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LEPTOSPIROSIS AS A CAUSE OF "WHITE SPOT" KIDNEYS IN SOUTH AFRICAN PIG ABATTOIRS

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ABSTRACT

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The incidence of isolation of *Leptospira* sp. from the kidneys of slaughter pigs with "white-spot" macroscopic lesions was determined. Histology and general bacteriology were performed. *Leptospira pomona* was isolated from 19/21 kidneys showing macroscopic lymphocytic lesions.

INTRODUCTION

Leptospirosis in pigs in South Africa is caused mainly by *Leptospira interrogans* serovar *pomona* and occasionally by *L. canicola* (Van Rensburg, 1973). *L. pomona* causes an essentially chronic disease with few detectable clinical signs initially, apart from fever, conjunctivitis and anorexia (Field & Sellers, 1951). In an infected herd, the more noticeable effects will be abortion in sows and the birth of dead or weak piglets (Wrathall, 1975). Infection becomes localized in the kidneys and gives rise to the progressive development of a nephritis (Smith, Jones & Hunt, 1972). A macroscopic lesion characterized by grey-white focal lesions and often referred to as "white-spot" kidneys, may develop (Jubb & Kennedy, 1970).

As other bacteria have been described as the cause of similar kidney lesions (Larsen & Tondering, 1954; Weidlich, 1954; Jeffcott, Betts & Harvey, 1967), general bacteriology, special procedures for the isolation of *Leptospira* and histology were performed concurrently on "white spot" kidneys to determine the aetiology of the lesions observed in kidneys in South African abattoirs.

MATERIALS AND METHODS

Kidney samples

Twenty-one kidneys showing focal grey-white lesions were selected for examination at 2 local abattoirs (Fig. 1 & 2). Nine kidneys with normal macroscopic appearance were included for comparison. The selected kidneys were dissected aseptically, and small pinches of tissue were taken for leptospiral isolation. Pieces of the same kidney were also collected for general bacteriology and histopathology.

Bacteriological methods

Five small pinches of kidney tissue were taken from each kidney and, using sterile procedure, inoculated immediately into 5 tubes of EMJH semi-solid medium[1] containing 500 μg/mℓ of 5-flourouracil[2] (5-FU). The cultures were incubated at 29 °C in the dark for 4–5 days, subcultured in fresh EMJH semi-solid medium with 5-FU and examined at weekly intervals for growth under a dark-field microscope. Cultures were examined for 12 weeks before they were discarded as negative. Cultures showing good growth were sub-cultured onto EMJH liquid medium[1] without 5-FU, from which the organisms were typed. Isolates were tested against the following 8 commercial antisera[1]: *L. pomona, L. grippotyphosa, L. hyos (tarrasovi) L. mini, L. pyrogenes, L. hardjo, L. canicola* and *L. icterohaemorrhagiae (copenhageni)*, using the microscopic agglutination microvolume technique of Sulzer & Jones (1978).

General bacteriology was performed by culturing kidney samples on blood tryptose agar aerobically and duplicate BTA plates were incubated with the addition of 10 % CO_2. Samples were also plated onto MacConkey's agar plates.

Histological methods

Kidney samples were placed in 10 % buffered formalin and processed according to standard procedures. Sections from each kidney were cut at 4 μm and stained with haematoxylin eosin. The sections were also stained by the Warthin-Starry silver impregnation method (Young, 1969) in an attempt to demonstrate leptospires in the kidney sections.

RESULTS

Bacteriology

L. pomona was isolated from 19 of the 21 kidneys with macroscopic lesions (90 %). *L. pomona* was isolated from one of the 9 kidneys with normal macroscopic appearance.

The isolation of pyogenic or other potentially pathogenic bacteria and their association with lesions and leptospiral isolates is shown in Table 1. Bacteria isolated were, in order of frequency of isolation, *Escherichia coli* (5), *Streptococcus equisimilis* (5), *Corynebacterium pyogenes* (4), *Staphylococcus aureus* (1) *Streptococcus suis II* (1) and *Haemophilus parasuis* (1).

