INTRODUCTION

Leptospirosis is a zoonotic infectious disease highly important for global public health. It is caused by pathogenic species of the Leptospira spp. genus (DE BRITO et al., 2018). In horses, the infection is associated with recurrent uveitis (VERMA & STEVENSON, 2012), abortions, stillbirths, or weak neonate foals (BROUX et al., 2012; HAMOND et al., 2013; PINNA et al., 2014). Horses are incidental hosts for most serovars of Leptospira spp., such as Icterohaemorrhagiae, Canicola, Pomona, and...
Grippotyphosa (ELLIS, 2015), considered reservoirs for the serovar of Bratislava (PINNA et al., 2014) 2012.

Infections in horses generally occurred by direct contact with contaminated urine or placenta fluids of infected animals or indirectly from a contaminated environment (FAISAL et al., 2012). Even with an intense humoral response, the agent can survive and multiply, being eliminated through the urine for up to three months (YAN et al., 2010). In this context, leptospirosis in horses can represent a neglected zoonosis since horses of most regions are pets, presenting a very close relationship with humans of different age groups. In 2018, 237 cases of leptospirosis were confirmed in the State of Santa Catarina, Brazil, with five deaths, most involving men resident in urban areas (DIVE, 2019).

The seroprevalence of leptospirosis in horses varies considerably depending on the geographic location, risk factors, and studies serovars (CASELANI et al., 2012; LASTA et al., 2013; FINGER et al., 2014). The prevalence and serovars involved depend on the reservoirs present (PETRAKOVSKY et al., 2014) since they might undergo regional variations, which undoubtedly contributes to the difference between the data on prevalence between countries or regions (LOUREIRO et al., 2013).

The primary control strategy for leptospirosis in all endemic regions is diagnosis followed by vaccination of the animals. However, serogroup-specific immunity, the diversity of serovars, and the absence of seroepidemiological data from endemic areas represent a challenge for defining vaccinal antigens (MARTINS et al., 2017).

In this context, the knowledge on prevalent serovars is essential to understand the epidemiology of the disease, allowing the discovery of new carriers and possible reservoirs, and aid in defining vaccinal antigens to be used in the immunoprophylaxis strategies (ALVES et al., 2016; VIEIRA et al., 2018). In Santa Catarina, there haven’t yet been any studies on the prevalence of leptospirosis in horses, making it difficult to update the vaccinal strains to be used in infection control programs. Therefore, this study aimed to evaluate the presence of anti-\textit{L. interrogans} antibodies in serum samples of unvaccinated horses, identify the primary serogroups circulating in the Serra Catarinense region and investigate the possible risk factors associated with the infection.

**MATERIALS AND METHODS**

**Animals and samples**

The total number of horses in the State of Santa Catarina is of approximately 112,766, 32,451 (28.77%) located in the Serra Catarinense region (IBGE, 2017) and with 3,314 properties registered in the official organ (CIDASC). We estimated the sampling according to the methodology proposed by THRUSFIELD (1997). The calculation was done using the Epi Info statistical package, version 7.1.5. (CDC, 2015), based on the estimation of the horse herd of the Serra Catarinense region (n=32,451) for an expected prevalence of P<5%, based on preliminary regional studies on the population of unvaccinated animals.

For this study, we evaluated 207 serum samples from unvaccinated horses with no clinical signs or history of the disease from 26 herds of the Serra Catarinense region. The herds used originated from collaborating owners from different areas, with samples of at most ten animals from each property. The samplings occurred from November of 2017 to March of 2018. We collected the blood samples through venipuncture of the jugular vein using vacuum tubes with no anticoagulant, maintaining the samples under refrigeration at 10 ºC. We subsequently centrifuged the samples, dividing them into serum aliquots and freezing them at -20 ºC until analysis.

**Serological test**

We assessed the samples for the presence of anti-\textit{Leptospira} spp. antibodies through a microscopic agglutination test (MAT) using live antigens cultivated in liquid medium (EMJH), according to OIE (2015). We obtained a panel of antigens with 14 serogroups (18 reference serovars) from the Laboratory of Veterinary Microbiology of the Universidade Federal Fluminense (UFF/RJ). The panel consisted of serogroups (serovars) Australis (Bratislava, Australis), Autumnalis (Autumnalis), Cynopteri (Cynopteri), Bataviae (Bataviae), Canicola (Canicola), Grippotyphosa (Grippotyphosa), Panama (Panama), Icterohaemorrhagiae (Copenhageni, Icterohaemorrhagiae), Louisiana (Louisiana), Pomona (Pomona), Sejroe (Guaricura, Hardjobovis; Wolfii), Pyrogones (Pyrogones), Tarassovi (Tarassovi), and Ballum (Castellonis).

