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Occurrence of antibodies against *Leptospira* spp. in donkeys of São Paulo state

Ocorrência de anticorpos contra **Leptospira** *spp. em asininos no estado de São Paulo*

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ABSTRACT: Among the diseases that affect equines, bacterial diseases play an important role from a health and economic point of view, especially leptospirosis and brucellosis. The study aimed to provide information on the occurrence of anti-*Leptospira* spp. and anti-*Brucella abortus* antibodies in donkeys of São Paulo state. We found a frequency of 62.4% (53/85) antibodies against *Leptospira* spp. The donkeys were not seropositive for *Brucella* spp.

KEYWORDS: donkeys; serology; leptospirosis; brucellosis.

RESUMO: Entre as doenças que acometem os equídeos, as enfermidades bacterianas assumem um papel importante do ponto de vista sanitário e econômico, destacando-se a leptospirose e a brucelose. O estudo teve como objetivo fornecer informações sobre a ocorrência de anticorpos anti-*Leptospira* spp. e anti-*Brucella abortus* em jumentos no estado de São Paulo. Estimou-se que 62,4% (53/85) dos animais apresentavam anticorpos anti-*Leptospira* spp. Os jumentos estudados não foram sororreagentes contra a *Brucella* spp.

PALAVRAS-CHAVE: jumentos; sorologia; leptospirose; brucelose.

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Leptospirosis is an infectious disease caused by spirochete bacteria of the genus *Leptospira*, which affects domestic and wild animals, with humans at the end of the chain of transmission. Wild animals, mammals (rodents, herbivores, insectivores, carnivores), birds, reptiles, and amphibians have been reported as carriers or reservoirs of *Leptospira* for humans and domestic animals. They are distributed worldwide, being highly prevalent in tropical and sub-tropical countries, where the temperature and humidity conditions favor their development. Acute leptospirosis outbreaks in equids are poorly documented, even though the disease is known worldwide. Cases of uveitis and relapsing iridocyclitis culminating in blindness have been linked to serological results for serovar Pomona. In Brazil, few reports have described isolations, such as the study by GIORGI et al. (1981), which revealed the first isolation of *Leptospira* spp. from aborted horse fetus, probably due to serovar Icterohaemorrhagiae. Serological surveys have been carried out in populations of donkeys, and the three performed in Brazil showed that the incidence of animals seropositive to *Leptospira* spp. varied from 25.53 to 85% (BEZERRA et al., 2010; OLIVEIRA FILHO et al., 2014; SHIMABUKURO et al., 2001).

In equids, *Brucella abortus* causes brucellosis, which mainly leads to suboccipital or interscapular bursitis, called "bad withers." It can also result in abortion in the last trimester of pregnancy and compromise reproductive and osteoarticular systems or have no symptoms. Equids are considered accidental hosts of the disease and usually contract it from other species such as cattle and pigs. Bad withers caused by brucellosis poses a public health risk, so infected animals should be euthanized. Several countries conducted serological surveys for antibodies to *Brucella spp.* in populations of donkeys, with incidence levels ranging from 0 to 40%. However, most studies found low levels of positivity (less than 10%) (ABO-SHEHADA, 2009; ACOSTA-GONZÁLEZ et al., 2006; DORNELES et al., 2013; GIDEL et al., 1974; SADIQ et al., 2013; TEL et al., 2011).

Donkeys have notable importance in the production of hybrid animals. The male donkey breeds with a horse mare to produce mules and donkeys, which are used as work animals on farms because of their hardiness and stamina. For this reason, these animals should be free from contagious diseases.

Given the importance of donkeys to humans as a source of draft power for different activities, the objective of this study was to provide information on the occurrence of antibodies to bacterial agents affecting donkeys of São Paulo state, Brazil.

Between 2009 and 2010, a total of 85 serum samples were collected from healthy donkeys from 5 properties located in Pirassununga, Barretos, Colina, and Araçatuba, São Paulo state, Brazil. Female donkeys had continuous access to pasture, while stallions were housed in stalls. At the time of collection, no animal had a history of clinical signs of brucellosis and leptospirosis.

