from the plate wells.

We considered samples with 50% agglutination to be reactive. Samples reactive at the initial dilution were assayed with serial, 2-fold dilutions from the original 1/100 dilution, as recommended by the OIE (2008).

To determine the prevalence, we considered as positive the animals reagents to one or more serovars against *Leptospira* spp. To determine the most probable serovar, we considered only the one of highest titer, and disregarded the animals with equal titers against two or more serovars.

#### *Statistical analysis and database*

The data was maintained and the tables and graphs were constructed using Microsoft Excel® version 2007 Matched with each serum sample was information on the municipality where the animal originate, the date of sampling, sex, species, and results of the MAT. Frequencies of variables (gender, status) were compared by chi-square test or Fisher's exact test using the software R (R CORE TEAM, 2013). Confidence interval (CI) of the observed frequencies was calculated as described by Thrusfield (2005).

## **Results**

Regarding the 767 animals examined, 687 equidae (89.57%, CI 95%: 87.41%–91.73%) were found seropositive to at least one of the 24 serovars of *Leptospira* spp., with titers equal to or greater than 100. The probable infecting serovars were Patoc (9.91%), Butembo (9.13%), Australis (7.82%), and Bratislava (5.87%) (Table 1, Figure 1).

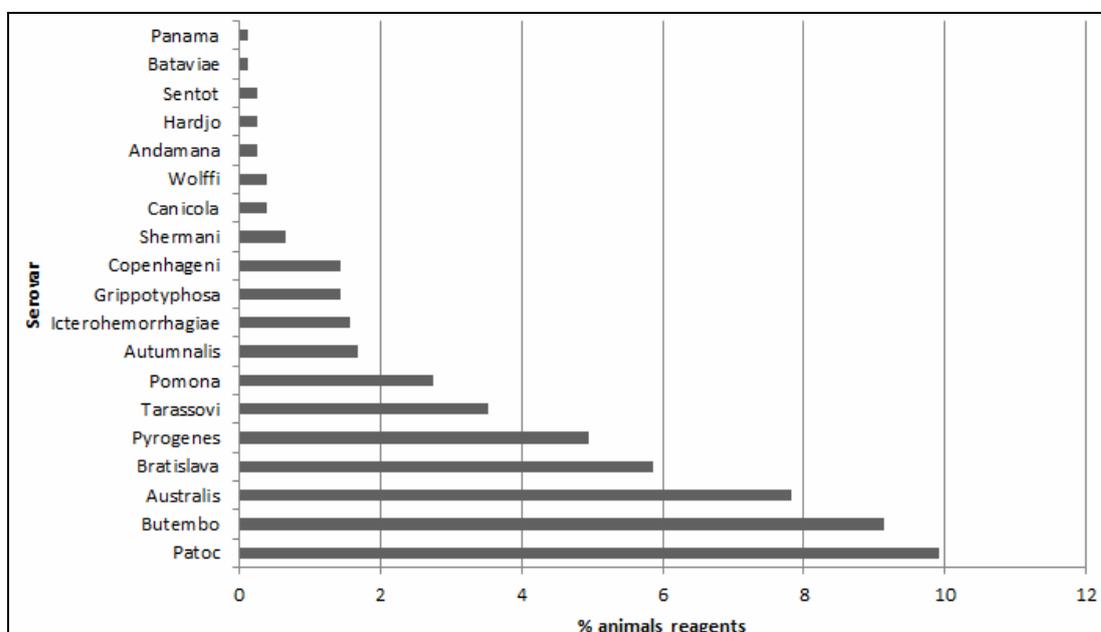
**Table 1.** Titer of agglutination considering only the biggest titer of serovar in microscopic agglutination testing of sera from and disregarding the animals with equal titers against two or more serovars in the microscopic agglutination test, among the 767 horses slaughtered between April and May 2013, São Gabriel, RS, 2013.