Histology

The kidneys from which *L. pomona* was isolated generally showed the same lesions, namely, subacute to chronic interstitial nephritis, characterized by varying degrees of fibrosis, and interstitial cellular infiltration. The areas of reaction varied from focal to extensive and coalescing. Lymphocytes were the predominant mononuclear cell types, while plasma cells were common. Sporadic neutrophils were present interstitially in some sections, hydropic degeneration of the tubular epithelium was seen in a few cases, and in other cases tubular dilatation with flattening of the epithelium predominated. Cell casts, mostly composed of neutrophils, were a common finding. Periglomerular and interstitial fibrosis were seen in many of the sections. In most of these the fibrosis was moderate to severe.

In 3 kidney sections, a moderate infiltration of neutrophils was observed in some interstitial foci. Another kidney, showing the typical histological changes, also had a mild infiltration of eosinophils interstitially.

No leptospires could be shown up in the section stained with Warthin-Starry stain.

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TABLE 1 The isolation of other bacteria in relation to macro- and microscopic kidney lesions and the isolation of *L. pomona*

Kidney No.	Bacterial isolate	Macro lesions	Micro lesions	*L. pomona* isolation
1	*E. coli*	+*	+	+
2	*E. coli*	+	+	+
3	*E. coli*	+	+	+
4	*E. coli*	+	+	+
5	*C. pyogenes*	+	+	+
6	*C. pyogenes*	+	+	+
7	*C. pyogenes*	+	+	+
8	*C. pyogenes*	+	+	+
9	*Staph. aureus*	+	+	+
10	*Strep. equisimilis*	+	+	+
11	*Strep. equisimilis*	+	+	+
12	*Strep. equisimilis*	+	+	+
13	*Strep. equisimilis*	+	+	+
14	*Strep. equisimilis*	+	+	+
15	—	+ (petechiae)	+	+
16	—	–	+	+
17	—	+	+	+
18	N/D**	+	+	+
19	N/D	+	+	+
20	N/D	+	+	+
21	*E. coli*	–***	+	–
22	*Strep. faecalis*	–	+	–
23	*Staph. epidermidis*	–	+	–
24		–	+	–
25		–	+	–
26	—	+ (petechiae)	+	–
27	*Strep. suis II*	+	+	–
28	*H. parasuis*	–	–	–
29	—	–	–	–
30	—	–	–	–

* + = present ** N/D = not done *** – = not present

L. pomona was not isolated from 10/30 kidneys in the survey, and only 2 of these 10 had macroscopic lesions. The one kidney had macroscopic petechiation, and microscopically showed red blood cells in the tubuli and hyaline degeneration of the tubular epithelium. Also present were focal necrosis in some glomerular tufts, accompanied by fibrin and neutrophils, and a few very small foci of interstitial lymphocyte infiltration. In the other kidney, a single connective tissue scar, extending from the capsule into the cortex, and a mild interstitial cellular infiltration was also present.

Of the 8 kidneys with normal macroscopic appearance, 3 appeared completely normal on histology. The remaining 5 kidneys had very mild, multifocal, mononuclear, interstitial reactions.

DISCUSSION

In this study, there appeared to be a good correlation between the isolation of *L. pomona*, the macroscopic "white-spot" lesion, and the histological lesions described by other authors in similar studies of *L. pomona* infections (Monlux, Seibold, Shalkop & Davis, 1952; Burnstein & Baker, 1954; Langham, Morse & Morter, 1958; Sleight, Langham & Morter, 1960; Hanson & Ferguson, 1970; Hanson & Tripathy, 1981). The histological lesions were also similar to those described by Michna & Campbell (1969) in their study on *L. canicola* and *L. icterohaemorrhagiae* infections in young pigs.