In summary, we added the antigenic suspension of live leptospires obtained from the respective serovars to serum diluted in series and incubated. The agglutination was examined using dark field microscopy (100 ×). We estimated the titers to be the highest serum dilution that agglutinated at least 50% of leptospires. We considered animals with a titre ≥100 as seroreagent. MAT was performed at the Laboratory of Animal Infectious Diseases of the Universidade Federal de Santa Catarina - UFSC, Curitibanos/SC.
**Epidemiological data**

We obtained the epidemiological data through a questionnaire applied to horse owners including information on the herd and possible risk factors associated with infection by *Leptospira* spp., such as animal breed, sex, rearing place (urban/rural), rearing system (extensive/semi-intensive/intensive), presence of rodents (yes/no), contact with wild animals (yes/no), contact with capybaras (*Hydrochoerus hydrochaeris*) (yes/no), contact with wild boars (yes/no), contact with cattle (yes/no), contact with sheep (yes/no), contact with pigs (yes/no), contact with dogs (yes/no), history of reproductive problems, uveitis or clinical disease diagnosed, or suspected of leptospirosis (yes/no), vaccination against leptospirosis (yes/no).

**Statistical analysis**

We recorded and analyzed the data generated by the interviews (independent variables) and the MAT (response variable) using the SAS statistical package (version 9.3, SAS Institute Inc., Cary, NC). Subsequently, we used descriptive statistics on all independent variables applied to the individual and tracked based on the response variable (positive or negative MAT). To estimate the risk of infection associated with the independent variables (qualitative variables), we used the logistic regression model (HOSMER & LEMESHOW, 1989) and multiple analysis. We presented the values for $p$ and considered them statistically significant when $P<0.05$.

**RESULTS**

The data from this study showed that 80% (21/26) of the studied herds were positive (with at least one seropositive animal) for infection by *Leptospira* spp. Although, previous regional studies by the present working group indicated an estimated prevalence of leptospirosis of 5% (unpublished data), the detailed study of this population revealed that 45.4% (94/207) of the horses were seropositive for *Leptospira* spp. The serogroups most frequently diagnosed MAT were Australis (Bratislava) 16.9% (35/207), Icterohaemorrhagiae (Icterohaemorrhagiae and Copenhageni) 14.4% (30/207), Grippotyphosa (Grippotyphosa) 5.31% (11/207), Sejroe (Hardjobovis and Guaricura) 3.8% (8/207), Ballum (Castellonis) 3.4% (7/207), Autumnalis (Autumnalis) 2.4% (5/207) Canicola (Canicola) 1.4% (3/207), Pomona (Pomona) 0.9% (2/207), and Tarassovi (Tarassovii) 0.5% (1/207), with titrations ranging from 100 (232 - 100%), 200 (45 - 19.40%) to 400 (4 - 1.72%) (Figure 1).

The logistic regression analysis showed an association between seropositivity for *Leptospira* spp. and qualitative variables, such as extensive rearing.
system (OR = 1.27; P<0.05), history of abortions or other clinical forms (OR = 2.56; P<0.01), and the presence of cattle (OR = 3.85; P<0.01) and capybaras (OR = 2.56; P = 0.06) (Table 1). When evaluating specific serovars, there was an association between the presence of capybaras (OR = 14.0; P<0.001) and pigs (OR = 21.76; P<0.001) with seropositivity to Australis (Bratislava). In animals seropositive to Icterohaemorrhagiae (Copenhageni and Icterohaemorrhagiae) there was an association with the history of abortion or other clinical forms (OR = 31.11, P<0.001) and the presence of sheep (OR = 9.28, P<0.001) (Table 1).

DISCUSSION

The data of this study showed 45.4% (94/207) of seropositivity of the horses tested for Leptospira spp., 80% (21/26) of the evaluated herds had at least one seropositive animal (titer ≥100). Considering that the samples were obtained from urban and rural properties, and equestrian leisure activities increasingly involve humans of different age groups (MENY et al., 2019), these data showed that leptospirosis is a neglected zoonosis due to the absence of specific control programs.

Other studies conducted in Brazil have shown substantial differences in the prevalence of serovars according to the geographic region (FINGER et al., 2014; OLIVEIRA FILHO et al., 2014; ALVES et al., 2016). According to FINGER et al. (2014), the most prevalent serogroup in the region of Curitiba (southern Brazil) was Icterohaemorrhagiae. OLIVEIRA FILHO et al. (2014) observed a higher prevalence of the serogroup Panama in the Paraíba region (Northeast Brazil) when they evaluated horses used in the study.

The most frequent serogroups diagnosed in the present study were Australis 16.9% (35/207), Icterohaemorrhagiae 14.4% (30/207), and Grippotyphosa 5.31% (11/207). The diversity of circulating serovars directly impacts vaccination programs since most commercial vaccines do not include all serogroups to which animals are exposed. Commercial vaccines included only the serogroups most frequently diagnosed, coinciding with those with the highest prevalence reported in the present study. However, when comparing the serogroups reported with those present in a complete commercial vaccine, indicated for Equidae, we found that it does not include serogroups Sejroe, Autumnalis, and Castellonis.

