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Diagnosis of *Leptospira* spp. Infection in Sheep Flocks in the State of Mato Grosso, Brazil

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ABSTRACT

Background: Leptospirosis has a worldwide distribution with high social and economic impact. It is caused by a variety of *Leptospira* spp. serovars, particularly in tropical regions. Various species of animals can be affected by *Leptospira* spp. including cattle, horses, sheep, goats and swine, and can act as a reservoir for human infection. In Brazil, sheep leptospirosis has been diagnosed in a variety of States, however, little is known about sheep flocks of Mato Grosso. Considering the marked growth of sheep industry in Mato Grosso State in past recent years, this study aimed to evaluate the frequency of serologically positive sheep for *Leptospira* spp. serovars in the State of Mato Grosso (Brazil).

Materials, Methods & Results: Blood samples of 346 sheep, which belonged to 16 sheep flocks of the medium northern region of Mato Grosso was obtained by jugular vein puncture. Serum was obtained by blood centrifugation and stored at -20°C until the test to be performed. The diagnosis was performed using the microscopic agglutination test. Serovars Australis, Autumnalis, Bataviae, Bolívia, Castellonis, Celledoni, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Lagoa, Norma, Panama, Pomona, Sejroe, Shermani, Szwajizak, and Wolffi were tested. Reactions were considered positive with minimum titration of 1:100. Considering all serovars, 54.9% of sheep were positive, and the serovar more frequently detected was Celledoni (22.8%), followed by Javanica (19.4%), Castellonis (16.5%), and Norma (15.3%), with predominance of titration of 100 for these serovars, while only one sheep had a titration of 1:800 detected for serovar Hardjo. All flocks (100%) were positive (at least one positive animal), with the frequency of animals positive to *Leptospira* spp. varying of 31.3% to 79.2% in flocks.

Discussion: Infection for *Leptospira* spp. in humans has a large social and sanitary impact. Under an animal health perspective, beyond sanitary impact, leptospirosis have a large economic impact and can act as important reservoirs for *Leptospira* spp. for human infection, especially in tropical conditions, which favor the persistence of agent in the environment. The occurrence of sheep leptospirosis has been diagnosed in several States of Brazil as Rio Grande do Sul, Paraíba, Rio de Janeiro, São Paulo and Maranhão, with frequency of herds positive for *Leptospira* spp. varying of 5.4% to 47.4%, however, the frequency of positive sheep in this study was higher than these previous reports in other Brazilian States. The most frequent serovar in sheep flocks have a great importance for prophylactic control. However, there were identified as main frequent serovar Celledoni and Javanica, which are considered unusual in sheep, and indicates a peculiar profile of ovine serovars for the region. Considering the high frequency of positive farms (100%) in Mato Grosso and the lack of studies about the occurrence of leptospirosis in this region, our results suggest the occurrence of risk factors that can favor the dispersion and survival of agent. Contact of the sheep evaluated in this study with other animal species such as cattle, dogs, and horses that can serve as a source for sheep infection or may become contaminated from sheep. Then, the profile of sheep infection by *Leptospira* spp. in the State of Mato Grosso has a peculiar profile of serovars, with high frequency of infection of animals and herds.

Keywords: small ruminants, leptospirosis, serology.

INTRODUCTION

Leptospirosis is an emerging infectious disease caused by several serovars of *Leptospira*. The disease has public health importance due to high incidence and severity of clinical manifestations in humans, in addition to the high economic impact for different animal species including cattle, horses, sheep, goats and swine [10].

Leptospira spp. survival for extended periods in environments with high temperature and humidity contribute for disease dispersion, especially in tropical regions [8]. In addition to these environmental conditions, the high population density and suitable reservoirs are determinant factors for disease occurrence and maintenance of the bacteria in the environment [11].

Thus, the broad climatic and environmental variations in Brazil are factors that contribute for a wide dispersion of infectious agents in different regions. This fact reflect in high variations of prevalence (5 to 47%), according the studies performed in the States of Paraíba, São Paulo, Maranhão, Rio Grande do Sul, and Rio de Janeiro [1-3,5,9].

The sheep industry in the State of Mato Grosso has experienced marked growth over the past recent years [4]. According the last official data, the sheep population in Mato Grosso is 307,948 animals [7], representing 1.7% of national population. Importantly, dispersion of infectious agents and disease of sheep has been poorly studied in this region. The present work aimed to evaluate the frequency of serovars of *Leptospira* spp. in sheep flocks in the State of Mato Grosso.

