ANTI-*Leptospira* spp. ANTIBODIES IN SEVERAL ANIMAL SPECIES ON THE SAME FARM

*ANTICORPOS ANTI-*Leptospira spp. *EM DIVERSAS ESPÉCIES ANIMAIS EM UMA PROPRIEDADE RURAL*

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ABSTRACT: Leptospirosis is a zoonotic disease that occurs worldwide, affecting pets and livestock that can be a source of infection for humans, considered therefore an important disease in public health. Thus, an investigation was made into the occurrence of anti-*Leptospira* agglutinins in several animal species on a farm located in the municipality of Uberlândia, state of Minas Gerais, Brazil. A total of 172 blood serum samples were collected from five species: cattle, dogs, goats, horses and sheep. The diagnosis was performed by means of the microscopic agglutination test (MAT), using a collection of 12 live antigens. Of the 172 animals evaluated, 62 (36%) were seroreactive, and the most frequent serovars in each species were: Hardjo (54.54%), Icterohaemorrhagiae (27.27%), and Autumnalis (18.18%) in cattle; Icterohaemorrhagiae (37.5%), Autumnalis (31%), Canicola (18.7%) and Hardjo (12.5%) in dogs; Grippotyphosa (75%) and Hardjo (25%) in goats; Icterohaemorrhagiae (50%), Grippotyphosa (37.5%) and Hardjo (12.5%) in horses, and Hardjo (34.78%); Grippotyphosa (26.08%); Bratislava (17.39%), Autumnalis (17.39%) and Icterohaemorrhagiae (4.4%) in sheep. It is believed that the occurrence of anti-*Leptospira* agglutinins can be traced back to a source of infection that is common to these species, or that the agent was probably transmitted between them because they live in close proximity to each other.

KEYWORDS: Serovars. Leptospirosis. Microscopic Agglutination Test.

INTRODUCTION

Leptospirosis, identified as one of the emerging infectious zoonosis of worldwide distribution, is caused by bacteria of the genus *Leptospira*, currently comprising 19 genospecies and more than 300 serological variants (ADLER; DE LA PEÑA MOCTEZUMA, 2010), which can affect domestic and wild animals as well as humans. The source of infection in humans is usually either direct or indirect contact with the urine of an infected animal, characterizing it as a high morbidity disease due to the easy dispersal of the agent in the environment, and is therefore a relevant disease in public health (FAINE et al., 1999; LEVETT, 2001).

The *Leptospira* spp. serovars that are present in a given region are associated with the presence of one or more maintenance host, which serve as natural reservoirs of the infection (BOLIN, 1996; LEVETT, 2001). The maintenance hosts are often wild species, and sometimes rodents, domestic animals and livestock. Contact with these hosts or with areas contaminated with their urine can cause infection in other species (HASHIMOTO et al., 2010).

Much of the resurgent international interest in leptospirosis stems from several human cases which have occurred in tropical regions, therefore, it is important to develop studies epidemiological studies to identify the main serovars involved, as well as the establishment of the related species in the transmission of this pathogen (LEVETT, 2001). In Brazil, the relationships between *Leptospira* spp. serovars and the preferred hosts vary according to region; however, in general, the predominant records are: Icterohaemorrhagiae and Copenhageni in synanthropic rodents; Grippotyphosa in marsupials; Hardjo and Wolffi in bovines; Pomona and Icterohaemorrhagiae in swine, Icterohaemorrhagiae in horses, sheep and goats; and Canicola, Icterohaemorrhagiae and Copenhageni in dogs (VASCONCELLOS, 1997; FAVERO et al., 2001; FAVERO et al., 2002; HASHIMOTO et al., 2010).

Due to the importance of leptospirosis in animals, not only because of the severity of disease but also its economic impacts and public health relevance, this study aimed to investigate the occurrence of anti-*Leptospira* agglutinins in various animals species on a farm located in the municipality of Uberlândia, MG, Brazil.

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CONTENT

The study was conducted in a farm in the municipality of Uberlândia, state of Minas Gerais (MG), Brazil, whose owner called in a veterinarian due to the occurrence of reproductive disorders in some species on the farm. The anamnestic history revealed that there was no control of rodents or any vaccination program against leptospirosis on the farm, and that several spontaneous abortions among cattle and sheep had been recorded.

This study was approved by the Ethics Committee on Use of Animals (CEUA) of the Federal University of Uberlândia – UFU, under protocol no. 015/08.

During the period of January 2008 to March 2009, 172 blood serum samples were collected from five species of domestic animals on the same farm. The property was selected for this study due the history of reproductive changes in sheep, it had a commercial kennel of Perdigueiro breed and several animal species living in close. The samples were taken from 16 cows, 27 dogs, 19 goats, 12 horses and 98 sheep.

