

THE ISOLATION OF *LEPTOSPIRA INTERROGANS* SEROVAR *POMONA* AND RELATED SEROLOGICAL FINDINGS ASSOCIATED WITH A MIXED FARMING UNIT IN THE TRANSVAAL

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ABSTRACT

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This is the first known isolation in the Republic of South Africa (RSA) of the serovar *pomona* from the organs of porcine foetuses as well as from the renal lymphnodes of slaughter pigs showing chronic nephritis. In addition, the serovar *pomona* was isolated from the kidneys of 87,5 % of the slaughter pigs examined. The success of these isolations was attributed in part to the refining of 2 existing isolation techniques which complement each other. Using the microscopic agglutination test, serum samples taken from the same farming unit showed evidence of antibodies to the serovar *pomona* in 89 out of the 170 bovines (52 %), 9 out of the 52 porcines (17 %), 2 of the 2 canines (100 %), 5 out of the 13 equines (38 %) and 2 out of the 152 ovines (1 %) that were tested. As far as is known, serological evidence of the serovar *pomona* in porcines, ovines, equines and canines has never previously been published in the RSA.

INTRODUCTION

Evidence of *Leptospira interrogans* serovar *pomona* (serovar *pomona*) in cattle, swine, sheep and horses has long been established elsewhere in the world (Faine, 1982). However, little has been published in this country regarding those animal species which may harbour this organism. Owing to the fastidious nature of this spirochete its isolation is restricted to well-equipped laboratories with trained personnel and it is probably for this reason that more work had not been done in this field until now. Up until 1986 the serovar *pomona* had been isolated in the RSA only from the urine of adult cattle (Herr, Riley, Nesor, Roux & De Lange, 1982) and from the kidneys of adult swine (Hunter, Van der Vyfer, Selmer-Olsen, Henton, Herr & De Lange, 1987). Serological evidence of the serovar *pomona* in the RSA has been reported only in cattle (Herr *et al.*, 1982). For this reason, an effort was made to determine whether leptospirae could be isolated from various porcine tissues by refining the isolation techniques previously used, and to determine whether leptospiral antibodies could be detected in species of animals other than those already described.

MATERIALS AND METHODS

Experimental animals

All animals tested in this project were derived from or found on a single mixed farming unit located in the south eastern Transvaal.

Bacteriology

Paired kidneys and renal lymphnodes were taken from 4 slaughter pigs which showed chronic kidney lesions. These were taken as sterilely as possible at an abattoir. Working in a biohazard cabinet and using sterile procedure, 1 g of tissue from each organ was then taken from a freshly cut surface. This tissue was homogenized by passage through a 14G needle and suspended in 9 ml of Sorensen's buffer (Sulzer & Jones, 1978). From the suspension 1,1 ml was withdrawn from which 0,1 ml was placed into 5 ml of semi-solid EMJH medium containing 0,5 mg of 5-fluorouracil per ml. The remaining 1 ml being added to a fresh 9 ml of Sorensen's buffer,

resulted in a suspension with a 1/100 tissue dilution. From this suspension, 1,1 ml was also withdrawn and the procedure repeated with tenfold dilutions until dilutions of 1/10 to a 1/10 000 of tissue were obtained. These were then incubated in the dark at 29 °C and examined weekly for growth. A parallel culture method, as described by Herr, Hunter & De Lange (1986) (consisting of direct placement of a small piece of tissue into selective media and then subculturing from these media after 3 days) was also carried out on each organ sample.

In addition to the above organ samples, 6 aborted porcine foetuses of 3 month's gestation, all from the same sow, were examined post-mortem within a biohazard cabinet. From each foetus, 1 cm³ of kidney, liver and approximately 0,1-0,5 ml of aqueous humour from both eyes were collected with sterile instruments. These organ samples were processed in the same way as described above.

Serology

The following animal numbers and species were bled, using 10 ml volume serum collecting vacuum tubes: 170 bovines, 153 ovines, 52 porcines, 13 equines and 2 canines. Each serum sample was then tested for antibodies against the serovar *pomona*, using the microscopic agglutination test (MAT) as described by Herr *et al.* (1986). Titres of 1/160 or greater to the serovar *pomona* were regarded as being positive.

Histopathology

A portion of each kidney and lymphnode collected from the pigs at the abattoir was placed in 10 % buffered formalin for histopathological examination, as described by Hunter *et al.* (1987).

RESULTS

Bacteriology

Serovar *pomona* was isolated from all 4 slaughter pigs examined. Successful isolation was confirmed within 10 days from 7 of the 8 kidneys and 4 of the 8 renal lymphnodes (Table 1).

Isolation of the serovar *pomona* was successful from 5 out of the 6 foetuses (the sow that aborted had an antibody titre of 1/5 120 to the serovar *pomona*). Serovar *pomona* was isolated from 5 out of the 6 kidneys, 2 livers and 2 samples of aqueous humour (Table 1).