SEROVAR	TITER							TOTAL		
	100	200	400	800	1600	3200	≥6400	N°	% <sup>a</sup>	% <sup>b</sup>
	N°	N°	N°	N°	N°	N°	N°	N°		
Patoc	03	26	27	11	07	01	01	76	9.91	18.86
Butembo	14	33	21	02	-	-	-	70	9.13	17.37
Australis	02	15	17	15	06	03	02	60	7.82	14.89
Bratislava	03	14	17	11	-	-	-	45	5.87	11.17
Pyrogenes	04	09	13	09	03	-	-	38	4.95	9.43
Tarassovi	02	06	09	08	01	-	01	27	3.52	6.70
Pomona	02	03	10	03	01	02	-	21	2.74	5.21
Autumnalis	04	03	03	01	02	-	-	13	1.69	3.23
Icterohemorrhagiae	04	05	03	-	-	-	-	12	1.56	2.98
Grippotyphosa	01	07	01	01	-	01	-	11	1.44	2.73
Copenhageni	02	04	03	01	-	-	01	11	1.44	2.73
Shermani	-	03	02	-	-	-	-	05	0.65	1.24
Canicola	02	01	-	-	-	-	-	03	0.39	0.74
Wolffi	-	01	01	01	-	-	-	03	0.39	0.74
Andamana	-	01	01	-	-	-	-	02	0.26	0.50
Hardjo	-	-	-	-	-	02	-	02	0.26	0.50
Sentot	01	01	-	-	-	-	-	02	0.26	0.50
Bataviae	-	01	-	-	-	-	-	01	0.13	0.25
Panama	-	01	-	-	-	-	-	01	0.13	0.25

a: Percentage (%) of total animals assayed (767)

b: Percentage (%) of total animals reactive (403).

**Figure 1.** Percentage (%) of equidae reactive to serovars of *Leptospira* spp. by microscopic agglutination test, considering only the serovar with the highest titer and disregarding the animals with equal titers against two or more serovars, among 767 horses slaughtered in the period April-May 2013, São Gabriel, RS, 2013.



Among the 687 reactive animals in this study, 593 (86.32%) had antibodies against more than one serovar, 94 (13.68%) were positive to only one, and two animals responded to up to 13 serovars.

Concerning the 767 equidae used in the study, 612 were from the Rio Grande do Sul State, and among these, 553 (90.36%) were seropositive to at least one serovar of *Leptospira* spp. Among the 112 animals from the State of Paraná, the frequency of antigen-reactive sera was 85.71%, and in the state of Santa Catarina, the frequency was 88.37% among the 43 animals studied. Fisher's exact test

( $p = 0.2795$ ) confirms the idea that there was no significant difference between seropositivity by state.

The probable serovar for a serum sample was considered the antigen with the highest titer antibody response (Table 2, Figure 2). Rio Grande do Sul had a high prevalence of seropositive animals to serovar Patoc (10.95%), Butembo (9.31%), and Australis (9.64%). In Santa Catarina, the most common serovars were Patoc (11.63%), Butembo (9.3%), and Tarassovi (6.98%). In equidae from the state of Paraná, the predominant serovars were Butembo (8.03%), Patoc and Tarassovi, with 3.57%.

**Table 2.** Distribution of equidae that were reactive to *Leptospira* antigen in the microscopic agglutination test in accordance by state of origin, considering only the serovar with the highest titer antibody reaction and disregarding the animals with equal titers against two or more serovars, among the 767 equines slaughtered in the period from April to May 2013, São Gabriel, RS, 2013.

SEROVAR	State							
	Rio Grande do Sul		Santa Catarina		Paraná		Total	
	Nº	% <sup>a</sup>	Nº	% <sup>b</sup>	Nº	% <sup>c</sup>	Nº	% <sup>d</sup>
<b>Patoc</b>	67	10.95	05	11.63	04	3.57	76	9.91
<b>Butembo</b>	57	9.31	04	9.30	09	8.03	70	9.13
<b>Australis</b>	59	9.64	-	-	01	0.89	60	7.82
<b>Bratislava</b>	41	6.70	01	2.32	02	1.78	45	5.87
<b>Pyrogenes</b>	35	5.72	-	-	03	2.68	38	4.95
<b>Tarassovi</b>	20	3.27	03	6.98	04	3.57	27	3.52
<b>Pomona</b>	19	3.10	01	2.32	01	0.89	21	2.74
<b>Autumnalis</b>	10	1.63	-	-	03	2.68	13	1.69
<b>Icterohaemorrhagiae</b>	10	1.63	-	-	02	1.78	12	1.56
<b>Copenhageni</b>	10	1.63	-	-	01	0.89	11	1.44
<b>Grippotyphosa</b>	07	1.14	01	2.32	03	2.68	11	1.44
<b>Shermani</b>	05	0.82	-	-	-	-	05	0.65
<b>Wolffi</b>	02	0.33	-	-	01	0.89	03	0.39
<b>Canicola</b>	03	0.49	-	-	-	-	03	0.39
<b>Sentot</b>	02	0.33	-	-	-	-	02	0.26
<b>Hardjo</b>	01	0.16	-	-	01	0.89	02	0.26
<b>Andamana</b>	02	0.33	-	-	-	-	02	0.26
<b>Panama</b>	01	0.16	-	-	-	-	01	0.13
<b>Bataviae</b>	01	0.16	-	-	-	-	01	0.13

