

## The effects of the leptospiral infection on reproductive performance in sows

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### ABSTRACT

A serologic survey was conducted among 76 sows randomly selected from a single farrow-to-finish swine herd, located on the Ibiúna municipality, state of São Paulo, Brazil, to investigate associations between *Leptospira* spp. seropositivity and reproductive performance. For detection of anti-leptospires antibodies, the microscopic agglutination test (MAT) was carried out using live cultures of 22 pathogenic and two saprophytic *Leptospira* spp. serovars. Questionnaires with open and closed questions were administered to the manager of the herd for collection of information about the sows included in the study and the reproductive performance of the subsequent offspring. The following variables were evaluated: age of sows, total number of piglets born, number of piglets born alive, number of weak piglets, number of weaned piglets, number of mummified fetuses, number of stillbirths, mass of piglets at birth, mass of piglets at weaning, weaning to service interval (WSI), and occurrence of return to heat, mummified fetuses and stillbirths. The frequency of seropositivity for at least one *Leptospira* spp. serovar was 18.4%. The reactant serovars and respective frequencies were Bratislava (33.3%), Hardjobovis (33.3%), Shermani (19.1%), Icterohaemorrhagiae (9.5%) and Grippotyphosa (4.8%). Seropositivity for *Leptospira* spp. was associated with prolonged WSI ( $P<0.001$ ), decreased number of piglets born ( $P = 0.001$ ), decreased number of piglets born alive ( $P<0.001$ ), decreased number of weaned piglets ( $P = 0.003$ ), low mass of the piglets at birth ( $P<0.01$ ), and increased number of stillbirths ( $P = 0.001$ ). Seropositive sows had also significant relative risk ( $P = 0.003$ ) of stillbirths.

**Key words:** *Leptospira* spp., seropositivity, reproductive performance, sows

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## Introduction

The production and productivity indices of swine herds can be influenced by several factors, such as 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 zoonose spread world-wide (RATHINAM et al., 1997). Leptospires are important etiological agents of reproductive disorders in swine and, although they can cause lesions in several organs, 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 in many tropical countries, it might therefore be expected that *Leptospira* spp. infections cause fewer obvious symptoms of reproductive failure due to immunity. Pigs can be infected by several leptospiral serovars, the particular ones depending on the occurrence of reservoir-hosts, environment, and climate in the particular area (FAINE et al., 1999).

The association between seropositivity of sows to *Leptospira* spp. and their reproductive performance has been shown in several studies. Pomona and Tarassovi serovars were reported by PRITCHARD et al. (1985) as causing abortions, stillbirths and the birth of weak piglets. Bratislava serovar has been associated with parameters indicating subfertility (VAN TIL and DOHOO, 1991; HATHAWAY and LITTLE, 1981; MOUSING et al., 1995) and a reduced number of piglets born per litter (FRANTZ et al., 1989). HATHAWAY (1985) stated that serovars Hardjo and Canicola have been incriminated as causing reproductive disorders in swine. The Icterohaemorrhagiae serogroup causes acute illness in piglets (usually with spontaneous recovery), and an association with reproductive problems in adult swine has been suspected (FERREIRA NETO et al., 1997; HATHAWAY, 1985). BOQVIST et al. (2002) observed associations between seropositivity for serovar Tarassovi and the number of piglets born dead per litter, and between seropositivity for serovar Grippotyphosa and prolonged weaning to service interval (WSI).

As a contribution to understanding leptospiral infection of sows, the aim of our study was to assess whether sows with antibodies against *Leptospira* spp. serovars have more impaired reproductive performance than sows with no *Leptospira* spp. antibodies.

## Materials and methods

Seventy-six sows were randomly selected from a single farrow-to-finish swine herd, located on the Ibiúna municipality, state of São Paulo, Brazil. The herd was not submitted to any type of intervention such as antibiotic therapy or immunoprophylaxis for leptospirosis or rodent control. All pigs in the herd were of the Large White and Landrace breeds. The total number of sows was approximately 160 and the management

system was by gestation in pens and confinement of piglets through weaning to fattening and finishing phases.