Of the 34 organs processed, the serovar *pomona* was isolated by the direct inoculation method from 15 organs (44 %). By using serial dilutions, a further 15 % (5

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TABLE 1 Isolations of serovar *pomona* using direct inoculation and dilution methods

PART A Isolation results from foetal tissues

| Animal No. | Left kidney | | | | | Liver | | | | | Eye fluid | | | | |
|------------|-------------|---|---|---|---|-------|---|---|---|---|-----------|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| a | + | - | + | + | + | + | - | + | - | - | + | - | - | - | - |
| b | + | - | - | - | - | + | - | - | - | - | c | - | - | - | - |
| c | + | - | + | + | - | - | - | - | - | - | + | - | - | - | - |
| d | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - |
| e | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| f | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - |

PART B Isolation results from adult slaughter pigs

| Animal No. | Left kidney | | | | | Right kidney | | | | | Left lymphnode | | | | | Right lymphnode | | | | |
|------------|-------------|---|---|---|---|--------------|---|---|---|---|----------------|---|---|---|---|-----------------|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 | 0 | 1 | 2 | 3 | 4 |
| A | + | - | - | - | - | + | - | - | - | - | + | - | - | - | - | - | - | + | + | + |
| B | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| C | + | - | - | - | - | + | - | - | - | - | - | - | + | + | - | - | - | - | - | - |
| D | + | - | - | - | - | + | - | - | - | - | - | - | + | - | - | - | - | - | - | - |

Legend

- + = a positive serovar *pomona* isolation
- = no isolation
- c = contaminated culture
- 0 = direct culture of tissue
- 1 = 1/10 tissue dilution
- 2 = 1/100 dilution
- 3 = 1/1 000 dilution
- 4 = 1/10 000 dilution

TABLE 2 Serological findings of the serovar *pomona* on a mixed farming enterprise

| Species | No. tested | No. with pos. titres (>1/160) | Mean titre (1/X) | % positive |
|---------|------------|-------------------------------|------------------|------------|
| Bovine | 170 | 89 | 6 626 | 52 |
| Equine | 13 | 5 | 13 696 | 38 |
| Porcine | 52 | 9 | 39 040 | 17 |
| Ovine | 153 | 2 | 160 | 1 |
| Canine | 2 | 2 | 960 | 100 |

organs) successful isolations was achieved. One organ showed contamination and no leptospire were isolated from 13 organs (38 %) (Table 1).

Serology

Positive titres to the serovar *pomona* were recorded for 5 out of the 13 (38 %) equines, 9 out of the 52 (17 %) porcines, 2 out of the 2 (100 %) canines, 2 out of the 153 (1 %) ovines and 89 out of the 170 (52 %) bovines. The mean titres of each species were equines = 1/13 696, porcines = 1/39 040, ovines = 1/160, canines = 1/960 and bovines = 1/6 629 (Table 2).

Histopathology

The kidneys of the four slaughter pigs showed a chronic, multifocal, prominent, lymphocytic, interstitial nephritis. All the lymphnodes examined showed active lymphoid proliferation. The use of the Warthin-Starry staining technique demonstrated the presence in the kidneys of organisms which resembled leptospire.

DISCUSSION

Bacteriology

Our findings are the first reported isolations in the RSA of the serovar *pomona* from the renal lymphnodes of adult pigs and the organs of 3-month-old porcine foetuses. In addition, successful isolation of the serovar *pomona* from 'white spotted' kidneys of adult swine confirm the findings of Hunter *et al.* (1987). The success of our isolations appears to have been enhanced when serial dilutions of tissue specimens were used. This probably had a dilution effect on any contaminants that were competing with the *Leptospira* and/or any toxins that may

have been released from organ tissues as a result of autolysis. Serial dilutions were responsible for the successful isolation from 2 kidneys and 3 lymphnodes which otherwise would have been said to be negative had direct culture methods alone been used. The most successful isolations took place at the 1/100 and 1/1 000 tissue dilutions (Table 1). This concept of diluting tissue specimens is not new but is based on a modification of the technique developed by Sulzer & Jones (1978). However, our findings show that it is important that serial dilutions be done in conjunction with the direct culture of undiluted tissue suspensions, as the two complement each other.

It is currently assumed that porcine carcasses with kidneys that show chronic lesions are free of *Leptospira* once those kidneys have been removed. It is thought that in the chronic stages of the disease the leptospire are confined to the protective environment of the proximal tubules where they cannot be reached by antibodies (Faine, 1982). However, the isolation of the serovar *pomona* from the renal lymphnodes of those kidneys with chronic nephritis questions these assumptions and makes one ask whether the organism can also occur in other organs besides lymphnodes, despite the chronicity of the kidney lesions. A more detailed study, however, is necessary before this question can be answered.

Serology

This is the first time in the RSA that serological evidence of the serovar *pomona* in equines, canines, ovines and porcines has been published. (Unpublished laboratory records at the VRI Onderstepoort, however, do show serological evidence of the serovar *pomona* in porcines dating back to 1980 and in 2 dogs recorded in

1985). This study has therefore shown that, since a number of domestic animal species have acquired the serovar *pomona* in the RSA, the finding is of practical significance, especially in the case of mixed farming activities.

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