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Prevalence of anti–*Leptospira* spp. antibodies among donkeys in the semi-arid northeastern region of Brazil

Prevalência de anticorpos anti-*Leptospira* spp. em jumentos no semiárido região nordeste do Brasil

Prevalencia de anticuerpos anti-*Leptospira* spp. en burros en la región del semiárido de Brasil

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Abstract
Leptospirosis is a zoonotic disease that causes financial and significant problems for herds. The objective was to define a prevalence of Leptospira spp. in serovar samples from 145 stray donkeys from the Brazilian semiarid region using the microscopic agglutination test (MAT), 24 serovars of Leptospira spp. Of the reactive animals, 34 of 145 (23%) had titers between 100 and 3,200. The serovars identified in the sera with high frequency were Kennewicki and Bratislava. In these animals serovars, Pyrogenes, Tarassovi, Canicola, Guaricura, Icterohaemorrhagiae, Grippotyphosa and Copenhageni were also used, showing animals with a lower rate of positive reactivity. This result indicates that the animals studied may be in contact with serovars Kennewicki and Bratislava, since wild animals, rodents and swine may harbor these serovars. Thus, the lack of adoption of protected sanitary measures may favor the occurrence of infection in the sampled animals, prevailing in the herd studied.
Keywords: Donkeys; Equids; Leptospirosis; Serology, Diseases infection.

Resumo
A leptospirose é uma zoonose que causa problemas financeiros e significativos para os rebanhos. O objetivo foi definir uma prevalência de *Leptospira* spp. em amostras de sorovares de 145 jumentos errantes do semiárido brasileiro por meio do teste de aglutinação microscópica (MAT), 24 sorovares de *Leptospira* spp. Dos animais reativos, 34 dos 145 (23%) apresentaram títulos entre 100 e 3.200. Os sorovares identificados nos soros com alta frequência foram Kennewicki e Bratislava. Nestes animais também foram utilizados os sorovares Pyrogenes, Tarassovi, Canicola, Guaricura, Icterohaemorrhagiae, Grippotyphosa e Copenhageni, apresentando animais com menor índice de reatividade positiva. Este resultado indica que os animais estudados podem estar em contato com os sorovares

Kennewicki e Bratislava, uma vez que animais silvestres, roedores e suínos podem abrigar esses sorovares. Assim, a falta de adoção de medidas sanitárias protegidas pode favorecer a ocorrência de infecção nos animais amostrados, prevalecendo no rebanho estudado.
Palavras-chave: Jumentos; Equídeos; Leptospirose; Sorologia; Doenças infecciosas.

Resumen
La leptospirosis es una zoonosis que causa importantes problemas económicos al ganado. El objetivo fue definir una prevalencia de *Leptospira* spp. en muestras de serovares de 145 burros extraviados de la región semiárida brasileña mediante la prueba de aglutinación microscópica (MAT), 24 serovares de *Leptospira* spp. De los animales reactivos, 34 de 145 (23%) tenían títulos entre 100 y 3200. Los serovares identificados en los sueros con alta frecuencia fueron Kennewicki y Bratislava. En estos animales también se utilizaron los serovares Pyrogenes, Tarassovi, Canicola, Guaricura, Icterohaemorrhagiae, Grippotyphosa y Copenhageni, mostrando animales con menor tasa de reactividad positiva. Este resultado indica que los animales estudiados pueden estar en contacto con los serovares Kennewicki y Bratislava, ya que los animales salvajes, los roedores y los cerdos pueden albergar estos serovares. Así, la falta de adopción de medidas sanitarias protegidas puede favorecer la ocurrencia de infección en los animales muestreados, prevaleciendo en el rebaño estudiado.
Palabras clave: Burros; Equinos; Leptospirosis; Serología; Enfermedades infecciosas.

1. Introduction

Spirochetes of the genus *Leptospira* infect a wide variety of domestic and wild animal species, as well as humans, who are considered incidental hosts (Musso & La Scola, 2013; Pinto et al., 2016; Chadsuthi et al., 2017; Vieira et al., 2017). *Leptospira* spp. are grouped into 23 species, 30 serogroups, and >300 serovars according to their phenotypic characteristics and gene expressions. They have been classified as group I (pathogenic), group II (moderately pathogenic), and saprophytic *Leptospira* spp. (World Customs, 2007; Fouts et al., 2016).

Leptospirosis, a cosmopolitan zoonotic disease that leads to significant economic losses in herds, is caused by a wide variety of *Leptospira* spp. The infection is widely disseminated in Latin America, and the uncertainty of control and prevention programs is directly reflected in high incidence and mortality rates (Petrakovsky et al., 2014).

Infection by *Leptospira* spp. affects many animals, including donkeys, as already described and identified in studies with the serovars Icterohaemorrhagiae, Ballum, Pomona, Hardjo and Canicola (Ali & Safarmashaei 2012; Hajikalaei et al, 2005; Esquivel et al., 2018). The severity of the disease is related to the serovars involved in the infection, which can present the disease in a subclinical way, whereas in endemic areas many species can play a role of reservoir (Fouts et al., 2016).