It is apparent from work done on *L. pomona* by these authors that the virulence of the strains will determine the severity of the histological lesions. There is agreement that the acute stage of infection is characterized by the absence of macroscopic lesions, very mild histological lesions, mainly tubular degeneration and mild interstitial nephritis. Large numbers of leptospires could be demonstrated at this stage. The acute stage then passes over into the sub-acute and chronic stages in which fibrosis and extensive mononuclear infiltration are evident and leptospires are not readily detected. The kidneys in our study appeared therefore to be in the chronic stage.

Most of the kidneys from which leptospires were isolated were reported to be accompanied by severely enlarged renal lymph nodes, none of which were submitted for histology. However, this is consistent with the findings of Langham *et al.* (1958), who found enlarged renal lymph nodes 76 and 146 days post-infection. It would seem that renal lymph node enlargement is not necessarily an indication of acute leptospiral infections in pigs. This fact should be borne in mind during meat inspection at abattoirs.

The typical "white-spot" kidney lesion referred to has been attributed to infections with bacteria other than *Leptospira* sp. Larsen & Tondering (1954) described lymphocytic interstitial nephritis in pig kidneys which they ascribed to ascending urinary tract infections caused by *E. coli* and *Staph. aureus*. Jeffcott *et al.* (1967) attributed interstitial nephritis and fibrosis to concomitant isolations of *E. coli* and various streptococci, while Weidlich (1954) described purulent embolic nephritis caused by *Corynebacterium* sp., *E. coli, Strep.* sp. and *Staph.* sp.

In our study, 3 kidneys from which *L. pomona* were isolated showed purulent foci. One of these yielded a *Staph. aureus* isolate, while the other 2 yielded *Strep. equisimilis* on culture. It is therefore possible that these bacteral species could have been responsible for the purulent reaction (Hanson & Ferguson, 1970).

The 5 microscopically normal kidneys which showed mild interstitial lymphocytic reactions yielded *E. coli, Strep. faecalis* and *Staph. epidermidis* on culture. This would seem to support the findings of Larsen & Tondering (1954) and Jeffcott *et al.* (1967) that *E. coli*, streptococcal and staphylococcal species may cause interstitial nephritis. However, these lesions were extremely mild and showed no macroscopic lesions.

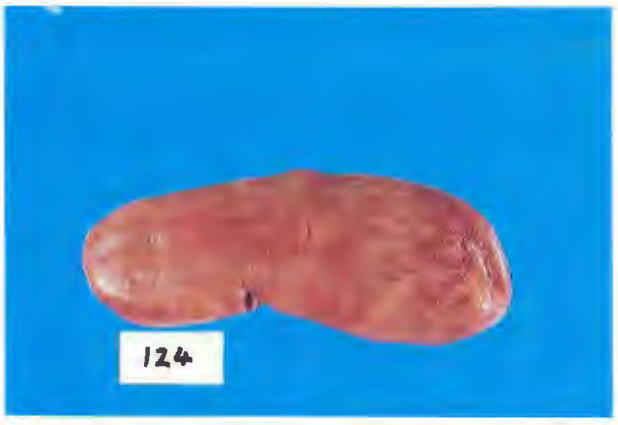

FIG. 1 The typical greyish-white multifocal lesions of the kidneys examined in the survey. Kidney 124 shown in the photograph corresponds to kidney 11 referred to in the text

FIG. 2 The cut surface of kidney 124 seen in Fig. 1

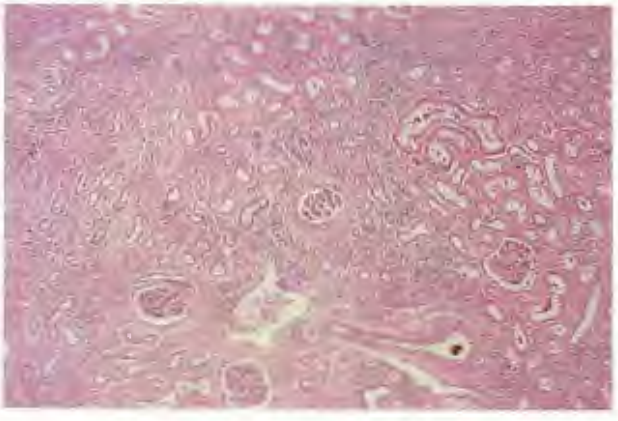

FIG. 3 Typical histopathological picture of kidneys from which *L. pomona* was isolated. The section shows focal mononuclear cell infiltration, interstitial and periglomerular fibrosis and tubular dilatation: HE ×160

