Brazilian Journal of Microbiology (2002) 33:174-177
ISSN 1517-8382

LEPTOSPIROSIS IN SLAUGHTERED SOWS: SEROLOGICAL AND HISTOPATHOLOGICAL INVESTIGATION

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Submitted: June 13, 2001; Returned to authors for corrections: August 02, 2001; Approved: June 26, 2002

ABSTRACT

Swine leptospirosis is recognized world wide as an important cause of reproductive failure. Serum and histo-pathological examinations have been constantly used to diagnose this disease. This study was carried out on slaughtered sows from Northern Paraná State to compare serum findings with kidney, liver, ovary and uterus histo-pathological examinations. The microscopic serum-agglutination carried out on 36 animals detected 24 with titles $\geq$ 100 and 12 negative (< 100). The histopathological examinations made on livers, kidneys, ovaries and uterus stained with hematoxylin-eosin, were carried out on 16 randomly chosen animals, 11 from the groups with $\geq$ 100 serological titles and five from the negative group. The results showed that all the 16 animals had indicative lesions in the liver or in one of the kidneys. There were no lesions in the ovaries or uterus. Microscopic serum-agglutination title readings between 20 and 80 were detected in animals of the group considered negative with title < 100.

Key words: leptospira, swine, diagnostic.

INTRODUCTION

Leptospirosis is a zoonosis of economic significance in swine all over the world (16), not only because of abortions and stillbirths, but also because of the high death rates caused by the icterohaemorrhagiae serovar (19). Losses caused by leptospirosis result from the localization of the bacteria in the host that during the leptospiremia period diffuse throughout the animal organism, including the genital tract (9,10,11). At this phase, leptospira can be found in the liver, lungs, eyeballs and sometimes in the central nervous system. The concept that the kidney is the main location of persistent leptospira and the main maintainer of the chronic disease state has been questioned by new findings in swine infected by bratislava serovar, which was isolated from the oviduct and uterus of sows that aborted and from the reproductive tract of boars (11). In cattle, hardjo serovar was isolated from the reproductive tract of non-pregnant cows (12). In the microscopic serum-agglutination test (SAM) the 100 titer is considered positive (21), but Ellis *et al*. (11) isolated leptospira from the reproductive tract of sows with titers ranging from 10 to 30.

In swine slaughterhouses, the macroscopic indication of leptospirosis is the visualization of multifocal white spots in the renal parenchyma. These lesions are the reflection of interstitial nephritis, commonly found in renal infections by leptospiras (18,26). However, the white spots are not good indicators of infection, because 7.5% of the swine examined by Chappel *et al.* (5) with visually normal kidneys were also infected. Under the microscope, lesions found as nephritis with vacuolar degeneration and tubular epithelium necrosis, hemorrhaged areas of the lungs and hepatic necrosis, are not considered pathognomonic (2).

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Sows slaughtered in Northern Paraná State were studied to compare the *Leptospira* spp serological results with the histopathological observations in the kidney, liver, ovary and uterus.

MATERIALS AND METHODS

Material collection

Blood and kidney, liver, uterus and ovary fragments from 36 sows were collected in a slaughterhouse located in Northern Paraná State from January to July 2000. The blood was collected in test tubes during the animal bleeding and identified with the carcass number in the slaughter line. The fragments samples were obtained during the evisceration and individually placed on sterile Petri dishes which were also identified by the corresponding carcass number and then sent to the Laboratory of Veterinary Anatomic Pathology of the State University of Londrina (UEL).

Microscopic serum-agglutination test (SAM)

The 36 obtained sera were placed in sterile flasks and kept at -20ºC in the Leptospirosis Laboratory at Preventive Medicine Departament-UEL. The SAM was carried out following Ryu (23). Live cultures of 22 *Leptospira* spp. serovars were used: australis (Ballico), bratislava (Jez bratislava), autumnalis (Akiyami A), butembo (Butembo), fortbragg (Fort Bragg), castellonis (Castellon 3), bataviae (Van Tienen), canicola (Hond Utretcht), whitcombi (Whitcombi), cynopteri (3522 C), sentot (Sentot), grippotyphosa (Moskva V), hebdomadis (Hebdomadis), copenhageni (M 20), icterohaemorrhagiae (RGA), panama (CZ 214 K), pomona (Pomona), pyrogenes (Salinem), wolffi (3705), hardjo (Hardjoprajitno), shermani (1342 K) and tarassovi (Perepelitsin). The cultures were kept from 5 to 10 days at 28ºC in EMJH medium enriched with sterile inactivated rabbit serum. All sera were initially diluted at 1:100 and those that presented 2+ or higher agglutination at this dilution were considered positive. They were then serially diluted until the maximum positive dilution was determined. Among those animals whose sera showed negative results at the 1:100 dilution but presented lesions suggestive of leptospira found in the histo-pathological exams, five were randomly chosen and diluted further at 1:10, 1:20, 1:40 and 1:80 (6,11,13). Reading was carried out in a dark field microscope according to Myers (21).