For the study of brucellosis, we used the buffered acidified antigen test. In this test, an antigen suspension was diluted to 8% in a buffer solution, pH 3.65. Acidification enhances IgG1 agglutination (which is lower at neutral pH), destroys nonspecific agglutinins, and reduces IgM reactivity, thereby decreasing cross-reactivity with other etiological agents. This test is qualitative and ideal for screening herds.

Leptospirosis was determined by the microscopic seroagglutination technique with live antigens, used to measure agglutinin levels of all serum samples. Live cultures of *Leptospira* spp. 24 serovars were kept in liquid-modified EMJH medium, with 7 − 10 days of growth, free from contamination and autoagglutination, and with an approximate density of 100 to 200 microorganisms per microscopic field, at 400X magnification. The reaction was performed in polystyrene 96-well microtiter plates, using 50 µL of serum diluted 1:50 initially in Sorensen's phosphate-buffered saline (pH 7.4) and adding 50 µL of antigen, yielding an initial dilution of 1:100. To determine the final titer of antileptospiral agglutinins in each serum, 2-fold serial dilutions were prepared in Sorensen's phosphate buffered saline (pH 7.4). The microplates were incubated at 28 − 30°C for three hours, followed by reading with dry dark-field microscopy, at 100X magnification, revealing the formation of clumps. The serum titer was considered the reciprocal of the highest dilution that showed 50% agglutinated *Leptospira*.

We found leptospirosis antibodies against the following serovars: Icterohaemorrhagiae in 35 (41.2%) animals, Pomona in 4 (4.7%), Grippotyphosa in 26 (30.6%), Australis in 2 (2.4%), Autumnalis in 2 (2.4%), Shermani in 4 (4.7%) Canicola in 1 (1.2%), Wolffi in 1 (1.2%), Hardjo in 18 (21.2%), Bratislava in 23 (21.2%), Bataviae in 1 (1.2%), and Copenhageni in 3 (3.5%), with the total of seropositive animals being 62.4% (53/85) .The results suggest that leptospirosis also affects donkeys, as reported in several papers, and should be better studied because of its important zoonosis and for the close relationship between humans and this animal species. The high incidence observed in this study may be explained by the fact that these animals have a low livestock value in the study area. Therefore, donkeys are not kept in stables and have greater access to pastures (flooded and watery areas), close to the excretions of other reservoir species for leptospirosis, for example, rodents and cattle (OLIVEIRA FILHO et al., 2014).

Positivity for the serovars Icterohaemorrhagiae and Grippotyphosa suggest direct or indirect contact with excretions of rodents and other wildlife since they are natural hosts of these serovars (LANGONI et al., 2004; HASHIMOTO et al., 2007; CHIARELI et al., 2008; OLIVEIRA FILHO et al., 2014).

Cattle generally act as reservoirs for the serovar Hardjo (ARAÚJO, 2010). Equids are regarded as accidental hosts for this serovar (CHIARELI et al., 2008). This study considered

that the animals that were positive for this serovar were likely bred in close contact with cattle.

The serovar Bratislava also showed a high incidence in clinically asymptomatic animals. This result corroborates those reported by various authors, suggesting that horses can serve as a reservoir for this serovar (KITSON-PIGGOT; PRESCOTT, 1987; BARWICK et al., 1998; AGUIAR et al., 2008; BÅVERUD et al., 2009).

The absence of animals seropositive for *Brucella* spp. among the donkeys in this study indicates that infection was not present or the prevalence was low in this population. Although the donkeys demonstrated an infection development similar to that observed in horses, it is difficult to assess the true impact of infection by *Brucella* spp. in this species or their importance in the epidemiology of brucellosis (DORNELES et al., 2013). The direct comparison between frequencies of seropositive animals found in serological surveys in Brazil and different countries and the results obtained in this study is useful, mainly due to differences in observational studies, serological tests, diagnostic cut-offs, sample size, and representativeness.

Positive serological results for *Leptospira* spp. suggest that donkeys could be an active or potential reservoir for this disease and they may be transmissible to other domestic livestock. In addition, donkeys might constitute a dormant threat for the direct infection of humans (leptospirosis).

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