a: Percentage (%) of the number of animals assayed from the state (612)

b: Percentage (%) of the number of animals assayed from the State (43)

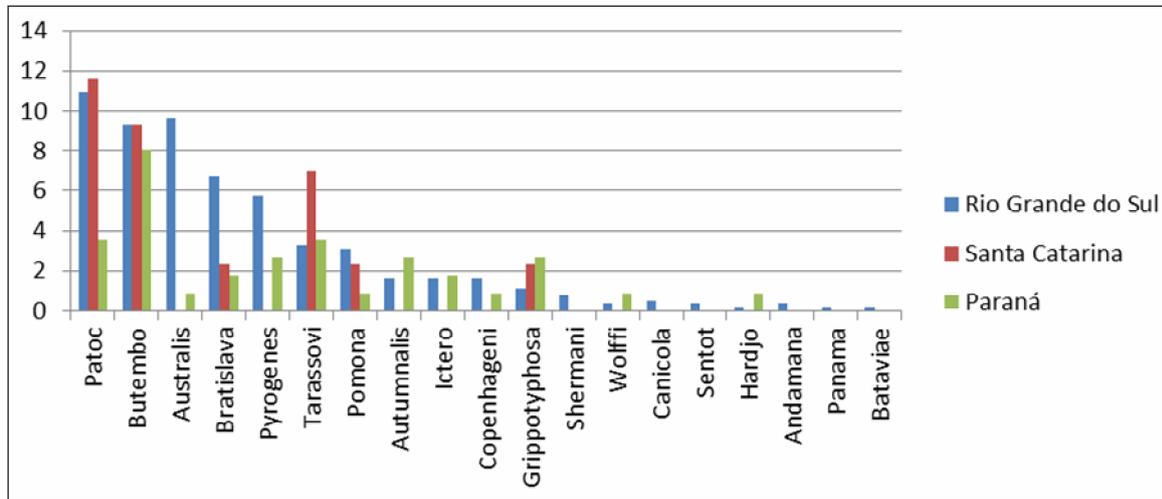
c: Percentage (%) of the number of animals assayed from the State (112)

d: Percentage (%) of the total number of animals assayed (767).

We observed that all (100%) of the 45 municipalities had seropositive animals against one or more serovars of *Leptospira* (Table 3). The municipalities of Rio Grande do Sul were those with the highest percentage of reactive animals. All equidae coming from the cities of Caçapava do

Sul, Capão Cipó, Cristal, Dom Pedrito, Itacurubi, Pinheiro, Piratini, Quaraí, Rosário do Sul, São Gabriel, Santa Maria, Santa Vitória do Palmar, Santo Ângelo, São Sepé, São Vicente do Sul, Taquari, Unistalda, Vacaria, and Vila Nova do Sul that were tested by MAT were antibody positive for one more *Leptospira* serovar.

**Figure 2.** Percentage (%) of equidae reactive by microscopic agglutination test to serovar-specific *Leptospira* spp. antigens by state of origin of the 767 animals slaughtered in the period April-May 2013, São Gabriel, RS, 2013.