Histopathologic examination

From the total of 36 animals, 11 from the group with a SAM title ≥ 100 and five from the negative animals at the 1:100 dilution were randomly sampled for the histopathologic tests. The fragments of the organs collected were fixed in buffered formol at 10% and embedded in paraffin. Five mm thick cuts were stained by the hematoxylin-eosin method (HE).

RESULTS

Twenty-four (66.67%) of the 36 samples obtained were considered positive at 1:100 dilution. Antibodies against the icterohaemorrhagiae serovar were detected at a higher frequency (79.16%). Among the five negative sera at 1:100 dilution, antibodies against the icterohaemorrhagiae serovar were detected in two sera at the 1:80, two sera at 1:40 and in one serum at 1:20 dilution. Antibodies against the castellonis, grippotyphosa, pomona and fortbragg serovars were also detected in these animals (Table 1).

All the 16 sows histopathologically analyzed showed lesions suggestive of leptospirosis (11 from the group with ≥ 100 SAM titers and five from the group with < 100 SAM titers (Table 2).

The histological alterations observed were: periportal inflammatory infiltration (Fig. 1), hepatic necrosis (Fig. 1), mononuclear infiltrate in the renal parenchyma, vacuolar degeneration in the tubular epithelium and renal mononuclear inflammatory infiltration (Fig. 2). The highest number of alterations was detected in the liver. Among the animals with ≥ 100 SAM

Table 1. Number of animals with ≥ 100 and < 100 SAM titers with histological alterations suggestive of *Leptospira* spp detected in the kidney, liver, ovaries and uterus of 16 sows discarded at a slaughterhouse in Northern Paraná State.

	Antibody titers against serovars				
Animal	icterohaemorrhagiae	fortbragg	castellonis	grippotyphosa	pomona
01	80	-	-	-	-
02	40	-	-	-	-
03	20	20	20	-	-
04	80	-	80	80	-
05	40	-	40	-	40

SAM = microscopic serum-agglutination.

Table 2. Histological alterations suggestive of *Leptospira* spp observed in 16 sows at a slaughterhouse in Northern Paraná State.

	SAM ≥ 100*	%	SAM < 100**	%	Total	
Presence of histological alterations	11	68.75	5	31.25	16	100
Ausence of histological alterations	00	00	00	00	00	00
Total	11	68.75	5	31.25	16	100

* = animals with ≥ 100 SAM titers; ** = animals with < 100 SAM titers; % = percentage in relation the total number of samples; SAM = microscopic serum-agglutination.

titles, 27.2% presented hepatic and renal lesions, and 63.6% showed only hepatic alterations. SAM negative samples at 1:100 dilution showed 40% hepatic lesions. No significant histological alterations were observed in the ovary or uterus (Table 3).

Table 3. Animals with < 100 SAM titers with histological alterations suggestive of leptospirosis at a slaughterhouse in Northern Paraná State.

SAM	Tissue					
	kidney	liver	kidney/liver	ovary	uterus	Total
≥100	01	07	03	00	00	11
<100	01	02	02	00	00	05
Total	02	09	05	00	00	16

SAM = microscopic serum-agglutination.

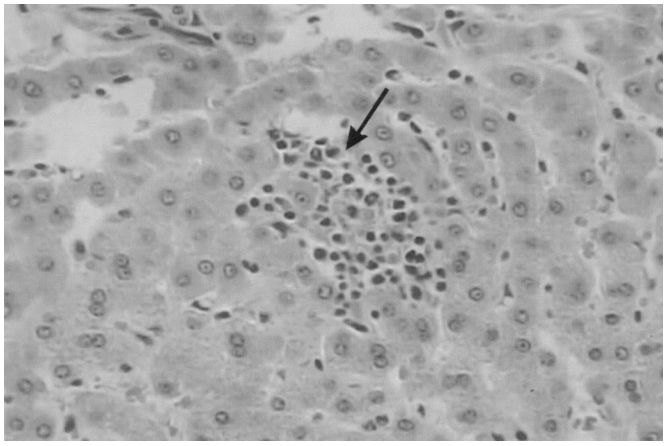

Figure 1. Liver; pig. Necrosis and inflamatory mononuclear cell infiltrates (arrow). H.E. stain. X 40.

