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**Occupational Exposure to Pathogenic
Leptospira from Sheep Carcasses in a New
Zealand Abattoir**

**A dissertation presented
in partial fulfilment of the requirements
for the degree of Masters in Veterinary Studies at
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Abstract

This research was undertaken in response to a rising trend in the incidence of notified human leptospirosis cases, particularly in meat workers, between 2000 and 2005 in New Zealand. Meat workers constituted the highest proportion (65% during 2004) of notified leptospirosis cases. Sheep either alone or in combination with other animals constituted the second most important animal contact source of human infection (after cattle). Further justification of this research was due to the fact that a number of cases were reported from meat workers employed in sheep-only abattoirs and concerns were raised that sheep may be a significant source of human infection.

A longitudinal study was undertaken to determine the serological and cultural prevalence of two of the most commonly diagnosed serovars, *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona. Lines of sheep and individual sheep were systematically randomly sampled at a sheep-only abattoir in Feilding from May 2004 to June 2005. In addition, an assessment of the risk of occupational exposure of meat workers to carcasses shedding live leptospire was carried out using a stochastic model. The association between white-spotted kidney lesions and the serological and cultural prevalence of leptospirosis in sheep was determined along with the diagnostic value of these lesions to predict serological and culture status at the line and individual carcass levels.

The study showed that the prevalence of lines with sheep seropositive to one or both serovars was 44% (95% CI 35–54), corresponding to 45% (95% CI 35–55) of farms. This indicates that nearly half the sampled farms had been exposed to infection previously. The overall individual serological prevalence in the sample of 15,855 sheep processed was 6% (95% CI 5–7). Lambs born in the 2003–2004 season had a significantly higher serological prevalence to one or both serovars at the line and individual animal levels compared with lambs born in the 2004–2005 season, suggesting a strong seasonal effect. The serological prevalence of Hardjobovis was significantly higher than Pomona at the line and individual animal levels. The overall isolation rates of live leptospire from seropositive kidneys of Hardjobovis and Pomona were 22% and 17% respectively, and 1% from seronegative carcasses. From a purposively selected line (suspected of being from a farm with active leptospirosis) all

13 kidneys of seropositive carcasses were culture positive indicating a high risk of exposure of meat workers to leptospire in such a situation. Kidneys from seropositive carcasses were significantly more likely to return culture positive compared with kidneys from seronegative carcasses. The assessment of daily risk of exposure of meat workers indicated moderate risk for eviscerators and meat inspectors ranging from 3–11 (95% CI 0–22) and 6–18 (95% CI 1–34) carcasses potentially shedding live leptospire respectively, and a high risk for offal-handlers that ranged from 18–54 (95% CI 7–91) shedding carcasses.

The results from the third study showed that the prevalence of white spotted kidneys was 16% and 91% at the individual sheep and line levels, respectively. Carcasses with white spotted kidneys were 5.2 times (95% CI 3.9–7.1) more likely to test seropositive to one or both serovars, but lesions were poor predictors of serological status as judged by test sensitivity and positive predictive values. Furthermore, a positive linear association between white-spot kidney lesion scores and seropositivity to either or both serovars was evident. Consideration of lesion status of lines rather than for individual animals resulted in higher test sensitivity but still suffered from a low positive predictive value. Leptospire were isolated from 5% (95% CI 4–8) kidneys that were cultured. There was no statistically significant association between white spotted kidney lesion scores and culture test results in the survey data; however, a significant linear positive association was evident when culture data from a purposively sampled farm was merged with the survey data.

We conclude that the processing of sheep in sheep-only abattoirs constitutes a definite exposure risk of meat workers to leptospirosis and that exposure risks ranged from moderate to high degrees depending on type of duties performed on the slaughter room floor. Furthermore, since grossly visible white-spotted kidney lesions were positively associated with serological and cultural prevalence of disease it would be advisable for meat workers to take extra care when processing lines with a high prevalence of carcasses with these lesions to reduce the risk of infection. This recommendation is made despite the poor predictability of serological and cultural status of these lesions.

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Table of Contents

ABSTRACT	III
ACKNOWLEDGEMENTS	V
TABLE OF CONTENTS.....	VII
LIST OF FIGURES	IX
LIST OF TABLES.....	XI
CHAPTER 1 INTRODUCTION	13
CHAPTER 2 LITERATURE REVIEW	17
2.1 INTRODUCTION	19
2.2 TAXONOMY	19
2.3 MORPHOLOGY AND PROPERTIES	21
2.4 SOURCES AND TRANSMISSION ROUTES OF INFECTION	22
2.5 PATHOGENESIS.....	24
2.6 CLINICAL MANIFESTATIONS	26
2.6.1 Clinical signs in sheep and goats	27
2.6.2 Clinical signs in cattle.....	28
2.6.3 Clinical signs in pigs.....	29
2.6.4 Clinical signs in horses, companion, and wild animals	29
2.6.5 Clinical signs in humans	30
2.7 PATHOLOGY	31
2.8 DIAGNOSIS	33
2.8.1 Direct examination of clinical specimens.....	34
2.8.2 Culture	34
2.8.3 Serology	35
2.8.4 Molecular tests.....	36
2.9 TREATMENT, CONTROL AND PREVENTION	36
2.10 LEPTOSPIROSIS IN NEW ZEALAND.....	38
2.11 CONCLUSION.....	44
CHAPTER 3 PREVALENCE OF PATHOGENIC <i>LEPTOSPIRA</i> IN SHEEP IN A SHEEP-ONLY ABATTOIR IN NEW ZEALAND.....	57
3.1 INTRODUCTION	61
3.2 MATERIALS AND METHODS.....	63
3.3 RESULTS	66
3.4 DISCUSSION	74
CHAPTER 4 ASSESSMENT OF OCCUPATIONAL EXPOSURE RISK TO LEPTOSPIROSIS IN A SHEEP-ONLY ABATTOIR IN NEW ZEALAND	83
4.1 INTRODUCTION	87

4.2	MATERIALS AND METHODS	88
4.3	RESULTS	91
4.4	DISCUSSION	97
4.5	CONCLUSION.....	99
CHAPTER 5 ARE WHITE-SPOT KIDNEY LESIONS IN SHEEP ASSOCIATED WITH LEPTOSPIROSIS?.....		101
5.1	INTRODUCTION.....	105
5.2	MATERIALS AND METHODS	106
5.3	RESULTS	107
5.4	DISCUSSION	111

List of Figures

Figure 3.1: Map of the districts of New Zealand showing the location of nearest towns or localities of farms of origin (triangular point) of slaughtered sheep sampled in a sheep-only abattoir (Abattoir in Feilding) during the period 18 May 2004 to 14 June 2005 in New Zealand.....	67
Figure 3.2: Frequency histogram showing lines sizes of slaughtered sheep sampled in a sheep only abattoir during the period 18 May 2004 to 14 June 2005.....	68
Figure 3.3: Frequency histogram showing the percentages of within-line serological prevalence to either Hardjobovis or Pomona or both in slaughtered sheep sampled in a sheep-only abattoir during the period 18 May 2004 to 14 June 2005.	68
Figure 3.4: Time series plots showing monthly moving averages of seroprevalences of serovar Hardjobovis in Cohort 1 lambs († and dashed line) and Cohort 2 lambs (◆ and solid line) in a New Zealand abattoir. Error bars (—) show the standard errors of the point estimates at calendar week. No sampling was carried out between July and October 2004.....	72
Figure 3.5: Time series plots showing the monthly moving averages of seroprevalences of serovar Pomona in sheep of Cohort 1 lambs († and dashed line) and Cohort 2 lambs (◆ and solid line) slaughtered in a New Zealand abattoir. Error bars (—) show the standard errors of the point estimates at calendar week. No sampling was carried out between July and October 2004.	72
Figure 4.1: A scenario tree outlining the pathways leading to infected kidneys potentially shedding live leptospire and subsequent exposure of meat workers to leptospirosis.....	90
Figure 4.2: Histogram of numbers of sheep slaughtered over different calendar months of the study period from 18 May 2004 to 14 June 2005 at a sheep-only abattoir in New Zealand.....	92
Figure 4.3: Means and 95% confidence intervals (error bars) of the monthly seroprevalence of leptospiral antibody positive slaughter lines (lines were considered positive if one or more carcasses were seropositive to either one or both Hardjobovis or Pomona serovars) in sheep at a sheep-only abattoir in New Zealand during the study period from 18 May 2004 to 14 June 2005. No samples were collected between July and October 2004.....	92
Figure 4.4: Means and 95% confidence intervals (error bars) of the monthly seroprevalence of leptospiral antibody positive carcasses at a sheep-only abattoir in New Zealand during 18 May 2004 to 14 June 2005. No samples were collected between July and October 2004.	94
Figure 4.5: Frequency distribution of the daily risk of exposure to live leptospiral organisms for an eviscerator from carcasses of Cohort 1 (empty bar ☺) and Cohort 2 (filled bar †) at a sheep-only abattoir in New	

Zealand from 18 May 2004 to 14 June 2005 (5000 model runs per cohort).....	94
Figure 4.6: Frequency distribution of the daily risk of exposure to live leptospiral organisms for a meat inspector from carcasses of Cohort 1 (empty bar ☺) and Cohort 2 (filled bar !) at a sheep-only abattoir in New Zealand from 18 May 2004 to 14 June 2005 (5000 model runs per cohort).....	96
Figure 4.7: Frequency distribution of the daily risk of exposure to live leptospiral organisms for an offal-handler from carcasses of Cohort 1 (empty bar ☺) and Cohort 2 (filled bar !) at a sheep-only abattoir in New Zealand from 18 May 2004 to 14 June 2005 (5000 model runs per cohort).....	96
Figure 5.1: Frequency histogram of within-line prevalences of carcasses with one or more WSK lesions on either one or both kidneys in sheep sampled at a New Zealand abattoir during 18 May 2004 to 14 June 2005.	108
Figure 5.2: Random-effect model-adjusted relative risks (◆) and 95% CI (—) for seropositivity in animals with zero, 1 to 5, and >5 white spot lesions on one or both kidneys in randomly sampled slaughtered sheep during the period 18 May 2004 to 14 June 2005.....	112
Figure 5.3: Crude relative risks (◆) and 95% CI (—) for culture positivity in animals with zero, 1 to 5, and >5 white spot lesions on one or both kidneys in sheep during the period 18 May 2004 to 14 June 2005.	112

List of Tables

Table 2.1: Leptospiral serovars isolated in New Zealand and their taxonomy.....	21
Table 3.1: Seroprevalences, shown as percentages with Fleiss adjusted 95% confidence intervals in brackets, of lines and sheep with positive titres of 1:48 or greater to serovars Hardjobovis and Pomona in two cohorts of slaughtered sheep sampled in a sheep-only abattoir during 18 May 2004 to June 2005 in New Zealand.	70
Table 3.2: Seroprevalences, shown as percentages with Fleiss adjusted 95% confidence intervals in brackets, of lines and sheep with positive titres of 1:48 or greater to serovars Hardjobovis and Pomona in two regions in slaughtered sheep sampled in a sheep-only abattoir during 18 May 2004 to June 2005 in New Zealand.	70
Table 3.3: Results of a logistic regression model with line included as a random effect showing model-adjusted predicted mean seroprevalences and 95% confidence intervals (expressed as percentages) of either Hardjobovis or Pomona or both for the explanatory variables tested in the model.	73
Table 4.1: Results of sensitivity analyses showing degrees of correlation (Spearman rank correlation coefficient) between each variable and output (daily exposure risk of a meat worker to carcasses potentially shedding live leptospores) during processing periods of Cohort 1 and Cohort 2 sheep at sheep-only abattoir in New Zealand.....	95
Table 5.1: Test operating characteristics (chi-square test <i>P</i> -values, sensitivity, specificity, positive and negative predictive values with 95% confidence intervals) for occurrence of white spotted kidneys in individual lambs and lines of lambs at slaughter evaluated for gold standard serology and culture status with prevalence ratio estimates (PR), test (TPr) and lesion (DPr) prevalences.	110

Chapter 1

Introduction

The incidence of notified human leptospirosis in New Zealand has shown a rising trend over the recent years with an average annual incidence rate of 4.4 per 100,000 persons after a long term decline trend to a minimum of 2.9 per 100,000 persons observed in 1996–1998. The recent trend also showed that meat workers constituted the highest proportion of notified human leptospirosis, surpassing dairy and pig farm workers who represented the highest risk occupational group in the past. Furthermore, contact with sheep either alone or in combination with other animals constituted the second most important animal contact source for human infection. A number of cases were also reported from meat workers employed in sheep-only abattoirs which raised concerns that sheep may be a significant source of human infection.

These issues coupled with paucity of epidemiological information of leptospirosis in sheep have motivated this work to determine whether or not processing of sheep constituted source of human infection. This thesis provides firstly an update of literature review on leptospirosis with particular emphasis on leptospirosis in sheep and man in New Zealand presented in Chapter 2. It is followed by three descriptive and analytical studies either submitted or prepared for publication in peer-reviewed journals.

Chapter 3 provides results of longitudinal study undertaken to determine serological and cultural prevalence of the two most commonly diagnosed pathogenic *Leptospira*, serovars Hardjovavis and Pomona in a sheep-only abattoir in New Zealand and determined whether or not processing of sheep presented exposure risk of meat workers to leptospirosis. Recognising that processing of sheep constituted exposure risk of meat workers to leptospirosis, daily exposure risk of these workers to carcasses shedding live leptospires was assessed using a stochastic model in Chapter 4.

Since white-spotted kidney lesions are frequently observed in both natural and experimental infection of animals with leptospires, there has been a particular interest of a possible value of white-spotted kidney lesions for indicating current or past leptospirosis episodes in slaughtered cattle and pigs. In addition, meat inspectors and abattoir workers generally assumed that white-spots kidney lesions are related to leptospiral infection which has generated particular interest to study association between these lesions and leptospires

infection. This study therefore took the opportunity to study the association between white-spotted kidney lesions and leptospires infection and evaluated the diagnostic value of the lesion to predict serological and cultural status of carcasses and lines of slaughtered sheep in Chapter 5. These studies have generated valuable information and understanding of leptospirosis in slaughtered sheep and the exposure risk of meat workers processing sheep to pathogenic leptospires. It is also hoped that the findings and information generated from these studies could be effectively utilised for related future studies in devising appropriate control strategies to minimise human infection in sheep processing abattoirs.

Chapter 2

Literature Review

2.1 Introduction

Leptospirosis is a disease characterised by an acute febrile illness occurring in animals and man caused by the pathogenic species of genus *Leptospira* belonging to the family Leptospiraceae, in the order Spirochaetales (Faine et al 1999). It is prevalent worldwide, in both developing and industrialised countries across temperate and tropical regions, and in both urban and rural settings. It is considered to be re-emerging and the most widespread zoonotic disease of global importance (Vinetz 1997; Organization 1999; Levett 2001; Vinetz 2001; Bharti et al 2003; Meites et al 2004). The disease is primarily an occupational zoonosis, affecting farmers, veterinarians, abattoir workers, meat inspectors, sewer workers, rice field workers, sugar cane cutters, banana farmers, and other occupational groups who acquire infection either directly or indirectly from infected animals. Leptospirosis incidence is significantly higher in developing countries with warm tropical climates than in temperate regions, attributed mainly to longer survival of leptospires in the warm and humid environments. These conditions favour establishment of high prevalences in maintenance hosts, such as small mammals, domestic and wild animals. In addition, there is a greater exposure rate of humans to infected animals in developing countries. In temperate regions, the disease is seasonal, with peak incidence occurring in summer or fall when it is warm and humid (Faine et al 1999; Levett 2001). This disease is also an important occupational zoonosis in developed countries, such as Denmark (Holk et al 2000), Germany (Jansen et al 2005), USA (Campagnolo et al 2000b; Meites et al 2004), New Zealand (Blackmore et al 1979; Thornley et al 2002).

2.2 Taxonomy

The family Leptospiraceae currently comprises three genera; *Leptospira*, *Leptonema* and *Turneria* (Faine et al 1999). Before 1989, the genus *Leptospira* was divided into two species, *Leptospira interrogans*, (pathogenic strains) and *Leptospira biflexa* (saprophytic strains) on the basis of growth at 13°C and in the presence of 8-azaguanine (225 µg/ ml) and failure of the latter to form spherical cells in 1M NaCl (Johnson and Faine 1984).

Currently, the two major types of classification system for genus *Leptospira* are based on the phenotypic and genotypic characteristics (Faine et al 1999; Levett 2001). According to phenotypic classification, *Leptospira interrogans* is divided into more than 200 pathogenic serovars and *Leptospira biflexa* into more than 60 serovars, defined by agglutination after cross-absorption with homologous antigen. Two strains are considered to belong to different serovars if more than 10 per cent of homologous titres remains in at least one of the two antisera on repeated testing (Levett 2001). Furthermore, for diagnostic and epidemiological convenience, serovars which share antigens on controlled cross absorption and agglutination are grouped into serogroups, but serogroups on their own have no taxonomic status (Faine et al 1999).

According to the genotypic classification system, the genus *Leptospira* is divided into 18 genomospecies based on DNA-DNA homology measured by DNA hybridization, where DNA from different leptospire species were hybridised and homology ratios calculated under various conditions of stringency. The recently organised 18 genomospecies are: *L. alexanderi*, *L. biflexa*, *L. borgpetersenii*, *L. fainei*, *L. inadai*, *L. interrogans*, *L. kirschneri*, *L. meyeri*, *L. noguchii*, *L. santarosai*, *L. weilii*, *L. wolbachii*, *L. parva*, genomospecies 1, genomospecies 3, genomospecies 4, genomospecies 5 (Brenner et al 1999; Faine et al 1999).

The genomospecies classification is not useful clinically, as it does not correspond to the system of serogroups and both pathogenic and non-pathogenic serovars occur within the same species. In addition, a number of serovars can belong to more than one genomospecies. While the molecular based classification system is taxonomically correct, its limitations and the impracticality of DNA/DNA hybridization for routine identification at the species level require clinical laboratories to retain serological classification for routine clinical and epidemiologic studies until simpler DNA-based identification methods are developed and validated (Levett 2001).

Of the more than 200 pathogenic serovars occurring worldwide, only six belonging to two species, are known to be endemic in New Zealand. Two further serovars, Canicola and Australis have been isolated from humans only (Hathaway 1981; Thornley et al 2002;

Bruere 2003). Of the two genotypes of Hardjo (Hardjoprajitno and Hardjobovis), only Hardjobovis has been identified in New Zealand and Australia (Robinson et al 1982; Ramadass et al 1990). The taxonomy of serovars (adapted from Brenner et al (1999) known to occur in New Zealand is provided in the Table 1.1.

Table 2.1: Leptospiral serovars isolated in New Zealand and their taxonomy

Genomospecies	Serovar	Serogroup
<i>L. borgpetersenii</i>	Balcanica	Sejroe
<i>L. borgpetersenii</i>	Ballum	Ballum
<i>L. borgpetersenii</i>	Hardjobovis	Sejroe
<i>L. borgpetersenii</i>	Tarassovi	Tarassovi
<i>L. interrogans</i>	Copenhageni	Icterohaemorrhagiae
<i>L. interrogans</i>	Pomona	Pomona

2.3 Morphology and properties

The genus *Leptospira* consists of spirochaetes that are flexible, helicoidal in shape, with one or both ends hooked or straight and a sub-terminal flagella located at each end. They are usually 6 to 20µm long and 0.1µm in diameter and can be seen only under dark field microscopy and are not easily stained with routinely used stains (Faine et al 1999). The outer envelope of the characteristic double membrane of leptospire is composed of protein, lipid and lipopolysaccharides (LPS) moieties. Various notable antigens associated with outer envelope LPS are important for serovar identification and a target for naturally acquired immunity. The inner envelope is the cell wall made of a peptidoglycan complex and is separated from the outer envelope by a periplasmic space. Cell contents include fibrillar material, nuclear material, mesosomes and occasionally electron-dense round unidentified inclusions. The two flagellae located at each end of leptospire are locomotor organs and are similar in general structure to flagellae of other gram-negative bacteria. They have a hooked proximal end and disc rotor insertion, intercalated with the layers of the cell wall. A hooked region penetrates the wall, outside of which the flagellum is a central core, presumably made of protein, arranged either in linear, coiled or globular form,

and surrounded by an outer sheath. Some flagellar antigens react with immunoglobulin produced by infected or immunized animals (Faine et al 1999).

Leptospire are motile with characteristic translational and nontranslational movement and vary from microaerophilic to obligate aerobic. However, the initial undefined concentration of CO₂ was found to be essential. They utilise ammonium salts as a nitrogen source, unsaturated fatty acids as a carbon source and purines as protein source They grow in simple media enriched with vitamins (vitamins B₂ and B₁₂ are growth factors). Long chain fatty acids are the sole carbon source and are metabolized by β-oxidation. Optimum growth occurs at pH 7.2 to 7.6 and 20°C to 30°C, respectively. Non-pathogenic leptospire can grow at 11°C to 13°C, whereas pathogenic do not grow at 13° C. Growth usually occurs in 6 to 14 days, but is variable (Faine et al 1999; Levett 2001).

Leptospire do not withstand drying and cannot survive in contaminated water or urine. They can survive freezing temperatures in a protein-containing environment, such as in lyophilised cultures and withstand cryopreservation at approximately -70° C for long time in liquid nitrogen, in frozen tissue block sections at approximately -20° C, and in kidneys on sale in butcher shops (Peet et al 1983; Faine et al 1999). Leptospire do not survive temperatures above 41°C to 42°C (in a laboratory culture medium) and therefore are presumably destroyed in febrile animals at these temperatures. They cannot withstand acidic conditions of pH 6.8 or lower, but may survive in alkaline conditions up to pH 7.8 to 7.9 (Faine et al 1999).

