Final Report Executive Summary

Epidemiology and Control of Leptospirosis in Farmed Deer in New Zealand.

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1. Introduction.

The research presented in this report and thesis was in response to the Deer Industry's desire for robust data on the national distribution and on-farm epidemiology of leptospiral infections, as well as the effectiveness of vaccination in reducing risk to animals and humans and the influence of maternally derived antibody on vaccine response. This research investigated the epidemiology and distribution of infection associated with serovars Hardjobovis, Pomona, and Copenhageni in farmed deer. Serology, bacteriology, and pathology were employed as determinants of infection and vaccine efficacy.

Leptospiral spirochaete bacterial infections are an emerging health problem in human and veterinary medicine, having an important economic impact on production animal industries, and being one of the most frequent zoonoses in the human population (Plank and Dean 2000; Levet 2001). Evidence from surveys of free-ranging and farmed deer indicates that *Leptospira* have been prevalent in many deer populations internationally (Ayanegui-Alcérreca et al 2003). Leptospirosis in farmed deer in New Zealand has been reported intermittently for about 25 years (Ayanegui-Alcérreca et al 2003). Nevertheless, not much was known of its prevalence, epidemiology, subclinical effects, prevention and its significance to human health (Wilson et al 1998). New Zealand has a unique temperate pastoral farming system for deer, often carried out in association with sheep and cattle farming, suggesting that the epidemiology of leptospirosis in deer may have unique characteristics in this country.

2. Chapter 1: Deer Leptospirosis reviews (International and New Zealand).

Leptospira infection in feral and farmed deer occurs worldwide and can be a significant medical and economic problem. The literature on leptospirosis in deer available from 1957 to 2005 included reports from 16 countries. Forty five (68.2%) were from wild deer, 16 (24.3%) were from farmed deer with nine of those from New Zealand, three were experimental infection of captive deer, and two were from a zoo. The majority of reports are from red (29.5%), white-tailed (16.5%), fallow (17.7%), and roe deer (9.5%). Most reports (72.7%) are based on serology only, with evidence for 19 serovars, with the most common serovars reported being Pomona (26.2 %), Hardjobovis (14.9%), Copenhageni (14.7%), Grippotyphosa (10.5%) and Ballum (6.3 %). However, only serovars Hardjobovis, Pomona, Copenhageni and Roumanica have been cultured from deer. Disease, culture and gross and/or histopathological lesions were described in 18 (27.2%) reports involving red, white-tailed, wapiti and Sika deer, following either natural or artificial challenge, and associated with serovars Pomona, Hardjobovis, Copenhageni, Roumanica and Ballum.

The present work also reviewed current knowledge of leptospirosis in farmed deer in New Zealand. There have been several reports in farmed deer in New Zealand, and more recently, human cases linked to farmed deer. Most knowledge evolves from serological studies with culture evidence from some, suggesting infection is widespread, and there are several reports of clinical disease, occurring as both individual cases and outbreaks.

Based on the published literature from New Zealand, serovar Pomona appears to produce more severe clinical disease than serovar Hardjobovis, although the relative risk has not been determined. Disease is usually manifest by haemolysis, jaundice, renal lesions, haemoglobinuria and often sudden death with few pathological signs. Kidney lesions are commonly observed at slaughter and evidence suggests many are associated with leptospiral infections, where Hardjobovis is the predominant serovar which appears to cause less severe kidney lesions than Pomona. Mixing of young stock from several sources appears to be a significant risk factor for outbreaks. Serological evidence of serovars Hardjobovis, Pomona and Copenhageni has been reported with serovar Hardjobovis having the highest prevalence either individually or mixed with serovar Pomona. Infection and disease caused by serovar Copenhageni and others appear uncommon. It is the opinion of the authors that leptospirosis is under-diagnosed and under-reported in the New Zealand farmed deer industry.

Little data exists on the efficacy of vaccines in producing an antibody response, and no data exists on the effect of vaccination on shedding of leptospiral organisms.

3. Chapter 3: A longitudinal retrospective serological investigation of leptospirosis on deer farms.

Serovars Hardjobovis, Pomona and Copenhageni had been previously isolated from farmed deer in New Zealand, but little was known of the epidemiology of these infections. This retrospective seroepidemiological analysis utilised a serum bank from a 2-year longitudinal observational study undertaken in 1992-3 involving 16 deer farms from the Southern North Island. The aim was to evaluate the pattern of the leptospiral infection in the farmed deer. The association between seroconversion and the significant effect variables farm, age, sex, year and season were analysed by logistic regression ($p<0.05$).