Blood was collected from sows with sow-matings during the period corresponding to a maximum of 90 days and a minimum of seven days before the bleedings (FERREIRA NETO et al., 1997). Blood was collected from the cranial vena cava in 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 -20 °C until further analysis. The animals received humane care as outlined in the "Guide for Care and Use of Laboratory Animals" of the National Research Council (ANONYM., 1996).

For detection of anti-leptospires antibodies, the microscopic agglutination test (MAT) was carried out following GALTON et al. (1965) and COLE et al. (1973). 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 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. Then they were 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.

Questionnaires with open and closed questions were administered to the herd's manager for collection of information about the sows included in the study and the reproductive performance of the subsequent offspring. Data on the following parameters were collected: age of sows, total number of piglets born, number of piglets born alive, number of weak piglets, number of weaned piglets, number of mummified fetuses, number of stillbirths, mass of piglets at birth, mass of piglets at weaning, weaning to service interval (WSI), and occurrence of return to heat and abortions.

Age of sows, total number of piglets born, number of piglets born alive, number of weaned piglets, number of mummified fetuses, number of stillbirths, mass of piglets at birth, mass of piglets at weaning and WSI were compared between seropositive and seronegative sows by Mann-Whitney U test (ZAR, 1999), as all variables presented a non-normal distribution by the Kolmogorov-Smirnov test. The statistical software package SPSS, version 13.0, was used for the analysis. Relative risks of return to heat, stillbirth and mummified fetuses for seropositive sows were calculated using the software Epi Info version 6.0. P-values  $\leq 0.05$  were considered significant.

## Results

Of the 76 sows, 14 (18.4%) were seropositive for at least one *Leptospira* spp. serovar. The reactant serovars and respective frequencies were: Bratislava (33.3%), Hardjobovis (33.3%), Shermani (19.1%), Icterohaemorrhagiae (9.5%) and Grippotyphosa (4.8%) (Table 1).

The median and respective 1<sup>st</sup> and 3<sup>rd</sup> quartiles of the following variables were: age of the sows = 24.6 months (21.9; 27.9); number of piglets born = 11.0 (10.0; 11.0); number of piglets born alive = 10.0 (9.0; 11.0); number of weaned piglets = 9.0 (8.0; 10.0); number of mummified fetuses = 0.0 (0.0; 0.0); number of stillbirths = 0.0 (0.0; 1.0); mass of piglets at birth = 1.69 (1.62; 1.74); mass of piglets at weaning = 6.50 (6.19; 6.77); weaning to service interval (WSI) = 5.0 (4.0; 5.7). Return to heat, mummified fetuses and stillbirths were reported in eight (10.5%), two (2.6%) and 22 (28.9%) of the sows, respectively. There was no occurrence of abortions or weak piglets.

The comparisons between the seropositive and seronegative sows regarding the characteristics of the subsequent offspring are presented in Table 2. Sows that were seropositive had a longer ( $P<0.001$ ) WSI compared with seronegative ones (7.0 and 5.0 days, respectively), decreased number of piglets born ( $P = 0.001$ ), decreased number of piglets born alive ( $P<0.001$ ), decreased number of weaned piglets ( $P = 0.003$ ), increased number of stillbirths ( $P = 0.001$ ) and low mass of piglets at birth ( $P = 0.01$ ).

The frequencies of return to heat, stillbirth and mummified fetuses in seropositive and seronegative sows are presented in Table 3. Only the relative risk of stillbirths in seropositive sows was significant ( $P = 0.003$ ).