2.4 Sources and transmission routes of infection

The main sources of leptospiral infection for humans are small feral or domestic mammals and it is thought that every known species of mammals and marsupial can carry and excrete leptospire (Faine et al 1999). From an epidemiological perspective, it is important to know that three main types of leptospire-host relationship exist in nature, maintenance or

reservoir¹, spillover² and aberrant³ or incidental hosts. Reservoir hosts are vital for the persistence of leptospire in the environment. Endemic infection occurs in reservoir hosts and the cycle of infection is most commonly maintained by direct transmission. In this host, infection usually occurs at young age and the prevalence of chronic urinary excretors increases with the age. Spillover hosts acquire infection through direct or indirect contact with reservoir hosts and while infection may often be severe the duration of leptospire excretion is often self-limited in these hosts. It is possible that animals may be reservoir hosts of some serovars but spillover hosts of others (Faine et al 1999; Levett 2001).

Small mammals are often important reservoirs and a source of infection to humans and domestic animals such as cattle, pigs, and dogs. Dairy cattle are reservoir hosts of serovars Hardjo, Pomona, and Grippotyphosa, pigs for serovars Pomona, Tarassovi, or Bratislava and dogs for serovar Canicola (Hathaway 1981; Faine et al 1999). The general perception is that sheep are not considered to be reservoir hosts for any serovar (Blackmore et al 1982; Hathaway et al 1982), although other studies have shown some evidence of sheep acting as reservoirs for at least serovar Hardjo (Gordon 1980; Cousins et al 1989; Gerritsen et al 1994).

Independent transmission cycles for different serovars between ecologically associated rodents and farm animals have been described, where serovars commonly affecting domestic animals differ from those affecting rodents and other small animals (Faine et al

¹ A maintenance or reservoir host is one which maintains infection persistently in the population, and often either does not get disease, or there is only mild disease, or only young animals are clinically infected while adults are immune or only subclinically infected.

² A spillover host is one which is susceptible to infection if exposed, and excretes the agent and transmits infection to other hosts, but would not maintain infection within the species in the long term unless there is constant or intermittent replenishment of infection from a reservoir host species. So if exchange of infection with reservoir hosts is eliminated, infection will sooner or later die out in spillover hosts. Commonly, spillover hosts suffer much more severe disease than reservoir hosts do, and disease affects a wider range of age groups than in reservoir hosts. There can be cascades of spillover hosts, with infection in one such host species spilling over into a second, and so on. However, if the reservoir host source is removed, the spillover cascade will eventually dry up. Controlling a disease in spillover hosts will only work for the short term, unless further transmission from reservoir hosts is prevented.

³ An aberrant host is one which is only rarely infected, commonly suffers severe disease, and usually does not excrete sufficient virus to transmit to other hosts. Aberrant hosts are therefore unimportant in the epidemiology of the disease, but may be severely affected. Man is an aberrant host for leptospirosis.

1999). The rate of transmission is influenced by many factors, such as the degree of contact between reservoir hosts and spillover hosts, population density, size of animals, urine output, duration and concentration of leptospire excretion in urine, environmental conditions such as climates, temperature, moisture content, pH and the chemical composition of the medium on to which leptospire are excreted in urine. Most leptospirosis outbreaks in animals (Smith and Armstrong 1975) and humans (Trevejo et al 1998; Kupek et al 2000) are associated with rainfall and surface flooding.

Transmission can be direct or indirect. Direct transmission of infection occurs through transplacental, sexual contact, suckling from an infected mother and via the genital tract (Ellis et al 1986; Faine et al 1999), infected semen or embryos used for artificial insemination, and *in vitro* fertilisation (Bielanski and Surujballi 1998; Faine et al 1999). Direct transmission is the main pathway for dairy and pig farmers, veterinarians, meat inspectors and abattoir workers (Faine et al 1999) through direct handling of animals or animal products. The main route of entry of leptospire is via abrasions or cuts in the skin and the conjunctiva or mucous membranes of the alimentary or respiratory tracts. Infection may also be mediated through intact skin after prolonged immersion in water (Faine et al 1999; Levett 2001). Indirect transmission of infection commonly occurs through contact with contaminated surface water, sewage, slaughterhouse drainage fluids, drain water, and mud and soil.

2.5 Pathogenesis

The pathogenesis of leptospirosis is similar, if not identical, for every animal species known to be infected, including humans (Faine et al 1999). Leptospire do not localise at the site of entry, but spread rapidly via lymphatics to the blood stream, from where they circulate to all tissues. Motility of leptospire may be important in initial infection and spread of organisms from the site of entry to organs such as lung, liver, kidney, eye and brain. Avirulent leptospire are rapidly cleared from the blood stream by reticulo-endothelial phagocytosis, whereas pathogenic leptospire can evade this process. Leptospire replicate exponentially in the blood stream and associated tissues with doubling times of approximately 8 hours.

The characteristic primary lesions involve damage to the walls of small blood vessels, leading to leakage and extravasations of cells and haemorrhages. They may be followed by other lesions produced as a result of secondary effects (Faine et al 1999). There is a paucity of information available on actual mechanisms by which leptospire cause disease. Nonetheless, certain virulence factors described below are thought to be important in the pathogenesis, toxin production, adhesion, and immune mechanism (Faine et al 1999; Levett 2001).

The pathological changes resulting from endotoxins, haemolysins (sphingomyelinase produced by serovars Ballum, Hardjo, Pomona, and Tarassovi) and protein cytotoxins (produced by serovars Pomona and Copenhageni), as well as glycolipoproteins with cytotoxic activity (produced by serovars Copenhageni and Canicola) have been demonstrated either in vitro or in vivo (Faine et al 1999; Levett 2001). Attachment of virulent leptospire to renal epithelial cells in vitro and enhancement of attachment by sub-agglutinating concentration of homologous antisera has also been demonstrated. In human patients with renal failure, hypokalaemia accompanied by lower levels of serum sodium and higher serum concentration of aldosterone and cortisol compared with normal or acute tubular necrosis control patients was observed. Elevated peptidoglycan levels resulting from leptospire induced TNF alpha production in monocytes consistent with murine tumor necrosis are associated with severity of disease (Faine et al 1999). Cytolytic activity results from the action of phospholipases on erythrocytes and other cell membranes containing phospholipids and LPS activity causes haemorrhages and coagulation defects, which are features of severe forms of leptospirosis, due to thrombocytopenia and independent fibrinolysis consistent with disseminated intravascular coagulopathy. Lipopolysaccharides from virulent leptospire cause greater cytotoxicity and aggregation of platelets with release of ATP and serotonin. In fatal cases of human leptospirosis with pulmonary haemorrhages, thrombocytopenia results from platelet aggregation and adhesion to vascular endothelium, where there may be evidence of leptospiral antigen. Lipopolysaccharides are highly antigenic and react in ELISAs (Enzyme-linked immunosorbent assays) or immunodiffusion tests and sensitise erythrocytes to immune agglutination or lysis. Epitopes in LPS are apparently involved in agglutination and it is

clearly an antigen to which there is relevant infection response. The same epitopes identified by polyacrylide gel electrophoresis (PAGE) react both with the monoclonals known to be protective in animal experiments and with convalescent sera, showing that patients gradually develop clinically relevant protective antibodies reacting with identified epitopes of LPS (Faine et al 1999).

Disappearance of organisms from the blood coincides with production of antibodies in the immune phase. A factor that influences the severity of symptoms comes from inflammation associated with production of immune complexes. Generalized infection in humans and horses may cause autoimmune uveitis. Autoimmunity also influences the progression of renal lesions in dogs (Faine et al 1999). In experimental studies in guinea pigs, it was observed that leptospiral antigen localizes in the interstitium of kidney, while immunoglobulins G (IgG) and complement 3 (C3) were deposited in the glomeruli and in the walls of small blood vessels (Yasuda et al 1986). Sensitised individuals harbour leptospires in the anterior chamber of the eye, which is an immunologically privileged and isolated site and subsequent contact with leptospires can cause auto-immune inflammation of the uveal tract, a condition known as “moon-blindness” in horses (Faine et al 1999).

Immunity to leptospirosis is primarily humoural and is specific for homologous or closely related serovars. It is usually long lasting and protective even at low levels of antibody. Immunity to leptospirosis is primarily due to antibody against LPS antigen, however, investigation of vaccine efficacy in cattle and swine demonstrated that antibody against LPS alone was not sufficient for protection from infection and disease. Cell mediated immunity, although not detected in cattle naturally infected with serovar Hardjo, may be a critical component in vaccine-induced protection in cattle (Faine et al 1999).

2.6 Clinical manifestations

Leptospirosis is generally characterised by acute febrile illness in both humans and animals and its manifestation varies from subclinical to overt and serious forms. Regardless of the manifestation, chronic renal carrier states are inevitable and play a vital role in maintaining leptospires in the environment and the epidemiology of the disease. Leptospires may persist

in kidneys by adhering to the proximal renal tubular epithelial cell border for periods of weeks to years and lifetimes, and are shed in urine either continuously or intermittently.

In general, the incubation period in animals ranges from 3 to 7 days in the acute form. It is characterised by fever, listlessness, loss of appetite, irritability, congestion of mucous membrane conjunctiva, and sometimes diarrhoea. Signs of haemorrhage and jaundice may be observed followed by recovery or death. In recovered animals, weight loss, runting in young animals, and chronic renal failure eventually leading to death may be observed.

2.6.1 Clinical signs in sheep and goats

The incubation period ranges from 4 to 6 days. The commonly observed clinical signs in acute leptospirosis in sheep are marked anorexia, depression, breathlessness and fever (0.5°C to 2°C) lasting for 4 to 5 days. Haemoglobinemia and haemoglobinuria with dark red urine colour may be observed in some animals (Vermunt et al 1994b; Faine et al 1999). Abortions and still births in pregnant animals and neonatal or lamb mortalities occur following infection with serovar Hardjo (Beamer 1953; McCaughan et al 1980; Ellis et al 1984) and serovar Pomona (Smith and Armstrong 1975; Davidson and Hirsh 1980). Acute haemolytic anaemia accompanied by depression, dyspnoea, tachycardia and high lamb mortality rates in lambs due to infection with serovar Pomona have been reported (Smith and Armstrong 1975; Davidson and Hirsh 1980; Vermunt et al 1994a; Vermunt et al 1994b). Fever, haemolytic anaemia, and haemoglobinuria were observed in an experimental study of ovine leptospirosis with serovar Pomona (Morse 1957). Clinical leptospirosis in sheep is commonly due to serovar Pomona in Australia (Sullivan 1974), America (Beamer 1953; Davidson and Hirsh 1980) and New Zealand (Vermunt et al 1994a; Vermunt et al 1994b). Agalactia associated with serovar Hardjo infection in sheep has also been reported (McKeown and Ellis 1986).

Subclinical infection with seroconversion, leptospiruria and focal interstitial nephritis has been reported in experimental infection studies of sheep with serovars Balcanica or Hardjo (Durfee and Presidente 1979; Hathaway and Marshall 1979). In a similar study, when sheep were infected with serovar Hardjo, the clinical manifestations were sufficiently mild to pass

unnoticed in the field (Andreani et al 1983). Experimentally infected sheep in this study developed pyrexia that lasted for 2 to 6 days and leptospiremia lasting 2 to 6 days after 4 to 8 days post inoculation was observed in all infected animals. Leptospire were isolated from the urine of two ewes on 35th days post inoculation but there was no spread of infection to control sheep. Clinically normal lambs with high microscopic agglutination test (MAT) titres were reported in New Zealand during outbreaks of serovar Pomona (Vermunt et al 1994b). Acute or carrier infections in sheep and goats are reported relatively rarely (Faine et al 1999).

2.6.2 Clinical signs in cattle

Acute leptospirosis in calves and dairy cattle can result from infection with a range of serovars, particularly Pomona. Commonly observed clinical signs include high fever, haemolytic anaemia, haemoglobinuria, jaundice, pulmonary congestion, and occasionally meningitis and death. In dairy cattle transient fever with a precipitous drop in milk production ('milk drop syndrome') lasting for 2 to 10 days is commonly reported (Faine et al 1999). The udder will be soft and flabby and milk yellow in colour with colostrum like consistency. The somatic cell count will be high. This condition occurs most commonly with Hardjoprajitno infection, but may also occur from infection with Hardjobovis or other serovars. When cattle are infected by serovars which are not adapted, additional symptoms such as agalactia with small quantities of blood-tinged milk may be seen and abortion storms may occur. Recovery usually takes up to 10 days and cows that show a significant drop in milk production usually do not return to full production in the affected lactation (Faine et al 1999). Recovered cattle may show suboptimal growth and significant renal lesions at slaughter leading to discounting or condemnation of carcasses. The subclinical form of the milk drop syndrome may also occur in lactating cows infected with serovar Hardjoprajitno where infection is endemic (Dhaliwal et al 1996)

Chronic leptospirosis in pregnant cattle associated with serovars Hardjo or Pomona causes abortion, stillbirth, or birth of premature and weak infected calves. Retention of foetal membranes following abortion is also common. Congenitally infected new borne calves are often weak and affected by degeneration of the liver or kidneys or both. Interestingly,

chronic infection with Hardjo may cause abortion weeks to months after initial infection. This makes diagnosis difficult as the antibody titres in the dam will be low or falling at the time of abortion. Although the evidence is not conclusive, infertility, as measured by prolonged calving intervals, increased numbers of services-per-conception and early embryonic loss, is thought to be due localisation of leptospire in the uterus and oviduct of Hardjo infected cattle. A significant number of cattle infected with Hardjo but not detectable serologically respond well to antibiotic therapy (Faine et al 1999)

2.6.3 Clinical signs in pigs

Pigs show similar symptoms to those described for sheep and cattle. The common clinical manifestations seen in young pigs with acute leptospirosis are fever, rapid onset of weakness, anorexia, congestion of conjunctiva, jaundice, and convulsions. Body temperature rises by 0.5°C to 1.5°C. Haemorrhages, haematuria, and jaundice accompanied by signs of kidney failure followed by death may be seen in newborn and young piglets. Pregnant sows, newborn and young piglets are most susceptible to acute leptospirosis, whereas adult and non-pregnant pigs may act as asymptomatic carriers. In pregnant sows, abortion, stillbirths or birth of weak or sick piglets, commonly appearing 14 to 60 days after exposure may be the only signs of infection. Recovered pigs show suboptimal growth and shed high number of leptospire in the urine. Other signs include decreased farrowing rates, repeat breeding, and delayed return to oestrus suggestive of early embryonic death in infection with serovars such as Bratislava (Faine et al 1999).

2.6.4 Clinical signs in horses, companion, and wild animals

Other animal including horses, common pets and wild animals show symptoms similar to those seen in other animals. Infected horses exhibit varying degree of fever, accompanied by anorexia, conjunctival suffusion, mucosal petechiae, haemoglobinuria, anaemia, jaundice, depression and weakness lasting 5-18 days. Pregnant mares may abort and leptospire may be found in foetal tissues, placentas, and urine of dams. Up to 45% of horses may develop the condition called 'moon blindness' 2 to 8 months after initial infection as a result of recurrent iridocyclitis, or uveitis (Faine et al 1999).

2.6.5 Clinical signs in humans

The usual incubation period in humans is 5 to 14 days, but may extend from 2 to 30 days. Clinical manifestations in most types of leptospirosis in humans are rapid onset of headache, fever, myalgia, sometimes with rigors, accompanied by nausea with or without vomiting, conjunctival suffusion, a transient skin and mucosal rash, photophobia and other signs of meningism. Progression of disease depends on the infecting serovar, the infective dose, nutrition of the patient and access to immediate medical attention (Faine et al 1999). Contrary to earlier belief that the distinct clinical syndromes produced were determined by the specific serogroup, recent and more intensive studies demonstrated no such association (Levett 2001; Vinetz 2001; Bharti et al 2003). It was rather determined by the ecology of reservoir hosts within geographic regions. Severe leptospirosis in humans is frequently associated with serovars Icterohaemorrhagiae, Copenhageni, Bataviae where 5 to 15 percent of patients infected with these serovars develop the severe icteric or typical Weil's disease syndrome (Faine et al 1999). Leptospirosis in humans is often biphasic in nature with a week long septicaemia phase followed by an immune phase with antibody production and shedding of leptospores in urine. Most complications of leptospirosis comes from localisation of leptospores in tissues coinciding with the immune phase, usually in the second week of illness (Levett 2001). The frequency of reported symptoms in 381 notified cases of leptospirosis in Australia (1 January 1998 to 30 June 1999) were headache (73.5%), severe fever (66.7%), chills (66.4%), sweats (66.4%), myalgia (68.0%), nausea/vomiting (55.9%), arthralgia (48.0%), back pain (33.3%), conjunctival suffusion (21.0%), mild fever (24.1%), renal involvement (15.7%), respiratory involvement (15.5%), vision disturbance (9.7%), diarrhoea (6.6%), rash (6.0%), and pulmonary haemorrhage (3.9%) (Smythe et al 2000)

Levett (2001) described two forms of human leptospirosis, anicteric (mild form) and icteric. Most human infections are anicteric with subclinical or mild severity, and patient may not seek medical advice. The asymptomatic or subclinical form of leptospirosis in humans is common where the disease is endemic (Bharti et al 2003). Common symptoms of anicteric leptospirosis are febrile illness of sudden onset, headache, chills, myalgia, abdominal pain, conjunctival suffusion, and occasionally transient skin rashes. Symptoms

subside after about one week when antibodies develop. The fever may recur 3 to 4 days after an initial remission. There may be severe headache, symptoms very similar to and easily confused with those of dengue fever in tropical regions, retro-orbital pain, and photophobia. Very severe myalgia affecting the lower back, thighs, and calves is also common and aseptic meningitis is reported in about 25 per cent of cases, mainly in young children. Cases of anicteric leptospirosis are seldom fatal, but a few deaths associated with pulmonary haemorrhage have been reported.

Icteric leptospirosis is more severe and its course is more rapid than the anicteric form. Severe cases are often detected late in the course of the disease, thereby contributing to the high mortality rate of 5% to 15%. Jaundice, acute renal failure and pulmonary haemorrhages may be observed. Pulmonary haemorrhage may be severe enough to cause death and is considered the major complication of leptospirosis, which may be accompanied by coughing, dyspnoea, haemoptysis and other respiratory disorders. Cardiac involvement is common both in icteric and anicteric leptospirosis with mortality reaching up to 54% in severe cases with myocarditis. Myocarditis appeared to be strongly associated with pulmonary symptoms in the few fatal cases of anicteric leptospirosis. Other complications include foetal death and abortion in pregnant women, rhabdomyolysis, thrombocytopenic purpura, reactive arthritis, epididymitis, Guillain-Barré syndrome and chronic uveitis (Levett 2001). Encephalitis-induced coma has been reported in some patients (Dimopoulou et al 2002) and a fatal intra-cerebral haemorrhage due to leptospirosis was reported in a 47 year old sewerage drain worker (Theilen et al 2002) and a case fatality ranged from 1% to 7.9% in China, 0.8% in Brazil, 0.7% to 13.9% in India and 5% in Thailand (Vinetz 2001).

2.7 Pathology

Histopathological changes observed due to leptospirosis are similar in animals and humans. They are primarily related to vasculitis, endothelial damage of small blood vessels, followed by extravasations of fluid, leptospire, erythrocytes, infiltration of inflammatory cells such as neutrophils, monocytes, histiocytes, and plasma cells (Faine et al 1999; Levett

2001). Endothelial damage is caused by leptospiral toxins. Gross pathological findings are jaundice of varying degree depending on the severity of the disease. Petechial or larger haemorrhages are commonly observed over extensive parts of the body organs and tissues including subcutaneous tissues and fat, peritoneal, pleural, pericardial and meningeal membranes. Pathological changes are most notably seen in the kidneys, liver, heart and lungs, although other organs may be affected depending on severity of infection. Lymph nodes are enlarged and haemorrhagic. In severe cases of pulmonary haemorrhage in humans, pulmonary congestion, haemorrhages and infiltration of alveolar spaces by monocytes and neutrophils are observed. In the interalveolar septa, leptospire may be seen lodged within the endothelial cells. The heart appears pale and flaccid and pericardial effusion may be present. Petechial haemorrhages may be seen on epicardium and endocardium. Interstitial myocarditis with infiltration of predominantly lymphocytes, plasma cells, and coronary arteritis and some cases of toxic myocarditis leading to death have been reported. The gross structure of the liver is not usually altered but liver cells become irregular, swollen and degenerated with intrahepatic cholestasis. Kupffer cells become engorged and contain leptospire. Skeletal muscles show degeneration and focal necrosis of isolated muscle fibres occurs, with infiltration of histiocytes, neutrophils, and plasma cells. In the brain, focal extravasations and haemorrhages, subarachnoid perivascular cuffing and small areas of capillary thrombosis and ischaemic necrosis may be seen. Ischaemic damage may also be observed in the spinal cord (Faine et al 1999; Levett 2001).

Notable histopathological changes in almost all cases of leptospirosis in animals and humans are observed in the kidneys. The kidneys may be swollen, yellow-green (patients with jaundice) with pin-point haemorrhages on the kidney surfaces. Vascular changes always precede the ischaemic renal damage, leading to necrosis of cells of the proximal convoluted tubules. The cortex is ischaemic and the medullary vessels are dilated with medullary haemorrhages. Histological examination of kidneys of infected carriers may show interstitial nephritis but renal pathology may be absent in chronic carriers (Bharti et al 2003).