Five (5) mL of blood were collected aseptically from each animal into sterile tubes without anticoagulant. After clot retraction, the samples were centrifuged at 3,000 x g, and the serum separated into aliquots and stored in plastic Eppendorf tubes at -20º C until the serological examination.

The diagnosis was established by means of the microscopic agglutination test (MAT), using a collection of 12 live antigens that included the serovars: Autumnalis, Australis, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Tarassovi and Wolffi, cultivated in leptospiral EMJH culture medium plus 10% sterile rabbit serum at the Laboratory of Infectious Diseases of the Faculty of Veterinary Medicine – UFU. The blood serum samples that agglutinated at least 50% of the leptospira at a dilution of 1:100 were considered seroreactive. The seroreactive samples were then titrated up to 1:6400, diluted in a geometrical ratio of two. The highest dilution that showed at least 50% of agglutinated leptospira in the microscopic field was considered the final titer. The data were evaluated using descriptive analysis with simple percentage calculation (THRUSHFIELD, 2004).

Of the 172 animals evaluated, 62 (36%) were seroreactive to one or more *Leptospira* spp. serovars, showing titers that varied from 100 to 3200. The serovars with the highest occurrence on the farm were Hardjo (29%), Icterohaemorrhagiae (22.6%), Grippotyphosa (19.4%) and Autumnalis (17.7%). None of the animals were seroreactive to the serovars Bataviae and Tarassovi. Table 1 lists the MAT results according to the species, number of seroreactive animals, and most frequent serovars.

Table 1. Frequency of seroreactive animals and serovars found per species on the same farm in Uberlândia, MG, Brazil in 2009.

SPECIES	Frequency of seroreactive animals for *Leptospira* spp.		SEROVARS
	N° POS./n	%	
Bovine	11/16	68.75	Autumnalis; Bratislava; Hardjo; Hebdomadis; Icterohaemorrhagiae; Wolffi
Canine	16/27	59.25	Autumnalis; Bratislava; Canicola; Grippothyphosa; Hardjo; Icterohaemorrhagiae; Pomona; Wolffi
Caprine	4/19	21.05	Autumnalis; Grippothyphosa; Hardjo; Wolffi
Equine	8/12	66.66	Autumnalis; Bratislava; Hardjo; Icterohaemorrhagiae; Pomona; Wolffi
Sheep	23/98	23.46	Australis; Bratislava; Grippothyphosa; Hardjo; Icterohaemorrhagiae; Wolffi

Among the 62 seroreactive animals, 41 (66%) presented antibodies to more than one *L. interrogans* serovar. In these cases of coagglutination, the serovar with the highest titer was considered reactive. Therefore, the most frequent serovars in the various animal species were: Hardjo (54.54%), Icterohaemorrhagiae (27.27%), and Autumnalis (18.18%) in cattle; Icterohaemorrhagiae (37.5%), Autumnalis (31%) Canicola (18.7%), and Hardjo (12.5%) in dogs; Grippotyphosa (75%) and Hardjo (25%) in goats; Icterohaemorrhagiae (50%), Grippotyphosa (37.5%) and Hardjo (12.5%) in horses; and Hardjo (34.78%), Grippotyphosa (26.08%); Bratislava (17.39%), Autumnalis (17.39%), and Icterohaemorrhagiae (4.4%) in sheep.

In leptospirosis, there is no host specificity for the various serological variants of *Leptospira* spp. but there are some so-called preferred hosts and others that are accidental hosts (FAINE et al., 1999). The fact that the serovars of the genus *Leptospira* spp. are not species specific has led to the frequent discovery of the different serovars that comprise the genus, which affect different animal species.

Likewise, it is also common to find two or more animal species that can manifest the disease simultaneously in the same region or farm (ZAKERI et al., 2010; HASHIMOTO et al., 2010). This indicates that the coexistence of various species on the same farm and the lack of compliance to health standards contribute to the establishment of possible contamination among them, giving rise to various clinical manifestations characteristic of leptospirosis in the different affected species.

The serovar Hardjo, which is most often found in cattle, leads to economic losses by reducing reproductive efficiency, causing spontaneous abortion and premature birth of calves, as well as a decline in production (LILENBAUM; SOUZA, 2003). This serovar has been detected frequently in Brazil's cattle herds, with a rate of 51.5% of seroreactive cattle (FAVERO et al., 2001). The practically homogeneous distribution of this serovar appears to be independent of the different conditions in each region, the type of herd operation and the breeding practices (CASTRO et al., 2008).