Table 1. Number of samples with titers to five *Leptospira* spp. serovars obtained by the microscopic agglutination test (MAT) in 76 serum samples from sows from a single farrow-to-finish swine herd, located on the Ibiúna municipality, state of São Paulo, Brazil

Serogroup	Serovar	Titer of agglutinins			Total (%)
		100	200	400	
Shermani	Shermani	4			4 (19.1)
Australis	Bratislava	4	1	2	7 (33.3)
Grippotyphosa	Grippotyphosa	1			1 (4.8)
Sejroe	Hardjo (Hardjobovis)	1	3	3	7 (33.3)
Icterohaemorrhagiae	Icterohaemorrhagiae	2			2 (9.5)

Table 2. Comparisons of seropositive and seronegative sows to *Leptospira* spp. regarding the characteristics of the subsequent offspring in a single farrow-to-finish swine herd, located on the Ibiúna municipality, state of São Paulo, Brazil

Variables	Seropositive sows			Seronegative sows			P-value
	N	Median	1 <sup>st</sup> ; 3 <sup>rd</sup> quartiles	N	Median	1 <sup>st</sup> ; 3 <sup>rd</sup> quartiles	
Age of sows (months)	14	25.3	24.6; 28.0	62	22.9	21.1; 28.2	0.286
Total piglets born	14	9.5	8.0; 10.0	61	11.0	10.0; 12.0	0.001
Nº of piglets born alive	14	8.5	5.8; 9.0	61	11.0	10.0; 11.0	< 0.001
Nº of weaned piglets	13	7.0	5.5; 9.0	62	9.0	8.0; 10.0	0.003
Nº of mummified fetuses	14	0.0	0.0; 0.0	62	0.0	0.0; 0.0	0.246
Nº of stillbirths	14	1.0	0.0; 2.3	62	0.0	0.0; 0.0	0.001
Mass of piglets at birth (kg)	14	1.63	1.53; 1.65	62	1.70	1.63; 1.75	0.01
Mass of piglets at weaning (kg)	14	6.52	6.18; 6.93	62	6.49	6.19; 6.74	0.80
Weaning to service interval (days)	14	7.0	5.0; 12.0	62	5.0	4.0; 5.0	< 0.001

Table 3. Relative risks (RR) and respective confidence intervals (95% CI) of mummified fetuses, stillbirths and return to heat in *Leptospira* spp. seropositive sows from a single farrow-to-finish swine herd, located on the Ibiúna municipality, state of São Paulo, Brazil

Variables	Frequency in seropositive sows	Frequency in seronegative sows	RR	95% CI	P-value
Mummified fetuses	1/14	1/62	4.43	0.29 - 66.59	0.336
Stillbirths	9/14	13/62	3.07	1.65 - 5.71	0.003
Return to heat	3/14	5/62	2.66	0.72 - 9.83	0.159

## Discussion

In this study, we identified associations between *Leptospira* spp. seropositivity and impaired reproductive performance of sows. The study showed that *Leptospira* spp. seropositive sows presented a prolonged WSI and a decreased number of piglets born, compared with the seronegative, which is corroborated by the results of BOQVIST et al. (2002). Other parameters associated with seropositivity were number of piglets born alive, number of weaned piglets, number of stillbirths and mass of the piglets at birth. FERREIRA NETO et al. (1997) did not observe any association among these variables and the presence of antibodies against serovar Icterohaemorrhagiae.

The analysis of the data suggests that there was an association between the proportion of stillbirths and the presence of antibodies against *Leptospira* spp. (Table 3). This is corroborated also by the median number of stillbirths (Table 2). Stillbirths, abortions and the birth of weak piglets of reduced viability are primary signs of chronic leptospirosis in pigs and it is the aspect of leptospirosis that can cause considerable economic losses (ELLIS, 1999).

There were coagglutinations among *Leptospira* spp. serovars. 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 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.