MATERIALS AND METHODS

Sampling

By random sampling, 16 sheep flocks of the medium northern region of Mato Grosso were selected, including the municipalities of Sinop, Vera, Santa Carmen, and Ipiranga do Norte. One-year-old or older rams and ewes were sampled, resulting in 346 samples.

Blood collection and serology

Sampling was performed by external jugular vein puncture using vacuum tubes without anticoagulant. Blood samples were centrifuged, and sera were stored in triplicates at -20°C until further testing.

Serologic diagnosis was performed by using the microscopic agglutination test (MAT), which was performed at the Instituto Desidério Finamor (Porto Alegre, Brazil). Samples that resulted in a minimum of

50% of agglutinated *Leptospira* by microscopic field, with a minimum titration of 1:100, were considered positive. When positive on minimum titration, samples were sequentially diluted (1:200, 1:400, and 1:800). Antigens of serovars Australis, Autumnalis, Bataviae, Bolívia, Castellonis, Celledoni, Grippotyphosa, Hardjo, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Lagoa, Norma, Panama, Pomona, Sejroe, Shermani, Szwajizak, and Wolffi were used.

RESULTS

All tested flocks were positive for at least one serovar (100%) and the frequency of positive sheep per flock varied from 31.3% (5/16) to 76.5% (13/17), as detailed on Table 1.

In total, 54.9% of sheep were positive with minimal titration. The serovar more frequently detected was Celledoni (22.8%), followed by Javanica (19.4%), Castellonis (16.5%) and Norma (15.3%), with predominance of titration of 100 for these serovars, while only one sheep had a titration of 1:800 detected for serovar Hardjo. The serovar Autumnalis, Batavie, Shermani, and Szwajizak were not detected in any of the evaluated sheep (Table 2).

DISCUSSION

Leptospirosis results in large social and economic impact, especially in tropical conditions that favor the persistence of agent in the environment, and consequently transmission of disease [11]. Considering the high epidemiologic importance of reservoirs, the diagnosis of leptospirosis in sheep has been largely neglected. A high frequency of positive farms (100%) and individual sheep (54.9%) was observed in this study, which is the first seroepidemiological study of exposure to *Leptospira* spp. in sheep in the state of Mato Grosso, which supports the hypothesis that sheep may serve as an important reservoir of the agent.

The microscopic agglutination test (MAT) is the most largely used method of diagnosis for screening of leptospirosis. Sheep flocks of different Brazilian regions were evaluated by this technique, resulting in frequencies of 34.2% in Rio Grande do Sul [5], 5.4% in Paraíba [1], 47.4% in Rio de Janeiro [9], 23.0% in São Paulo [2] and 32.0% in Maranhão [3]. In spite of the broad variation in prevalences observed among different States, the frequency of positive sheep in this study was higher than these previous reports in other Brazilian states.

Table 1. Detailed information by flock diagnosed with *Leptospira* spp. on medium northern region of Mato Grosso State, Brazil.