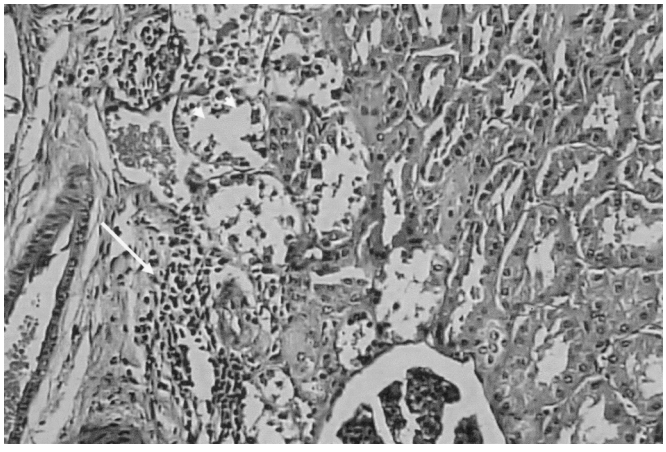

Figure 2. Kidney; pig. Inflamatory mononuclear cell infiltrates (arrow) and tubular epithelial necrosis (arrowheads). H.E. stain. X 20.

DISCUSSION

In this study, most of the sows discarded at slaughter showed antibody titers ≥ 100 against the icterohaemorrhagiae serovar. This is in line with the report of Van Til and Dohoo (24), who also observed in sows sent for slaughter icterohaemorrhagiae serovar antibodies at a higher frequency than the pomona and bratislava serovars antibodies, which are traditionally associated with swine (4,7,14,17,20).

The results of the histo-pathological exams carried out on the 11 animals from the serologic ≥ 100 positive titer group showed hepatic and renal lesions. The detected lesions were mononuclear infiltration in the renal parenchyma, hepatic necrosis and periportal inflammatory infiltration and vacuolar degeneration of the renal epithelium, which are leptospirosis characteristics as described by Baskerville (2).

The observed tissue alteration, mainly nephritis, are similar to those described by Baker *et al.* (1) in swine reactant to the icterohaemorrhagiae serovar. The hepatic observations in this study are inline with those described in dogs by Bishop *et al.* (3), indicating that the infection caused by leptospira produces necrosis in the hepatic cells and stimulates the inflammatory response.

No lesions were detected in the ovaries or uterus of the 16 sows (11 from the ≥ 100 titer group and 5 from the < 100 titer group). Girio *et al.* (15) did not observe any type of genital/urinary alteration in 17% of the female reactive to the icterohaemorrhagiae serovar, while renal and reproductive system lesions were reported in 32.9% and 50.1% of the females, respectively. Santa Rosa *et al.* (22) reported that the localization of the icterohaemorrhagiae serovar in the genital/urinary system without causing lesions may be associated to the renal carrier condition.

The hepatic and renal lesions detected in histo-pathological exams carried out on five animals from the SAM < 100 titer group were similar to those observed in another 11 animals belonging to the SAM ≥ 100 titer group. In this group was found, further on lesions, antibodies titles with 80, 40 and 20. This result was related, mainly, against icterohaemorrhagiae serovar.

The serologic results obtained from animals with < 100 titers are in line those of Ellis *et al.* (11), who isolated leptospira from one sow considered SAM negative at 1:100 dilution, 147 days after the abortion. Also, Ellis *et al.* (8) did not observe a relationship between the presence of antibodies and the state of the carrier in a cattle herd infected by the hardjo leptospira. Vasconcellos *et al.* (25) studied the influence of the swine serum concentration on the SAM using *Leptospira biflexa* strain Buenos Aires as antigen. The results indicated that serum concentration increased the sensibility, but reduced the specificity of the method, causing inespecific serological reactions. This also may have occurred with the SAM results of the sows groups at a slaughterhouse in Northern Paraná State with <100 titles (five animals).

These results show that slaughter sows with negative (< 100) SAM titles may have histological lesions suggestive of leptospira infection.

RESUMO

Leptospirose em matrizes suínas de abatedouro: investigação sorológica e histopatológica

A leptospirose suína é reconhecida mundialmente como uma importante causa de doença reprodutiva. Os exames sorológicos e histopatológicos têm sido constantemente utilizados para o diagnóstico dessa enfermidade. Este trabalho foi realizado em matrizes suínas de descarte da região norte do Estado do Paraná com o objetivo de comparar os achados sorológicos com os exames histopatológicos de rim, fígado, ovário e útero. A soroaglutinação microscópica realizada em 36 animais, detectou 24 com títulos considerados positivos (≥ 100) e 12 negativos (< 100). Os exames histopatológicos realizados em cortes de fígados, rins, ovários e úteros corados pela hematoxilina-eosina, foram realizados em 16 animais escolhidos aleatoriamente (11 do grupo com título sorológico ≥ 100 e cinco do grupo <100). Os resultados mostraram que todos os 16 animais tinham lesões histológicas sugestivas no fígado ou em um dos rins. Os ovários e úteros examinados não apresentaram lesões. Foram detectados títulos entre 20 a 80 na soroaglutinação microscópica nos cinco animais do grupo com título < 100.

Palavras-chave: leptospira, suíno, histopatologia, diagnóstico.

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