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SCIENTIFIC COMMUNICATION

FREQUENCY OF ANTI-LEPTOSPIRES AGGLUTININS IN SOWS FROM A SWINE HERD IN THE IBIÚNA MUNICIPALITY, STATE OF SÃO PAULO, BRAZIL

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ABSTRACT

A serologic survey was conducted among 164 sows from a single farrow-to-finish swine herd located in the Ibiúna municipality, state of São Paulo, Brasil, to determine the frequency of anti-leptospires agglutinins. For detection of anti-leptospires antibodies, the microscopic serum-agglutination test (MAT) was carried out using live cultures of 22 pathogenic and two saprophytic *Leptospira* spp. serovars. The most frequent serovar was found crossing the results of frequency and titer of agglutinins, and sera presenting equal titers for two or more serovars were not considered for this analysis. Of the 164 sows, 27 (16.5%) were seropositive for at least one *Leptospira* spp. serovar. The most frequent serovar was Hardjo (Hardjobovis), with 13 (54.2%) reactant sera. Other reactant serovars and respective frequency were: Shermani (16.6%), Bratislava (12.5%), Autumnalis (12.5%) and Icterohaemorrhagiae (4.2%).

KEY WORDS: *Leptospira* spp., swine leptospirosis, seropositivity, sows.

RESUMO

FREQÜÊNCIA DE AGGLUTININAS ANTI-LEPTOSPIRAS EM MATRIZES SUÍNAS DE UM REBANHO DO MUNICÍPIO DE IBIÚNA, ESTADO DE SÃO PAULO, BRASIL. Com o objetivo de determinar a freqüência de aglutininas anti-leptospiras, foi realizado um inquérito sorológico em 164 matrizes de uma granja suína de ciclo completo localizada no Município de Ibiúna, Estado de São Paulo, Brasil. Para a detecção de anticorpos anti-leptospiras, foi utilizada a técnica de soroaglutinação microscópica (SAM), utilizando-se culturas vivas de 22 sorovares patogênicos e dois sorovares saprófitos de *Leptospira* spp. Para a determinação do sorovar mais provável, foram considerados o título de aglutininas e a freqüência de soros reagentes, e soros que apresentaram títulos iguais para dois ou mais sorovares foram excluídos desta análise. Das 164 matrizes suínas, 27 (16,5%) foram soropositivas para pelo menos um dos sorovares empregados. O sorovar mais provável foi o Hardjo (Hardjobovis), com 13 (54,2%) soros reagentes. Também foram constatadas reações sorológicas para os seguintes sorovares: Shermani (16,6%), Bratislava (12,5%), Autumnalis (12,5%) and Icterohaemorrhagiae (4,2%).

PALAVRAS-CHAVE: *Leptospira* spp., leptospirose suína, soropositividade, porcas.

The production and productivity indices of swine herds can be influenced by several factors including genetic, environmental, nutritional, toxic, management and infectious. Among infectious diseases, leptospirosis occupies an important position. This infection, considered as reemerging in some countries, is a worldwide spread zoonose (RATHINAM et al., 1997). Leptospires are important etiological agents of reproductive disorders in swine and although they can cause lesions in several organs, they preferentially localize in the kidneys, where they multiply and are eliminated through the urine (FAINE et al., 1999). Leptospiral infection in pigs causes fetal death, abortion, infertility, and birth of weak piglets. Abortions are often restricted to periods of declining immunity in the sow population (ELLIS, 1999). In endemically infected areas, such as found in many tropical countries, it might therefore be expected that *Leptospira* spp. infections cause fewer obvious symptoms of reproductive failure due to immunity.

The epidemiology of swine leptospirosis is potentially very complicated, since swine can be infected by any of the pathogenic serovars. Fortunately, only a small number of serovars will be endemic in

any particular region or country. Furthermore, leptospirosis is a disease that shows a natural nidality, and each serovar tends to be maintained in specific-maintenance hosts. Therefore, in any region, pigs will be infected by serovars maintained by pigs or by serovars maintained by other animal species present in the area. The relative importance of these incidental infections is determined by the opportunity that prevailing social, management, and environmental factors provide for contact and transmission of leptospires from other species to pigs (ELLIS, 1999).

Throughout the world, the *Leptospira* spp. serovars more frequently isolated from swine are Pomona, Tarassovi, Bratislava, Grippothyphosa and, with smaller predominance, Icterohaemorrhagiae and Canicola (FAINE et al., 1999). The first isolations of leptospires in Brazilian swine were accomplished by GUIDA (1947 / 48) in São Paulo state. GUIDA et al. (1959), SANTA ROSA (1962), SANTA ROSA et al. (1970), SANTA ROSA et al. (1973), CORDEIRO et al. (1974) and OLIVEIRA et al. (1980) described isolations of the serovars Canicola, Pomona, Icterohaemorrhagiae and Hyos.

The present study was designed to assess the frequency of sows with antibodies against *Leptospira* spp. serovars in a swine herd in the Ibiúna municipality, state of São Paulo, Brazil.

All sows (n = 164) from a single farrow-to-finish swine herd located on the Ibiúna municipality, state of São Paulo, Brazil, were utilized. The herd was not submitted to any type of intervention such as antibiotic therapy or immunoprophylaxis for leptospirosis or rodent control, and there were commercial and breeding activities on the herd. All pigs in the herd were of the Large White and Landrace breeds. The management system was of gestation in pens and confinement of piglets through weaning to fattening and finishing phases.