We also demonstrated in this study that horses reared extensively (OR = 1.27; p <0.05) and in contact with cattle (OR = 3.85; p <0.01) are more likely to have seropositivity for leptospirosis. ELLIS (2015) reported that cattle are considered the primary hosts of serovars Hardjo, Pomona, and Grippotyphosa, representing a reservoir and potential risk factor for infection of other animal species, such as sheep and horses that often cohabit in the same environments (COUSINS et al., 1989; SILVA et al., 2007).

Despite considering leptospirosis as subclinical in horses (HAMOND et al., 2012), the data in this study showed that there is a higher risk of infection (OR = 2.56; p <0.01) in properties with a history of clinical signs of the disease, such as abortion, the birth of weak foals, and repetition of heat, especially when considering the serogroup Icterohaemorrhagiae (OR = 9.28; p <0.001). PINNA et al. (2014) also observed a higher risk of infection for seropositive animals to serogroups Icterohaemorrhagiae and

| Serological standard (Positive serology in MAT) | Qualitative variables | OR | IC 95% - Risk | P values |
|-----------------------------------------------|-----------------------|----|---------------|----------|
| Australis                                     | Extensive rearing system | 1.27 | 0.52 | 3.13 | p<0.05 |
|                                               | Contact with cattle    | 3.85 | 1.69 | 8.77 | p<0.01 |
|                                               | Disease history        | 2.56 | 1.27 | 5.16 | p<0.01 |
|                                               | Contact with capybara  | 2.07 | 0.96 | 4.45 | p=0.06 |
|                                               | Contact with capybara  | 14.00 | 3.39 | 57.86 | p<0.001 |
|                                               | Contact with pigs      | 21.76 | 7.29 | 64.94 | p<0.001 |
| Icterohaemorrhagiae                           | Disease history        | 31.11 | 6.37 | 151.90 | p<0.001 |
|                                               | Contact with sheep     | 9.28 | 3.13 | 27.48 | p<0.001 |
Australis in a study conducted in horses with a history of reproductive problems.

PETRAKOVSKY et al. (2014) reported that a wider variety of serogroups circulating in a region is associated with a more diverse fauna when compared to an area with few animal hosts. In this study, we found animals reagent to a wide variety of serogroups such as Australis, Icterohaemorrhagiae, Grippotyphosa, Sejroe, Ballum, Autumnalis, Canicola, Pomona, and Tarassovi, suggesting that there are several species potentially reservoirs to Leptospira spp. in the region.

The results reported in this study showed a higher risk of the disease in horses in contact with capybaras (OR = 2.07; p = 0.06). Despite the many species of wild animals acting as reservoirs for leptospires, their role as a source of infection for animals and humans is still unclear (VIEIRA et al., 2018). The capybaras in the Serra Catarinense region have intense proliferation due to low predation, found even in an urban area. In Brazil, capybaras are also studied as reservoirs to leptospires, having already been identified as seroreagent to different serovars, such as Icterohaemorrhagiae, Copenhageni, Pomona, Castellonis, Grippotyphosa, Hardjo, Canicola, and Bratislava (LANGONI et al., 2016).

Results of this study showed that contact with pigs (OR = 21.76; p < 0.001) and sheep (OR = 9.28; p < 0.001) increased the chance of seroreagent horses by representatives of the Australis and Icterohaemorrhagiae serogroup, respectively. According to PINTO et al. (2016), the Bratislava serovar (Australis serogroup) is considered adapted and maintained by horses and pigs, allowing the determination of reproductive alterations, or present as subclinical. Sheep, in turn, can act as maintenance or accidental hosts, depending on the region and rearing conditions, mainly for serovars Hardjo, Autumnalis, Icterohaemorrhagiae, and Pomona; and consequently, representing a source of infection to cattle and horses (MELO et al., 2010).

We did not include the presence of rodents (rats, mice) and dogs as risk factors in the analysis because they are present in all properties. In the case of dogs, PINTO et al. (2016) identified that the most frequent serovars are Canicola and Icterohaemorrhagiae; and therefore, may represent a possible agent reservoir for horses and humans both in urban and rural areas. Rodents should always be considered in the transmission chain since they represent an important reservoir for different Leptospira spp. in nature, especially for serogroups Icterohaemorrhagiae and Ballum (FAISAL et al., 2012; HOUWERS et al., 2011).

CONCLUSION

The findings presented in this study showed a high frequency of horses reagent to Leptospira spp. in the Serra Catarinense region, revealing the need for emergency vigilance measures to control this important zoonosis in horse herds. Furthermore, the presence of different animal species occupying the same spaces suggests the environment as a determining factor in the infection epizootiology and variability of circulating serovars. The research also highlighted the need for new studies focusing on updating the vaccinal strains commercially used, given that most do not contemplate all serovars circulating in all regions.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This research follows the norms issued by the National Council on Animal Experimentation Control (CONCEA) and was previously approved by the Ethics Commission on Animal Use by the Universidade do Estado de Santa Catarina (CEUA/ UDESC), with CEUA protocol nº 4299250816.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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