We observed that all (100%) of the 45 municipalities had seropositive animals against one or more serovars of *Leptospira* (Table 3). The municipalities of Rio Grande do Sul State were those with the highest percentage of reactive animals. Equidae coming from the cities of Caçapava do Sul, Capão Cipó, Cristal, Dom Pedrito, Itacurubi, Pinheiro, Piratini, Quaraí, Rosário do Sul, São Gabriel, Santa Maria, Santa Vitória do Palmar, Santo Ângelo, São Sepé, São Vicente do Sul, Taquari, Unistalda, Vacaria, and Vila Nova do Sul.

In the municipalities of Paraná, Ampere had the greatest number of reactive animals (97.73%), and

in the State of Santa Catarina, the city of Lages had a highest prevalence of *Leptospira* positive animals (95.45%).

The MAT showed that out of 407 males and 360 females studied, 85.26% (CI 95%: 82.82% – 88.70%) of male equids were reactive to *Leptospira* and 94.44% (CI 95% 92.07% – 96.81%) of females were reactive (Table 4). Chi-square testing showed that there was highly significant difference ( $p = 5.4444 \times 10^{-5}$ ) between the proportion of positive males and females.

**Table 3.** Municipalities, number (N°), and percentage (%) of equidae antibody reactive to at least one serovar of *Leptospira* spp. by microscopic agglutination test (MAT), among the 767 horses slaughtered between April and May 2013, São Gabriel, RS, 2013.

State/Municipality	Reactives		Non-reactives		Total
	N°	%	N°	%	
<b>Rio Grande do Sul</b>					
Alegrete	53	94.64	03	5.36	56
Bagé	45	71.43	18	28.57	63
Caçapava do Sul	15	100.00	00	0.00	15
Cacequi	15	88.24	02	11.76	17
Canguçu	16	88.89	02	11.11	18
Capão Cípó	01	100.00	00	0.00	01
Cristal	02	100.00	00	0.00	02
Cruzeiro do Sul	20	95.24	01	4.76	21
Dom Pedrito	02	100.00	00	0.00	02
Encruzilhada do Sul	08	80.00	02	20.00	10
Itacurubi	10	100.00	00	0.00	10
Itaqui	25	89.29	03	10.71	28
Lavras do Sul	12	70.59	05	29.41	17
Maçambará	26	92.86	02	7.14	28
Pinheiro Machado	11	100.00	00	0.00	11
Piratini	02	100.00	00	0.00	02
Pelotas	03	75.00	01	25.00	04
Quaraí	22	100.00	00	0.00	22
Rosário do Sul	03	100.00	00	0.00	03
Santa Cruz do Sul	06	54.55	05	45.45	11
Santa Maria	16	100.00	00	0.00	16
Santa Vitória do Palmar	40	100.00	00	0.00	40
Santana da Boa Vista	08	80.00	02	20.00	10
Santana do Livramento	30	88.24	04	17.76	34
Santiago	23	82.14	05	17.86	28
Santo Ângelo	08	100.00	00	0.00	08
Santo Antônio das Missões	05	100.00	00	0.00	05
São Borja	40	97.56	01	2.44	41
São Francisco de Assis	08	80.00	02	20.00	10
São Gabriel	14	100.00	0	0.00	14
São Lourenço do Sul	07	87.50	01	12.50	08
São Sepé	2	100.00	0	0.00	02
São Vicente do Sul	16	100.00	00	0.00	16
Taquari	06	100.00	00	0.00	06
Unistalda	11	100.00	00	0.00	11
Vacaria	21	100.00	0	0.00	21
Vila Nova do Sul	01	100.00	00	0.00	01
<b>Santa Catarina</b>					
Bandeirante	09	90.00	01	10.00	10
Lages	21	95.45	01	4.54	22
Saltinho	05	83.33	01	16.67	06
São Miguel do Oeste	03	60.00	02	40.00	05
<b>Paraná</b>					
Ampére	43	97.73	01	2.27	44
Nova Laranjeiras	17	85.00	03	15.00	20
Paulo Frontin	15	62.50	09	37.50	24
São Mateus do Sul	21	87.50	03	12.50	24
<b>Total</b>	<b>687</b>	<b>89.57</b>	<b>80</b>	<b>10.43</b>	<b>767</b>

**Table 4.** Number and percentage of sera from 767 equidae reactive to at least one serovar of *Leptospira* spp. of microscopic agglutination testing that were slaughtered between April and May 2013, São Gabriel, RS, 2013.