In all domestic animals, the consistent lesions seen in kidneys are varying number of white spots, 1 mm to 5 mm in diameter on subcapsular surfaces on gross examination representing multifocal interstitial nephritis (MFIN) which is common in subacute and chronic infection (Smith and Armstrong 1975; Davidson and Hirsh 1980; McCaughan et al 1980; Baker et al 1989; Wilson et al 1998; Faine et al 1999). However, some chronic carriers have grossly-normal kidneys (Faine et al 1999). Kidneys may be enlarged, pale brown and friable in consistency.

Microscopically, cortical cellular necrosis, petechiae and echymotic haemorrhages particularly in the glomeruli and the proximal convoluted tubules are observed. Leptospire may be seen within renal tubular epithelium on microscopic examination using Warthin-Starry silver stain.

The main pathological findings associated with fatal cases of leptospirosis are a varying degree of jaundice, widespread haemorrhages and anaemia, with bloodstained exudates and urine (Vermunt et al 1994b; Faine et al 1999). Perivascular cuffing and haemorrhages in the brain and vacuolation of surfaces of endometrial cells in the uterus may be observed. While there is clear evidence of genital infection and venereal transmission are described, there is limited information describing pathological changes in the associated organs and tissues (Faine et al 1999).

2.8 Diagnosis

Choice of the most appropriate diagnostic test depends on whether the objective is confirmation of disease or epidemiological surveillance and whether the disease process is acute or chronic. Diagnostic tests commonly applied for leptospirosis are: a) direct examination of clinical specimens for organisms, b) culture and isolation of leptospire from clinical specimens, c) serological diagnosis, and d) molecular diagnosis. General clinical laboratory findings such as erythrocytes sedimentation rate, liver function tests and urine analysis are useful for initial guidance.

2.8.1 Direct examination of clinical specimens

Dark-field microscopic examination of clinical specimens is useful in early infection (leptospiremic phase), usually between 3 to 7 days. Leptospirems with their characteristic shape and movement visualised under dark-field microscopy offer a quick diagnostic method but it suffers from poor sensitivity and specificity (Faine et al 1999) as approximately 10^4 leptospirems/ml are necessary for one cell per field to be visible under dark-field microscopy (Turner 1970). Staining methods such as silver staining, the Warthin-starry staining of histopathological tissue specimens, immunofluorescence staining of bovine urine, water, and soil, immunoperoxidase staining of blood and urine, and immunohistochemical methods increase sensitivity.

2.8.2 Culture

Culture and identification of leptospirems is the definitive test of diagnosis. Blood, cerebrospinal fluid (first 5 to 10 days), urine (usually from 1 to 3 weeks after onset of symptoms) and tissues such as kidney sections can be cultured in leptospirems selective media. Specimens collected for culture should not contain antibiotic residues, tissue autolysis should not be advanced, and in the case of urine, an optimum pH should be maintained. Specimens should be transported refrigerated in suitable transport medium to prevent overgrowth of other bacteria. Commonly used selective media are EMJH (tween-albumin), Korthof, Stuart and Fletcher media. Selective media containing sodium sulfathiazole (50 μ g/ml), neomycin sulphate (5 μ g/ml) and cycloheximide (0.5 μ g/ml) reduce the risk of contamination and improve recovery rates of leptospirems. For tissue culture such as kidney sections EMJH containing tween 80/40/lactalbumin hydrolysate, or with 0.15% agar and 100 μ g/ml to 200 μ g/ml of 5-fluorouracil and 0.4% to 1.0% rabbit serum give best growth even for fastidious and slow growing serovars such as Bratislava. Inoculated culture media are incubated at $29^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and examined at weekly to fortnightly intervals for up to 3 to 6 months before declaring a negative result. Culture method does not identify multiple serovars in mixed infections and further serological or molecular techniques are required to identify the serovars involved (Faine et al 1999).

2.8.3 Serology

The reference serological diagnostic test for leptospirosis is the microscopic agglutination test (MAT). It is the most commonly used diagnostic test for humans and animals (Faine et al 1999). The MAT detects agglutinating antibodies in serum that react with live antigen suspensions of leptospires, and in general has high sensitivity and specificity (Cumberland et al 1999). Agglutinating antibodies (IgM) usually appear in blood within 3 to 10 days after the onset of symptoms but delayed appearance for as long as 3 to 4 weeks after initial infection has been reported (Faine et al 1999). The antigen used should include a range of serovars representative of all serogroups and those known to exist in the local environment. The antibody titres to locally isolated leptospiral antigen are often found to be relatively higher than titres to laboratory stock strains of serovars within the same serogroup.

The MAT is a serogroup-specific assay, contradictory to a widely held earlier belief that it was serovar specific. A high degree of cross-reaction may occur between different serogroups, especially in the acute-phase (mostly IgM antibody) while the convalescent phase (mostly IgG antibody) is associated with comparatively high serogroup specificity using the MAT (Levett 2001). Microscopic agglutination test positive samples are identified by a fourfold or greater rise in titres between paired sera collected 5 to 10 days apart. A titre of 1:100 or greater in previously negative samples is taken as confirmatory evidence of leptospirosis in areas where the disease is endemic. Antibody levels usually decline over weeks or months, although in some human cases they may persist for 2 to 10 years. The MAT is the appropriate test for epidemiological serosurvey with titres of 1:25 (Blackmore et al 1984) to 1:40 and more for Hardjo (Bey and Johnson 1986) required for evidence of past infection. For herd level testing, at least 10 animals from each herd or 10 per cent of the herd, whichever is greater should be tested (OIE 2004).

Methods developed to overcome the disadvantages of the MAT include the complement fixation test, the macroscopic agglutination test, microcapsule agglutination, the Potac slide agglutination test, the indirect haemagglutination test, sensitised erythrocyte lysis, counterimmunoelectrophoresis, and various types of ELISAs (Faine et al 1999; Levett 2001). The IgM-ELISA has higher sensitivity than the MAT early in the acute phase

(Swart et al 1983; Thierrmann and Garrett 1983; Cumberland et al 1999). Although, ELISA methods are reasonably sensitive they lack the serovar specificity of the MAT. Some studies that evaluated ELISA methods includes diagnoses of Hardjo in sheep (Adler et al 1981; Cousins et al 1991), Hardjo and Pomona in cattle (Thierrmann 1983; Cho et al 1989), a competitive ELISA for detection of Pomona antibodies in bovine sera (Surujballi and Mallory 2001), monoclonal antibody-based dot-blot ELISA for detection of *Leptospira* species in bovine urine (Suwimonteerabutr et al 2005)

2.8.4 Molecular tests

Molecular diagnostic tests include DNA probes, DNA-DNA hybridization analysis, polymerase chain reaction (PCR) based strategies and molecular typing such as digestion of chromosomal DNA by restriction endonucleases (REA), restriction fragment length polymorphism (RFLP), ribotyping, and pulsed-field gel electrophoresis (PFGE) (Faine et al 1999; Levett 2001). Some of the studies that evaluated PCR-based diagnostic methods include magnetic immuno-capture PCR assay for detection of serovar Hardjo in cattle (Taylor et al 1997), PCR in dogs (Harkin et al 2003), PCR in humans (Merien et al 1995; Merien et al 2005). Bajani et al (2003) evaluation of commercially available rapid serologic tests for diagnosis of leptospirosis in humans.

2.9 Treatment, control and prevention

Treatment of leptospirosis in both humans and animals involves chemotherapy with appropriate antibiotics and supportive management depending on the severity and conditions of patients. Doxycycline 100 mg twice daily for 7 days reduces the duration and severity of illness in anicteric leptospirosis in humans. Doxycycline given orally at the rate of 200 mg weekly is used chemoprophylactically in humans in high risk environment. Penicillin has also been used but with mixed results (Faine et al 1999; Levett 2001). A randomised clinical trial of doxycycline prophylaxis in endemic areas of the North Andaman islands of India found no difference in infection rates using MAT, but a significant protective effect in reducing morbidity and mortality (Sehgal et al 2000).

Treatment with streptomycin (dihydrostreptomycin) at various doses in cattle and pigs reduced clinical illness, and prevented abortion as well as the carrier state. Amoxicillin is an effective treatment for Hardjo infection in cattle (Faine et al 1999; Marshall and Manktelow 2002).

Control and prevention of leptospirosis in animals requires a holistic approach that incorporates a range of strategies. It includes tracing and identification of sources of infection, control of spread of infection among reservoir hosts, and reducing direct and indirect contact between reservoir and spillover hosts. For control in humans, occupational hygiene and safety measures, education and awareness of high risk populations, and instituting mandatory disease notification and surveillance systems are required (Faine et al 1999). Immunisation of domestic animals is a critical component adopted routinely in many countries with dual aims of protecting animals as well as reducing the incidence in humans by reducing the incidence in animals. Typically, polyvalent vaccines that protect against common locally occurring serovars are used because vaccinal immunity is serovar specific, or will produce only incomplete protection against closely related members of the same serogroup. Vaccination of sheep against leptospirosis is not routinely practiced in New Zealand, whereas cattle are routinely vaccinated using bivalent vaccines against serovars Hardjo and Pomona. Pigs are routinely vaccinated against serovars Pomona, Tarassovi and sometimes Bratislava. Vaccination of pregnant animals confers passive immunity in newborns for several months. Conflicting results emerged from a vaccination trial in cattle with several vaccinated animals harbouring leptospires in the kidney and uterus. Nevertheless, vaccination of dairy cattle and pigs was successfully implemented in New Zealand and was clearly beneficial (Marshall 1987; Faine et al 1999).

Currently, there is no effective leptospiral vaccine available for human use, although some countries like China, Russia, and Cuba have developed vaccines for human use. Cuba has claimed a 100% protection of people from the use of trivalent vaccines containing serovars Canicola, Icterohaemorrhagiae, and Pomona and reported no side-effects amongst 100,000 persons who were vaccinated. Problems generally encountered in the development of effective leptospiral vaccines for humans are short-term and incomplete protection, lack of cross-immunity between serovars coupled with wide range of serovars infecting humans,

particularly in tropical developing countries, and the possibility of inducing autoimmune disease such as uveitis (Bharti et al 2003).

2.10 Leptospirosis in New Zealand

The first reported leptospirosis outbreaks in New Zealand were in 1952 in calves and humans (Bruere 1952), in humans, calves and pigs (Kirschner et al 1952), and in sheep (Hartley 1952). All were attributed to serovar Pomona. Outbreaks of leptospirosis have caused calf mortality, haemoglobinuria and abortion in dairy cattle (Te Punga and Bishop 1953; Salisbury 1954) and lamb mortality along with leptospirosis related clinical symptoms in sheep (Webster and Reynolds 1955). A serological investigation was carried out in pigs for *Leptospira* by Russell and Hansen (1958). Epidemiological studies from 1970s onwards involved a wide range of domestic and wild animal hosts. Only six serovars, belonging two genomospecies of the more than 200 pathogenic serovars of *Leptospira* known to occur worldwide were identified as endemic in New Zealand. Serovars Australis and Canicola have been only isolated from human patients and are not considered to be endemic. Cattle are recognised as a reservoir host for serovar Hardjobovis, pigs for serovars Pomona and Tarassovi, brushtail possums (*Trichosurus vulpecula*) for serovar Balcanica, black rats (*Rattus rattus*) for serovar Ballum, brown or Norway rats (*Rattus norvegicus*) for serovar Copenhageni, and possibly farmed deer for Hardjobovis and Pomona. Sheep, goats, horses, camelids, dogs including humans have been generally considered to be spillover hosts in this country (Hathaway 1981; Marshall and Manktelow 2002).

2.10.1 Leptospirosis in sheep

There is limited information available on the epidemiology and transmission dynamics of leptospirosis in sheep in New Zealand. The few studies were carried out mostly in the late 1970s and early 1980s (Hodges 1974; Ris 1975; Blackmore et al 1976; Hathaway and Marshall 1979; Marshall et al 1979; Mackintosh et al 1981; Blackmore et al 1982). Leptospirosis in sheep was first reported in 1952, about the same time as the first reported outbreaks in cattle and pigs due to serovar Pomona in the Gisborne and Manawatu districts

(Hartley 1952). Sporadic outbreaks of leptospirosis in sheep due to serovar Pomona were subsequently reported (Salisbury 1954; Webster and Reynolds 1955; Vermunt et al 1994a; Vermunt et al 1994b) and in all these clinical outbreaks of leptospirosis in sheep, only serovar Pomona was identified. These studies, however, did not report whether or not the diagnostic tests included serology against serovar Hardjo titres, and co-infection with Hardjo, even if not clinically important, may have been overlooked.

Later studies that included serology against serovar Hardjo titres showed that the prevalence of Hardjo was widespread in their study populations (Ris 1975; Blackmore et al 1982). In a retrospective survey of 449 sheep sera collected since 1968 from 12 flocks in the southern half of the North Island (Ris 1975), approximately 71% of sera had positive Hardjo titres (MAT titres of 1:100 or greater) and within-flock prevalence ranged from 75% to 100% in 9 of 12 flocks. The other three flocks also had moderately high within-flock prevalence of Hardjo positive titres, ranging from 30% to 42%. This study provided the first evidence of widespread Hardjo infection at high prevalences in sheep without causing overt disease.

Blackmore et al (1976) in a serological study that included breeding bulls, sheep and wildlife (rats, rabbits, hedgehogs) around an artificial breeding centre found 5 of 28 sheep kidneys were culture positive and four of these sheep also had positive MAT titres. Bahaman et al (1980) reported the first isolation of serovar Hardjo from the kidneys of one- to two-year old sheep in a sample of 180 sheep from 12 flocks submitted to two meat works. Two of three isolates were serovar Hardjo.

Detailed serological and cultural prevalence found in this survey of 928 sheep from 45 lines in the Manawatu were; 20% of sheep had positive MAT titres (1:48 or greater) to serovar Hardjo, 3.8% to Pomona, 2.6% to Tarassovi, 2.3% to Copenhageni and 2.7% to Ballum (Blackmore et al 1982). A follow-up serological survey of the flock 18 months later from which three culture isolates were made, recorded serological prevalences of serovar Hardjo in 44% and 84% of hoggets and ewes respectively, while all lambs were seronegative. All urine samples were negative for leptospires when examined under dark field microscopy. This is the only study that provides insight into the transmission dynamics in a sheep flock

that showed significant seroconversion when lambs become hoggets, and hoggets became ewes. There was circumstantial evidence that infection was acquired from cattle and it seemed that although sporadic infection of sheep with serovar Hardjo could occur, sheep were unlikely to act as a reservoir host. However, studies in other countries also found a high serological prevalence of Hardjo in sheep (Hoare and Claxton 1972; Gordon 1980; McCaughan et al 1980; Hathaway et al 1982; Cousins and Robertson 1986) along with evidence that sheep are a potential reservoir host of serovar Hardjo (Cousins and Robertson 1986; Cousins et al 1989; Gerritsen et al 1994) on the basis of high infection rates in flocks that had no contact with cattle, and prolonged leptospiruria lasting for more than 11 months.

Hathaway and Marshall (1979) conducted a study of experimental infection in sheep with serovar Hardjo and Balcanica over a three week period to gain an insight into whether sheep could act as a reservoir host for these serovars. They showed that infection was readily established with both serovars without overt disease in all inoculated animals. Leptospiruria and renal infection were observed in all sheep infected with serovar Hardjo for up to 3 weeks of the study period. In contrast, leptospiruria was observed during the second week in only one sheep inoculated with serovar Balcanica and kidney cultures from the Balcanica inoculated sheep were all negative. A similar study of experimental infection of 8 ewes with serovar Balcanica produced a similar result (Mackintosh et al 1981), with all animals seroconverting by 14 days post-inoculation. Leptospiruria was observed in only one sheep, 18 days post inoculation, and culture of kidney from this sheep at 50 days post inoculation yielded a positive result. None of the inoculated sheep showed clinical signs of disease beyond a transient rise in temperature.

Only one study has evaluated the efficacy of vaccination. It used the Hardjo component of a bivalent Pomona/Hardjo vaccine against serovar Hardjo in sheep and reported significant but incomplete protection in terms of kidney culture results, where only 2 of 9 (22%) vaccinated animals were culture positive, while 10 (100%) control sheep were culture positive (Marshall et al 1979). Two vaccinated sheep did not produce agglutinating antibodies, suggesting they resisted artificial challenge due to presence of protective non-humoural antibodies. Prior to this study, Webster and Reynold (1955) evaluated the

efficacy of a heat-killed whole culture Pomona vaccine in sheep and reported that vaccination resulted in strong and lasting immunity as none of vaccinated sheep showed leptospiruria, whereas 3 of 8 controls showed leptospiruria for up to 3 months. They noted that the majority of sheep naturally infected with Pomona can excrete the organisms for up to 2 months with an extreme case of 9 months in a single sheep and demonstrated urinary excretion in asymptomatic sheep.

In general, even though prevalence of serovar Hardjo is high in sheep (Ris 1975; Blackmore et al 1982), sporadic outbreaks of clinical leptospirosis are associated with serovar Pomona infection (Salisbury 1954; Webster and Reynolds 1955; Vermunt et al 1994a; Vermunt et al 1994b). Some sheep remain asymptomatic despite positive MAT titres to Pomona in natural (Vermunt et al 1994b) and experimental infections (Hodges 1974). Overt leptospirosis was most commonly observed in lambs with mortality and morbidity approaching 18% and 100% in lambs respectively (Vermunt et al 1994b), and the majority of adult sheep showed no clinical evidence of infection.

2.10.2 Leptospirosis in humans

Leptospirosis is the most frequently notified occupational zoonotic disease in New Zealand, and the incidence is relatively high compared with other developed countries with temperate climates (Thornley et al 2002). The major source of infection in humans is predominantly of domestic animal origin, with clearly definable high risk occupational groups, consisting of livestock farmers, veterinarians, meat processing workers and forestry-related personnel (Schollum and Blackmore 1982; Thornley et al 2002). After the first report of human leptospirosis in 1951 in New Zealand (Kirschner and Gray 1951), the disease was made notifiable under the Health Act 1956, and all acute human leptospirosis cases reported by medical practitioners are recorded (Thornley et al 2002). Leptospirosis is also recognized as an important occupationally acquired infectious disease of farmers and meat workers under the Occupational Safety and Health Services (OSH) and Accident Compensation Corporation (ACC). The number of notifications increased from 759 during 1956–1960 to 1660 in 1961–1965, and there was a further increase to 3019 cases during

1966–1970. The highest number (860) of leptospirosis cases in humans was notified in 1971 (Christmas et al 1974a).

After the introduction of widespread vaccination of dairy cattle and pigs along with educational and publicity campaigns on the measures to protect against leptospirosis in late 1970s and early 1980s, the number of human cases notified to the Department of Health dropped (Marshall 1987). In particular, the annual incidence of leptospirosis in dairy farm workers dropped from 110 per 10,000 between 1970 and 1980 to 45 per 10,000 after widespread vaccination of dairy cattle. No significant confounding factors, such as weather patterns and changes in farming practices were identified that explained this reduction in number of cases (Marshall 1987). The annual incidence of leptospirosis cases then declined to a minimum level of 2.9 per 100,000 persons in 1996-1998 (Thornley et al 2002).

Recently, the annual incidence has shown an increasing trend with an average annual incidence rate of 4.0 per 100,000 persons (laboratory confirmed) over the period 2001-2003 (Baker and Lopez 2004). Previously, the majority of leptospirosis cases were notified in dairy farm and pig farm workers (Christmas et al 1974a; Blackmore and Schollum 1982b; Schollum and Blackmore 1982; Thornley et al 2002), but data from the 3 consecutive years since 2002 indicated that meat workers (freezing workers, butchers, or meat inspectors) now constituted the highest proportion of notified leptospirosis cases (Sneyd and Baker 2003; Anonymous. 2004, 2005). Meat workers constituted 48.9%, 46.1%, and 64.7% of notified leptospirosis cases amongst the recorded occupations in 2002, 2003 and 2004 respectively, whereas the corresponding percentages for farm workers were 41.2%, 35.3%, and 28.4%. The annual incidence of leptospirosis was highest among meat processing workers (164 per 100,000), followed by livestock farm workers (92 per 100 000) and forestry-related workers (24 per 100,000) for the period 1990–1998 (Thornley et al 2002).

The most common serovars consistently affecting humans were Hardjo, followed by Pomona, and Ballum (Christmas et al 1974b; Mackintosh et al 1980; Thornley et al 2002; Sneyd and Baker 2003; Anonymous. 2004, 2005) and infections with other serovars were rarely recorded (Thornley et al 2002). There were significant differences in the distribution

of infecting serovars among the main occupational groups. Serovar Hardjo was most commonly detected in farm workers, Pomona in meat workers, and Ballum in forestry-related workers (Thornley et al 2002; Baker and Lopez 2004). A cross-sectional survey of dairy farm workers in the Manawatu region by (Mackintosh et al 1980) revealed 34% of persons milking cows had leptospiral titres of $\geq 1:24$ or greater. Seventy three percent of these people had titres to Hardjo, 44% to Pomona and 6% to both serovars. The prevalence of positive titres to Ballum and Copenhageni was 8%. Risk factors with a significant positive correlation with leptospiral titres were time spent in the shed during milking, the wearing of shorts, the keeping of pigs for sale, the number of years the individual had been working on a dairy farm, and the type of milking shed and herd size.

Baker and Lopez (2004) reported associations between serovars in notified cases and patient contact with animals prior to illness for the period 2001–2003. Of the 90.4% (321 of 355) of notified cases that had information on contact with animals or animal products prior to their illness, 47.0% reported unspecified contact with a farm, 23.4% reported contact with cattle only, 9.3% with cattle in combination with other animals, 5.9% with sheep only, 4.4% with sheep in combination with other animals, 2.5% with pigs only, and 1.2% with rodents only. This study indicated that most human infections were associated with contact with cattle, either alone or in combination with other animals. Sheep were the second most important contact animal, either alone or in combination with other animals. In patients that had contact with sheep or sheep in combination with other animals, both serovars Hardjo and Pomona occurred in similar proportions, while Hardjo predominated in those that had contact with cattle. For the first time sheep were implicated as a potential source of human infections, contrary to previously held perceptions that they were not an important source of human leptospirosis (Baker and Lopez 2004). Serovar Pomona predominated in patients who had contact with pigs, supporting an earlier serological study of 65 persons working or resident on 41 pig farms (Schollum and Blackmore 1982).

