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OCCURRENCE OF ANTIBODIES AGAINST *Leptospira* spp. IN HORSES OF THE URBAN AREA OF LONDRINA, PARANÁ, BRAZIL

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SUMMARY

A total of 320 horses were studied in this paper, both male and female, between two and 17 years of age, which were used for traction of wagons in the urban area of the municipality of Londrina (PR). These animals were kept, after their daily work, in abandoned areas or plots, in the outskirts of the urban area of the city. When these animals were attended by the veterinarians, between 1996 and 2005, none of them presented symptoms suggesting leptospirosis. The most frequent reasons for the visit were loss of weight, unwillingness for work, parasitism, laminess, and wounds. Microscopic Seroagglutination Test (SAM), with 22 *Leptospira* serovars, was performed in sera sample from all these animals. The aim of this study was to investigate the occurrence of antibodies against *Leptospira* spp. in horses from the urban area of Londrina (PR). From the samples tested, 214 (66.88%) were considered positive, with titers between 100 and 3200, being that 49 (22.90%) presented antibodies against a single serovar of *Leptospira*, and 165 (77.10%) samples presented antibodies against two or more serovars simultaneously, where in 88 (53.33%) it was possible to characterize the most likely probable serovar. Antibodies against the serovar Icterohaemorrhagiae were detected in 32 (23.36%) animals.

KEYWORDS: Leptospirosis, Horses; Serological diagnosis; Antibodies.

INTRODUCTION

Leptospirosis is one of the most widespread zoonoses in the world. In Brazil it is considered an endemic disease, posing serious risks to public health (FIGUEIREDO *et al*., 2001). Despite being known as a serious disease, in horses the majority of infections by leptospira seem to be asymptomatic (HATHAWAY *et al*., 1981; ELLIS *et al*., 1984).

Animals living in the outskirts of urban areas, where sanitary and infrastructure conditions are poor, next to dumps, open-sky sewage systems, and in contact with other animal species, are considered at risk for leptospirosis (GENOVEZ, 1996). Among the various species considered as maintenance host animals of *Leptospira* spp. (BARWICK *et al.* 1997), horses can be considered important reservoirs for the transmission of this microorganism to other animals and even to men (ROMERO *et al*. 1993).

Leptospirosis in horses can be caused by different *Leptospira* serovars. However, in Brazil there have been prevalences of antibodies against the serovar Icterohaemorrhagiae (LILENBAUN, 1998; LANGONI *et al*., 2004). Rodents are considered hosts for serovar Icterohaemorrhagiae, and are seen as a source of infection not only for other animals but also for human beings (SANTA ROSA *et al*., 1980; FAINE, 1982).

The aim of this paper was to investigate the occurrence of antibodies against *Leptospira* spp. in horses used for pulling wagons in the urban area of Londrina (PR).

MATERIALS AND METHODS

A total of 320 animals, males and females, aged between two and 17 years of age, used for traction of wagons in the urban area of Londrina (PR), which were assisted in an extension program named "Projeto Carroceiro", between 1996 and 2005 in the Veterinary Hospital (HV) of Universidade Estadual de Londrina (UEL), were studied. None of the animals assisted in the Project presented symptoms suggesting leptospirosis, and the most frequent reasons for the assistance were weight loss, unwillingness for work, parasitism, laminess, and wounds. These animals were kept, after their daily work, in derelict areas or plots, situated in the outskirts of the urban area of the city.

A blood sample was taken from each animal when it was being seen as part of the project. After the extraction of the sera, each sample was kept in sterile container and stored at -20 °C until its usage in the Microscopical Seroagglutination Test (SAM), in the Leptospirosis Laboratory of Preventive Veterinary Medicine Department at UEL.

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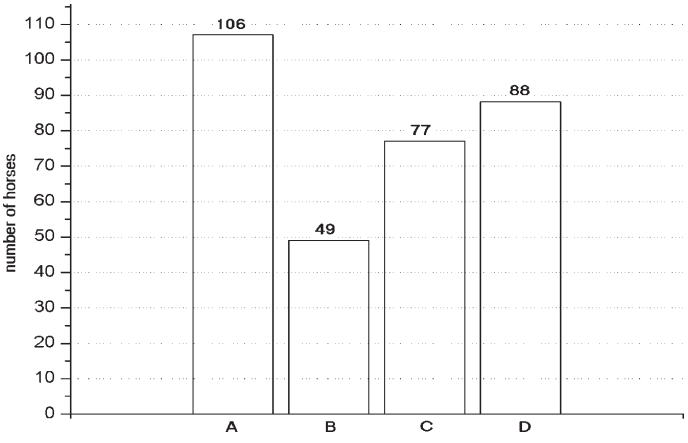
In order to detect antibodies against *Leptospira* spp., all sera samples were tested against 22 reference serovars: Australis, Bratislava, Autumnalis, Butembo, Fortbragg, Castellonis, Bataviae, Canicola, Whitcombi, Cinoptery, Grippothyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi and Sentot and were stored at 28° C for five to 10 days in media EMJH (DIFCO®-USA), modified by the addition of rabbit sera. The samples presenting agglutination of 2+ or more in the dilution 1:100 were considered positive, being those considered positive further diluted in a series manner and examined until the determination of maximum positive dilution (MYERS, 1985).