Sex	Reagents	Frequency (%)	Confidence Interval (95%)
Male	347	85.26	82.82%–88.70%
Female	340	94.44	92.07%–96.81%
Total	687	89.57	87.41%–91.73%

## Discussion

The prevalence (89.57%, CI 95%: 87.41%–91.73%) of animals reactive to *Leptospira* by MAT was high. This high antibody prevalence may be explained by the fact that, in contrast to the beef market, there are no specific farms in Brazil for horsemeat production, making it difficult to control hygiene and sanitation where these animals are maintained.

Another factor that might help to explain the high prevalence of antibodies against *Leptospira* spp. among the slaughtered horses is the fact that they were working animals in urban centers, living in harsh environments and are constantly in contact with other species, such as rodents, dogs, cattle, and pigs.

A small number of *Leptospira* antibody-positive horses have been reported in other studies with horses slaughtered in slaughterhouses. Evers et al. (2012), observed in 398 serum samples of slaughtered equids in the State of Paraná, 30.9% (123) were seropositive to at least one serovar of *Leptospira* spp. Rocha et al. (2004) tested sera from 145 horses from slaughterhouses in Portugal, and found that 37% of animals were seropositive to at least one serovar by MAT. Evers et al. (2012) found that the main risk factors associated with the high frequencies of reactivities animals to *Leptospira* was the movement of horses, environmental factors, rodents as maintenance hosts, in addition to direct contact with *Leptospira* contaminated urine. Infections by the serovars Patoc (9.91%) and Butembo (9.13%) were most common in this study. The high prevalence of antibodies against Patoc was

unexpected, because equines do not serve as the maintenance hosts for this serovar.

There are few reports on the frequency of the Patoc serovar in horses in Brazil. This serovar belongs to the species *Leptospira biflexa*, and it is grouped with the non-pathogenic strains of *Leptospira*. Antigens of this serovar are often used in serological investigations due their cross-reactivity with pathogenic serovars and their value in detecting antibodies earlier in the course of infection (AGUIAR et al., 2008). Thus, this serovar can be used as a serological indicator and is used as a MAT antigen.

Silva et al. (2010) surveyed domestic and wild animals for antibodies against *Leptospira* spp. in from March to October 2008. They found that sera from 15 of 97 (15.46%) horses reacted to the serovar Patoc, the most found in these animals. Favero et al. (2002) found Patoc seropositive horses in the states of Paraíba (45%), Rio Grande do Sul (47.7%), and Mato Grosso (33.4%). Although there are few reports, reactions against serovar Patoc are common among the animals studied, confirming the utility of using antigens of this serovar as a diagnostic tool in serological investigations of leptospirosis.

In Brazil, prevalences against the different serovars in horses vary, most studies have found the highest prevalence of seroreactivity to Icterohaemorrhagiae. In this study, only 1.56% of the animals were seropositive for Icterohaemorrhagiae. Higher frequencies of reactive sera were found in three farms with reproductive problems in the state of Rio de Janeiro, where Lilenbaum (1998) found 43.40% of animals responsive to the

Icterohaemorrhagiae serovar. This result was similar to that reported by Viegas et al. (2001), who found antibodies in 53.1% of horses in the state of Bahia. Langoni et al. (2004) also found this serovar to be predominant in 520 (37.01%) animals. Linhares et al. (2005) reported that 56 (68.28%) of samples from the micro region of Goiania were seropositive for Icterohaemorrhagiae; Hashimoto et al. (2007) found 32 (23.26%) and Caselani et al. (2012) found 10 (10.75%) seropositive animals in their studies.

As working animals with exposures to the environment, such as contact with domestic garbage and food waste and contact with rodents, antibody evidence that the Icterohaemorrhagiae serovar is circulating suggests the need for health management programs for these animals. This serovar is associated with human disease, posing a potential public health problem. Therefore, prevention and control measures in animals are extremely important to break the disease cycle. In Brazil, the only vaccine available for leptospirosis in horses includes antigens of this serovar among the 12, and it is part of an important preventive method.