In general, the incidence of leptospirosis is much higher in males than in females, but this difference was not statistically significant for meat workers (Thornley et al 2002; Baker and Lopez 2004). Workers in 29 to 59 year old age group were most commonly affected. Leptospirosis cases have been reported in most geographic regions including Tairāwhiti,

Hawkes Bay, South Canterbury, Northland, Nelson-Marlborough, Waikato, Midcentral, WestCoast, Taranaki, Bay of Plenty, Wanganui and Southland (Baker and Lopez 2004). The district health boards of Hawke's Bay, Tairāwhiti, Nelson-Marlborough, WestCoast, and South Canterbury have consistently reported high rates of leptospirosis (Sneyd and Baker 2003; Anonymous. 2004; Baker and Lopez 2004; Anonymous. 2005). A significant positive linear correlation was also found between leptospirosis incidence and the ratio of dairy cattle numbers to human population in each territorial authority (Thornley et al 2002)

A early 1980's serological survey of 1215 meat inspectors (representing more than 70% of meat inspectors employed in New Zealand at time) and 1248 meat workers from 6 meat works (3 abattoirs processing sheep and cattle, 2 processing sheep, cattle and pigs, and one processing pigs only) showed 9.5% of meat inspectors and 4.1% of meat workers were seropositive (MAT titre of 1:24 or greater) with titres compatible with occupational exposure to domestic stock (Blackmore and Schollum 1982a). The subgroup of meat workers working on the slaughter floor had the highest prevalence of leptospiral titres (10.4%) and the inspection and processing of pigs constituted the greatest risks for leptospirosis. There was no significant difference in seroprevalence between meat workers on the slaughter floor and meat inspectors, even though the overall prevalence in meat workers (4.1%) was approximately half that of meat inspectors (9.1%), further supporting the evidence that working on the slaughter floor constituted the greatest risk. Serovar Pomona was predominant in both meat inspectors and meat workers followed by serovars Hardjobovis and Tarassovi. This study also demonstrated that leptospiral titres can persist for 10 years or more in the most people (50%) with medically confirmed leptospirosis. Blackmore et al (1984) in their longitudinal study of meat inspectors reported a seroconversion rate of 4.4% per annum.

2.11 Conclusion

This literature review has summarised the current knowledge on important aspects of leptospirosis, such as latest taxonomy being followed, sources and transmission routes of infection, pathogenesis, and various clinical manifestations in animals and humans. In addition, this review has outlined the currently applied diagnostic tests for leptospirosis and

the existing epidemiological information of leptospirosis in animals and man, with more emphasis given to the disease in sheep and humans in New Zealand. It is clear from this review that the epidemiological information available on leptospirosis in sheep at both farm and slaughter levels is limited and dated. The available information indicates that infection with serovars Hardjo and Pomona (serovar Hardjo being more common than serovar Pomona) predominates in both sheep and humans in New Zealand. Recent reports of rising trend of leptospirosis in meat workers with a good proportion of leptospirosis cases being reported in meat workers employed in sheep-only abattoirs, along with the reports of sheep either alone or in combination with other animals being the second most important contact animal of human leptospirosis patients, points to the likely role of sheep as a source of human infection in New Zealand. However, there is a paucity of information about whether the serovars involved in meat workers employed in sheep-only abattoirs are directly related to exposure to sheep and whether sheep are reservoir hosts for any serovar or serovars in New Zealand. If sheep are shown to be a reservoir host in New Zealand, as suggested by studies in other countries (sheep as a reservoir host for serovar Hardjo), it would have a significant impact, given more than 30 million sheep population and about 28 million being slaughtered every year. As the majority of farms in New Zealand are mixed animal species farming and as cattle and pigs are recognised reservoir hosts for serovars Hardjobovis and Pomona respectively, there is a compelling need to update the knowledge on inter-species transmission dynamics of leptospirosis to understand the epidemiological status of sheep for maintaining infection in New Zealand.

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Chapter 3

Prevalence of pathogenic *Leptospira* in sheep in a sheep-only abattoir in New Zealand

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ABSTRACT

AIM: To determine the prevalence of two most commonly diagnosed pathogenic leptospires, serovars Hardjobovis and Pomona in sheep in a sheep-only abattoir in New Zealand and determine the isolation rate of live leptospires from sheep kidneys.

METHODS: A longitudinal study of serological and kidney culture prevalences of serovars *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona in lines of sheep and individual sheep systematically randomly selected at a sheep-only abattoir during May 2004 to November 2004 and again December 2004 to 14 June 2005, plus a cross-sectional study of prevalence in a purposively selected line of sheep from a flock with evidence of an outbreak of leptospirosis.

RESULTS: The prevalence of lines with animals MAT (microscopic agglutination test) seropositive to one or both serovars in 95 lines of sheep sampled from 89 farms was 44.2% (95% CI 34.6–54.2) and 44.9% (95% CI 35.0–55.3) of farms showed evidence of previous exposure to the disease. The overall individual serological prevalence in the sample of 15,855 sheep processed was 5.7% (95% CI 4.9–6.7). The serological prevalence of Hardjobovis was significantly higher than Pomona both at line (33% and 4% respectively) and individual (5% and 1% respectively) levels. Lambs born in the 2003–2004 season were 2.9 times (95% CI 2.0–4.2) and 9.2 times (95% CI 6.6–13.0) more likely to test seropositive to either one or both serovars at line and individual animal levels respectively than lambs born in the 2004–2005 season, indicating a strong seasonal effect. Line size was not a significant risk factor for serostatus. A low but persistent seroprevalence of Hardjobovis in both cohorts of sheep suggested a low endemic level to this serovar, whereas for Pomona infection appeared to be sporadic. The overall isolation rates of live leptospires from kidneys were 8 of 37 (21.6%) Hardjobovis and 1 of 6 (16.7%) Pomona seropositive carcasses, and 5 of 499 (1%) seronegative carcasses. All 13 kidneys of seropositive carcasses from a purposively sampled line were culture positive, indicating a high risk of exposure of meat workers in outbreak situations. Kidneys of MAT seropositive carcasses were 21.7 times (95% CI 7.6–61.9) more likely to test culture positive than

kidneys of seronegative carcasses. In general, the results indicated that 13 of every 1000 sheep slaughtered were potentially shedding live leptospires.

CONCLUSIONS: The study demonstrated the presence of a definite risk of occupational exposure of meat workers in a sheep-only slaughterhouse to the two most commonly diagnosed pathogenic *Leptospira* serovars.

KEY WORDS: Leptospirosis, sheep, meat workers, slaughterhouse, Hardjobovis, Pomona

List of abbreviations

CI = Confidence interval; MAT = Microscopic agglutination test; NPV = Negative predictive value; PPV = Positive predictive value.

3.1 Introduction

Leptospirosis is a re-emerging and widespread zoonotic disease of global importance (Levett 2001; Vinetz 2001; Bharti et al 2003). It is the most frequently notified occupational zoonotic disease in New Zealand, with almost all cases being acquired directly through contact with domestic animals and animal products (Thornley et al 2002; Baker and Lopez 2004). The annual incidence rate of notified human leptospirosis cases has shown a rising trend over the recent years, with an average annual incidence rate of 4.4 cases per 100,000 persons in 2001–2003, reversing the long term decline trend observed since 1980s to a minimum average annual rate of 2.9 per 100,000 persons in 1996–1998. High risk occupational groups for leptospirosis include livestock farmers, meat processing workers, veterinarians and forestry-related personnel (Schollum and Blackmore 1982; Thornley et al 2002). Until recently, the majority of notified leptospirosis cases were predominantly in dairy and pig farm workers (Christmas et al 1974a; Blackmore and Schollum 1982a; Schollum and Blackmore 1982; Thornley et al 2002), but data for 2002 to 2004 indicate that workers in abattoirs have the highest risk for notified leptospirosis, accounting for 49%, 46% and 65% of notified cases amongst these occupations in 2002, 2003 and 2004, respectively (Sneyd and Baker 2003; Anonymous. 2004, 2005). Serovars Hardjobovis, Pomona, and Ballum, in that order, are the most common serovars diagnosed in humans (Thornley et al 2002; Sneyd and Baker 2003; Anonymous. 2005) and of the genotypes Hardjoprajitno and Hardjobovis, only Hardjobovis has been identified in New Zealand and Australia (Robinson et al 1982; Ramadass et al 1990). Significant differences in the distribution of serovars infecting the main occupational groups have been observed with serovar Hardjobovis most commonly detected in livestock farm workers, Pomona in meat workers, and Ballum in forestry-related workers (Thornley et al 2002; Baker and Lopez 2004).

Contact with cattle alone (23% of cases), or in combination with other animals (9%) was involved in most human infections (Baker and Lopez 2004), and sheep alone (6%) or in combination with other animals (4%) were considered the second most important source of infection. Unspecified contact with a farm was reported in 47% of cases. Hardjobovis and Pomona occurred in similar proportions in patients that had contact with sheep, or sheep in

combination with other animals, whereas Hardjobovis predominated in those that had contact with cattle. These findings, along with reports of increasing incidence of leptospirosis in meat workers, and cases in meat workers employed in sheep-only abattoir have raised concerns that sheep may have become a significant source of human infection. Sheep have not been considered to be reservoir hosts for any of the serovars in New Zealand or important sources of human infection (Blackmore et al 1982; Blackmore and Schollum 1982a; Hathaway et al 1982) but studies in other countries (Gordon 1980; Cousins et al 1989; Gerritsen et al 1994) have suggested that sheep may be a reservoir host for serovar Hardjo.

Currently, there is limited information available on the epidemiology and transmission of leptospirosis in sheep in New Zealand and inferences rely for the most part on studies carried out in the late 1970s and 1980s (Ris 1975; Hathaway and Marshall 1979; Marshall et al 1979; Blackmore et al 1982). These studies and those from other countries (Hoare and Claxton 1972; Gordon 1980; McCaughan et al 1980; Hathaway et al 1982) demonstrated high seroprevalences of Hardjo in sheep. However, despite observed high seroprevalences of Hardjo, overt outbreaks of leptospirosis in sheep in New Zealand have been sporadic and attributed only to Pomona (Thornton 1994; Vermunt et al 1994a; Vermunt et al 1994b).

As diseases are brought under control, epidemiological mechanisms that were relatively unimportant in the early stages of control programmes often become more important. While cattle contact is still associated with many human cases, sheep contact now appears to constitute a greater proportionate risk of disease for humans than it did in earlier times and effort to better understand the epidemiology of the disease in sheep and the current risk to humans from sheep are now warranted. The objectives of this study were to determine the current serological and cultural prevalences of the two commonly diagnosed serovars, *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona in slaughtered sheep at a sheep-only abattoir in New Zealand as a first step in assessing the risk of transmission of leptospirosis from sheep to abattoir workers.

3.2 Materials and Methods

Data collection

Sera and kidneys were sampled at weekly intervals from sheep slaughtered at a sheep-only abattoir from 18 May 2004 to 14 June 2005, excluding the period from 1 July to 31 October 2004. Sheep sampled during the period 18 May 2004 to 9 November 2004 consisted of 2003–2004 late-season lambs (lambs born in August to September 2003, hereafter referred to as Cohort 1), and those sampled from 6 December 2004 to 14 June 2005 consisted of lambs born in the 2004–2005 season (lambs born in August to September 2004, hereafter referred to as Cohort 2). During the study period, the abattoir processed an average of 25 to 40 lines and approximately 9,416 to 21,728 sheep per week. Sheep came from the Central North Island, South Auckland, Hawkes Bay, Wairarapa, Marlborough and Canterbury (Figure 3.1). On each collection day, 3 to 5 lines (each line mostly represented a consignment from a single farm) were selected and 30 sheep from each line were selected by systematic random sampling. Approximately 10 ml of blood was collected from each animal and carcasses were individually identified at this point. Kidneys of seropositive Cohort 2 animals were cultured for isolation of leptospires along with 509 single kidneys from the first 15 carcasses of each of 34 lines. Kidneys were collected in pre-sterilised plastic sachets and transported under refrigerated conditions. On occasions where white-spot lesions were present in the pair of kidneys from a carcass, the kidney with the greatest number of lesions was cultured.

An additional 30 sera and kidney samples were systematically taken from a purposively selected line of 500 lambs from a farm where an outbreak of leptospirosis was suspected two weeks prior to the sampling date because 12 of 500 carcasses were condemned for icterus at inspection.

Serology

Sera collected from 18 May 2004 to 9 November 2004 were submitted to the Leptospirosis Research Unit in the Institute of Veterinary, Animal and Biomedical Sciences, Massey

University, Palmerston North and tested for *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona using the standard method of microscopic agglutination test (MAT) described by Faine (1982). The antigens used for MAT were 4 to 14 days-old cultures of locally isolated strains of Hardjobovis and Pomona which contained approximately 1 to 2×10^8 organisms/ml based on the culture density on the McFarland nephelometer scale. Eight of two-fold serial dilutions covering the range from 1:24 to 1:3072 serum-antigen mixtures were prepared in a 96 wells flat-bottomed serology plates. The plates were then placed in re-sealable plastic bags and incubated at room temperature (20°C to 30°C) for 1.5 to 4 hours after which the degree of agglutination and end-point titre were determined by examination under dark-field microscopy. The end-point titre was the lowest dilution at which approximately 50% or more of the organisms were agglutinated and titre of 1:48 or greater were considered positive for both Hardjobovis and Pomona serovars.

From 6 December 2004 onwards, serology was conducted by Gribbles Veterinary Pathology Laboratory, Palmerston North. Sera were submitted to the laboratory on the same day of collection and results were available within 1 to 2 days. The same standard method of MAT was used for serology except that the two-fold serial dilutions of serum-antigen mixtures used were from 1:50 on and end-point titre of 1:50 or greater were considered positive.

Culture

Kidneys were cultured as soon as serology results were available, mostly within 2 to 3 days after sample collection dates. Kidneys were cultured in EMJH (Ellinghausen-McCullough-Johnson-Harris) medium containing 5-Fluorouracil as described by Ellinghausen and McCullough (1965); Johnson and Harris (1967) and Faine et al (1999). All kidneys were cultured in the Leptospirosis Research Unit. Kidney surfaces were sterilized by swabbing with alcohol followed by flaming with a Bunsen burner. A 10 to 15g section of kidney that included portions of white-spot kidney lesion (when present) and extending from the renal cortex to the medulla was aseptically removed. It was then homogenised in 50 to 100ml of standard sterile normal saline using a stomacher. A 100µl aliquot of this suspension was

inoculated to 5ml of the medium described earlier. From this inoculated medium, a 100 μ l aliquot was transferred to another 5ml of medium followed by further transfer of 100 μ l from it to the final 5ml of the medium. All three inoculated media bottles were then incubated at 28°C to 30°C and examined every 1 to 3 weeks for presence of leptospire by dark-field microscopy. Cultures that showed no growth after 6 month's incubation period were declared negative.

Statistical analyses

Univariate and bivariate analyses

Data were checked with descriptive statistics for data entry errors, and observations with outlying or missing values. The serological prevalences of Hardjobovis and Pomona serovars in the study populations were estimated at line and individual animal levels. Lines were considered positive if one or more carcasses tested seropositive to either or both of the two serovars. Differences in serological prevalences of the two serovars in the two cohorts of sheep were compared using prevalence ratios at line and individual animal levels. Trends in the serological prevalences of the two serovars over the calendar months of the study period were examined separately for the two cohorts by plotting time series of four-weekly moving averages. Differences in serological prevalences between sheep originating from the North and South Islands were also determined at line and individual animal levels. A crude association between positive MAT titre (a titre of 1:50 or greater) and culture test result was also estimated along with the positive predictive value (PPV) for positive MAT titres (proportion of carcasses with positive MAT titre that were kidney culture test positive). McNemar-test was used to test for statistically significant differences in carcass prevalence between serovars; the difference in carcass prevalence between cohorts was compared by chi-square test. Prevalences were reported as point estimates with Fleiss adjusted 95% confidence intervals (Fleiss 1981) in brackets.

Multivariate analyses

A multivariable random effects logistic regression model was fitted to account for within-line clustering of carcasses when assessing the differences in the model adjusted predicted

mean seroprevalences of either one or both serovars in the two cohorts of sheep sampled over different calendar months and adjusted for the effect of line size. Both serovars were combined for the analyses since the prevalence of serovar Pomona was low in the study population. The explanatory variables included in the model were cohort-calendar month as an interaction term, and line size. All variables were included as categorical variables and line size was categorised as four categories based on the quartile distribution with cut-points values of 72, 121, and 220 corresponding to the 25th, 50th, and 75th percentiles, respectively. The linearity of line size with the outcome variable on a logit scale was assessed by plotting the regression coefficients against the mid-points of the four quartiles (Hosmer and Lemeshow 2000) and as there was no linear relationship, it was fitted as categorical variable. The logistic regression model was fitted by a Generalised Linear Mixed Model using Proc GLIMMIX procedure in SAS version 9.1 for windows (SAS Institute, Inc., Cary, NC). Predicted least-squares mean prevalence (predicted population margins) for each categorical variable, adjusted for other variables in the model were obtained using the option LSMEANS statement in Proc Glimmix. The options ILINK, DIFF and CL in LSMEANS statement were used to obtain the predicted mean prevalence on the scale of the data, and to compare the differences in means to determine which groups differed significantly, and to obtain 95% confidence intervals, respectively. In all analyses, statistical significance was assessed at the 5% level using two sided tests.

3.3 Results

Descriptive statistics

The study population comprised 15,855 sheep of mixed breeds in 95 lines from 89 farms and came from at least 27 different localities from 11 districts of New Zealand (3 South Island and 8 North Island). Seventeen percent of this population was sampled in this study. The majority of farms of origin were located close to the abattoir (Figure 3.1). A total of 82 lines (86%) comprising 2,374 (86%) sheep originated from the North Island and 13 lines comprising 384 (14%) sheep came from the South Island. A total of 2,758 serum samples were tested for antibodies titres against serovars Hardjobovis and Pomona. The number of sheep per line ranged from 30 to 550 and the majority of lines comprised of 30 to 250

sheep (Figure 3.2). Line sample sizes ranged from 6% to 100% of sheep in a line (mean 30%, median 24%) and approximately two-thirds of samples comprised less than 40% of the line.

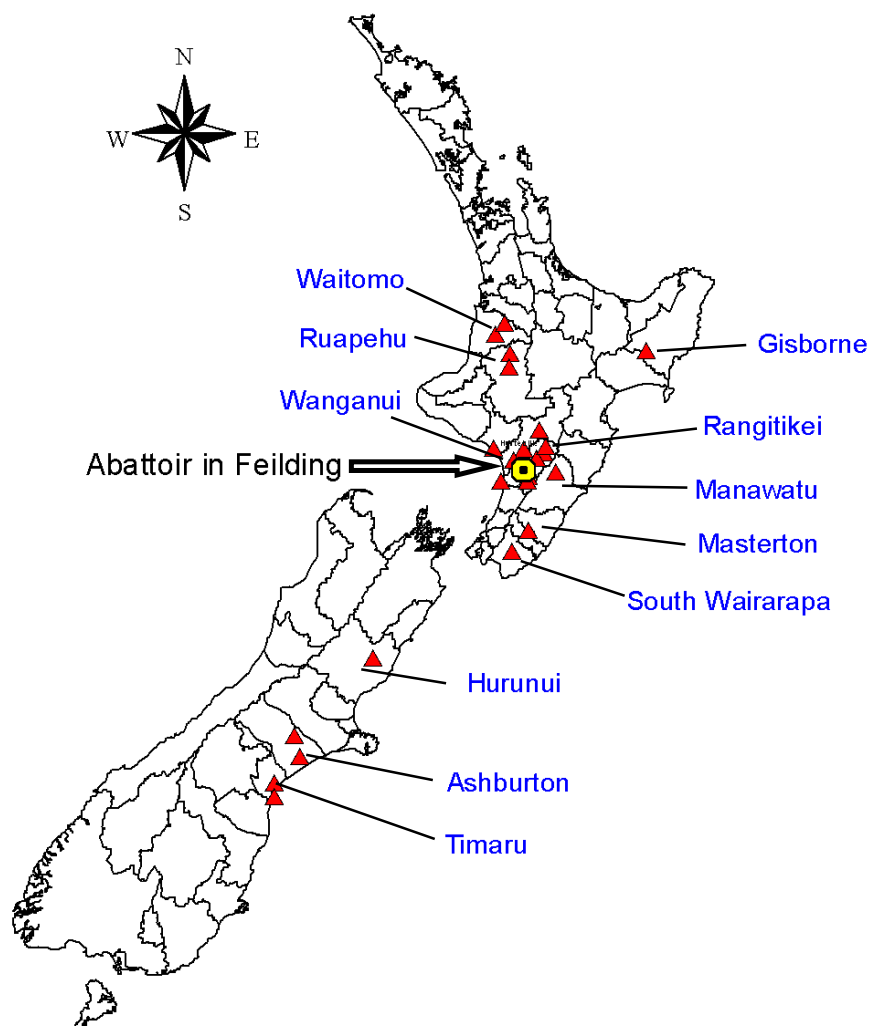


Figure 3.1: Map of the districts of New Zealand showing the location of nearest towns or localities of farms of origin (triangular point) of slaughtered sheep sampled in a sheep-only abattoir (Abattoir in Feilding) during the period 18 May 2004 to 14 June 2005 in New Zealand.