Wandering animals such as donkeys are a reality in northeastern Brazil, the fact that they feed on garbage and food scraps increases the probability of the risk of coming into contact with the urine of rodents, which are the main reservoirs of leptospires (Bory et al., 2019). Studies with infectious agents in donkeys are scarce. Thus, the objective was to define the prevalence of *Leptospira* spp. in serum samples from 145 stray donkeys raised in the semi-arid region of northeastern Brazil.

2. Methodology

We collected blood samples from 145 donkeys, between 2015 and 2016, in the municipality of Apodi (5.6625°S and 37.7988°W), located in the state of Rio Grande do Norte, a semi-arid region of northeastern Brazil. The donkeys were kept on a farm that rescued homeless donkeys found wandering in the region. A basic physical examination was performed. Blood samples were collected by jugular venipuncture into sterile vacuum test tubes and centrifuged at 3,000 × *g* for 10 min. Sera were removed and stored at –20°C in 1.5-mL microtubes for subsequent serologic analysis. Sampling was performed in compliance with the Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and approved by the Bioethics Committee of Escola de Medicina Veterinária at Universidade Federal da Bahia (Protocol 16/2013).

Serologic analysis was performed using the microscopic agglutination test (MAT) (Petrakovsky et al., 2014). Starting from a dilution of 1:100, sera were diluted in phosphate-buffered saline (PBS), and geometric dilutions of up to 3,200 were

prepared with sera that showed >50% agglutination. We used 24 live strains, including serovars Hebdomadis, Panama, Guaricura, Hardjo-prajitno, Bratislava, Autumnalis, Castellonis, Bataviae, Australis, Canicola, Cynopteri, Djasiman, Grippotyphosa, Copenhageni, Icterohaemorrhagiae, Sejroe, Javanica, Ballum, Kennewicki, Pomona, Pyrogenes, Hardjo-bovis, Wolffi, and Tarassovi. The strains were from the collection of Laboratório de Doenças Transmissíveis at Universidade Federal de Campina Grande (Paraíba, Brazil) and were originally supplied by Fiocruz Bahia (Salvador, Bahia, Brazil; Copenhageni), Fiocruz Rio de Janeiro (Rio de Janeiro, Brazil; Grippotyphosa, Icterohaemorrhagiae, Ballum, Wolffi), and The Institut Pasteur (Paris, France; Hebdomadis, Bataviae, Australis, Canicola, Cynopteri, Djasiman, Sejroe, Javanica, Kennewicki, Pomona, Pyrogenes, Hardjo-bovis, Tarassovi). Previously known positive and negative serum of each serovar were employed as controls in all reactions.

3. Results

Thirty-four of the 145 tested donkeys (23%) had anti–*Leptospira* spp. antibodies. Serogroups Pomona (27 of 34; 79%), Icterohaemorrhagiae (2 of 34; 6%), Australis (2 of 34; 6%), Tarassovi (1 of 34; 3%), Canicola (1 of 34; 3%), and Sejroe (1 of 34; 3%) were detected in this order of frequency. Among 34 positive donkeys, 20 (59%) had positive reactions to >1 serovar, and high titers were found against various serovars (Table 1).

Table 1 *Leptospira* spp. serovar antibody titers identified by microscopic agglutination test (MAT) in free-roaming donkeys from Brazil's semi-arid region.

Animal	MAT titers to leptospiral serovars								
	Kennewicki	Bratislava	Pyrogenes	Tarassovi	Canicola	Guaricura	Icterohaemorrhagiae	Grippotyphosa	Copenhageni
1	–	–	–	–	–	100	–	–	–
2	800	100	–	–	–	–	–	–	–
3	400	100	–	100	–	–	–	–	–
4	400	–	–	–	–	–	–	–	–
5	400	200	–	–	–	–	–	–	–
6	400	–	–	–	–	–	–	–	–
7	800	200	–	–	–	–	–	–	–
8	200	–	–	–	–	–	–	–	–
9	200	–	–	–	–	–	–	–	–
10	200	–	–	–	–	–	–	–	–
11	800	400	–	–	–	–	–	–	–
12	800	200	–	–	–	–	–	–	–
13	800	–	–	–	–	–	–	–	–
13	–	–	–	200	–	–	–	–	–
14	800	400	–	–	–	–	–	–	–
15	800	200	–	–	–	–	–	400	–
16	400	200	–	–	–	–	–	–	–
17	800	200	–	–	–	–	–	–	–
18	–	–	100	–	–	–	–	–	200
19	800	200	–	–	–	–	–	–	–
20	200	–	–	–	–	–	–	–	–

21	3,200	400	200	–	–	–	–	–	–
22	–	100	–	–	–	–	–	–	–
23	1,600	800	–	–	–	–	–	–	–
24	1,600	400	–	–	–	–	–	–	–
25	–	–	–	–	100	–	–	–	–
26	200	–	100	–	–	–	–	–	–
27	1,600	800	–	–	–	–	–	–	–
28	800	200	–	–	–	–	–	–	–
29	200	–	–	–	–	–	–	–	–
30	400	200	–	–	–	–	–	–	–
31	800	200	–	–	–	–	–	–	–
32	–	100	–	–	–	–	–	–	–
34	–	–	–	–	–	–	100	–	–

Dash (–) = no data. Source: Authors.