The most frequent reactant serovars in this study were Bratislava and Hardjobovis. Serovar Bratislava has emerged as 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 and CASSELLS, 1990; BOLIN et al., 1991; SCHÖNBERG et al., 1992; GUMMOW et al., 1999) and seropositivity has been associated with impaired reproductive performance of sows (VAN TIL and DOHOO, 1991; HATHAWAY and LITTLE, 1981; MOUSING et al., 1995; FRANTZ et al., 1989). The occurrence of the serovar Hardjobovis in this study was surprising because this serovar is maintained by cattle world-wide, 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 Icterohaemorrhagiae has also been associated with impaired reproductive performance in sows (VAN TIL and 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*). Serovar Grippotyphosa has previously been isolated in clinical cases of abortions (HANSON et al., 1971; MILLER et al., 1990) and seropositivity has been associated with prolonged weaning to service interval (WSI) (BOQVIST et al., 2002). This serovar is maintained by wildlife hosts (ELLIS, 1999), particularly by rodents, which probably explains the low frequency (4.8%) in this study. Serovar Shermani, which was the third 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.

It is likely that the sows in the study have developed a degree of immunity to leptospires as the tropical climate, management and housing practices favor *Leptospira* spp. infection. This might explain why no abortions and no weak piglets were recorded in this study, as these usually occur in previously uninfected animals or in animals with declining immunity, rather than in circumstances where infection is endemic (BOQVIST et al., 2002). It might be suggested that the sows in this study developed less dramatic signs of *Leptospira* spp. infection (such as prolonged WSI, decreased number of piglets born, decreased number of piglets born alive, decreased number of weaned piglets and low mass of the piglets at birth) due to endemic exposure to leptospires.

### Conclusions

The results obtained in this study suggest that *Leptospira* spp. infection, indicated by seropositivity, had a negative impact on the reproductive performance of sows, characterized by prolonged WSI, decreased number of piglets born, decreased number of piglets born alive, decreased number of weaned piglets, low mass of the piglets at birth, increased number of stillbirths and significant relative risk of stillbirths.

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**SAŽETAK**

U svrhu određivanja povezanosti između seropozitivnosti na leptospire i reproduksijske sposobnosti krmača provedeno je serološko istraživanje na 76 nasumce odabranih krmača s jedne farme zatvorenog tipa, smještene u okrugu Ibiuna u pokrajini Sao Paulo u Brazilu. Za dokazivanje specifičnih protutijela rabljena je mikroskopska aglutinacija sa živim kulturama 22 patogena i dva saprofitska serovara leptospira. Radi prikupljanja podataka upućeni su upitnici upravitelju farme s pitanjima o krmačama uključenima u istraživanje i o reproduksijskoj sposobnosti njihova potomstva. Procjenjivane su sljedeće varijable: dob krmača, ukupan broj oprasene prasadi, broj živooprasene prasadi, broj nevitalne prasadi, broj odbite prasadi, broj mumificiranih plodova, broj mrtvooprasene prasadi, masa prasadi pri prasenju, masa pri odbiću, razdoblje od odbića do bucanja i pojava ponovnog bucanja, te broj mumificiranih plodova i mrtvooprasene prasadi. 18,4% krmača bilo je serološki pozitivno za barem jedan serovar leptospira. Dokazana su protutijela za serovarove Bratislava (33,3%), Hardjovobis (33,3%), Shermani (19,1%), Icterohaemorrhagiae (9,5%) i Grippotyphosa (4,8%). Seropozitivnost za leptospire bila je povezana s produženim razdobljem od odbića do prvog bucanja ( $P<0,001$ ), sa smanjenim brojem oprasene prasadi ( $P = 0,001$ ), sa smanjenim brojem živooprasene prasadi ( $P<0,001$ ), smanjenim brojem odbite prasadi ( $P = 0,003$ ), s malom masom prasadi pri prasenju ( $P<0,01$ ), te s povećanim brojem mrtvooprasene prasadi ( $P = 0,001$ ). U serološki pozitivnih krmača također je ustanovljen znatan relativni rizik ( $P = 0,003$ ) da će oprasiti uginulu prasad.

**Ključne riječi:** *Leptospira* spp., seropozitivnost, reproduksijska sposobnost, krmače

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