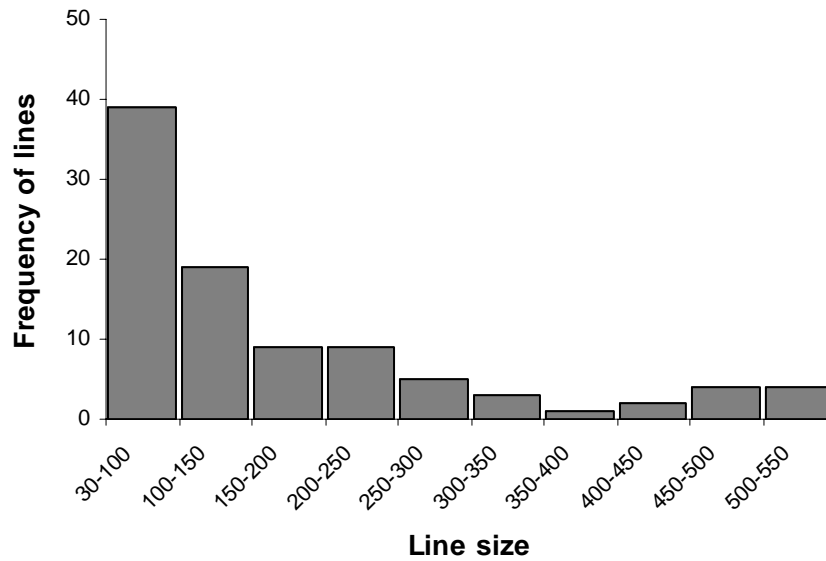


Figure 3.2: Frequency histogram showing lines sizes of slaughtered sheep sampled in a sheep only abattoir during the period 18 May 2004 to 14 June 2005.

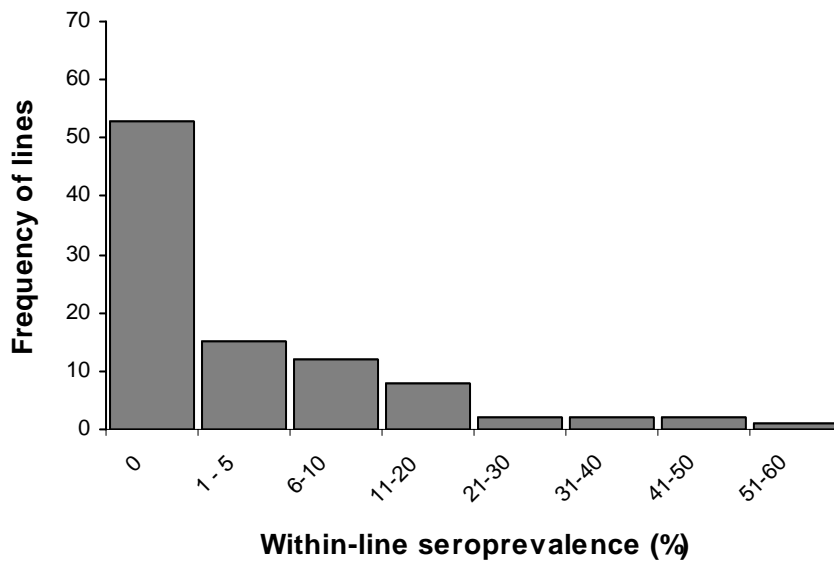


Figure 3.3: Frequency histogram showing the percentages of within-line serological prevalence to either Hardjoberg or Pomona or both in slaughtered sheep sampled in a sheep-only abattoir during the period 18 May 2004 to 14 June 2005.

Serology

The overall seroprevalence of lines with positive titres to either one or both serovars was 44.2% (95% CI 34.6–54.2) and 44.9% (95% CI 35.0–55.3) of farms had seropositive sheep. A total of 158 of 2,758 sheep (5.7%, 95% CI 4.9–6.7) were seropositive to one or both serovars. The distributions of line and individual animal prevalences of the two serovars of Cohort 1 lambs and Cohort 2 lambs are shown in Table 3.1. Titres of serovar Hardjobovis ranged from 1:48 to 1:25,600 in Cohort 1 lambs and from 1:48 to 1:12,800 in Cohort 2 lambs. For serovar Pomona, titres ranged from 1:48 to 1:12,800 in Cohort 1 lambs, and 1:48 to 1:1600 in Cohort 2 lambs. Only 9 (1.5%) Cohort 1 lambs had positive titres to both serovars.

The prevalence of serovar Hardjobovis positive lines (32.6%) was higher than for serovar Pomona positive lines (4.2%) (Table 3.1). The within-line seroprevalence of Hardjobovis in positive lines ranged from 3 to 60% in Cohort 1 lambs and from 3 to 16% in Cohort 2 lambs, and for Pomona from 3 to 40% in Cohort 1 lambs and 3 to 6% in Cohort 2 lambs, respectively. Overall, within-line seroprevalences for most seropositive lines were <10%, indicating a low degree of within-line clustering (Figure 3.3).

Line prevalences of both serovars were higher in Cohort 1 lambs than in Cohort 2 lambs. Lines of Cohort 1 lambs were 2.9 times (95% CI 2.0–4.2) more likely to test positive to either one or both serovars than Cohort 2 lamb lines and 3.2 times (95% CI 2.1–4.8) times more likely to test positive to Hardjobovis than Cohort 2 lines. A similar trend was observed with serovar Pomona, where Cohort 1 lines were 4.2 times (95% CI 1.4–12.5) more likely to test positive than Cohort 2 lines.

At the individual animal level, the prevalence of serovar Hardjobovis (4.6%) was higher than for Pomona (0.8%) (Table 3.1). Cohort 1 lambs were 9.3 times (95% CI 6.4–13.4) and 14.4 times (95% CI 5.9–34.9) more likely to test positive to Hardjobovis and Pomona respectively, than Cohort 2 lambs. Overall, Cohort 1 lambs were 9.2 times (95% CI 6.6–13.0) more likely to test positive to either one or both serovars than Cohort 2. There were

Table 3.1: Seroprevalences, shown as percentages with Fleiss adjusted 95% confidence intervals in brackets, of lines and sheep with positive titres of 1:48 or greater to serovars Hardjobovis and Pomona in two cohorts of slaughtered sheep sampled in a sheep-only abattoir during 18 May 2004 to June 2005 in New Zealand.

	Sample size	Hardjo only (+ve)	Percentage (95% CI)	Pomona only (+ve)	Percentage (95% CI)	Hardjo & Pomona	Percentage (95% CI)	Hardjo or Pomona	Percentage (95% CI)
Prevalence of lines with titres									
Cohort 1 lambs	21	13	61.9 (40.9 – 79.2)	1	4.8 (0.8 – 22.7)	5	23.8 (10.6 – 45.1)	19	90.5 (71.1 – 97.3)
Cohort 2 lambs	74	18	24.3 (16.0 – 35.2)	3	4.1 (1.4 – 11.3)	2	2.7 (0.7 – 9.3)	23	31.1 (21.7 – 42.3)
Both cohorts	95	31	32.6 (24.0 – 42.6)	4	4.2 (1.6 – 10.3)	7	7.4 (3.6 – 14.4)	42	44.2 (34.6 – 54.2)
Serological prevalence in sheep									
Cohort 1 lambs	619	90	14.5 (12.0 – 17.5)	16	2.6 (1.6 – 4.2)	9	1.5 (0.8 – 2.7)	115	18.6 (15.7 – 21.8)
Cohort 2 lambs	2139	37	1.7 (1.3 – 2.4)	6	0.3 (0.1 – 0.6)	0	0.0 (0.0 – 0.2)	43	2.0 (1.5 – 2.7)
Both Cohorts	2758	127	4.6 (3.9 – 5.5)	22	0.8 (0.5 – 1.2)	9	0.3 (0.2 – 0.6)	158	5.7 (4.9 – 6.7)

Table 3.2: Seroprevalences, shown as percentages with Fleiss adjusted 95% confidence intervals in brackets, of lines and sheep with positive titres of 1:48 or greater to serovars Hardjobovis and Pomona in two regions in slaughtered sheep sampled in a sheep-only abattoir during 18 May 2004 to June 2005 in New Zealand.

	Sample size	Hardjo only (+ve)	Percentage (95% CI)	Pomona only (+ve)	Percentage (95% CI)	Hardjo & Pomona	Percentage (95% CI)	Hardjo or Pomona	Percentage (95% CI)
Prevalence of lines with titres									
North Island	82	26	31.7 (22.6 – 42.4)	4	4.9 (1.9 – 11.9)	6	7.3 (3.4 – 15.1)	36	43.9 (33.7 – 54.7)
South Island	13	5	38.5 (17.7 – 64.5)	0	0.0 (0.0 – 22.8)	1	7.7 (1.4 – 33.3)	6	7.3 (3.4 – 54.2)
Both Islands	95	31	32.6 (24.0 – 42.6)	4	4.2 (1.6 – 10.3)	7	7.4 (3.6 – 14.4)	42	44.2 (34.6 – 54.2)
Serological prevalence in sheep									
North Island	2374	115	4.8 (4.1 – 5.8)	19	0.8 (0.5 – 1.2)	8	0.3 (0.2 – 0.7)	142	6.0 (5.1 – 7.0)
South Island	384	12	3.1 (1.8 – 5.4)	3	0.8 (0.3 – 2.3)	1	0.3 (0.0 – 1.5)	16	4.2 (2.6 – 6.7)
Both Islands	2758	127	4.6 (3.9 – 5.5)	22	0.8 (0.5 – 1.2)	9	0.3 (0.2 – 0.6)	158	5.7 (4.9 – 6.7)

no significant differences between the North and South Islands in seroprevalences of serovars Hardjobovis and Pomona at line or individual animal levels (Table 3.2).

Time series plots of seroprevalences of Hardjobovis and Pomona serovars for both cohorts are shown in Figures 3.4 and Figure 3.5. Apart from a slight rise during April and May 2005, there was no appreciable upward trend in the seroprevalence of Hardjobovis in Cohort 2 lambs. This contrasted with serovar Hardjobovis where there was a sharp rise in seroprevalence in Cohort 1 lambs during May, June and November 2004. There was no apparent trend in seroprevalence for serovar Pomona over time in Cohort 2 lambs, whereas it trended downwards in Cohort 1 lambs during May, June and November 2004. The low but persistent seroprevalence of serovar Hardjobovis in both cohorts of sheep observed throughout the study period suggests low level endemicity in the study population. For serovar Pomona, except for those lambs sampled in May and June 2004, the prevalence was negligible during other periods and indicates only sporadic Pomona infection in the study population.

There were no significant differences in the predicted mean prevalences of the two serovars combined among Cohort 2 lambs sampled over different calendar months, after accounting for the effects of line size and line-level clustering (Table 3.3). Statistically significant differences in predicted mean prevalences were observed between Cohort 1 and Cohort 2 lambs sampled in the corresponding months of May and June of 2004 and 2005, with the significantly higher prevalences in Cohort 1 clearly indicating a seasonal affect (Table 3.3). There were no statistically significant differences between the predicted mean prevalences of two serovars combined in the Cohort 1 lambs sampled in May and those sampled in June and November 2004. However, the predicted mean prevalences were significantly higher in the Cohort 1 lambs sampled in November 2004 than in those sampled in June 2004. In general, there was not much variation in seroprevalences over time and the differences appear to have been related to a cohort seasonal effect. There was no significant association between line size and serostatus of carcasses after adjusting for clustering effects of line and calendar month covariates in the model (Table 3.3).

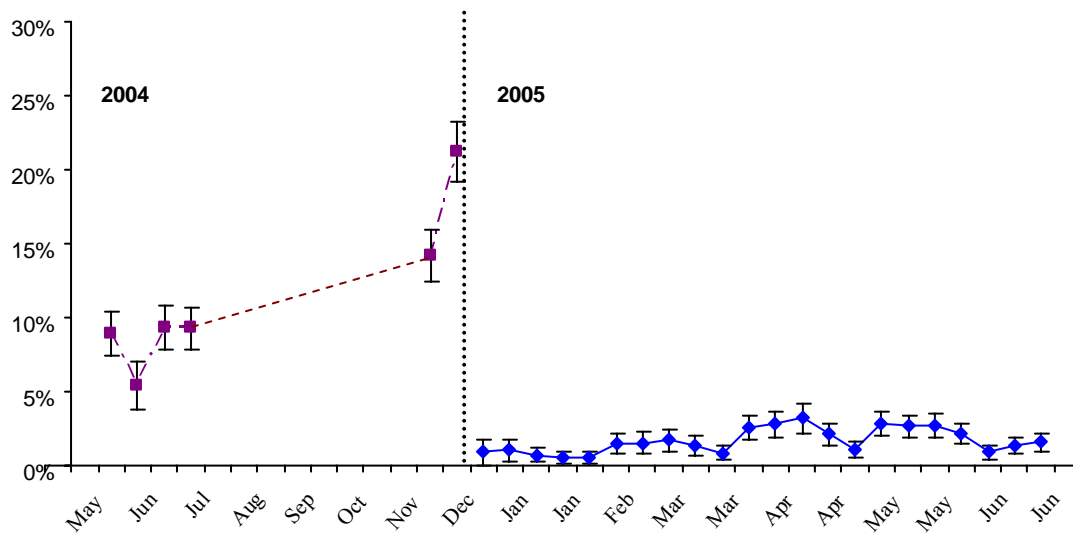


Figure 3.4: Time series plots showing monthly moving averages of seroprevalences of serovar Hardjobovis in Cohort 1 lambs (◻ and dashed line) and Cohort 2 lambs (◈ and solid line) in a New Zealand abattoir. Error bars (—) show the standard errors of the point estimates at calendar week. No sampling was carried out between July and October 2004.

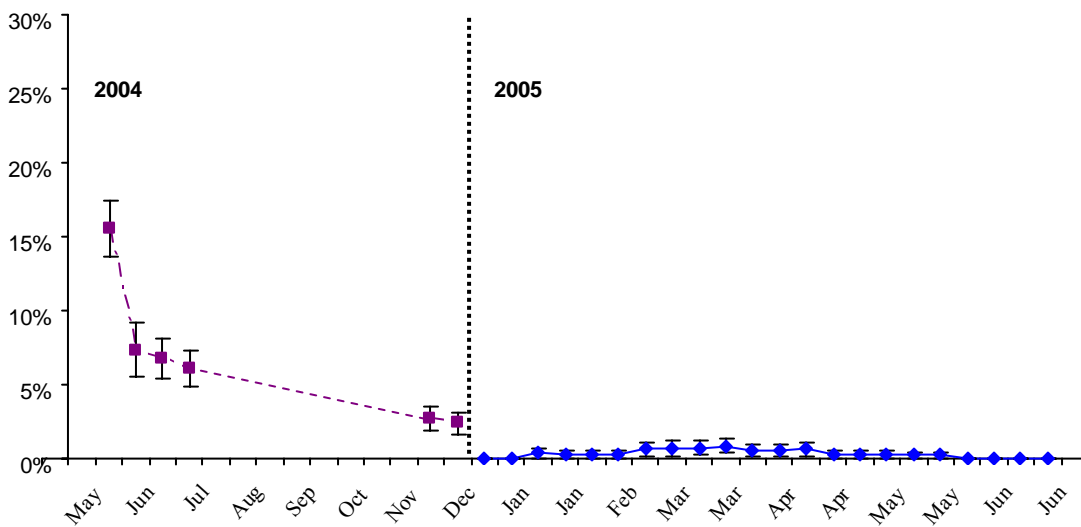


Figure 3.5: Time series plots showing the monthly moving averages of seroprevalences of serovar Pomona in sheep of Cohort 1 lambs (◻ and dashed line) and Cohort 2 lambs (◈ and solid line) slaughtered in a New Zealand abattoir. Error bars (—) show the standard errors of the point estimates at calendar week. No sampling was carried out between July and October 2004.

Table 3.3: Results of a logistic regression model with line included as a random effect showing model-adjusted predicted mean seroprevalences and 95% confidence intervals (expressed as percentages) of either Hardjobovis or Pomona or both for the explanatory variables tested in the model.

Variable	Total (N)	Positive (n)	Least-square mean prevalence (%)	95% CI (%)
a) Cohort -calendar months				
Cohort 1 lambs				
May 2004	90	18	13.3 ^{b c}	3.4 – 40.0
June 2004	319	36	8.6 ^b	4.1 – 17.0
November 2004	210	61	26.3 ^c	12.8 – 46.4
Cohort 2 lambs				
December 2004	190	2	1.0 ^a	0.2 – 5.9
January 2005	300	2	0.7 ^a	0.1 – 3.1
February 2005	203	6	1.8 ^a	0.5 – 6.5
March 2005	355	12	2.4 ^a	0.9 – 5.9
April 2005	419	13	2.4 ^a	1.0 – 5.4
May 2005	432	4	0.8 ^a	0.3 – 2.7
June 2005	240	4	1.6 ^a	0.5 – 5.8
c) Line size (categorical)				
Line size (30 - 72)	740	37	2.3 ^a	1.1 – 4.8
Line size (75 - 121)	656	46	3.3 ^a	1.6 – 6.7
Line size (125 - 220)	680	49	3.7 ^a	1.9 – 7.0
Line size (224 - 550)	682	26	2.5 ^a	1.2 – 5.1

^{a b c} Differences of least square mean prevalence with different letter superscripts within column and variable are statistically significant ($P < 0.05$)

Kidney culture test results and MAT titres

Of 37 and 6 kidneys from seropositive Hardjobovis and Pomona lambs, eight (22%) and one (17%) were culture positive, equating to 21% overall. Of 509 kidneys which were cultured irrespective of serostatus, 5 of 9 Hardjobovis and one of one Pomona seropositives were culture positive, and 5 of 499 (1%) of seronegatives to both serovars were culture positive. The serovars of the culture isolates as yet have not been identified.

There was a strong association between MAT titres and culture results. Kidneys from carcasses with MAT titres of >1:50 were 21.7 times (95% CI 7.6–61.9) more likely to be culture test positive than animals with negative MAT titres. However, the PPV was 21.4% (95% CI 11.7–35.9), indicating a low degree of predictability which is most likely related to the low prevalence. The NPV was 99.0% (95% CI 97.7–99.6).

Serology and culture results of a line of lambs from a farm suspected of having a recent outbreak of leptospirosis

Of 30 of 500 lambs sampled from the purposively selected line, 13 were seropositive to Pomona and culture test positive, and none were seropositive to Hardjobovis. In addition, 3 of 5 kidneys from seronegative carcasses with white-spotted kidney lesions were culture positive, indicating a very high risk of exposure for meat workers to live leptospire when lambs are processed during or soon after an outbreak of leptospirosis in the flock.

3.4 Discussion

This study provides up to date information on the prevalence of the two most commonly diagnosed pathogenic *Leptospira*, viz. serovar Hardjobovis and Pomona, in randomly sampled sheep in a sheep-only abattoir in New Zealand. It was conducted over 13 months and demonstrated that about one half of the sampled lines and sheep farms in the study population had been previously exposed to infection with either or both Hardjobovis or Pomona. The high prevalences of Hardjobovis at line (32.6%) and individual animal (4.6%) levels indicated that infection with Hardjobovis was common, in contrast to the lower 4.2% and 0.8% corresponding levels for Pomona. Sampling was not conducted during the sheep processing off-season extending from July to October 2004 in order to obtain a reasonably unbiased estimate of farm prevalence. During this period, traders commonly source sheep from other farms and regions, and subsequent mixing of sheep would be expected to increase the risk of infection. Because of the single abattoir focus, extrapolation of the results of this study to other New Zealand sheep farms cannot be fully justified, despite the study population coming from several regions of the country. More studies are now

required to determine the spatial pattern of the disease and determine the consistency of the high prevalence of infected flocks observed in this study. Nevertheless, the results are consistent with earlier reports of high flock and within flock prevalences of Hardjibovis in sheep in New Zealand (Ris 1975; Blackmore et al 1982), and in other countries (McCaughan et al 1980; Hathaway et al 1982).

The seroprevalences of Hardjibovis (4.6%) in Cohort 2 lambs which were approximately 3–10 months old and Cohort 1 lambs (14.5%) aged 9–16 months are comparable and consistent with an earlier study (Blackmore et al 1982) that recorded seroprevalences of 2.8% in animals up to 1-year-old and 22.9% in 1 to 2-year-olds. In that study the seroprevalence of Pomona was relatively higher in both age groups in the earlier study and there was a high incidence of Hardjo seroconversion over time in a longitudinal study of an infected farm. Higher prevalences in older lambs and replacement hoggets and ewes indicated that the exposure of farmers arising from a high number of infected sheep may be similar to that of abattoir workers whose exposure probably arises from their close contact with kidneys and urine. In our study, there were no significant differences in seroprevalences of Hardjibovis in Cohort 2 lambs over time but significantly higher prevalences were recorded in Cohort 1 lambs sampled in May and June 2004 than in Cohort 2 lambs in the corresponding months in 2005. This strong seasonal effect may have been due to high rainfalls accompanied by widespread surface flooding in February 2004 and relatively less rainfall in 2005. The likely association between rainfall with surface flooding and poor drainage and leptospirosis outbreaks in sheep has been commented on in earlier reports of the disease in New Zealand (Hartley 1952; Vermunt et al 1994b) and in the USA (Smith and Armstrong 1975). However, since many risk factors, other than rainfall with surface flooding and poor drainage may also contribute to infection, further studies of the disease in sheep are required to determine the respective roles of risk factors other than those associated with periods of high rainfall.