All 16 farms and 51.8% of individual animals were seropositive for Hardjobovis at an antibody titre cut point (dilution) of 1:24. Data confirmed that infection with this serovar occurs through the first year of life and is persistent with an endemic pattern. The cycle of infection was similar between years with the highest prevalence in older deer, and lowest prevalence in 4-month-old deer. No reproductive losses or clinical disease was recorded during the years of sample collection. This observation, coupled with high seroprevalence, parallels the findings from cattle in New Zealand, where kidney invasion and potential as a zoonosis rather than animal disease are the primary concern for Hardjobovis. However, sub-clinical production losses remained undetermined.

Four of the farms (25%) and 24.6% of individual animal were seropositive to Pomona, using a titre cut point of 1:48. The pattern within and between farms resembles a sporadic or epidemic infection. Thirteen percent of farms and 10.5% of individual animals were seropositive for Copenhageni. Most titres to Copenhageni were considered to be cross-reactivity to serovar Pomona.

Re-sampling of seven of the herds in 2004 showed all remained seropositive to Hardjobovis. Two were seropositive to Pomona, both of which were seronegative in 1992-3. Two herds seropositive to Pomona in 1992-3 were seronegative in 2004. Data supports the proposal that deer are maintenance hosts for serovar Hardjobovis, and accidental hosts for serovar Pomona and Copenhageni.

4. Chapter 4: Longitudinal observations of *Leptospira* in naturally infected commercial farmed red deer herds.

This study set out to evaluate infection *Leptospira borgepetersenii* sv Hardjobovis and/or *Leptospira interrogans* sv Pomona and Copenhageni in commercial deer herds based on: serological evidence, urine Dark Field Microscopy (DFM) observations, urine and kidney cultures and pathology findings to validate serology. Samples were collected approximately 6 weekly intervals, and the data was used to establish an understanding of the epidemiology of a disease. The study focused largely on rising 1-year-old deer. Three commercial farms from the Southern North Island of New Zealand were selected based on serological evidence of infection with these serovars. Farm 1 (Hardjobovis positive) and Farm 2 and 3 (Hardjobovis and Pomona positive). The farms had no recorded history of vaccination against *Leptospira*. Observations spanned from June 2003 to December 2004 and a total of 160 weaners of both sexes were included.

Culture confirmed serovars Hardjobovis and Pomona only. When combined with serological evidence from this and other studies, this suggests that infection with Copenhageni is not common in farmed deer.

This study showed that serovar Hardjobovis had the highest seroprevalence in deer at 1-2 years of age (Prevalence range (PR) 7.5% to 57.5%) and a geometric mean of the titre (GMT) of 50.8. Titres ranged from 1:0 to 1:3072. Despite no clinical evidence of disease, there were mild kidney lesions and high urine shedding and kidney culture rates. Infection followed an annual endemic cycle with an increasing proportion of animals becoming infected during the first 12 months of age. All these features indicate a host-adapted relationship with this serovar in farmed deer.

Infection with serovar Pomona was characterised by high prevalence within infected herds (PR 15.0%-97.5%; GMT 147.0). Titres ranged from 1:0 to 1:12,228. Clinical evidence of disease and severe kidney lesions were observed. There was a lower culture rate than for Hardjobovis, which is typical of epidemic infection. These observations suggest that farmed deer can act as a maintenance population for Pomona (i.e. at the herd level), and an accidental host at the individual animal level.

The proportion of urine samples DFM positive ranged from 0-60.0% between samplings. Each farm and sampling period was different ($p < 0.001$). Data shows that shedding in urine can persist for more than nine months. Sixty eight percent of the kidneys showed moderate to severe gross lesions. The most frequent lesions were lymphocytic infiltrations and periglomelular damage. The mildest lesions were in kidneys from Farm 1 (Hardjobovis only) with the most severe lesions from Farm 3, with significant difference between farms ($p = 0.002$). Odds ratios for the relationship between a live *Leptospira* (DFM in urine or culture from kidney of urine) and seropositivity ranged between 2.99 to 1.7.