A significant presence of the serovar Hardjo (34.78%) was also detected in the sheep flock, with titers ranging from 400 to 3200. The main complaints regarding reproductive disturbances in this species were: spontaneous abortion, stillbirth and reproductive failure. Some authors consider that sheep act as accidental hosts, becoming infected by serovars commonly found in other domestic and wild animals (ELLIS, 1994, FAINE et al., 1999). On the other hand, surveys have shown that the infection of sheep by *Leptospira* sp seems to be common and, in most cases, is associated with the presence of the serovar Hardjo (MELO et al., 2010), which is the one the most responsible for reproductive losses in cattle and also causes the largest number of spontaneous abortions in sheep (HERRMANN et al., 2004).

The frequency of anti-*Leptospira* agglutinins found in this study showed the lowest rates in sheep (23.47%) and goats (21.05%). Studies have shown that leptospirosis occurs less frequently in sheep and goats than in cattle, swine and horses (CICERONI et al., 2000). However, in the absence of treatment, infected sheep may be able to develop chronic kidney infection and to release bacteria into the environment, thereby transmitting them to other animals in the herd, as well as to individuals in close contact with them (ZAKERI et al., 2010).

With regard to the frequency of the serovar Icterohaemorrhagiae (37.5%) in dogs, similar results have also been found in other studies (JOUGLARD; BROD, 2000; QUERINO et al., 2003), which identified it as one of the most prevalent. The significant frequency of this serovar highlights the importance of controlling the rodent population, since they are the maintenance hosts of this serovar (BOLIN, 1996; QUINN et al., 2005).

The occurrence of serovar Autumnalis in dogs, as found in a study conducted in domiciliated dogs from the same municipality (CASTRO et. al., 2011), is a warning regarding the existing commercial vaccines on the market, which do not include this serovar. It should be noted that vaccines against leptospirosis promote an immune response against the LPS of these bacteria, and are thus restricted to antigenically related serovars (PETERSEN et al*.,* 2001; BHARTI et al*.,* 2003). There is no proven cross-immunity among serovars, which highlights the importance of continued research into new vaccines for canine leptospirosis, aiming at more effective and lasting vaccine coverage.

The fact that the farm's facilities are in close proximity, the close interaction among the species, and the farm's hygiene-sanitary management, are probably risk factors for transmission of the microorganism among the species. According to Bolin (1996), the *Leptospira* spp. serovars that are present in a region are associated with the presence of one or more maintenance hosts that serve as natural reservoirs of infection. The presence of the serovar Hardjo in dogs may be due from their proximity to cattle and sheep, since this serovar normally circulates in these populations.

Among the evaluated species, the second highest prevalence of seroreactivity was found in horses (66.66%), most of which presented anti-*Leptospira* antibodies against the serovar Icterohaemorrhagiae. This serovar is one of those responsible for acute leptospirosis, with symptoms of bleeding, severe liver failure, uremia-related abortion, and other reproductive disorders (QUINN, 2005; LINHARES et al., 2005).

It is believed that the occurrence of anti-*Leptospira* agglutinins in the evaluated species may be ascribed to a common source of infection for these species, such as infected wild animals or rodents, as well as areas contaminated with their urine. Conversely, the agent may have been transmitted among the animal species due to their close cohabitation.

RESUMO: A leptospirose é uma zoonose de ampla distribuição mundial, que acomete animais de estimação e produção, os quais podem ser fonte de infecção para a espécie humana, considerada, portanto uma relevante doença em saúde pública**.** Desta forma, objetivou-se investigar a ocorrência de aglutininas anti-*Leptospira* em diversas espécies animais em uma propriedade rural localizada no Município de Uberlândia-MG. Foram colhidas 172 amostras de soro sanguíneo provenientes de cinco espécies animais: bovinos, caninos, caprinos, equinos e ovinos. Estabeleceu-se o diagnóstico pela técnica de Soroaglutinação Microscópica (SAM) com uma coleção de 12 antígenos vivos. Dos 172 animais avaliados, 62 (36%) foram reagentes, as sorovariedades mais frequentes em cada espécie foram: Hardjo (54,54%), Icterohaemorrhagiae (27,27%), e Autumnalis (18,18%) nos bovinos; Icterohaemorrhagiae (37,5%), Autumnalis (31%), Canicola (18,7%) e Hardjo (12,5%) e nos caninos; Grippothyphosa (75%) e Hardjo (25%) nos caprinos; Icterohaemorrhagiae (50%), Grippothyphosa (37,5%) e Hardjo (12,5%) nos equinos e Hardjo (34,78%); Grippothyphosa (26,08%); Bratislava (17,39%), Autumnalis (17,39%) e Icterohaemorrhagiae (4.4%) nos ovinos. Acredita-se que a ocorrência de aglutininas anti-*Leptospira* possa estar relacionada com uma fonte de infecção comum para as espécies, ou ainda que a transmissão do agente tenha ocorrido entre espécies animais, provavelmente devido à convivência habitacional próxima.

PALAVRAS-CHAVE: Sorovares. Leptospirose. Soroaglutinação Microscópica.

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