The analysis of the results considered as the more likely the serovar presenting the highest titer. The animals which presented reaction to titers equal to two or more serovars were excluded from this analysis, and considered positive for *Leptospira* spp. (FÁVERO *et al.*, 2002).

RESULTS

From the 320 horses studied, 214 (66.88%) were considered positive, presenting titers between 100 and 3200 for one or more serovars. Among the animals which presented seroreaction, 49 presented antibodies against only one serovar, and 165 presented antibodies against two or more serovars, where in 88 it was possible to characterize the most likely serovar (Fig. 1).

Serovar Icterohaemorrhagiae was the most likely serovar in 32 animals, followed by Grippothyphosa in 18, Hebdomadis in 15, Castellonis in 14, Pyrogenes in 11, Fortbragg in 10, Pomona in nine, Sentot in seven, Canicola in six, Butembo in four, Bratislava in three, Hardjo in three, Shermani in two and Australis, Autumnalis and Bataviae in one each (Table 1).



A. Negative Results; B. Positive for only one serovar; C. Positive for more than one serovar (most likely serovar not identified); D. Positive for more than one serovar (most likely serovar identified).

Fig. 1 - Microscopical Seroagglutination Test (SAM), performed with 22 *Leptospira interrogans* serovars, in sera samples of 320 horses used for traction of wagons in the urban area of Londrina (PR) between years 1996 and 2005.

Titers obtained against serovar Icterohaemorrhagiae ranged from 100 to 800, Grippothyphosa from 100 to 3200, Hebdomadis from 100 to 3200, Castellonis from 100 to 1600, Pyrogenes from 100 to 1600, Fortbragg from 200 to 3200, Pomona from 200 to 1600, Sentot from 100 to 800, Canicola from 100 to 800, Butembo from 100 to 200, Bratislava 100 to 800, Hardjo from 800 to 3200, Shermani from 200 to 400, Australis with 100, Autumnalis with 100 and Bataviae with 400.

Table 1
Most likely serovars and titers detected in the Microscopical Seroagglutination Test (SAM), performed with 22 *Leptospira interrogans* serovars in 137 seroreagent horses used for traction of wagons in the urban area of Londrina (PR) between the years 1996 and 2005

	Serological titers							
Serovars	100	200	400	800	1600	3200	Total	(%)
Icterohaemorrhagiae	12	12	07	01	-	-	32	23.36%
Grippothyphosa	03	02	03	07	01	02	18	13.14%
Hebdomadis	03	02	03	02	03	02	15	10.95%
Castellonis	02	06	03	02	01	-	14	10.22%
Pyrogenes	03	04	02	01	01	-	11	8.03%
Fortbragg	-	01	05	02	01	01	10	7.30%
Pomona	-	03	02	03	01	-	09	6.57%
Sentot	01	02	03	01	-	-	07	5.11%
Canicola	01	01	03	01	-	-	06	4.37%
Butembo	03	01	-	-	-	-	04	2.92%
Bratislava	01	01	-	01	-	-	03	2.19%
Hardjo	-	-	-	01	-	02	03	2.19%
Shermani	-	01	01	-	-	-	02	1.46%
Australis	-	-	01	-	-	-	01	0.73%
Autumnalis	-	01	-	-	-	-	01	0.73%
Bataviae	-	-	01	-	-	-	01	0.73%
Total	29	37	34	22	08	07	137	100.00%

DISCUSSION

The results from the SAM in the seroreagent horses of this paper suggest the existence of different sources of infection in the areas used for keeping these animals after their daily working period. These places, characterized by abandoned plots or areas in the outskirts of the urban region, presented basic conditions for the breeding of rodents, and other horses, cattle, canines, and even marsupials were also found in these areas. According to FAINE (1982), *Leptospira* spp. infection is directly related to the handling characteristics and environmental factors which expose the animals to risk factors of infection and for ROBERTS (1969), the main source of transmission of horses leptospirosis is the direct contact with urine contaminated with *Leptospira* spp. In this paper, the percentage of seroreagent horses was of 66.88. LANGONI *et al.* (2004) examining sera samples of horses in the states of São Paulo, Goiás and Mato Grosso do Sul found antibodies against leptospira in 54.00% while LINHARES *et al.* (2005) found 45.05% in Goiás. These results indicate that leptospirosis in horses occurs with considerable frequency, varying according to the region studied and the possible sources of infection.