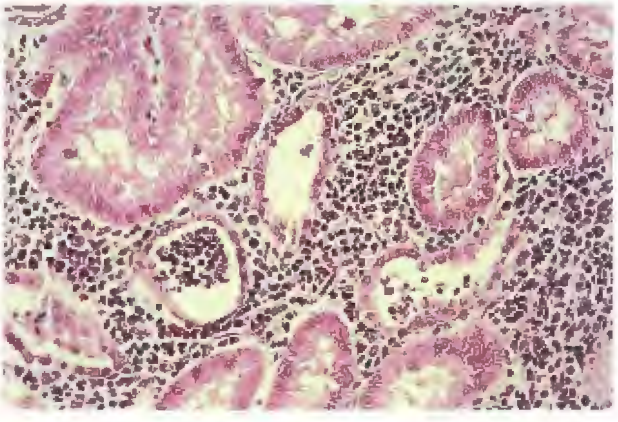

FIG. 4 Interstitial mononuclear cell infiltration and tubular degeneration. A cellular cast can be seen in the tubule with atropic epithelium: HE ×640

In our study, the interstitial lesions caused by these bacteria, therefore, did not represent a differential diagnosis for the gross "white-spot" lesions caused by *L. pomona*. The presence of eosinophils in one kidney with lesions otherwise typical for leptospirosis we attribute to possible exposure to allergens or parasites. *Strep. suis II* was isolated from the kidney which had a single connective tissue scar and a mild lymphocytic reaction. As *Strep. suis II* can cause meningitis, arthritis or septicaemia (Clifton-Hadley, 1983), its presence in the kidney was probably via haematogenous spread.

H. parasuis, primarily a normal respiratory inhabitant (Kilian, Frederiksen & Biberstein, 1981), was isolated from a kidney with a completely normal appearance. Its presence in the kidney can be regarded as most likely an incidental finding.

An attempt was made to estimate the age at which the pigs in this study, mostly porkers, were being infected by *L. pomona*. Sleight *et al.* (1960) recorded the presence of macroscopic lesions 7 days post-infection, while Burnstein & Baker (1954) first saw macroscopic lesions 3 weeks after infection. Lesions may therefore appear between 7–21 days. The lesions in our study correlated more closely with the lymphoreticular phase described by Michna & Campbell (1969), since advanced fibrosis was present. According to these authors this stage, is seen approximately 5 weeks post-infection. We estimate that infection occurs a few weeks post-weaning.

Vaccination has been used to control the appearance of these kidney lesions at slaughter. However, the work of Carghill & Davos (1981) in pigs, and Killinger, Taylor, Huhn, Hanson & Mansfield (1976) in cattle has shown that although vaccination reduced the incidence and severity of the renal lesions, it did not entirely prevent renal leptospirosis. Nevertheless, vaccination of piglets at weaning, combined with the vaccination of sows at each breeding cycle (Faine, 1982; York, 1975) will significantly reduce the level of infection in a piggery.

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REFERENCES

BURNSTEIN, T. & BAKER, J. A., 1954. Leptospirosis in swine caused by *Leptospira pomona*. *Journal of Infectious Diseases*, 94, 53–64.

CARGHILL, C. F. & DAVOS, D. E., 1981. Renal leptospirosis in vaccinated pigs. *Australian Veterinary Journal*, 57, 236–238.

CLIFTON-HADLEY, F. A., 1983 *Streptococcus suis* Type 2 infections. *British Veterinary Journal*, 139, 1–5.