Kidney lesions, active urine shedding, isolates and serology are proof of active infections with Hardjobovis and Pomona in farmed deer. Data establishes the capacity of farmed deer to sustain the infectious cycle, and gives evidence of the role of deer as an important contributor to the epidemiology of infection to other animals and humans.

5. Chapter 5: Studies of a Trivalent Leptospiral Vaccine in Deer.

This is the first longitudinal study of a leptospiral vaccine in commercially farmed deer, and reports on the descriptive data from six vaccine trials conducted over two years on four deer farms from the lower North Island of New Zealand. Farms were selected based on on-farm serological screening for serovars *Leptospira borgepetersenii* Hardjobovis and/or *Leptospira interrogans* Pomona and Copenhageni using the MAT and clinical history. Farm 1, Hardjobovis positive, was used one year. Farms 2 3 were Hardjobovis and Pomona positive and were studied two and one year, respectively. Farm 4 was non-infected, and was studied over two years. Data from weaners on Farms 2 and 4 in Year 2 were the progeny of hinds that were part of maternal immunity trials (Chapter 6).

The vaccine used was the trivalent commercial vaccine "Leptavoid-3" (Schering-Plough Animal Health Ltd, New Zealand, licensed for deer) containing serovars Hardjobovis, Pomona and Copenhageni. A total of 427 deer were used, 50 to 75% of the deer in each group (vaccine and control) were vaccinated and were balanced by sex where possible. Blood was collected from all animals at approximately 6-weekly intervals and as many urine samples as possible were collected from females. Blood, and kidneys were collected from slaughtered deer, predominantly males.

No vaccination site lesions or other untoward responses were observed following vaccination. Serovars Hardjobovis and Pomona, but not Copenhageni, were confirmed by culture in the infected herds, thus serological results for the two former serovars represent a composite of vaccine and natural challenge. Responses suggest that strains in the trivalent vaccine used are homologous with those causing natural infection.

Vaccination produced antibodies in non-infected deer against the three serovars investigated. Vaccination enhanced immunity in infected herds, resulting in a reduction in the proportion of animals shedding leptospiral organisms in urine by 44% (crude vaccine efficacy), and the proportion of kidneys that were culture positive from 52.9% to 23.8% (crude vaccine efficacy 55%), despite on-going natural challenge from non-vaccinated animals in the herds. No difference in gross lesions between vaccinated and control deer were observed.

In one Pomona-infected herd for which data were available, the weaning percentage from vaccinated hinds was 97%, compared with 88% from non-vaccinated deer ($p=0.04$), and weight in rising 1-year-old deer with evidence of infection was 3.7kg less than those without evidence of infection ($p=0.37$).

6. Chapter 6: Serological responses to a Trivalent Leptospiral Vaccine in pregnant hinds and progeny.

This study investigated the antibody response to a trivalent commercial leptospiral vaccine given to pregnant hinds >2-years of age in both infected and non-infected herds, passive immunity in their progeny and subsequent active immunity from 12 weeks of age. This was to establish the pattern of maternally derived antibody and possible interference with vaccination of progeny. Vaccination at 12 weeks of age was chosen based on previous data suggesting that deer can be exposed to infection early in life (Chapters 3 and 4).

Pre-calving vaccination produced enhanced passive immunity in progeny, probably as a result of a combination of natural and vaccine stimulated antibody in the hind and active immunity in progeny exposed to infection at an early age.

The results showed a response in pregnant adult hinds to both the sensitiser and booster doses with titres persisting in more than 40% for at least 100 days and in 20-40% after one year without natural challenge. Regression analyses indicated that progeny of vaccinated hinds were 2.7 to 5.4 times as likely to be seropositive than progeny of non-vaccinated hinds.

There was a higher seroprevalence in progeny of vaccinated hinds in both infected and non-infected herds in Year 1 when hinds received a two-dosage vaccine schedule, than in progeny of vaccinated hinds from farm 4 in year 2, receiving only one booster. There was no detectable maternally derived antibody interference with vaccination of progeny at about 12-16 weeks of age. The vaccine produced similar seroprevalence and GMT patterns to those resulting from natural infection in hinds and progeny and a sustained antibody response in progeny.

This study has shown that pre-calving vaccination of hinds with this multi-strain leptospiral vaccine boosted the specific antibody titres in the hind, leading to elevated antibody levels in progeny providing immunity in both infected and non-infected herds through colostral transfer. It may be inferred that these elevated antibody levels provide a degree of immunity to leptospirosis in progeny and these may have contributed to the greater survival to weaning reported in Chapter 5 above. These data suggest that optimum immunity from vaccination would require annual booster vaccination of previously immunised hinds, followed by vaccination of progeny at about 12 weeks of age.