The generally very low prevalence of Pomona is consistent with earlier studies (Blackmore et al 1982; Vermunt et al 1994a; Vermunt et al 1994b) and provides more evidence of a sporadic nature of Pomona infection in sheep. A high exposure rate of sheep to Hardjibovis is not surprising, given that most New Zealand sheep farms are mixed-species enterprises

that also run cattle and/or deer, both of which are established maintenance hosts for *Hardjobovis* (Hathaway 1981; Wilson et al 1998; Marshall and Manktelow 2002). Sheep have not been considered to be maintenance hosts for any serovars in New Zealand in the past (Blackmore et al 1982; Hathaway et al 1982), but more recent studies in other countries have shown some evidence of sheep as a potential maintenance host for serovar *Hardjo* (Cousins et al 1989; Gerritsen et al 1994). The high seroprevalences of *Hardjobovis* and *Pomona* that were found in asymptomatic mobs of hoggets, 2-tooths and ewes in 5 New Zealand flocks, all with a history of a clinical outbreaks of leptospirosis in lambs attributed to *Pomona* infection (Dorjee et al 2005), are indicative of continued within-flock transmission of *Hardjobovis*. Persistence of asymptomatic *Hardjobovis* infection in both young and adult sheep, is suggestive of either established reservoir status or gradual host-adaptation of *Hardjobovis* in sheep. More work is warranted on the issue of maintenance or spillover host status of sheep because of their potential or already established role as a significant source of infection to other livestock and humans, given the national flock size of about 30 million female sheep of breeding age and the potential for human exposure during slaughter processing of about 28 million annually. It also raises issues for control programmes since controlling a disease in spillover hosts is generally not effective in the long term if transmission from maintenance hosts is not prevented. Vaccination of sheep for leptospirosis is not routinely practised in New Zealand. Vaccine efficacy in sheep in the face of continued challenge by other livestock, wildlife or environmental sources under New Zealand farming conditions has not yet been established

Line size did not influence the serostatus of lines or carcasses possibly but the possibility that sizes of lines at slaughter did not accurately reflect original farm mob size cannot be discounted. There were no significant differences between the seroprevalences of the two serovars at line and at individual animal levels in sheep from the North or South Islands but the sample size for the South Island was relatively small and no general inference should be made on this issue.

The overall culture isolation rate of 21% and 1% of leptospires from seropositive and seronegative carcasses observed in random samples of slaughtered sheep provided further evidence of sheep as a potential source of leptospirosis in meat workers. The 100% and

60% culture isolation rates of live leptospires from kidneys of seropositive and seronegative carcasses from a line from an outbreak farm points to a considerably greater risk of exposure of meat workers when sheep are sent for slaughter either during or soon after an outbreak when potentially infective leptospires are highly likely to be in the urinary tracts of recently infected sheep. Clinical outbreaks coinciding with animals going for slaughter appear to be rare events, given the overall low isolation rates of leptospires observed in this study, but it is clear that procedures aimed to prevent sending potential shedders of leptospires for slaughter during or soon after outbreaks need to be developed and instituted.

More accurate information on culture isolation rates would have been obtained if kidney cultures had been performed from the start of the study instead of December 2004, after which time the prevalence of seropositive lambs was relatively low. Simple extrapolation of test results from cultures to risk of human exposure is difficult since the sensitivity of kidney culture for leptospires is generally considered to be low and the various steps in the processes involved in exposure to live leptospires and successful initiation of infection are not well understood at this stage.

The positive association between levels of MAT titre and culture isolation rate is consistent with recent infection. The use of a minimum MAT titre of 1:48 as the cut-point in sheep as recommended by Blackmore et al (1982) gives an optimum balance of high sensitivity and specificity and it is unlikely that low sensitivity of the test during the first one to two weeks after initial infection would have appreciably altered the results of this study. Changing test laboratories half-way through the study was not likely to have biased the serology results since both laboratories followed the same protocol for MAT testing and used locally isolated strains of Hardjobovis and Pomona serovars as antigens and employed almost identical cut-points for positive.

The culture isolates have not yet been identified, but those from seropositive carcasses are expected to be the same serovar as was detected by serology. Isolates from seronegative carcasses could be either Hardjobovis or Pomona (if they were recently infected), or of some other, such as Balcanica, Tarassovi, Copenhageni and Ballum which have been

identified in sheep in New Zealand (Blackmore et al 1982). All culture isolates have been retained for comparison with human isolates to determine whether they are of same genotype. In general, if 44.2% lines are seropositive, and 12.8% of carcasses are seropositive within positive lines, and 22.2% of kidneys from seropositive carcasses are culture positive, then approximately 13 of every 1,000 sheep (the study abattoir processes about 2,000 sheep daily) are potentially shedding live leptospire. An additional risk and a moderately high exposure frequency comes from the about 1% of seronegative carcasses which may be shedding live leptospire. Despite the low (5.7%) overall serological prevalence observed at individual animal level, the risk to meat workers would appear to be moderately high, in view of the large numbers of sheep slaughtered annually.

The study provides strong evidence of an underlying risk to meat workers of exposure to live leptospire during processing of sheep carcasses, which is increased when sheep come from farms with recent clinical or unrecognised outbreaks of leptospirosis. The risk of exposure from lamb carcasses appears to increase with the age of lambs at slaughter but the overall risk will depend largely on the number and frequency of slaughtering of lambs and older age groups of sheep. More work is needed to understand the risk to abattoir workers and other persons working with sheep and it is important at this relatively early stage of the understanding of leptospirosis in sheep not to rush to conclusions or introduce a host of interventions that may not be effective. This does not mean that some measures may not be helpful if introduced now but rather that an open mind should be kept until more information is forthcoming. The study also highlighted a need for farm-level serological surveys to evaluate sources of infection and host-adaptation of *Hardjovis* in sheep, because of the major implications it would have for future control of leptospirosis in livestock and humans in New Zealand.

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Chapter 4

Assessment of Occupational Exposure Risk to Leptospirosis in a Sheep-only Abattoir in New Zealand

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ABSTRACT

AIMS: To determine the risk of workers in a sheep-only abattoir exposed to carcasses shedding *Leptospira* serovars Hardjobovis and Pomona.

METHODS: As described previously, serological and culture prevalences of serovars Hardjobovis and Pomona were determined at line and carcass level from systematic-randomly sampled carcasses in a sheep-only abattoir from May 2004 to June 2005. Assessments of daily exposure risk of meat workers to carcasses shedding live leptospire were restricted to meat workers handling kidneys (eviscerator, meat inspector, and offal-handler). A risk assessment for each group of meat worker was carried out for the processing periods May to November 2004 (Cohort 1 sheep) and December 2004 to June 2005 (Cohort 2 sheep). A stochastic model using beta-distribution for various input parameters was developed to assess the daily exposure to carcasses shedding live leptospire. Sensitivity analysis used Spearman rank correlation coefficients between the frequency of shedding leptospire and all input parameters.

RESULTS: The abattoir processed 25 to 45 lines corresponding to 9,416 to 21,728 sheep per week. Each eviscerator, meat inspector and offal-handler processed an average of 225, 374 and 1123 sheep per day, respectively. A total of 2,758 serum samples were tested for titres against two serovars which represented 15,855 sheep of mixed breeds in 95 lines originating from 89 different farms around New Zealand. Of these, 19 of 21 (90.5%, 95% CI 71.1–97.4) lines of Cohort 1 sheep and 23 of 74 (31.1%, 95% CI 21.7–42.3) lines of Cohort 2 sheep included one or more sheep with positive titres against either one or both serovars. At animal level, a total of 43 of 633 (6.8%, 95% CI 5.9–9.0) Cohort 1 sheep and 115 of 566 (20.4%, 95% CI 17.2–23.9) Cohort 2 sheep within positive lines were seropositive. Overall, 42 of 95 lines (44.2%, 95% CI 34.6–54.2) corresponding to 158 of 2,758 sheep (5.7%, 95% CI 4.2–6.7) had titres against either one or both serovars. Kidneys from 8 of 37 Hardjobovis (21.6%, 95% CI 11.4–37.2) and 1 of 6 Pomona (16.7%, 95% CI 3.0–56.4) seropositive carcasses were culture test positive with an overall isolation rate of 20.9% (95% CI 11.4–35.2). A total of 5 of 499 kidneys (1.0%, 95% CI 0.4–2.3) from randomly selected seronegative carcasses were culture test positive. Median daily exposure

risks for eviscerators were 11 (95% CI 3–22) and 3 (95% CI 0–9) shedding carcasses during processing periods of the Cohort 1 and Cohort 2 sheep, respectively, and that for meat inspectors were 18 (95% CI 7–34) and 6 (95% CI 1–14) shedding carcasses during the corresponding processing periods, respectively. The corresponding exposure rates for offal-handlers were 54 (95% CI 28–91) and 18 (95% CI 7–34) shedding carcasses, respectively. Sensitivity analyses indicated that culture isolation rates from both seropositive and seronegative carcasses and number of sheep processed by each meat worker per day had the greatest influence on the response variable of the models.

CONCLUSIONS: The study revealed that processing of sheep in the sheep-only abattoir was associated with regular exposure of meat workers to viable leptospiral pathogens. The exposure risk ranged from moderate for eviscerators and meat inspectors (3-11 infectious carcasses per day) to high for offal-handlers (18-54 infectious carcasses per day).

KEY WORDS: Leptospirosis, occupational, exposure, risk, assessment, meat workers, sheep.

List of abbreviations:

CI = Confidence interval; EMJH = Ellinghausen-McCullough-Johnson-Harris;

MAT = Microscopic agglutination test.

4.1 Introduction

Leptospirosis is recognised as the most important occupationally acquired zoonotic disease in New Zealand. The annual incidence of the disease has shown a rising trend over the recent five years, reversing the long term decline observed since 1980s to a minimum of level of 2.3 per 100,000 persons in 1999 (Marshall 1987; Anonymous. 2000; Thornley et al 2002; Anonymous. 2004; Baker and Lopez 2004; Anonymous. 2005). The average annual incidence of the disease was 4.0 per 100,000 persons over the period 2001–2003 and this is considered to be relatively high compared with other developed countries with temperate climates (Thornley et al 2002; Baker and Lopez 2004). Amongst the high risk occupational groups, meat workers constituted the highest proportion of notified leptospirosis cases for the three consecutive years since 2002 (Sneyd and Baker 2003; Anonymous. 2004, 2005). Meat workers constituted 48.9%, 46.1% and 64.7% of the notified leptospirosis cases amongst the recorded occupations in 2002, 2003 and 2004 respectively, whereas the corresponding percentages of farm workers were 41.2%, 35.3%, and 28.4% respectively, thus surpassing the rate of farm workers who represented the highest proportion until then.

Baker and Lopez (2004) reported associations between serovars in notified cases and patient contact with animals prior to illness for the period 2001–2003. Their study indicated that most human infections were associated with contact with cattle, either alone or in combination with other animals. Sheep were the second most important contact animal, either alone or in combination with other animals, thus highlighting the importance of sheep as a source of human infection. In patients that had contact with sheep or sheep in combination with other animals, the majority of cases were caused by serovars Hardjobovis and Pomona and both these serovars occurred in similar proportions. The only serological survey comprising 1,215 meat inspectors and 1,248 meat workers carried out in early 1980's from six meat processing plants showed 9.5% of meat inspectors and 4.1% of meat workers were seropositive with titres compatible with occupational exposure to domestic stock (Blackmore and Schollum 1982). To our knowledge, no study was conducted to assess the frequency of exposure of meat workers to leptospirosis in any abattoir, although meat workers were commonly reported to be affected with leptospirosis throughout the

world (Sullivan 1974; Blackmore et al 1979; Blackmore and Schollum 1980; Gsell 1990; Smythe et al 2000; Thornley et al 2002; Baker and Lopez 2004; Jansen et al 2005).

The objective of this study was to assess the risk of human exposure to sheep carcasses that were potentially shedding live leptospire, *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona in a sheep-only abattoir in New Zealand using a stochastic model. The basic information gained from this study could be used for further comprehensive risk assessment of occupational leptospirosis for meat workers employed in sheep processing abattoirs in New Zealand.

4.2 Materials and Methods

Data

Serum and kidney samples were collected from sheep selected by systematic random sampling from randomly selected slaughter lines of sheep in a sheep-only abattoir in New Zealand. Selections of lines and carcasses have been described in detail in Chapter 3. Briefly, samples were collected between 18 May 2004 and 14 June 2005, excluding the period from 1 July to 31 October 2004. The slaughter lambs born in August to September 2003 are referred to as Cohort 1; they were slaughtered and sampled from 18 May to 9 November 2004. Lambs born in August to September 2004 were sampled from 6 December 2004 to 14 June 2005 (Cohort 2). The MAT (microscopic agglutination test) was used to measure serum-antibody against serovars Hardjobovis and Pomona with a positive cut-off titre of 1:48 or greater. Lines were considered positive if one or more carcasses were seropositive to either one or both serovars. From December 2004 onwards kidneys from seropositive Cohort 2 lambs were cultured in EMJH (Ellinghausen-McCullough-Johnson-Harris) medium containing 5-fluorouracil and examined by dark field microscopy for the presence of leptospire at one to three weekly intervals. In addition, a total of 509 kidneys from 34 Cohort 2 lambs were cultured by selecting the first 15 carcasses irrespective of their serostatus by systematic random sampling technique from a subset of samples. This provided the culture isolation rate from seronegative carcasses.

Data on numbers of monthly slaughter of sheep, the number of processing staff and the type of their works including meat inspectors present during the study period were retrieved from the abattoir database. Since only sera and kidneys were sampled, the assessments of the risk of exposure to live leptospiral organisms were restricted to those groups of meat workers having physical contact with kidneys. This included the following processing types: eviscerators, meat inspectors and offal handlers. The abattoir processed sheep in two shifts per day each with a different group of meat workers.

Risk assessment

The seroprevalence data for serovars Hardjobovis and Pomona were combined as the prevalence of Pomona was low. Thus a sero-positive carcass was defined as a carcass being seropositive to either or both serovars. In addition, assessment of the exposure risk for each processing type was estimated for processing periods of the Cohort 1 and Cohort 2 lambs separately, as there was a marked difference in seroprevalence between the cohorts (Chapter 3). The combination of prevalences of sero-status with culture status at line and carcass levels resulted in conditional probabilities for the culture status of a carcass depending on sero-status shown in Figure 4.1. The total probability of carcasses that were potentially shedding live leptospores was calculated as the sum of probabilities of kidney culture positives from seropositive and seronegative carcasses within seropositive lines and seronegative carcasses within seronegative lines. Estimates of culture isolation rates from seropositive and seronegative carcasses obtained in Cohort 2 were also applied to Cohort 1 lambs for which no culture data were collected. This assumed that the likelihood of culture isolation from seropositive and seronegative carcasses was the same in both cohorts. In this study, the sensitivity and specificity of the MAT and kidney culture test were assumed to be 100% as there was no information in literature on test characteristics.

To estimated daily frequencies of the exposure to carcasses potentially shedding live leptospores for the three processing types, a stochastic model was developed as follows:

$\chi = \text{Binomial}(n, p)$, where χ represented the binomially distributed daily exposure risk of a meat worker to carcasses shedding live leptospores; n represented the normally

distributed number of sheep processed by each worker per day (standard deviation of ± 50 sheep/day based on the abattoir data), and p the sum of probabilities of culture positives from seropositive and seronegative carcasses.

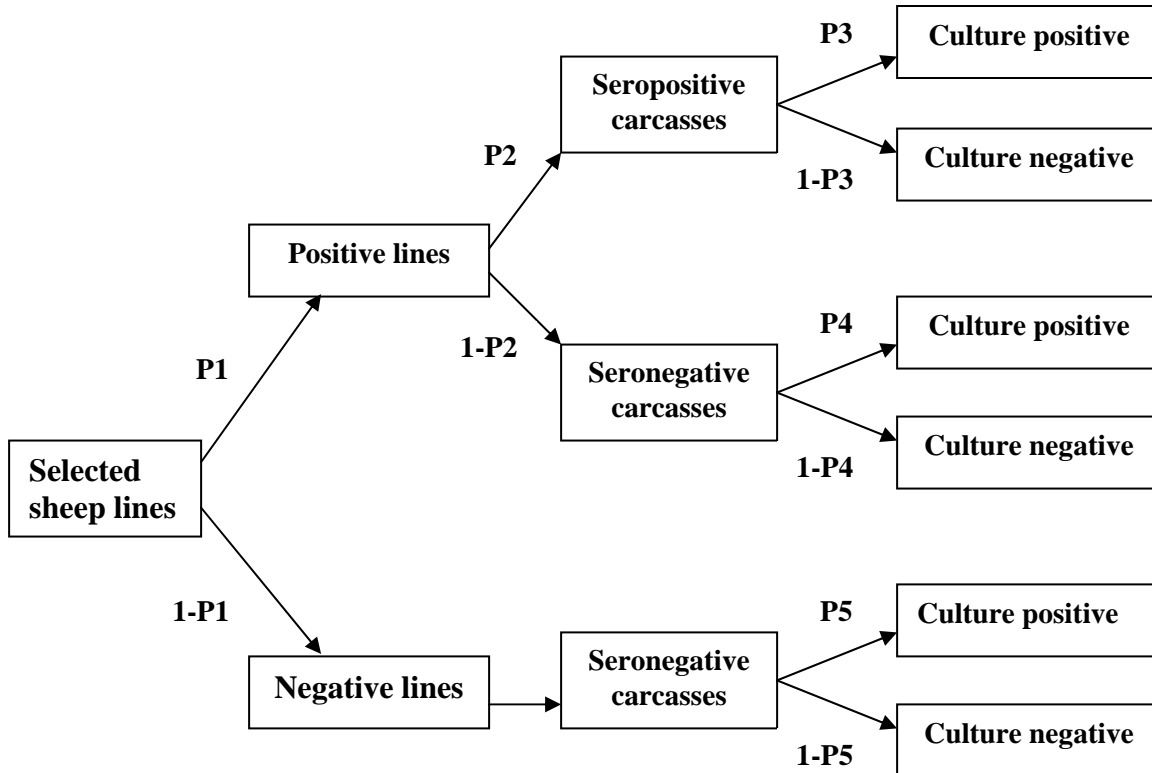


Figure 4.1: A scenario tree outlining the pathways leading to infected kidneys potentially shedding live leptospire and subsequent exposure of meat workers to leptospirosis.

The uncertainty about prevalences of seropositive sheep lines, seropositive carcasses and culture tests positives (p) were simulated using beta-distributions as follows: $p = Beta(x+1, n-x+1)$, where x = number of positives and n = number of samples (Murray 2002). Since the mean of a beta distribution is restricted to be between zero and one inclusively, the beta distribution was appropriate in modeling the uncertainty about the parameter p , the probability for a carcass being culture-positive. The outcome distribution was the posterior

distribution arising from uncertainties of several input parameters combined. The beta distribution may be regarded as a non-informative conjugate prior [$\text{beta}(1,1) = \text{uniform}(0,1)$] to a binomial likelihood function in terms of Bayesian inference (Vose 2000; Murray 2002).

In this model, uncertainty and variable components were simulated jointly. Simulations were performed using @RISK software version 4.5.5, 2004 (Palisade Corporation, New York, USA) which is an add-in program for Microsoft Excel (Microsoft Corporation, Seattle, Washington). Each simulation consisted of 5,000 iterations using the Latin Hypercube sampling method until convergence was achieved based on stable percentiles, means, and standard deviations (all changes in subsequent model runs $<1.5\%$). Medians and their 95% credible intervals were used to describe the daily exposure risk. A sensitivity analysis of the model was performed by calculating the Spearman rank correlation coefficients of the model response (the frequency of exposure of meat worker to carcass potentially shedding live leptospirae per day) associated with a standard deviation change in each of the model input variables.

4.3 Results

Descriptive statistics

During the study period, the abattoir processed 25 to 40 lines corresponding to 9,416 to 21,728 sheep per week. Numbers of sheep slaughtered over calendar months of the study period were more or less uniform with peak slaughter done during January to March 2005 and relatively lesser in number during off-season period of August to October 2004 (Figure 4.2). Average number of sheep processed by each eviscerator, meat inspector and offal-handler per day during the study period were 225, 374 and 1123, respectively. Number of sheep lines sampled per month ranged from 3 during May 2004 to 15 during months of April and May 2005 with an average value of 9 lines. The number of sheep sampled per month ranged from 90 during May 2004 to 432 during May 2005 with an average value of 276 sheep per month. A total of 2,758 serum samples were tested for antibodies against serovars Hardjobovis and Pomona, representing the study population of 15,855 sheep of

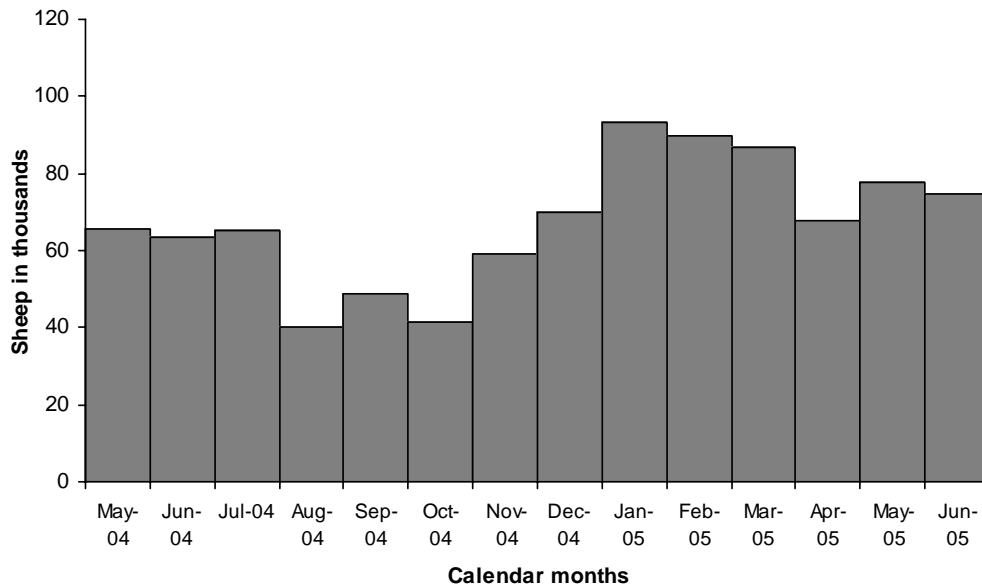


Figure 4.2: Histogram of numbers of sheep slaughtered over different calendar months of the study period from 18 May 2004 to 14 June 2005 at a sheep-only abattoir in New Zealand.