Other serovars occurred infrequently, including the following: Australis (7.82%), Bratislava (5.87%), Pyrogenes (4.95%), and Tarassovi (3.52%). The diversity of serovars in this study may reflect contact between equids and animals of different species in places where it is circulating, demonstrating the susceptibility of horses to bacteria in the genus *Leptospira*.

When the distribution of *Leptospira* spp. by state is considered, 553 of 612 (90.36%) animals from the State of Rio Grande do Sul were seropositive to at least to one serovar of *Leptospira* spp. One of the risk factors that may be associated with the high frequency of animals *Leptospira* seropositivity in the state of Rio Grande do Sul is agriculture, the predominant economic activity in the state. The cultivation of rice may attract rodents, which are maintenance hosts of *Leptospira*.

According to IBGE (2011b), out of the 20 major municipalities that produce rice in Brazil, except for Lagoa da Confusão in the state of Tocantins, all are from Rio Grande do Sul, contributing 21% to national production. This suggests that agriculture, especially rice cultivation, can favor the infection, since factors necessary for the survival of *Leptospira* are present, such as running water and carrier animals. Animals slaughtered in a slaughterhouse are often used in field activities, and so are exposed to the same risk factors as reported for humans, such as contact with soil and water contaminated by the urine of rodents on farms.

In this study, the serovars that prevailed in Rio Grande do Sul were Patoc (10.95%), Butembo (9.31%), and Australis (9.64%). A similar result was reported by Favero et al. (2002), with the Patoc serovar (47.7%) detected most often of the serovars. In contrast, Lasta et al. (2013), in a study of horses in that State, found Icterohaemorrhagiae the predominant serovar.

Among the 112 animals from the State of Paraná, the frequency of reactivities was also high, with 85.71% of animals seropositive *Leptospira*. This high frequency was also observed by Hashimoto et al. (2007) in the state of Paraná: when surveying horses from the urban area of Londrina, they observed that 66.88% of samples were considered positive by MAT for *Leptospira* spp. However, the most frequently occurring serovar was Icterohaemorrhagiae (22.36%). In the present study, the probable serovars of infection were Butembo (8.03%) followed by Patoc (3.07%) in this state.

In Santa Catarina, the frequency of positive animals was 88.37% among 43 animals. The most probable serovars of infection were Patoc (11.63%), Butembo (9.3%), and Tarassovi, with 6.98% of animals reactive by MAT. When serologically surveying cattle in this region, Favero et al. (2001) found that Patoc was one of the most common serovars in the state of Santa Catarina, demonstrating that different species are being exposed to the same

serovar and reinforcing the importance of proper hygiene and sanitary husbandry for the control of leptospirosis.

Diversity of *Leptospira* spp. was apparent across all regions represented in this study, with all (100%) municipalities serving as the source of seropositive animals against one or more serovars of *Leptospira*. Aguiar et al. (2008) suggested that anti-*Leptospira* spp. antibodies in horses is important, as these animals can act as carriers, releasing the bacteria in urine, contaminating the environment and increasing infections in humans and other animal species.

The prevalence of the disease in males and females observed in this study was 85.26% (CI 95%: 82.82%–88.70%) for males and 94.44% (CI 95%: 92.07%–96.81%) for females. Chi-square testing showed that there was a significant difference ( $p = 5.4444 \times 10^{-5}$ ) between the proportion of males and females that were MAT reactive. Results from this study contrast with the findings of Coiro et al. (2012), who found no significant difference in *Leptospira* exposure between male and female horses. Langoni et al. (2004), however, found a significant difference between the sexes, with 379 of 617 (61.43%) females positive and 32 of 71 (45.17%) males positive. Similar to this study, they found a higher prevalence in females, which the authors attribute to the different husbandry practices of the sexes.

## Conclusion

Infections with *Leptospira* spp. is circulating in the areas associated with this study, with a high frequency of reactive animals observed. These findings highlight the importance of studying leptospirosis in horses from slaughterhouses, as these animals are in direct contact with other animals, including humans. These working animals represent an important source of infection for other species. The fact that sera from these animals was reactive to serovars of different *Leptospira*

suggests that different control measures should be implemented in the various states of Brazil where animals in this study originated, in order to reduce the reservoirs of this pathogen.

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