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SCIENTIFIC COMMUNICATION

PREVALENCE OF ANTI-*LEPTOSPIRA* SPP. ANTIBODIES IN SWINE SLAUGHTERED IN THE PUBLIC SLAUGHTERHOUSE OF PATOS CITY, PARAÍBA STATE, NORTHEAST REGION OF BRAZIL

S.S. Azevedo[1], R.M. Oliveira[1], C.J. Alves[1], D.M. Assis[1], S.F. Aquino[1], A.E.M. Farias[1], D.M. Assis[1], T.C.C. Lucena[1], C.S.A. Batista[2], V. Castro[3], M.E. Genovez[3]

[1]Universidade Federal de Campina Grande, Centro de Saúde e Tecnologia Rural, Unidade Acadêmica de Medicina Veterinária, Av. Universitária, s/nº, CEP 58700-970, Patos, PB, Brasil. E-mail: ssazevedo_vet@yahoo.com.br

ABSTRACT

A serologic survey was conducted among 131 swine slaughtered in the public slaughterhouse of Patos city, Northeast region of Brazil, to determine the prevalence of anti-*Leptospira* spp. agglutinins. For serologic diagnosis of leptospirosis, the microscopic 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 131 swine analyzed, 44 were seropositive for at least one *Leptospira* spp. serovar, resulting in a seroprevalence of 33.6% (95% CI = 25.5% - 42.4%). The most frequent serovar was Pomona, with 38 (29.0%; 95% CI = 21.4% - 37.6%) reactant sera. Other reactant serovars and respective prevalence were: Pyrogenes (2.3%; 95% CI = 0.5% - 6.5%), Canicola (1.5%; 95% CI = 0.2% - 5.4%) and Shermani (0.8%; 95% CI = 0.02% - 4.2%). There was statistical difference in seroprevalence to serovar Pomona compared with others reactant serovars ($P < 0.0001$).

KEY WORDS: *Leptospira* spp., swine leptospirosis, seroprevalence, Patos City.

RESUMO

PREVALÊNCIA DE ANTICORPOS ANTI-*LEPTOSPIRA* SPP. EM SUÍNOS ABATIDOS NO MATADOURO PÚBLICO DE PATOS, ESTADO DA PARAÍBA, BRASIL. Com o objetivo de determinar a prevalência de aglutininas anti-*Leptospira* spp., foi realizado um inquérito sorológico em 131 suínos abatidos no matadouro público de Patos, Estado da Paraíba, Brasil. Para o diagnóstico sorológico de leptospirose, 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. Soros que apresentaram títulos iguais para dois ou mais sorovares foram excluídos desta análise. Dos 131 suínos, 44 foram soropositivos para pelo menos um dos sorovares empregados, resultando em uma soroprevalência de 33,6% (IC 95% = 25,5% - 42,4%). O sorovar mais provável foi o Pomona, com 38 (29,0%; IC 95% = 21,4% – 37,6%) soros reagentes. Também foram constatadas reações sorológicas para os seguintes sorovares: Pyrogenes (2,3%; IC 95% = 0,5% – 6,5%), Canicola (1,5%; IC 95% = 0,2% – 5,4%) e Shermani (0,8%; IC 95% = 0,02% –4,2%). Houve diferença significativa na soroprevalência para o sorovar Pomona em relação aos demais sorovares ($P < 0,0001$).

PALAVRAS-CHAVE: *Leptospira* spp., leptospirose suína, soroprevalência, Patos.

The production and productivity indices of swine herds can be influenced by 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 zoonosis (RATHINAM et al., 1997).

[2]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, São Paulo, SP, Brasil.
[3]Instituto Biológico, Centro de Pesquisa e Desenvolvimento de Sanidade Animal, São Paulo, SP, Brasil.

Leptospires are important etiological agents of reproductive disorders in swines. 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.

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, nowadays known as Tarassovi.

The present study was designed to assess the prevalence of anti-*Leptospira* spp. antibodies in swine slaughtered in the public slaughterhouse of Patos City, state of Paraíba, Northeast region of Brazil.

Swine slaughtered in the public slaughterhouse of Patos city, state of Paraíba, Northeast region of Brazil were analyzed. The number of samples was calculated taking into account the assumed prevalence for anti-*Leptospira* spp. antibodies of near 50%, to maximize the sample size and obtain a minimal confidence of 95%, and a statistical error of 10% (Thrusfield, 1995). Calculations were executed using EpiInfo version 6.04, resulting in a recommended sample size of 96 swine sera. A total of 131 blood samples were collected during October and November 2006, being 45 samples from male pigs and 86 from females. The collections were performed each Friday of all slaughtered pigs.