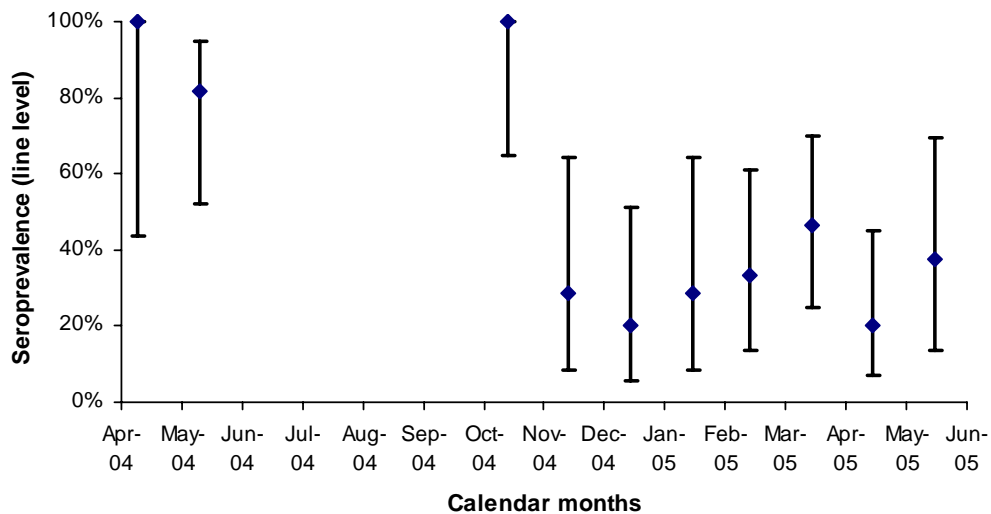


Figure 4.3: Means and 95% confidence intervals (error bars) of the monthly seroprevalence of leptospiral antibody positive slaughter lines (lines were considered positive if one or more carcasses were seropositive to either one or both Hardjobovis or Pomona serovars) in sheep at a sheep-only abattoir in New Zealand during the study period from 18 May 2004 to 14 June 2005. No samples were collected between July and October 2004.

mixed breeds in 95 lines from 89 different farms originating from 27 different localities of 11 districts in New Zealand. Monthly seroprevalences of lines with positive titres to either one or both serovars ranged from 20.0% during January and April 2005 to 100% during May and November 2004 (Figure 4.3). Overall 42 of 95 lines (44.2%, 95% CI 34.6–54.2) equivalent to 40 of 89 farms (44.9%, 95% CI 35.0–55.3) had one or more seropositive sheep. A total of 19 of 21 Cohort 1 (90.5%, 95% CI 71.1–97.4) and 23 of 74 Cohort 2 (31.1%, 95% CI 21.7–42.3) sheep lines had one or more sheep with positive titres.

Monthly seroprevalences of sheep with positive titres ranged from 0.7% in January 2005 to 29.0% in November 2004. The monthly prevalence during the study period 18 May 2004 to 14 June 2005 is shown in Figure 4.4. Overall, a total of 158 of 2,758 sheep (5.7%, 95% CI 4.2–6.7) were seropositive to either one or both serovars. Of these, 115 of 565 Cohort 1 sheep (20.4%, 95% CI 17.2–23.9) and 43 of 633 Cohort 2 sheep (6.8%, 95% CI 5.1–9.0) within seropositive lines were seropositive.

Kidneys from 8 of 37 Hardjobovis (21.6%, 95% CI 11.4–37.2) and 1 of 6 Pomona (16.7%, 95% CI 3.0–56.4) seropositive carcasses were culture test positive with an overall isolation rate of 20.9% (95% CI 11.4–35.2). In addition, of 509 kidneys cultured irrespective of their serostatus, 5 of 9 Hardjobovis (55.6%, 95% CI 26.7–81.1) and 1 of 1 Pomona (100%, 95% CI 20.7–100) seropositive carcasses were culture test positive, while 5 of 499 seronegative carcasses (1.0%, 95% CI 0.4–2.3) were also culture test positive.

Exposure risk estimates

The estimate of daily exposure risk for each eviscerator working in the abattoir to carcasses shedding live leptospire during the processing of lambs from Cohort 1 and Cohort 2 were 11 (95% credible interval 3–22) and 3 (95% credible interval 0–9) respectively. The frequency distribution of variation in the daily exposure risks of an eviscerator to carcasses shedding live leptospire are shown in Figure 4.5. For meat inspectors, the daily exposure risk to carcasses shedding live leptospire during processing of carcasses from Cohort 1 and Cohort 2 were 18 (95% credible interval 7–34) and 6 (95% credible interval 1–14) respectively (Figure 4.6). Similarly, the daily exposure risk of an offal-handler to carcasses

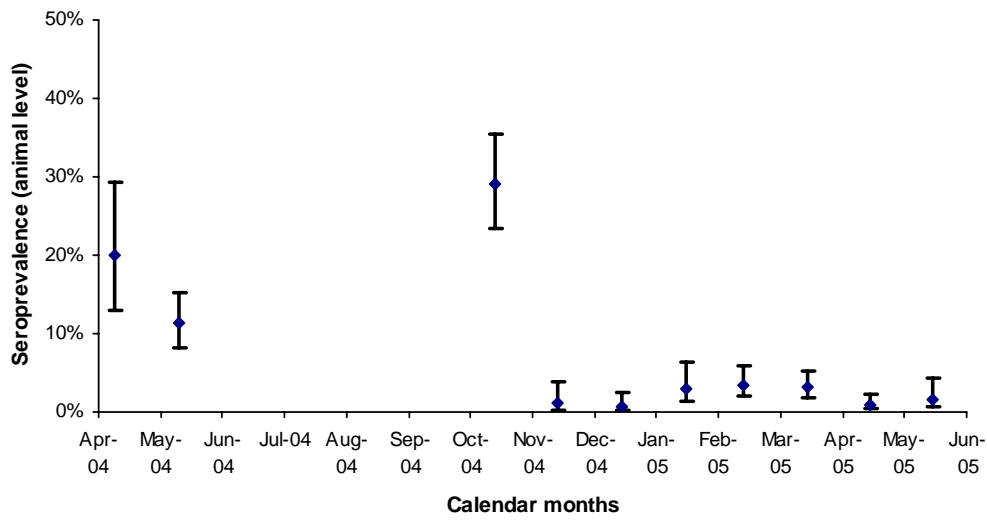


Figure 4.4: Means and 95% confidence intervals (error bars) of the monthly seroprevalence of leptospiral antibody positive carcasses at a sheep-only abattoir in New Zealand during 18 May 2004 to 14 June 2005. No samples were collected between July and October 2004.

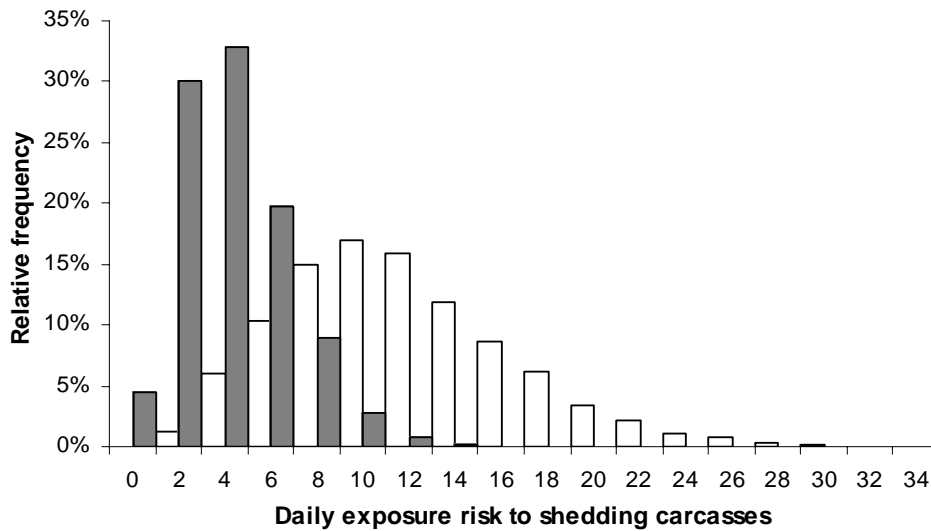


Figure 4.5: Frequency distribution of the daily risk of exposure to live leptospiral organisms for an eviscerator from carcasses of Cohort 1 (empty bar) and Cohort 2 (filled bar) at a sheep-only abattoir in New Zealand from 18 May 2004 to 14 June 2005 (5000 model runs per cohort).

potentially shedding live leptospires were 54 (95% CI 28–91) and 18 (95% CI 7–34) during processing periods of the Cohort 1 and Cohort 2 sheep, respectively (Figure 4.7).

Sensitivity analyses of exposure risk assessment models showed that the culture isolation rate from seropositive and seronegative carcasses, and the number of carcasses processed daily had a relatively large impact on the daily exposure risk of Cohort 1 sheep. For offal handlers however, only the culture isolation rate from seropositive carcasses impacted to a substantial degree on the daily exposure risk (Table 4.1). Results of sensitivity analyses of the exposure risk assessment for eviscerators and meat inspectors during processing of Cohort 2 suggested that the culture isolation rate from seronegative carcasses was more important than other input variables.

Table 4.1: Results of sensitivity analyses showing degrees of correlation (Spearman rank correlation coefficient) between each variable and output (daily exposure risk of a meat worker to carcasses potentially shedding live leptospires) during processing periods of Cohort 1 and Cohort 2 sheep at sheep-only abattoir in New Zealand

Variables	Spearman rank order correlation coefficient					
	Eviscerator		Meat Inspector		Offal-handler	
	Cohort 1	Cohort 2	Cohort 1	Cohort 2	Cohort 1	Cohort 2
Culture isolation rate from seropositive carcasses	0.47	0.14	0.59	0.14	0.76	0.20
Culture isolation rate from seronegative carcasses	0.18	0.42	0.22	0.51	0.25	0.72
Prevalence of seropositive carcasses within positive lines	0.12	0.08	0.18	0.12	0.22	0.11
Prevalence of seropositive lines	0.11	0.09	0.15	0.08	0.21	0.12
Number of carcasses processed by a meat worker/day	0.47	0.33	0.34	0.26	0.16	0.11

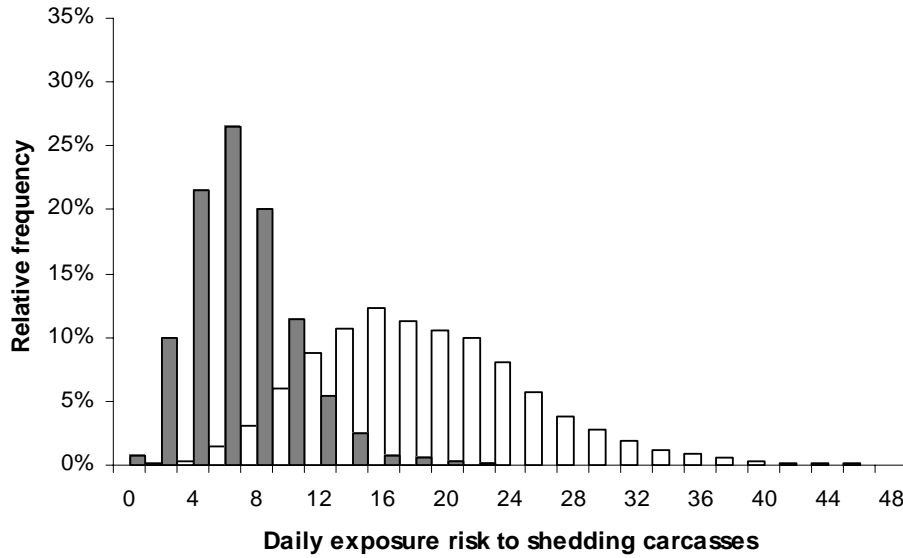


Figure 4.6: Frequency distribution of the daily risk of exposure to live leptospiral organisms for a meat inspector from carcasses of Cohort 1 (empty bar ☺) and Cohort 2 (filled bar ☹) at a sheep-only abattoir in New Zealand from 18 May 2004 to 14 June 2005 (5000 model runs per cohort).

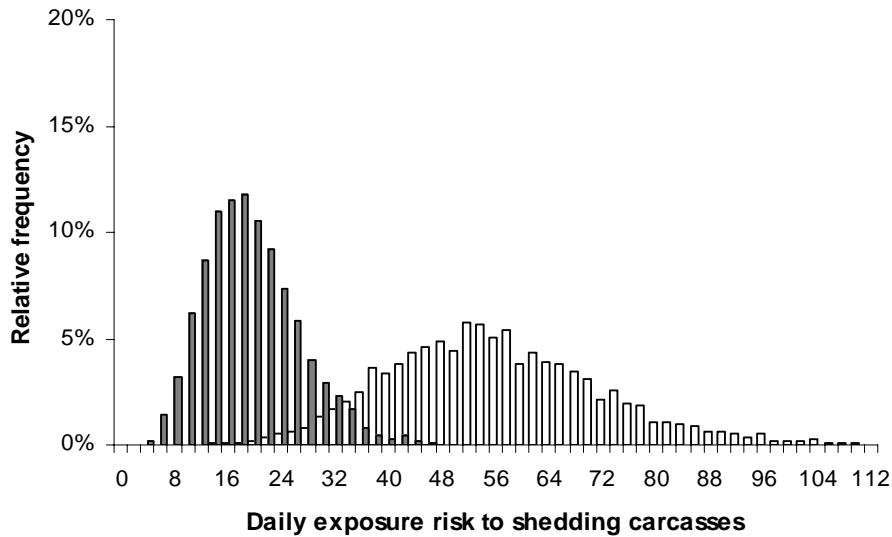


Figure 4.7: Frequency distribution of the daily risk of exposure to live leptospiral organisms for an offal-handler from carcasses of Cohort 1 (empty bar ☺) and Cohort 2 (filled bar ☹) at a sheep-only abattoir in New Zealand from 18 May 2004 to 14 June 2005 (5000 model runs per cohort).

4.4 Discussion

This study showed for the first time in New Zealand that processing of sheep in the abattoir at Feilding presented meat workers with a consistent exposure risk to leptospiral organisms in two subsequent years. The presence of live leptospires in kidneys exposes meat workers to the risk of infection with leptospires, especially while handling kidneys. It is unknown however, how many infections occur as a result of exposure. The estimates of exposure risk varied from moderate for an eviscerator and meat inspector to high for offal-handler depending upon the number of in-contact sheep carcasses per day. An offal-handler had the greatest exposure risk because only a single offal-handler is employed vis-à-vis five eviscerators and three meat inspectors during a typical shift in the abattoir. Therefore sheep may be an important source for leptospirosis in humans (Baker and Lopez 2004).

Exposure from sheep carcasses may constitute a similar occupational risk for human infection as reported from workers processing other livestock (Blackmore and Schollum 1982a). The later was a cross-sectional study at six meat processing plants (3 sheep and cattle processing plants, 2 sheep/cattle/pig processing plants, and 1 pig-only processing plant). The authors reported no significant difference between the serological prevalence in workers on slaughter floor and meat inspectors (Blackmore and Schollum 1982). While guidelines exist about the use of protectional clothing, meat workers do not always comply to wear hand gloves and face mask notwithstanding those workers with injuries may be more compliant. In addition, it was noticed that eviscerators normally make deep and wide incision on each kidney to enucleate kidney from capsule to enable meat inspectors to examine kidneys. Furthermore, there were reports that infection may be mediated through intact but dampened skin after prolonged immersion in water (Faine et al 1999; Levett 2001). Such practices may increase the likelihood of infection. It was also evident that exposure risk may vary from one season to another and in different months within season resulting from different infection prevalence in sheep. However, true occupational infection rates of workers at sheep-only slaughter plants are not known warranting further investigation.

For all analyses in this study, it was assumed that the sensitivity and specificity of MAT and culture test were 100% as there is no information in literature. In general, the MAT is considered to be reference test and its sensitivity and specificity are considered to be high as it does not cross-react with any other bacteria, other than leptospire belonging to the same serogroups. However, the sensitivity of culture test is generally considered low (Faine et al 1999). These assumptions might have reduced the true culture prevalence resulting in conservative estimates of occupational risk. Furthermore, samples from Cohort 1 carcasses were not tested by culture when the seroprevalence was much higher than in Cohort 2 carcasses. If prevalence was associated with the isolation rate from seropositive carcasses, the exposure risk would be expected higher in the first year of study. A higher isolation rate was observed in a line of sheep from a farm with a clinical outbreak of leptospirosis (Chapter 3)

The variability and uncertainty components of each risk model were simulated jointly, as second order modeling (where variability and uncertainty components of are modeled separately) was not justified due to only small differences in model outputs (Murray 2002). The differences were small probably because our risk models were relatively simple with a low degree of complexity. As expected, culture isolation rates from seropositive and seronegative carcasses and the number of sheep processed by each worker per day had the greatest impact on the daily exposure risk of meat workers. A system of rotating meat workers assigned for various tasks at different points on the slaughter floor may reduce the average individual exposure risk to leptospirosis.

As the study was restricted to a single sheep-only abattoir in New Zealand, extrapolation of these findings to other abattoirs may be done with caution. Factors like processing set up, processing speed, and the number of meat workers in each category of the three examined processing types may vary from one abattoir to another. Although, this study yielded a meaningful insight into daily frequency of exposure risk of meat workers to leptospirosis at a sheep-only abattoir, further research is required to determine to what extent exposure results in human infection and clinical human leptospirosis, in order to describe the risk of human occupational leptospirosis in sheep-only processing plants.

4.5 Conclusion

Based on sampling at a sheep-only abattoir and stochastic risk modeling, this study provided strong evidence for consistent human occupational exposure to live leptospira organisms throughout two years of study.

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Chapter 5

Are white-spot kidney lesions in sheep associated with leptospirosis?

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ABSTRACT

AIMS: To determine the association between white spots kidney lesions and serological and cultural prevalences of leptospirosis in sheep and the diagnostic value of these lesions in individual sheep and lines of sheep at slaughter as indicators of past or current episodes of leptospirosis.

METHODS: Diagnostic test evaluation of the value of grossly visible white spots in kidneys for predicting serological and culture test positivity to either Hardjobovis or Pomona or both in individual lambs and lines of lambs at slaughter were estimated. A fixed effect multivariable logistic regression model was fitted to determine whether or not within-line percentage of carcasses with WSK and line size were significant risk factors for serostatus of line. In addition, a random effect multivariable logistic regression was fitted to determine the differences in seroprevalence to either or both serovars amongst carcasses with different WSK lesion scores.

RESULTS: At the individual animal level, white spotted kidneys (WSK) were observed in 450/2,753 (16%) randomly sampled sheep at slaughter, of which 158 (5.7%) were seropositive to either or both serovars Hardjobovis and Pomona (Relative Risk 5.2, 95% CI 3.9–7.1) but the lesions were poor predictors of serological status with a test sensitivity of 51% (95% CI 43–59) and a positive predictive value of 18% (95% CI 14–22). Carcasses with high WSK lesion scores (>5 white spots or white mottling on one or both kidneys) were 6.1 times (95% CI 4.3–8.3) more likely to be seropositive to either one or both serovars than carcasses with low (1–5 white spots on one or both kidneys) lesion scores, but the test sensitivity and positive predictive value for those criteria were unacceptably low at 27% (95% CI 20–34) and 27% (95% CI % 21–35) respectively.

At slaughter line level, lesions occurred in one or more carcasses in 86/95 (91%) lines, of which 41/86 (47.7%) WSK positive lines and 1/9 (11.1%) WSK negative lines were seropositive. Of the 86/95 WSK positive lines, 31 (36.1%) and 4 (4.7%) were seropositive to serovars Hardjobovis and Pomona respectively, and 6 (7.0%) to both serovars. Consideration of lesion status of lines rather than for individual animals resulted in a higher

sensitivity of 98% (95% CI 87–100), but the positive predictive value was still low at 48% (95% CI 37–59). Carcasses with low and high lesion scores were 2.2 times (95% CI 1.4–3.3) and 4.4 times (95% CI 2.8–6.5) more likely to be seropositive than carcasses with no lesions. There were no significant differences between the predicted mean prevalences for each of the 4 categories of quartile derived line sizes and line serostatus was not significantly associated with the prevalence of WSK positive carcasses in lines, after adjusting for line size and cohort effects. Leptospire were cultured from one or more kidneys from 10/39 (25.6%) lines with WSK and 2/6 (33.3%) lines without WSK. Leptospire were isolated from 30/566 (5.3%) kidneys taken from carcasses in the randomly sampled lines and a farm suspected for a recent outbreak of leptospirosis. There was no statistically significant association between WSK lesion score and culture test results in the survey data, although a positive trend was observed. When culture data from a purposively sampled farm was merged to survey data, a significant positive linear association was evident. Carcasses with WSK lesion low and high scores were 2.9 times (95% CI 0.8–10.0) and 13.3 times (95% CI 6.7–26.8) respectively more likely to be culture positive than carcasses without WSK lesions but positive predictive values for tests with culture as the gold standard were all very low.