FAINE, S., 1982. Guidelines for the control of leptospirosis. WHO Offset Publication No. 67. Geneva: World Health Organisation.

FIELD, H. I. & SELLERS, K. C., 1951. *Leptospira icterohaemorrhagiae* infection in piglets. *Veterinary Record*, 63, 78–81.

HANSON, L. E. & FERGUSON, L. C., 1970. *In:* DUNNE, H. W. & LEMAN, A. D. (eds.) Diseases of swine (3rd ed.). Iowa, USA: Iowa State University Press.

HANSON, L. E. & TRIPATHY, D. N., 1981. *In*: LEMAN, A. D., GLOCK, R. D., MENGELING, W. L., PENNY, R. H. C., SCHOLL, E. & STRAW, B. (eds.). Diseases of swine (5th ed.). Iowa, USA: Iowa State University Press.

JEFFCOTT, L. B., BETTS, A. O. & HARVEY, D. G., 1967. Nephritis in sows. *Veterinary Record*, 81, 446–447.

JUBB, K. V. F. & KENNEDY, P. C., 1970. Pathology of domestic animals (2nd ed.). New York and London: Academic Press.

KILIAN, M., FREDERIKSEN, W. & BIBERSTEIN, E. L., 1981. *Haemophilus, Pasteurella* and *Actinobacillus*. London: Academic Press.

KILLINGER, A. H., TAYLOR, P. L., HUHN, R. G., HANSON, L. E. & MANSFIELD, M. E., 1976. Immunity to leptospirosis: renal changes in vaccinated cattle given challenge inoculum. *American Journal of Veterinary Research*, 37, 93–94.

LANGHAM, R. F. MORSE, E. V. & MORTER, R. L., 1958. Experimental leptospirosis. V. Pathology of *Leptospira pomona* infection in swine. *American Journal of Veterinary Research*, 19, 395–500.

LARSEN, N. B. & TONDERING, E., 1954. Nephritis interstitialis leucolymphocytaria hos svin. *Nordsk Veterinaer Medecin*, 6, 35–46.

MICHNA, S. W. & CAMPBELL, R. S. F., 1969. Leptospirosis in pigs: epidemiology, microbiology and pathology. *Veterinary Record*, 84, 135–138.

MONLUX, A. W., SEIBOLD, H. R., SHALKOP, W. T. & DAVIS, C. L., 1952. Leptospirosis in hogs. *North American Veterinarian*, 33, 467–469.

SLEIGHT, S. D., LANGHAM, R. F. & MORTER, R. L., 1960. Experimental leptospirosis: The early pathogenesis of *Leptospira pomona* infection in young swine. *Journal of Infectious Diseases*, 106, 262–269.

SMITH, H. A., JONES, T. C. & HUNT, R. D., 1972. Veterinary pathology (4th ed.). Philadelphia: Lea & Febiger.

SULZER, C. R. & JONES, W. L., 1978. Leptospirosis: methods in laboratory diagnosis. Revised edition. United States Department of Health, Education and Welfare. Centre for Disease Control, Atlanta, Georgia.

VAN RENSBURG, W. J. J., 1973. Isolasie van *Leptospira canicola* by varke en honde in Suid-Afrika. *Journal of the South African Veterinary Association*, 44, 435–436.

WEIDLICH, N., 1954. Zur Kenntnis der Embolisch-eitrigen Nierenentzundung des Schweines. *Zentralblatt für Vetinarmedizin*, 1, 455–468.

WRATHALL, A. E., 1975. Reproductive disorders in pigs. Farham Royal, Slough, England: Commonwealth Agricultural Bureaux.

YORK, C. J., 1975. Immunology and prophylaxis of leptospirosis. *Veterinary Medicine*, 52, 563–565.

YOUNG, B. J., 1969. A reliable method for staining spirochaetes in tissue sections. *Journal of Medical Laboratory Technology*, 26, 248–252.

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