Blood was collected before slaughter from the cranial vena cava into sterile vacuum tubes and stored on ice in a cooler during transport to the Laboratory of Transmissible Diseases of the Federal University of Campina Grande, Patos City, Paraíba State, Brazil. The sera were separated after clotting, centrifuged, and stored in sterile cryotubes at -20°C until further analysis.

For detection of anti-*Leptospira* spp. antibodies, the microscopic serum-agglutination test (MAT) (Faine et al., 1999) was carried out. Live cultures of 22 pathogenic and two saprophytic *Leptospira* spp. serovars were used: *Leptospira interrogans* serovars Australis, Bratislava, Autumnalis, Bataviae, Canicola, Sentot, Grippotyphosa, Hebdomadis, Copenhageni, Pomona, Pyrogenes, Wolffi and Hardjo (Hardjoprajitno); *L. borgpetersenii* serovars Castellonis, Whitcombi, Tarassovi, Javanica and Hardjo (Hardjobovis); *L. kirshneri* serovars Butembo and Cynopteri; *L. inadai* serovar Icterohaemorrhagiae; *L. noguchii* serovar Panama; *L. santarosai* serovar Shermani; and *L. biflexa* serovars Andamana and Patoc. The cultures were kept from five to 10 days at 28°C 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.

The prevalence of anti-*Leptospira* spp. antibodies was estimated from the ratio of positive results to the total number of swine examined, with the exact binomial confidence interval of 95% (Thrusfield, 1995), using the program EpiInfo, version 6.04. Differences in seroprevalence by reactant serovar were verified by Chi-square test (÷2) (Zar, 1999), with a significance level of 5%, using the software package MINITAB version 13.0.

Table 1 – Number, prevalence (%) and 95% confidence interval of samples with titers to four *Leptospira* spp. serovars obtained by the microscopic serum-agglutination test (MAT) in 131 serum samples from swine slaughtered in the public slaughterhouse of Patos city, Paraíba State, Northeast region of Brazil.

Serovar	Titer of agglutinins						Total	Prevalence (%)	95% CI
	100	200	400	800	1600	3200			
Pomona	2	9	6	10	6	5	38	29.0	21.4 - 37.6
Pyrogenes	3						3	2.3	0.5 - 6.5
Canicola		1	1				2	1.5	0.2 - 5.4
Shermani	1						1	0.8	0.02 - 4.2
Total	6	10	7	10	6	5	44	33.6	25.5 - 42.4

Of the 131 swine analyzed, 44 were seropositive for at least one *Leptospira* spp. serovar, resulting in a seroprevalence of 33.6% (95% CI = 25.5% - 42.4%). The most frequent serovar was Pomona, with 38 (29.0%; 95% CI = 21.4 - 37.6) reactant sera. Other reactant serovars and respective prevalence were: Pyrogenes (2.3%; 95% CI = 0.5 - 6.5), Canicola (1.5%; 95% CI = 0.2 - 5.4) and Shermani (0.8%; 95% CI = 0.02 - 4.2) (Table 1). There was statistical difference in seroprevalence to serovar Pomona compared with others reactant serovars ($P < 0.0001$).

The most frequent serovar in this study was Pomona. Ramos et al. (2006) examined pigs from farms of the Rio de Janeiro State, Brazil, and Pomona serovar was the second most frequent. This serovar has been reported as the most common serovar isolated from pigs worldwide, and its infection has been extensively studied and it provides a suitable model with which to illustrate general concepts of swine leptospirosis (Ellis, 1999). Azevedo et al. (2006) found serovar Hardjo (Hardjobovis) as the most frequent in sows from a swine herd in the Ibiúna municipality, State of São Paulo, Brazil, and Delbem et al. (2002), analyzing sera from pigs at slaughter in Northern Paraná State, Brazil, found Icterohaemorrhagiae as the most frequent serovar. These discrepancies may be explained by different cut-off points and antigens used in the serological test, however, the hypothesis of spread of a certain serovar in dependence of management and environmental factors and animal movement cannot be excluded (Faine et al., 1999).

Serovar Pyrogenes, the second most frequent serovar, is considered incidental for pigs. 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 by specific maintenance hosts. Therefore, in any region, pigs will be infected either 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).

Canicola was the third most frequent serovar in this study. Although organisms belonging to the Canicola serogroup have been recovered from swine (Ellis, 1999), little is known of the epidemiology of Canicola serovar infection in pigs. The dog is the recognized maintenance host for this serovar and is the probable vector whereby this serovar enters a piggery. Serovar Shermani, the fourth most frequent serovar, 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.

The high prevalence (33.6%) of anti-*Leptospira* spp. antibodies found in swine slaughtered in the public slaughterhouse of Patos City, Paraíba State, has potentially important implications for public health because slaughterhouse workers were exposed to the occupational risk of infection, and alerts for the importance of taking adequate sanitary cares at the slaughter to avoid the transmission of the infection.

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