Ciência Rural, Santa Maria, v.50:7, e20190483, 2020
http://doi.org/10.1590/0103-8478cr20190483
ISSNe 1678-4596
MICROBIOLOGY

Risk factors related to seropositivity for *Leptospira* spp. in horses in the Serra Catarinense region

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ABSTRACT: *Leptospirosis is a zoonotic infectious disease caused by the bacterium Leptospira spp. The consequences of infection in horses are unclear, and the serogroups involved vary depending on the region. This study aimed to evaluate the seropositivity of unvaccinated horses in the Serra Catarinense region and the possible risk factors associated with the infection. We used 207 horses from 26 properties, with no clinical history of infection and unvaccinated for leptospirosis. We submitted serum samples to the microscopic agglutination serum (SAM) to obtain the main reference serogroups. We considered animals with titre ≥100 as infected. The results of the serology revealed seropositivity in 45.4% (94/207) of the animals studied. The most frequent antibodies reportedwere those against the serogroups Australis 16.9% (35/207), Ichterhaemorrhagiae 14.4% (30/207), and Grippotyphosa 5.31% (11/207). Among the primary risk factors associated with the infection we reported extensive rearing system (OR = 1.27; P<0.05) and the presence of other animal species such as cattle (OR = 3.85; P<0.01) and capybaras (OR = 2.07; P=0.06). The findings presented in this study showed that leptospirosis is endemic in horses in the Serra Catarinense region, and revealed the need for emergency surveillance and control measures specific to this important zoonosis.*
Key words: *diagnosis, animal health, infectious diseases.*

Fatores de risco relacionados a soropositividade para *Leptospira* spp. em equinos na região da Serra Catarinense

RESUMO: *A leptospirose é uma doença infecciosa zoonótica, causada pela bactéria Leptospira spp. Em equinos, as consequências da infecção não são claras e os sorogrupos envolvidos variam dependendo da região. Sendo assim, este trabalho objetivou avaliar a soropositividade de Leptospira spp. em equinos não vacinados na região serrana Catarinense e estimar os possíveis fatores de risco associados à infecção. Para tal, foram estudados 207 equinos, de 26 propriedades, sem histórico clínico da infecção e não vacinados para leptospirose. As amostras de soro foram submetidas ao ensaio de soro aglutinação microscópica (SAM) para os principais sorogrupos de referência. Animais com título ≥100 foram considerados reagentes. Os resultados da sorologia revelaram uma soropositividade de 45,4% (94/207) para os animais estudados. Com maior frequência foram observados anticorpos contra os sorogrupos Australis 16,9% (35/207), Icterohaemorrhagiae 14,4% (30/207), e Grippotyphosa 5,31% (11/207). Dentre os principais fatores de risco associados à infecção observados, foram o sistema de criação extensivo (OR=1,27; P<0,05) e a presença de outras espécies animais como bovinos (OR=3,85; P<0,01) e capivaras (OR=2,07; P=0,06). Os achados apresentados neste estudo mostraram que a leptospirose em equinos é endêmica na região da serra Catarinense, e revelam a necessidade de medidas emergenciais de vigilância e controle específicas para esta importante zoonose.*
Palavras-chave: *diagnóstico, sanidade animal,doenças infecciosas.*

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* sp., such as *Icterohaemorrhagiae*, *Canicola*, *Pomona,* and

Received 06.27.19 Approved 04.01.20 Returned by the author 04.29.20
CR-2019-0483.R1

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-*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-*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), Lousiana (Lousiana), Pomona (Pomona), Sejroe (Guaricura, Hardjobovis; Wolffi), Pyrogenes (Pyrogenes), 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 (Tarassovi) 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

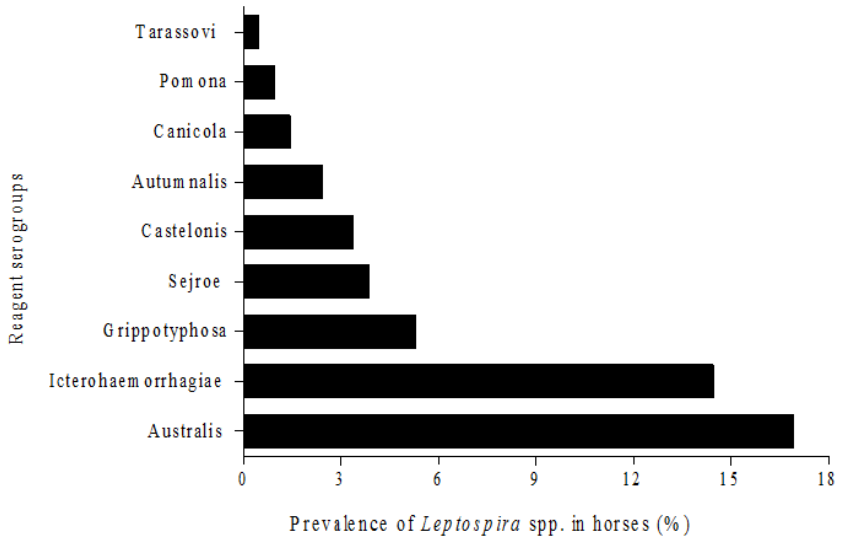

Figure 1 - Prevalence of Leptospira spp. in horses of the Serra Catarinense region in the State of Santa Catarina, Brazil. Samples of horse serum (n=207) collected from November of 2017 to March of 2018 and tested for 14 Leptospira spp. serogroups.

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

Table 1 - Risk factors associated with infection by *Leptospira* spp. in horses of the Serra Catarinense region I the state of Santa Catarina, Brazil. Samples of horse serum (n=207) collected from November of 2017 to March of 2018. The *odds ratio* (OR) and interval of confidence (IC) values are presented for horses with positive serology for *Leptospira* spp. and infection by the Australis or Icterohaemorrhagiae serogroups.

Serological standard	Qualitative variables	OR	IC 95% - Risk		
			Minimum	Maximum	P values
Leptospira spp. (Positive serology in MAT)	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
Australis	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, lcterohaemorrhagiae, 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 lcterohaemorrhagiae; 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 *lcterohaemorrhagiae* 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.

ACKNOWLEDGMENTS

This study was supported by the Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC), infrastructure support project for Universidade do Estado de Santa Catarina (UDESC) research groups, grants number 2017TR640 and for granting a scholarship for Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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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