Leptospirosis in sheep in southern Rio Grande do Sul, Brazil

Leptospirose em ovinos no sul do Rio Grande do Sul, Brasil

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ABSTRACT
Leptospirosis is a globally occurring zoonosis. The disease is often asymptomatic in sheep, but it is a major cause of reproductive losses. In Rio Grande do Sul state, commercial sheep herds total over 2,600,000 animals. In this light, our study aims to identify seroprevalence of anti-Leptospira antibodies in sheep in a slaughterhouse with state inspection level. Blood samples were taken at the time of bleeding at the slaughter line. Eighty-one serum samples were submitted to the Microscopic Agglutination Test (MAT), using a panel of 12 serovars. A total of 23 (28.39%) were reagents for at least one serovar. According to the most prevalent serovars (Autumnalis, Hardjo and Bratislava), grazing alongside cattle and the presence of rodents on the farm, may be influencing the transmission of the agent to the sheep. Although the prevalence was similar to the national average (26%), it is lower than that most recently described for the region (34.26%), which may indicate advances in technification and the elaboration of prevention policies.

Keywords: Sheep Leptospirosis, seroprevalence, slaughterhouse, MAT, zoonosis.

RESUMO
A leptospirose é uma zoonose de ocorrência global. Frequentemente, a doença é assintomática em ovinos, mas é uma das principais causas de perda reprodutiva. No estado do Rio Grande do Sul, a criação de ovinos é aproximadamente dois milhões e setecentos mil animais. Devido a essa importância, nosso estudo teve como objetivo estimar a soroprevalência de anticorpos anti-Leptospira em ovinos abatidas em um estabelecimento frigorífico com inspeção estadual. As coletas de sangue foram realizadas no momento da etapa de sangria na linha de abate. Oitenta e um soros foram submetidos ao Teste de Soroaglutinação Microscópica (MAT), usando um painel de 12 sorovares. Um total de 23 (28,39%) foram reagentes para pelo menos um sorovar. De acordo com os sorovares mais prevalentes (Autumnalis, Hardjo e Bratislava), pastar ao lado dos bovinos e a presença de roedores na propriedade, podem estar influenciando a transmissão do agente para os ovinos. Embora a prevalência tenha sido semelhante à média nacional (26%), ela é menor que o descrito mais recentemente para a região (34,26%), o que pode indicar avanços na tecnificação e elaboração de políticas de prevenção.

Palavras-chave: Leptospirose ovina, soroprevalência, frigorífico, MAT, zoonose.
INTRODUCTION

Leptospirosis is a globally occurring zoonosis caused by pathogenic bacteria of the *Leptospira* genus (Ellis, 2014). The disease is often asymptomatic in sheep, but abortions and lamb deaths may occur (Ciceroni et al., 2000; Carvalho et al., 2011). When the symptomatic disease occurs, common complications include sepsis, hemorrhaging, icterus, nephritis, hemoglobinuria, mastitis, and reproductive setbacks, such as fetal absorption, abortion, and weak newborns which usually die before one week (Alves et al., 2012). Leptospirosis in sheep herds is usually associated with grazing alongside cattle, where the smaller ruminant will infect themselves through direct or indirect contact with contaminated bovine urine (Escocio et al., 2010).

In Rio Grande do Sul, the southernmost state in Brazil, commercial sheep herds total over 2,600,000 animals (IBGE, 2017), that are mostly under technified. In this light, our study aims to identify seroprevalence of anti-*Leptospira* antibodies in sheep slaughtered at a commercial abattoir in southern Rio Grande do Sul, an important sheep rearing region in the state.

MATERIAL AND METHODS

This study was conducted in a slaughterhouse under state health inspection, operating in the city of Capão do Leão, in the southernmost state of Brazil (Rio Grande do Sul, RS). The studied population were sheep reared for, and slaughtered at slaughterhouse mentioned above. All animals that were approved for slaughter by the inspection agency were included, no exclusion criteria were applied. This study was approved by the ethics committee for animal experimentation of the Universidade Federal de Pelotas (CEEA-UFPel: 0678-2017).

Blood collections were carried out from May to October 2017, at the time of bleeding in the slaughter line. Sterile tubes were used, and the sera separated through centrifugation at 5000 xg for 10 minutes. Sera were kept at -20 °C until use. Kidneys were also collected in the slaughter line to attempt bacterial isolation (Silva et al., 2007). The left kidney was collected and processed upon arrival at the laboratory. Sterile fragments (0.5 cm$^3$) were macerated into EMJH media, left for one hour, and 500 µL were sub cultured into fresh EMJH media supplemented with 10% Difco™ *Leptospira* supplement. The cultivation attempts were observed weekly under dark field microscopy, for up to 10 weeks (unless contaminated).

