LEPTOSPIROSIS IN THE PIG

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SUMMARY

A study was made of leptospiral infection in pigs. The epidemiology of this disease and whether or not cell mediated immunity (CMI) is part of the immune response of pigs to *pomona* infection were investigated.

A number of serological techniques were used in this study. In a trial to measure the precision of the serological method, it was found that 93% of the sera tested varied by only $\pm$1 doubling dilution. An instrument was developed to aid the removal of samples from serology test-plates for darkfield examination and it was shown that the titres determined using this instrument were no different from those using the traditional capillary tube method. It was also found that the MA titres of sera from conventionally collected blood samples were the same as those estimated on sera collected from heart clots.

The isolation and identification of leptospires was used to define the epidemiology of leptospirosis in pigs. An efficient cultural method was developed, utilising a stomacher to homogenise kidneys. P80 medium was found to be superior to Stuart's and Fletcher's medium, with respect to the isolation of leptospires from pig kidneys.

A cultural and serological survey of leptospirosis was conducted in young and adult pigs from an abattoir. Serovar *pomona* was isolated from 38/84 (45%) of the kidneys from the young pigs, and from 1/65 (2%) of the adult kidneys. However 87% of the young pigs and 86% of the adult pigs were serologically positive at 1/12 to *pomona*. In this survey serovar *tarassovi* was isolated for the first time in New Zealand. In contrast to *pomona*, the prevalence of *tarassovi* culture-positive animals was 1/84 (1%) in the young group and 3/65 (5%) in the adult pigs. Twenty-one percent of the

young pigs and 25% of the adult pigs had MA titres to *tarassovi* of 1/12 or more. Although many of the sera reacted with serovars other than *pomona* and *tarassovi*, no other serovars were isolated. It was concluded that pigs in New Zealand are reservoir hosts for *pomona* and *tarassovi*, but that they do not become life-long carriers of these serovars. The MA titres to these serovars are maintained long after infection has been eliminated. It was also considered that *pomona* antibody cross-reacts with many other serovars. In this investigation it was found that the genus-specific microscopic agglutination test (MAT) using serovar *biflexa* (CDC) as antigen was of no value for detecting either infected pigs, or pigs which had titres to parasitic leptospires. It was also found that in many of the kidneys from which *pomona* was isolated either no gross leptospiral-like lesions were apparent, or only minor lesions were observed. It was concluded that infected kidneys that had no gross lesions constituted a potential public health risk.

Using abattoir samples, a number of tests were evaluated with respect to the ability to predict renal infection with serovar *pomona*. It was concluded that in a population with a moderate to high prevalence of infection, at a MA titre of 1/384 both the sensitivity and specificity will be approximately 85%. The usefulness of darkfield examination of urine and urine culture was limited because of low sensitivities of these parameters. In the sample collected from juvenile animals, 67% of the culture-positive animals had urine homologous MA titres of 1/4 or more, and 95% of the culture-negative animals were test-negative at this level. However, as older recovered animals were not included in this survey, it was considered that the specificity of this parameter was over-estimated.

The pattern of leptospiral infection in a pig herd was studied. This was a part of a collaborative study of leptospirosis infection in pigs, cattle and wildlife in this area. It was concluded that serovar *pomona* infection was endemic in this pig herd, and that the focus of infection was in those pigs aged between 6 and 12 months. The sera of the *pomona* infected pigs cross-reacted with many other serovars, and some marked paradoxical heterologous titres to *copenhageni* were observed. There was no evidence of current infection in the older sows nor in the cattle or wildlife in this area. It was concluded that pig to pig transmission of *pomona* infection occurred, and this was facilitated by the system of management, and the design of the buildings used to house the young stock.

Serological surveys were conducted in two other pig herds where serovar *tarassovi* had previously been isolated. There was serological evidence of extensive infection with both *pomona* and *tarassovi* in both herds. The younger animals were found to be the main reservoir of infection for both these serovars. There was no evidence of any reciprocal cross-protection between *pomona* and *tarassovi*.

A further serological survey of leptospirosis in pigs was conducted, using sera from 234 adult pigs selected from all major districts in New Zealand. It was concluded that infection with serovars *pomona* and *tarassovi* occurred commonly and it was estimated that 53% of the animals sampled had been infected with *pomona* and 33% with *tarassovi*. The prevalence of both *pomona* and *tarassovi* infection is higher in the North Island than in the South Island. *Pomona* infected herds are the most common, followed by herds infected by both *pomona* and *tarassovi*, with *tarassovi* infection alone being the least common. There was no evidence that infection with serovars other than *pomona* or *tarassovi* commonly occurs in pigs in New Zealand.

An abortion storm in pigs was investigated and shown to be due to *pomona* infection. In total 22% of the mated sows aborted. Leptospires were isolated from many tissues from the aborted piglets, but the most convenient tissue to use was vitreous humor. In these abortions a marked acute placentitis was observed. Antibiotic therapy did not prevent abortions.

In vitro lymphocyte transformation was used to investigate whether or not cell mediated immunity (CMI) was present in pigs that had been naturally infected with *pomona*. Lymphocyte microcultures from 18 pigs from a herd known to be endemically infected with *pomona* were prepared. Cells were stimulated with phytohaemagglutinin (PHA), a sonicated *pomona* extract and a sodium deoxycholate-derived *pomona* antigen. The time-responses of antigen stimulated cells over 144 hours were also studied. The PHA responses of the older pigs which had been infected with *pomona* were the same as those of the younger non-infected pigs. The activities of the non-stimulated cultures of these groups were also the same. The cells of all the animals were also transformed *in vitro* by the antigen extracts with dose-responses occurring in most cases. However the maximum responses of the pigs which had been infected with *pomona* were significantly greater than those which had not been infected. It was concluded that transformation in response to the *pomona* antigens had occurred in the cell cultures from animals which had been infected, suggesting that CMI is part of the immune response of the pig to *pomona* infection. It was also considered that the antigen extracts contained a low concentration of non-specific mitogens.

In a further study of these *in vitro* lymphocyte activities, the responses of B cell-depleted cultures to the *pomona* antigen extracts were investigated. The removal of B cells from porcine blood lymphocytes was achieved by nylon wool fractionation and confirmed by enumerating the cells exhibiting surface immunoglobulin. Although the

non-fractionated cells responded to both antigen extracts the eluted cells did not. Supplementation of cultures of eluted cells with adherent cells did not increase the level of transformation achieved. It was therefore concluded that the antigen-reactive cells in this *in vitro* system were probably B cells. Thus, the postulate that CMI is part of the immune response of the pig to infection with serovar *pomona* was not confirmed. However these findings were in accord with the observation that the essence of the resistance to infection with the parasitic leptospires lay with the ability of the animal to secrete antibody soon after infection.

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Some of the results in Chapter IX relate to serology performed in the Palmerston North Animal Health Laboratory, and I wish to thank Mr B L Stevenson for permission to quote these.

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