In 32 (23.36%) of the positive samples, antibodies against serovar Icterohaemorrhagiae were detected. The prevalence of serovar Icterohaemorrhagiae found in this paper suggests the participation of rodents in the transmission of the disease, since infected mice and rats are reservoirs for this serovar and also possible sources of infection (FAINE *et al.*, 1999). The unorderly urban growth and the existence of garbage scattered in derelict plots provide an ideal environment for the proliferation of the murine population, being an infection source of *Leptospira* spp. for other animal species and also for humans (CÔRTES, 1993).

Antibodies against serovar Grippotyphosa were found in 18 (13.14%) animals. This serovar has been frequently found in wild animals, suggesting their participation in the transmission of the disease (SANTA ROSA *et al*., 1975; LINS & LOPES, 1984). FÁVERO *et al.* (2002) despite finding greater prevalence of antibodies against serovar Grippotyphosa in sera from horses in the Mato Grosso do Sul State, have considered this serovar as having a low frequency in horses and suggested further investigation in order to identify the origin of the sources.

The percentage of seroreagent horses for serovars Hebdomadis (10.95%) and Castellonis (10.22%) found in this paper suggests the participation of bovines in the transmission of *Leptospira* spp. for the animals studied. In Brazil, serological data in cattle have detected antibodies against serovars Hebdomadis and Castellonis in low frequency (RODRIGUES *et al*. 1999; LANGONI *et al.* 2000). The owners of the animals in this study, who possessed their animals for a longer time, informed the researchers of the possibility of their horses living together with cattle in the derelict plots. However, a more detailed analysis of this situation was hampered due to the fact that some owners whose animals had been recently acquired had no detailed knowledge of previous living arrangement of their animals. In this paper, the presence of seroreagent horses for the other serovars (42.33%) indicates a possible contact with these *Leptospira* serovars, hosted by different species, in areas contaminated with urine. MYERS (1976) considered horses susceptible to several *Leptospira* serovars and for BOLIN (1996), the prevalence of serovars vary according to the region and the presence of maintenance hosts.

The results of this paper suggested that environmental factors such as poor sanitary conditions and the gathering of litter in the abandoned areas or plots used for the permanence of horses during the night have relationship with the high frequency of antibodies against *Leptospira* serovars detected in those animals. It must be considered, still, that 108 (50.47%) of the total seroreagent horses presented titers considered high (≥ 400) without presenting any symptoms. Sanitary measures applied to the environment and the spreading of information about leptospirosis are of utmost importance for the prophylaxis of the disease in the horses used for traction of wagons in the urban area of the city of Londrina (PR). The possibility of assymptomatic seroreagent animals eliminating *Leptospira* spp. through urine and the proximity of these animals to man indicate the need of further studies to determine the importance of these horses in the transmition of leptospirosis to man.

RESUMO

Ocorrência de anticorpos contra *Leptospira* spp. em eqüinos de área urbana de Londrina, Paraná, Brasil

Foram estudados 320 eqüinos, machos e fêmeas com idade entre dois a 17 anos, utilizados para tração de carroças na área urbana do município de Londrina (PR). Estes animais eram mantidos, após a jornada diária de serviço, em áreas ou terrenos baldios, localizados na periferia da área urbana do município. Por ocasião do atendimento, realizado entre 1996 e 2005, no Hospital Veterinário da Universidade Estadual de Londrina, nenhum dos animais apresentou sintomas sugestivos de leptospirose, sendo os motivos mais freqüentes, o emagrecimento, a indisposição para o trabalho, a verminose, problemas no sistema locomotor e feridas. A prova de soroaglutinação microscópica (SAM), com 22 sorovares de *Leptospira*, foi realizada em amostra de soro de todos os animais. O objetivo deste trabalho foi investigar a ocorrência de anticorpos contra *Leptospira* spp. em eqüinos da área urbana do município de Londrina. Foram consideradas positivas 214 (66,88%) amostras de soros com títulos entre 100 e 3200, sendo em 49 (22,90%) detectadas anticorpos contra um único sorovar de *Leptospira* e em 165 (77,10%) amostras com anticorpos contra dois ou mais sorovares simultâneamente, sendo em 88 (53,33%) destes caracterizado o sorovar mais provável. Anticorpos contra o sorovar Icterohaemorrhagiae foram detectados em 32 (23,36%) animais.

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