Blood was collected from the cranial vena cava into sterile vacuum tubes and stored on ice in a cooler during transport to the Faculty of Veterinary Medicine and Zootechny (FMVZ) of the University of São Paulo (USP), São Paulo, Brazil. The sera were separated after clotting, centrifuged, and stored in sterile cryotubes at -20º C until further analysis.

For detection of anti-leptospires antibodies, the microscopic serum-agglutination test (MAT) was carried out following GALTON et al. (1965). Live cultures of 22 pathogenic and two saprophytic *Leptospira* spp. serovars were used: Australis, Bratislava, Autumnalis, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Sentot, Grippotyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Panama, Pomona, Pyrogenes, Wolffi, Hardjo (Hardjobovis and Hardjoprajitno), Shermani, Tarassovi, Javanica, Andamana and Patoc. The cultures were kept from five to 10 days at 28ºC in EMJH medium enriched with sterile inactivated rabbit serum (ALVES et al., 1996). All sera were initially tested at 1:100 dilution and those that presented at least 50% of agglutination at this dilution were considered positive. They were then serially diluted until the maximum positive dilution was determined. The titer of antibodies was the reciprocal of the higher positive dilution that presented 50% of agglutination. The most frequent serovar was found crossing the results of frequency and titer of agglutinins. Sera presenting equal titers for two or more serovars were not considered for this analysis.

Of the 164 sows, 27 (16.5%) were seropositive for at least one *Leptospira* spp. serovar. The most frequent serovar was Hardjo (Hardjobovis), with 13 (54.2%) reactant sera. Other reactant serovars and respective frequency were: Shermani (16.6%), Bratislava (12.5%), Autumnalis (12.5%) and Icterohaemorrhagiae (4.2%) (Table 1).

The most frequent reactant serovar in this study was Hardjo (Hardjobovis). The occurrence of this serovar was surprising because it is maintained by cattle worldwide, and where cattle and pigs come in close contact, the opportunity arises for infection in pigs to occur, which is the exact opposite of the present study, whose sows came from a herd that utilize an intensive management system. Serovar Shermani, the second most frequent serovar in this study, was first isolated from spiny rats (*Proechimys semispinosus*) in Panama Canal Zone (SULZER et al., 1982) and seropositivity in sows has been described (GUERRA et al., 1986), however, clinical signs associated with this serovar in sows have never been reported.

Table 1 - Number of samples with titers to five *Leptospira* spp. serovars obtained by the microscopic serum-agglutination test (MAT) in 164 serum samples from sows from a single farrow-to-finish swine herd, located in the Ibiúna municipality, state of São Paulo, Brazil.

Serogroup	Serovar	Titer of agglutinins			Total (%)
		100	200	400	
Shermani	Shermani	4			4 (16.6)
Australis	Bratislava	2	1		3 (12.5)
Sejroe	Hardjo (Hardjobovis)	4	5	4	13 (54.2)
Icterohaemorrhagiae	Icterohaemorrhagiae	1			1 (4.2)
Autumnalis	Autumnalis	2	1		3 (12.5)

Serovar Bratislava has emerged as a major swine-maintained leptospiral infections in the last few years (Ellis, 1999), and has been isolated from clinical cases of reproductive disorders (Ellis et al., 1986; Bolin & Cassells, 1990; Bolin et al., 1991; Gummow et al., 1999) and seropositivity has been associated with impaired reproductive performance of sows (Van Til & Dohoo, 1991; Mousing et al., 1995). The frequency of antibodies against serovar Autumnalis found in the present study (12.5%) was higher than that observed by Guerra et al. (1986) and Faria et al. (1989).

Serovar Icterohaemorrhagiae has also been associated with impaired reproductive performance in sows (Van Til & Dohoo, 1991; Ferreira Neto et al., 1997) and the occurrence of this serovar in this study suggests its introduction to susceptible stock via an environment contaminated with infected rat urine, as the maintenance host for this serovar is the brown rat (*Rattus norvegicus*).

There were coagglutinations among *Leptospira* spp. serovars in this study. These coagglutinations might be interpreted as cross-reactions or miscellaneous infections (Ferreira Neto et al., 1997). The standard method for serologic diagnosis of leptospirosis is the microscopic serum-agglutination test (MAT), usually with a cut-off value at 100 (Faine et al., 1999). The MAT has severe limitations in the diagnosis of chronic infection in individual pigs. To the cut-off value at 100, the MAT sensitivity is believed to be only moderate (Mousing et al., 1995) and infected animals may have titers below the widely accepted minimum significant titer of 100, but the specificity is good (Ellis, 1999). Therefore, misclassification of serostatus probably occurred in this study, mainly false-negatives. However, the present experimental design did not permit the exclusion of this bias.

Low titers of leptospiral antibodies may be detectable for several years in some animals (Ellis, 1999). In this study, most titers were low (100), but it is not known whether these were rising or declining. It should be noted that MAT is an indirect test for diagnosis of leptospiral infection and that cross-reactions between serovars are a common feature (Faine et al., 1999). A direct way to detect infecting serovars is the isolation from clinical samples or other tests of leptospiral demonstration, such as immunostaining methods and molecular biology techniques (Faine et al., 1999). However, for practical reasons these direct methods are rarely used in veterinary epidemiological studies.

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