7. Chapter 7: Regional seroprevalence of Leptospirosis on deer farms in New Zealand.

A regional seroprevalence survey was performed to establish the distribution of infection by serovars Hardjobovis, Pomona and Copenhageni in farmed deer herds regionally throughout New Zealand. Results from 12 to 20 blood samples per herd from 110 unvaccinated red and red x wapiti deer farms are reported (total 2016 samples). Samples from 80 herds were collected on farms, and samples from 30 herds were collected from Deer Slaughter Premises (DSP). Most deer sampled on-farm were hinds 9- to 30-months of age while most sampled in DSPs were males in the same age range. Sampling took place between March 2003 and February 2005, with the majority collected in the latter part of 2004.

Eight two percent of herds showed serological evidence of infection. Hardjobovis was the predominant serovar at the herd and individual level (77.7% and 60.8%, respectively) using a titre cut point of 1:24. Pomona seroprevalence was 20% and 8.4% at the herd and animal levels, respectively, using a titre cut point of 1:48. Within-herd seroprevalence for both serovars ranged from 0 to 100%. Concurrent Hardjobovis and Pomona infections were observed in 16.4% of herds and 6.6% of individuals. No herds in this survey and only 1.2% of animals were seropositive for Copenhageni at cut point 1:48. No regional differences were shown. A concurrent survey indicated that approximately 80% of deer farms were concurrent with sheep and/or cattle.

Thus, seroprevalence of leptospirosis is high and evenly distributed in farmed deer herds thought New Zealand. These results confirm that risk of disease in deer and exposure of infection to humans and other livestock is widespread, suggesting that control of the disease needs to be considered at the national rather than regional level, and that on-farm strategies need to consider other ruminant species.

8. Chapter 8: Relationship between *Leptospira* serology, kidney lesions and culture.

This is the first investigation to test relationships between various potential means for diagnosis of leptospirosis and the risk that a deer was culture positive, therefore potentially a risk to humans exposed to kidneys from deer. The study reports data from 1164 non-vaccinated red and red x wapiti farmed deer from 82 lines at three Deer Slaughter Premises (DSP) in New Zealand in two sampling periods. In Period 1, (1992-1993) 53 herds from the southern North Island (Wilson et al 1998) were included. In Period 2, (2003-2004) 18 herds in Westland and 8 herds from the southern North Island were included. Blood and kidneys were collected.

In addition, blood and kidneys were collected from 197 deer from the three herds at the conclusion of epidemiological and vaccination studies presented in Chapters 4-6 (Farms 1, 2 and 3, respectively). One kidney from each was subjected to gross-histopathological examination and the second kidney used for culture.

Overall individual deer *Leptospira* seroprevalence was 74.14% (1164 samples) while the herd prevalence was 84.1%. Hardjobovis seroprevalence was 79.3% of herds and 67.3% of individuals. Pomona seroprevalence was 40.2% of herds and 25.2% of individuals. In three herds 3.4%-4.6% of individuals were positive to Copenhageni. Dual seropositivity to Hardjobovis and Pomona, and Hardjobovis and Copenhageni, was observed in 34.1% and 3.6% of the herds, respectively.

White spot lesions observed in the kidneys in this study are similar to multifocal chronic interstitial nephritis (MCIN) lesions reported in other species while not pathognomonic, are generally regarded as a indication of leptospirosis (Prescott 1992). White spots were observed in kidneys of more than 70% of herds and 28.7% of individual deer giving a Relative Risk (RR) of having lesions when seropositive of 1.8 for Hardjobovis and 1.6 for Pomona. The sensitivity of white-spots on kidneys as a predictor of Hardjobovis seropositivity was 25.5%, the specificity was 81.1%, the PPV (Positive Predictive Value) was 82.4%, and the NPV (Negative Predictive Value) was 23.8%. The sensitivity of white-spots on kidneys as a predictor of Pomona seropositivity was 35.1%, the specificity was 78.0%, the PPV was 33.1% and the NPV was 79.5%.