The serum samples were subjected to the Microscopic Agglutination Test (MAT). This was carried out according to World Health Organization recommendations, as previously
described (WHO, 2003; Faine et al., 1999). Samples were diluted 1:50, for a trial titer of 100. A total of 12 antigens were used, serovars: Canicola, Australis, Bataviae, Autumnalis, Bratislava, Copenhageni, Grippothyphosa, Hardjo, Icterohaemorrhagiae, Pomona, Pyrogenes, and Patoc I. These were kindly provided by the provided by the Coleção de Leptospira (CLEP)/Laboratório de Referência Nacional para Leptospirose (LNRNL), FIOCRUZ. Reactive samples were then titrated from 1:100 to 1:3200. When co-agglutination occurred, the highest titer was considered reactive.

3 RESULTS

A total of 81 sheep were included, from seven different herds and three different municipalities of the region (Capão do Leão; Arroio Grande; and Pedro Osório). Of these, 23 (28.39%) were seroreactive for at least one serovar, in the MAT. Patoc was the most prevalent serovar overall (n=8; 34.78%), and Autumnalis (n=4; 17.39%) and Hardjo (n=3; 13.04%) were the most prevalent, among the pathogenic serovars. Titters varied from 1:100 to 1:1,600. Complete results regarding seroreactivity and titters can be seen on table 1. Furthermore, though no contamination occurred in the isolation attempts, and all potential cultures were kept and observed for the full period, no isolate was obtained.

Table 1. Seroreactivity, according to serovar of highest titre, of sheep slaughtered at an abattoir in southern Rio Grande do Sul state.

| Serovar* | Title |
|----------|-------|
|          | 100   | 200  | 400  | 800  | 1600 | Total |
| Autumnalis | 1     | 2    | 1    |      |      | 4     |
| Bratislava  |       |      |      |      |      | 1     |
| Copenhageni | 1    | 1    |      |      |      | 2     |
| Grippothyphosa | 1   |      |      |      |      | 1     |
| Hardjo      |       |      |      |      | 1    | 2     |
| Icterohaemorrhagiae | 1 |      |      |      |      | 1     |
| Pomona      | 1     | 1    |      |      |      | 2     |
| Pyrogenes   |       |      | 1    |      |      | 1     |
| Patoc I     | 8     |      |      |      |      | 8     |
| **Total**   | **14**| **5**| **1**| **1**| **2**| **23**|

*Only reactive results considered.

4 DISCUSSION

In our study, and overall prevalence of 28.39% for anti-Leptospira antibodies was found in sheep slaughtered at Capão do Leão. This result was very close to the national average in sheep, which is 26% (Amorim et al., 2016) and 23% (Barbante et al., 2014), and slightly lower than the most recently described seroprevalence in Rio Grande do Sul state, of 34.78% (Hermann et al., 2004). However, in other regions of the country, low seroprevalence has been...
found, from 8.6% (Barbudo-Filho et al., 1999), to 3.5% (Azevedo et al., 2004), and 3% (Seixas et al., 2011; Amorim et al., 2016).

Regarding serovar specific reactions, Patoc I was by far the most prevalent in our study. Patoc I is a serovar of the *L. biflexa* species of nonpathogenic leptospiras. It is usually included in the MAT antigenic panel as a sentinel for possible cross reactions of serovars that are not present (Levett, 2001). In this light, it may be possible to assume that unusual serovars are likely circulating in the herds studied. The region of Pelotas, where Capão do Leão is situated, is known for its unusually high variability of circulating serovars, in humans and animals, and unexpected species and serovars of *Leptospira* are described circulating there, ever more frequently (Silva et al., 2007; Cunha et al., 2016).

Disregarding Patoc I, Autumnalis and Hardjo were the most prevalent serovars. Studies assessing seroprevalence in cattle are more frequent than in sheep, and these serovars are common in bovines of southern Rio Grande do Sul (Brod et al., 1995; Hermann et al., 2012). Considering that sheep and cattle are usually kept in the same pastures, grazing together and sharing the same watering opportunities, the circulation of the same serovars among these species is not surprising, and has been suggested before (Hermann et al., 2004). Furthermore, Autumnalis has also been implicated in ovine leptospirosis, and found as the most prevalent serovar in past studies (Araújo Neto et al., 2008; Higino et al., 2012), with some authors suggesting sheep as important risk factors for human infection with this serovar (Alves et al., 2012). Likewise, though the prevalence for serovar Copenhageni was relatively low in our study (8.69% of all reactives), the fact that it is circulating in the herds is worthy of note. This serovar has synanthropic rodents as its most important maintenance host (Zacarias et al., 2008), and is one of the most important causative agents of leptospirosis in humans (Silva et al., 2015).

**5 CONCLUSION**

Although the prevalence was similar to the national average, it is lower than that most recently described for the region, which may indicate advances in technification and the elaboration of prevention policies. Furthermore, cattle and rodents are likely associated with the circulating serovars in sheep herds.
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