Flock	City	Total of sheep per flock	Evaluated animals	Animal species with direct contact	Frequency of positive sheep	Positive serovars
1	Sinop	62	17 (27.4%)	Cattle	76.5%	Bolívia, Castellonis, Celledoni, Hardjobovis, Javanica, Lagoa, Norma, Panamá, Pomona and Wolffi
2	Sinop	100	26 (26.0%)	-	73.1%	Bolívia, Castelonis, Celledoni, Grippytyphosa, Hardjo, Hardjobovis, Hebdomadis, Javanica, Lagoa, Norma and Wolffi
3	Sinop	50	12 (24.0%)	-	41.7%	Bolívia, Castelonis, Celledoni, Hebdomadis, Javanica, Lagoa, Norma, Panama, Pomona
4	Santa Carmem	50	18 (36.0%)	cattle, dogs, and horses	44.4%	Bolívia, Castelonis, Celledoni, Hebdomadis, Javanica, Norma, Panama
5	Sinop	200	29 (14.5%)	cattle, dogs, and horses	58.6%	Bolívia, Castelonis, Celledoni, Grippytyphosa, Hebdomadis, Javanica, Lagoa, Norma, Panama, Pomona and Wolffi
6	Sinop	50	24 (48.0%)	Cattle	79.2%	Australis, Bolívia, Castelonis, Celledoni, Grippytyphosa, Hebdomadis, Javanica, Lagoa, Norma, Panama, Pomona and Wolffi
7	Santa Carmem	250	18 (7.2%)	-	61.1%	Bolívia, Castelonis, Celledoni, Grippytyphosa, Javanica, Norma, Panama, Pomona and Wolffi
8	Sinop	800	34 (4.2%)	-	32.4%	Bolívia, Castelonis, Celledoni, Hebdomadis, Javanica, Lagoa, Pomona and Wolffi
9	Vera	5.000	82 (1.6%)	Dogs	48.8%	Australis, Bolívia, Castelonis, Celledoni, Grippytyphosa, Hardjo, Hardjobovis, Hebdomadis, Icterohaemorrhagiae, Javanica, Lagoa, Norma, Panama, Pomona e Wolffi
10	Ipiranga do Norte	54	16 (29.6%)	-	31.3%	Castelonis, Celledoni, Hardjobovis, Javanica, Norma, Panama and Wolffi
11	Ipiranga do Norte	101	23 (22.8%)	Cattle	65.2%	Castelonis, Celledoni, Hardjo, Hardjobovis, Javanica, Norma, Panama, Pomona, Sejroe and Wolffi
12	Ipiranga do Norte	15	8 (53.3%)	cattle, dogs, and horses	50.0%	Bolívia, Castelonis, Celledoni, Panama and Wolffi
13	Ipiranga do Norte	12	9 (75.0%)	Cattle	66.7%	Castelonis, Celledoni, Hardjobovis and Javanica
14	Sinop	18	12 (66.7%)	cattle, dogs, and horses	50.0%	Castelonis, Celledoni and Javanica
15	Sinop	9	9 (100.0%)	cattle, dogs, and horses	55.6%	Castelonis, Celledoni, Javanica, Norma and Panama
16	Sinop	19	9 (47.4%)	Dogs	66.7%	Castelonis, Celledoni, Javanica and Norma

Table 2. Frequency of sheep serologically positive for *Leptospira* spp. serovars and maximum titration detected by microscopic agglutination test on medium northern region of Mato Grosso State, Brazil.

Serovar	Relative frequency	Absolut frequency	Titration			
			100	200	400	800
Australis	0.6%	2/346	1	1	0	0
Autumnalis	0.0%	0/346	0	0	0	0
Bataviae	0.0%	0/346	0	0	0	0
Bolívia	7.2%	25/346	20	4	1	0
Castelonis	16.5%	57/346	51	6	0	0
Celledoni	22.8%	79/346	61	18	0	0
Grippotyphosa	1.4%	5/346	4	1	0	0
Hardjo	2.3%	8/346	7	0	0	1
Hardjobovis	5.5%	19/346	17	2	0	0
Hebdomadis	3.8%	13/346	11	2	0	0
Icterohaemorrhagiae	0.3%	1/346	1	0	0	0
Javanica	19.4%	67/346	66	1	0	0
Lagoa	4.3%	15/346	12	3	0	0
Norma	15.3%	53/346	48	4	1	0
Panama	4.0%	14/346	13	1	0	0
Pomona	4.3%	15/346	13	2	0	0
Sejroe	0.3%	1/346	1	0	0	0
Shermani	0.0%	0/346	0	0	0	0
Szwajizak	0.0%	0/346	0	0	0	0
Wolffi	5.5%	19/346	18	1	0	0

Moreover, the high frequency of positive farms (100%) in Mato Grosso suggests the occurrence of risk factors that can favor the dispersion and survival of agent. In this context, the elevated rainfall in concentrated periods of the year in this region may favor the persistence of the agent, favoring infection [11].

Different serovars were more prevalent in different regions of Brazil. For instance, sorovar Autumnalis was the most frequent in the State of São Paulo [2], serovar Grippotyphosa in Maranhão [3], and serovar Hardjo in Rio Grande do Sul [5]. In this study, serovars Celledoni and Javanica were the most frequent, whereas these serovars have been rarely found previously in sheep [6]. The Javanica serovar in sheep is commonly associated with sharing pastures and premises with cattle [2]. Identification of uncommon serovars indicates a peculiar profile of ovine serovars for the region, emphasizing the importance of

the studies to determine the need for serovar-specific vaccination.

CONCLUSION

In conclusion, our results indicated 54.9% of positive individual sheep and 100% of positive sheep farms by the microscopic agglutination test (MAT), which was the first seroepidemiological study of leptospirosis in sheep in the State of Mato Grosso, Brazil.

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Ethical approval. All animal protocols have been reviewed and approved by the Ethics Committee on the Use of Animals of Embrapa Agrossilvipastoril (Animal use permit: Protocol 004/2015).

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

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