Serovar Kennewicki was detected most frequently among the positive sera; 27 of 34 (79%) positive donkeys were reactive to this serovar, followed by serovar Bratislava, to which 20 of 34 (59%) positive donkeys were reactive. Sera were also positive for serovars Pyrogenes (3 of 34), Tarassovi (3 of 34), Canicola (1 of 34), Guaricura (1 of 34), Icterohaemorrhagiae (1 of 34), Grippotyphosa (1 of 34), and Copenhageni (1 of 34). Antibody titers were highest to serovars Kennewicki (3,200) and Bratislava (800).

4. Discussion

Our finding of anti–*Leptospira* spp. antibodies in free-roaming donkeys in the semi-arid region of northeastern Brazil indicated that these animals were exposed to this bacterium. We detected anti–*Leptospira* spp. antibodies in 23% of 145 tested donkeys. Earlier studies have detected anti–*Leptospira* spp. antibodies in donkeys (e.g., Mexico: 78%; Iran: 41%; Morocco: 40%; Egypt: 7.7%). The most prevalent were serovars Icterohaemorrhagiae and Sejroe; Icterohaemorrhagiae and Ballum; Grippotyphosa and Hardjo; Pomona and Grippotyphosa, respectively (Samir et al., 2015; Ali & Safarmashaei, 2012; Benkirane et al., 2016; Esquivel et al., 2018). In São Paulo state in Brazil, a 2019 study found Icterohaemorrhagiae (41%) and Grippotyphosa (31%) in a group of 85 donkeys tested by the MAT (Lara et al., 2019).

Among the 34 animals that tested positive for *Leptospira* spp. antibodies, serovars Kennewicki and Bratislava showed the highest prevalence (i.e., 79% and 59%, respectively), with titers of 100–3,200. Serovar Kennewicki is found frequently in North American horses and is one of the most important causes of uveitis, as well as abortions and reproductive disorders in horses (Yan et al., 2010). Serovar Kennewicki has been isolated primarily in North American cattle, but it appears to be a cosmopolitan and easily adaptable serovar, given that studies in Latin America have isolated different strains in naturally infected humans, cattle, and sheep (Mori et al., 2017; Zarantonelli et al., 2018; Hamond et al., 2019).

Swine are the main reservoir for serovar Bratislava; clinical cases of the infection are characterized by abortion and infertility (Mori et al., 2017). Serovar Bratislava is the serovar reported most frequently in Latin America (Petrakovsky et al., 2014); pigs and donkeys shared the same space and the same source of food on the farm where this study was conducted. The presence of pigs on the farm is a relevant observation given that swine can be a source of infection for donkeys (Petrakovski et al., 2014; Mori et al., 2017).

Various wild animals have been reported as carriers and reservoirs of *Leptospira* spp., including opossums (*Didelphis albiventris* and *Didelphis marsupialis*), armadillos (*Euphractus sexcinctus*), rice rats and South American water rats (*Oryzomys* sp. and *Nectomys squamipes*), and wild boars (*Sus scrofa*) (Vieira et al., 2017). *Leptospira* spp. have been demonstrated in opossums and armadillo, which are frequently found in the semi-arid region of northeastern Brazil, and may be a source of infection for donkeys (Silva et al., 2015).

Previous investigations involving donkeys had not included serovar Kennewicki among the strains, and only one of the earlier studies added serovar Bratislava to the antigen panel. The use of autochthonous strains and a sample of each *Leptospira* spp. serogroup increases the efficacy of serologic detection considerably (Mori et al., 2017). However, the paucity of studies on donkeys in Brazil means that little is known about autochthonous strains and frequent serovars in donkeys, indicating the need for further investigations to fill this gap.

The MAT is the serologic gold standard for detection of anti–*Leptospira* spp. antibodies, and is the test used most frequently in epidemiologic studies; however, cross-reactions are expected between serovars from the same serogroup and must be considered (Chirathawon, 2014). *Leptospira* isolation is a difficult laboratory procedure, therefore high MAT antibody titers are useful to monitor the occurrence of *Leptospira* serovars. It is important to emphasize the high titers, and hence evidence of exposure, to the most frequent serovars observed in our study; serovar Kennewicki titers were 100–3,200; Bratislava titers were 100–800.

5. Conclusion

In this study, a high prevalence of antibodies against serovars Kennewicki and Bratislava was observed, indicating that donkeys may have been exposed to the agent through contact with animals considered reservoirs for these rorovars, such as swine, wild animals, and rodents. The lack of adoption of adequate sanitary measures may favor the occurrence of infection in the sampled animals, as the extensive breeding system prevails in the herd studied. It is considered that further studies with a greater number of samples are necessary to obtain the real risk of this serovar for the species studied.

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