Leptospires were isolated from 45/213 kidneys cultured (21.16 %). The culture prevalence was different between farms (2.5%, 8.6%, 39.3% and 31.3 % between farms and years). Significantly higher seroprevalence was observed in animals with cultures and kidney lesions than animals culture positive without kidney lesions (RR 2.15). The RR increased to 3 when both macro-microscopic lesions were observed.

The results confirmed a positive relationship between several diagnostic criteria and kidney culture result, suggesting that these are useful measures to predict the likelihood of a kidney being culture positive and hence a risk to humans exposed to deer kidneys. The bacteriological, pathological and serological findings reported here indicate that leptospiral infections are common in slaughtered farmed deer in New Zealand.

9. Chapter 9: General Discussion and Conclusion.

This study has helped define the epidemiological pattern of infection with leptospiral serovars Hardjobovis, Pomona and Copenhageni in farmed deer. The presence of kidney lesions, positive bacterial isolates and positive serology are proof of active infections with Hardjobovis and Pomona in farmed deer and their capacity to support the infectious cycle. Deer are likely to be an important contributor to the epidemiology of infection in other animals and humans at micro-ecosystem (farm) and macro-ecosystems (region or country) levels.

At the herd and individual animal levels the most prevalent serovar was Hardjobovis with some individual and dual infections with Pomona. There was little evidence of infection with Copenhageni. Hardjobovis appears to cause minimal disease, which was sub-clinical, characterised by mild kidney lesions, active bacterial shedding with a higher rate of seroconversion at 6-12 months of age. All these features indicate a host-adapted relationship with this serovar in farmed deer.

Evidence demonstrates that infections with serovar Pomona were present but at a lower herd and individual animal prevalence. Pomona infection resulted in high titres, was associated with severe and more frequent kidney lesions, and showed an endemic cycle. This suggests a non-host adapted relationship at the animal level. There was no age-related pattern for Pomona. The pattern within and between farms resembles a sporadic or epidemic rather than endemic infection. Most serovar Copenhageni titres were considered cross-reactivity to serovar Pomona with only a few titres possibly the result of the natural infection.

- Data support the proposal that deer are maintenance hosts for serovar Hardjobovis, and accidental hosts for serovar Pomona and Copenhageni.
- Data shows that shedding in urine can persist for more than 9 months.
- Serology is a reliable technique to identify infection at the herd level, since the identity of the isolates matched those in the serology results.
- Kidney lesions are a good indicator of infection at the herd level.
- Combining diagnostic techniques enhanced the chance of a correct diagnosis.
- The commercial trivalent vaccine used in this research produced an antibody response, and reduced leptospiruria and kidney lesions, therefore reducing the risk of leptospiral infections and disease in deer, and transmission to other livestock species and man.

- Results suggest that early vaccination of young animals and annual revaccination of all stock classes would be necessary to achieve effective herd immunity and reduce risk of disease and transmission.

- Vaccination reduced reproductive loss and by reducing infection rate, may produce a growth response in some circumstances. While these findings need to be replicated they suggest the impact of production losses associated with leptopsirosis may be under-estimated on New Zealand deer farms.

- This study has shown that pre-calving vaccination of hinds with this multi-strain leptospiral vaccine boosted the specific antibody titres in the hind, leading to elevated antibody levels in progeny providing immunity in both infected and non-infected herds through colostral transfer.

- Maternally derived antibody did not interfere with response to vaccination of progeny at about 12 weeks of age in infected or non-infected herds, and that an optimum immunity from vaccination would require annual booster vaccination of previously immunised hinds, followed by vaccination of progeny at about 12 weeks of age.

- The presence of white spots in the kidneys of farmed deer at slaughter was associated with a higher probability of having culture positive kidneys.

- The highest probability of a kidney being culture positive was when serology and pathology were combined as a diagnostic test particularly when Pomona was involved.

- There is a significant risk of exposure to people working in areas of the processing plants that can come into contact with kidneys additional to the risk of contact with urine *per se.*

- While serology, gross and histopathology are useful indicators that kidney culture may be positive, particularly at the herd level, evidence is presented that shows there is a risk, albeit lower, that kidneys without lesions and from serologically negative deer may carry leptospires in their kidneys.

- If no deer from a line of deer have kidney lesions, it could be assumed that there is a lower risk of the kidneys being infected. The daily risk of exposure to humans in slaughterhouses would vary between and within herds depending on the leptospiral infection status in the herd and the number of deer carcass handled daily by each worker.