CONCLUSIONS: White spots on sheep kidneys, as judged by test sensitivity and positive predictive values, are poor predictors of serological or culture positivity for leptospirosis. However their occurrence in individual sheep and in lines of sheep is positively associated with serological and culture evidence of exposure and condemnation of WSK is justified. Meat workers should take extra care when processing lines with high prevalences of white spotted kidneys to reduce their risk of exposure to infection.

KEY WORDS: Leptospirosis, sheep, meat workers, slaughterhouse, Hardjobovis, Pomona

List of abbreviations

CI = Confidence interval; MAT = Microscopic agglutination test; PPV= Positive predictive value; NPV = Negative predictive value; WSK = Kidneys with grey-white gross lesions of 1–3 mm diameter on the cortical surfaces.

5.1 Introduction

Focal interstitial nephritis in sheep presents grossly as grey-white lesions of 1–3 mm diameter on the cortical surfaces of kidneys and affected kidneys are commonly referred to as “white-spotted kidneys” (WSK) (Skilbeck et al 1988; Maxie 1993; Drolet et al 2002). Lesions are common in clinically healthy slaughtered pigs (Hunter et al 1987; Jones et al 1987; Baker et al 1989; Drolet et al 2002) and cattle (Prescott et al 1987; Maxie 1993; Yener and Keles 2001; Uzal et al 2002a; Uzal et al 2002b), and are the main reason for condemnation of kidneys. Although WSK are not pathognomonic for leptospirosis (Jeffcott et al 1967; Maxie 1993; Drolet and Dee 1999), they are frequently observed in natural and experimental infection with *Leptospira* spp. in cattle (Hadlow and Stoenner 1955; Amatredjo et al 1976), pigs (Cheville et al 1980; Jones et al 1987) and sheep (Marshall 1974; Durfee and Presidente 1979; Hathaway and Marshall 1979; McCaughan et al 1980; Ellis et al 1984; Vermunt et al 1994a; Vermunt et al 1994b). This association has led meat inspectors and slaughterhouse workers to assume that white spotted kidneys could be indicators of past or current leptospiral infection and kidneys are condemned as a consequence (Uzal et al 2002a). Leptospire have been isolated from kidneys of clinically healthy slaughtered sheep in New Zealand (Blackmore et al 1982), Australia (Gordon 1980) and Brazil (de Azevedo et al 2004) and clinically healthy slaughtered cattle (Prescott et al 1987; Uzal et al 2002a) and pigs (Peet et al 1983; Baker et al 1989; Campagnolo et al 2000a), and infected animals pose an occupational health risk of leptospirosis to meat workers.

The detection of cases of leptospirosis among meat workers in sheep-only slaughter plants in recent years (Baker and Lopez 2004) has led to renewed interest in the possible value of white-spotted kidney lesions for indicating current or past leptospirosis status of slaughtered cattle and pigs. Pig (Peet et al 1983; Hunter et al 1987; Jones et al 1987; Baker et al 1989; Drolet et al 2002; Boqvist et al 2003) and cattle (Prescott et al 1987; Skilbeck et al 1988; Yener and Keles 2001; Uzal et al 2002a) studies to date have not resolved the issue for those species and no such sheep studies have been conducted. If WSK were shown to be reliable indicators of leptospirosis at individual animal or line (farm) levels in sheep, then special precautions could be taken by meat workers during the processing of affected lines

to reduce risk of exposure to infection. The aim of this study was to determine the strength of any association between white-spotted kidneys and serological and culture positivity to the two most commonly diagnosed serovars, *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona in slaughtered sheep in New Zealand and the diagnostic value of these lesions to predict past or current episodes of leptospirosis in sheep.

5.2 Materials and Methods

Data collection

Serology, WSK and culture data were collected during a longitudinal study of leptospirosis at a sheep-only abattoir as described in Chapter 3. After removal of their capsules at the abattoir, kidneys from all but 5 of the 2,758 lambs sampled and tested serologically were scored as 0 for no visible white spots, low for 1–5 spots, and high for >5 spots or white-mottling on the kidney surfaces. When lesions were present on both kidneys the score was recorded for the kidney with the most lesions. Lines were considered WSK positive if one or more carcasses with WSK were present, else WSK negative. Kidneys of seropositive lambs in the 2003–2004 season cohort were cultured for leptospire along with 509 single kidneys from the first 15 carcasses of each of 34 lines. Culture data from a purposively sampled line from a leptospirosis outbreak farm were combined with culture data from the longitudinal study data to estimate associations between WSK lesion scores and kidney culture test results as there was few culture positives recorded in the longitudinal study.

Statistical analyses

Serology and culture results from Chapter 3 were used as gold standards for the evaluation of WSK at line and individual animal levels for predicting evidence of infection. Data for serovars Hardjobovis and Pomona were combined for the analysis since the prevalence of serovar Pomona was low. Sera were considered test positive if MAT titres to either or both serovars were 1:48 or greater and culture positivity was determined by culture of kidneys

(Chapter 3). Chi-square test *P*-values, sensitivities, specificities, PPVs, NPVs, and disease and test prevalences were calculated in DAG_Stat (Mackinnon 2000)

A fixed effect logistic regression model was fitted at line level to assess associations between within-line percentage of carcasses with WSK and line serostatus, adjusted for effects of cohort and line size, using the Proc Logistic procedure in SAS version 9.1 for Windows (SAS Institute, Inc., Cary, NC). Within-line percentage of carcasses with WSK was fitted as a continuous variable, and cohort, and line size categorised with cut-points values of 72, 121, and 220, corresponding to the 25th, 50th, and 75th percentiles respectively, as categorical variables in the model.

A random effect logistic regression model was also fitted to account for within-line clustering when testing for associations between WSK lesion scores and carcass serostatus. The explanatory terms, WSK lesion score, a cohort-calendar month interaction term, and line size were all fitted as categorical variables. The model was fitted by a Generalised Linear Mixed Model using Proc GLIMMIX procedure in SAS version 9.1 for windows (SAS Institute, Inc., Cary, NC) using a binomial error distribution and a logit link. Model derived odds ratios were converted to prevalence ratios using the method described by Zhang and Yu (1998).

5.3 Results

At the individual animal level, white spotted kidneys (WSK) were observed in 450/2,753 (16%) randomly sampled young sheep at slaughter, of which 158 (5.7%) were seropositive to either or both serovars Hardjobovis and Pomona (Relative Risk 5.2, 95% CI 3.9–7.1) but the lesions were poor predictors of serological status with test sensitivity of 51%(95% CI 43–59) and positive predictive value of 18%(95% CI 14–22) as shown in the Table 5.1. Carcasses with high (>5 white spots or white mottling on one or both kidneys) WSK lesion scores were 6.1 (95% CI 4.3–8.3) times more likely to be seropositive to either one or both serovars than carcasses with low (1–5 white spots on one or both kidneys) scores, but the

test sensitivity and positive predictive value for those criteria were low at 27% (95% CI 20–34) and 27% (95% CI % 20–35) respectively.

Within-line prevalences of carcasses with WSK ranged from 3–70% (mean = 6%, median = 13%) and most lines had 6–20% of lambs affected (Figure 5.1). Sample sizes of lines ranged from 6–100% (mean = 30%, median = 24%), and comprised <40% in two-thirds of all lines. There was no or little difference in sample size and within-line prevalences of WSK among lines sampled over time.

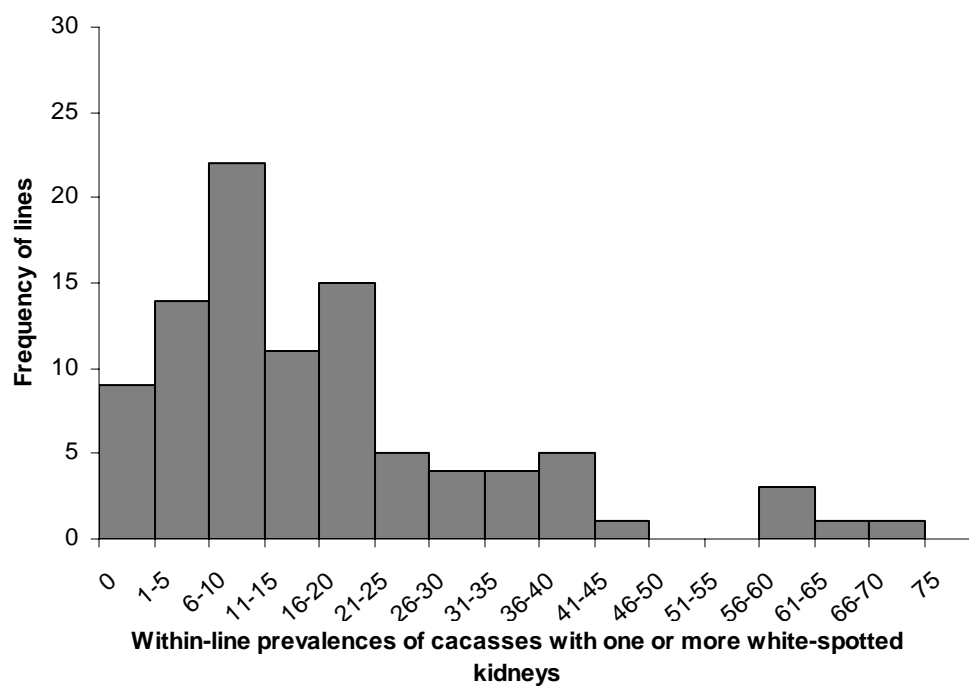


Figure 5.1: Frequency histogram of within-line prevalences of carcasses with one or more WSK lesions on either one or both kidneys in sheep sampled at a New Zealand abattoir during 18 May 2004 to 14 June 2005.

At slaughter line level, 86/95 lines (91%) had one or more carcasses with WSK, of which 41/86 (47.7%) WSK positive lines and 1/9 (11.1%) WSK negative lines were seropositive. Of the 86 WSK positive lines, 31 (36.1%) and 4 (4.7%) lines were seropositive to serovars Hardjobovis and Pomona respectively, and 6 (7.0%) to both. The only seropositive line without any WSK carcasses was seropositive to both serovars. The fixed effect logistic regression model (results not shown) indicated that line serostatus was not significantly associated with the prevalence of WSK positive carcasses in lines, after adjusting for line size and cohort effects.

Estimates with 95% confidence intervals for sensitivity, specificity, positive and negative predictive values, and prevalence ratios are shown in Table 5.1 with apparent and true disease prevalences for a range of diagnostic criteria tested against serology and culture test gold standards. Test characteristics with culture as the gold standard were not calculated for lines and lines with high vs. low WSK scores because chi-square tests *P*-values were ≥ 0.5 . With the exception of the high test sensitivity of 98% for WSK positive lines, where the positive predictive value was only 48%, test sensitivities and positive predictive values for all criteria tested against both gold standards were generally low.

Figure 5.2 illustrates the significantly positive linear associations between the relative risks of finding seropositives in carcasses with low and high WSK scores, that was evident after adjusting for the confounding effects of line size, line-level clustering, and a cohort-calendar-months interaction term, in the random effect model. Carcasses with low and high scores were 2.2 times (95% CI 1.4–3.3) and 4.4 times (95% CI 2.8–6.5) more likely to be seropositive than carcasses with no lesions. There were no significant differences between the predicted mean prevalence ratios for each of the 4 categories of quartile derived line sizes.

Table 5.1: Test operating characteristics (chi-square test *P*-values, sensitivity, specificity, positive and negative predictive values with 95% confidence intervals) for occurrence of white spotted kidneys in individual lambs and lines of lambs at slaughter evaluated for gold standard serology and culture status with prevalence ratio estimates (PR), test (TPr) and lesion (DPr) prevalences.

	P	T	F	F	T	Sensitivi	Specifici	PPV	NPV	PR	T	D
		P	P	N	N	ty	ty				Pr	Pr
Serology as the gold standard												
Animals with WSK vs. animals without WSK	<0.001	80	370	78	225	51 (43–58)	86 (84–87)	18 (15–22)	97 (96–97)	5.2 (3.9–7.1)	16.3	5.7
Animals with high vs. low WSK scores	<0.001	42	113	116	482	27 (20–34)	96 (95–96)	27 (21–35)	96 (95–96)	6.1 (4.3–8.3)	5.6	5.7
Lines with ≥1 WSK animals vs. lines without WSK animals	0.04	41	45	1	8	98 (87–100)	15 (8–27)	48 (37–58)	89 (57–98)	4.3 (0.7–27.6)	90.5	44.2
Lines with animals with high vs. lines with only low WSK score animals	<0.001	31	16	12	6	72 (57–83)	69 (56–80)	66 (51–78)	75 (61–85)	2.6 (1.6–4.5)	49.5	45.3
Lines with animals with ≥13.3 vs. lines with animals with <13.3 WSK	<0.001	30	19	12	4	71 (56–83)	64 (51–76)	61 (47–74)	74 (61–84)	2.3 (1.4–4.0)	51.6	44.2
Culture as the gold standard												
Animals with WSK vs. animals without WSK	0.3	35	65	11	469	21 (8–48)	88 (85–90)	4 (2–12)	98 (96–99)	1.9 (0.6–6.7)	12.4	2.6
Animals with WSK vs. animals without WSK**	<0.001	18	67	12	469	60 (42–75)	88 (84–90)	21 (14–31)	98 (96–99)	8.5 (4.2–17.0)	15.0	5.3
Animals with high vs. animals with low WSK scores**	<0.001	15	29	15	507	50 (33–67)	95 (92–96)	34 (22–49)	97 (95–98)	11.9 (6.2–22.6)	78	53

Note: TP=true positives; FP=false positives; FN=false negatives; TN=true negatives; PPV=positive predictive value; NPV=negative predictive value;

** Includes data from survey animals and a purposively selected line from a farm where a recent outbreak of leptospirosis was suspected

Leptospire were isolated from 14/548 (2.6%) survey sampled kidneys of which 2 came from 40 with low, 1 from 28 with high scores, and 11 from 480 with 0 scores. Of the 14 culture test positives, 8 were seropositive to Hardjobovis, 1 to Pomona, and 5 were from seronegative kidneys. The finding of no statistically significant association between WSK lesion scores and culture test results in the survey data ($P = 0.3$) rules out this criterion's use for predicting culture positivity.

When data collected purposively from the leptospirosis suspected-outbreak farm were added to the survey data, there was a positive linear association between WSK lesion scores and kidney culture results, wherein carcasses with low and high scores were 2.9 times (95% CI 0.8–10.0) and 13.3 times (95% CI 6.7–26.8) more likely to be culture positive than those without lesions (Figure 5.3). However, the low sensitivities and PPVs reported for WSKs as predictors of culture results were still strongly indicative of an unreliable test. A total of 13/30 lambs from the outbreak farm were seropositive to Pomona but none to Hardjobovis and all 13 were culture positive along with 3/5 kidneys from seronegative carcasses. One culture positive kidney had a zero score, one scored low and the others scored high.

5.4 Discussion

Predictive values are estimates of the probabilities that a disease is or is not present, depending on whether the test is positive or negative. The interpretation of the PPV of 18% when using the presence of WSK in individual animals for testing for evidence of disease exposure (Table 5.1) is that about only one in six animals with WSK would be seropositive and indicates that the test is unreliable for predicting disease exposure based on serology in individual animals. Predictive values are influenced by disease prevalence, in this case seropositivity of individual animals, but even though higher prevalences, such as those recorded for lines improved the PPVs for individual animals, they were still relatively low, and the corresponding NPVs were reduced. The low positive predictive values found for the study population may be partly due to low serological prevalence and the poor operating characteristics of the score-based test.

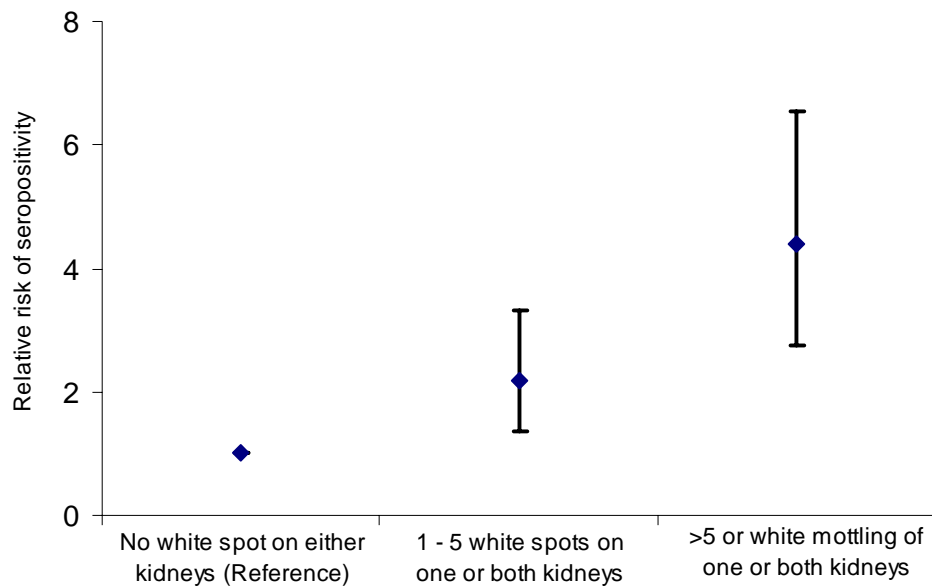


Figure 5.2: Random-effect model-adjusted relative risks (♦) and 95% CI (—) for seropositivity in animals with zero, 1 to 5, and >5 white spot lesions on one or both kidneys in randomly sampled slaughtered sheep during the period 18 May 2004 to 14 June 2005.

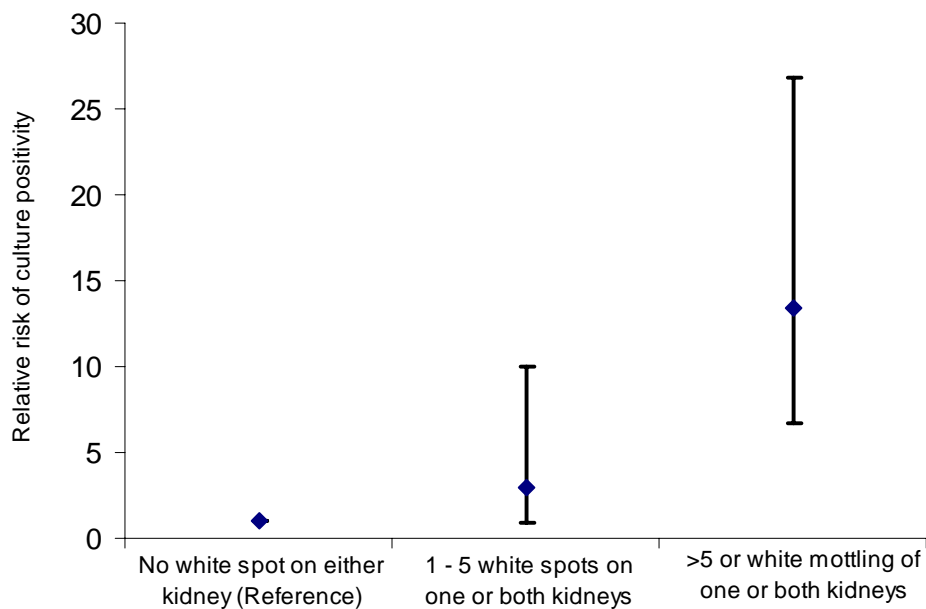


Figure 5.3: Crude relative risks (♦) and 95% CI (—) for culture positivity in animals with zero, 1 to 5, and >5 white spot lesions on one or both kidneys in sheep during the period 18 May 2004 to 14 June 2005.

The prevalence of carcasses with WSK was very high at line level and moderate at individual animal level, an indication that moderately high numbers of sheep in this study population were exposed to conditions that resulted in lesions. There was a statistically significant association between WSK positive lines and serology status, but not between lines and culture status. A poor predictability of WSK for culture positivity is not surprising, given that shedding is probably more likely in the early stages of infection and lesions would be expected to develop in the later stages. The most likely explanation for the 12 culture test positive animals with no lesions is early stage infection. It is also possible that seronegative animals with WSK could have been infected with serovars other than Pomona or Hardjobovis tested for in this study. The culture isolates have not yet been identified and uncertainties about possible involvement of other serovars should be resolved after that is done. It is unlikely that antibody titres would have sufficient time to decline to undetectable levels in the study animals because they were mostly lambs and the majority were less than one-year-old. The sensitivity of culture isolation for *Leptospira* spp. is considered to be high for shedders, but poor for all stages of infection and it is likely that the true prevalence of shedders was higher than the results indicated.

Focal interstitial nephritis can result from infectious agents other than *Leptospira* spp. (Jeffcott et al 1967; Maxie 1993; Drolet and Dee 1999), but interstitial nephritis associated with common bacteria in pigs generally only results in mild lesions that are not visible grossly (Hunter et al 1987). White spots appear only after 7–21 days after initial leptospirosis infection in pigs (Burnstein and Baker 1954; Sleight et al 1960) and may be absent in acute Pomona infections (Hunter et al 1987). Acute infections are characterised by very mild histological lesions, mainly tubular degeneration and mild interstitial nephritis.

The significantly higher within-line prevalence of WSK carcasses in seropositive lines than in seronegative lines found in this study is consistent with observations made in deer (Wilson et al 1998). However, the crude association found in this study not significant when adjusted for other covariates. Crude unadjusted measures of association at line level that are based on one or more carcasses may be too broad for useful and precise interpretation, because of possible influence from confounders such as line size, line-level

clustering, and calendar time of slaughter. However, the significant positive association between WSK scores and seropositivity, even after accounting for within-line clustering and covariates effects at carcass level is consistent with earlier studies in swine (Baker et al 1989) and cattle (Yener and Keles 2001). Baker et al (1989) found a statistically significant association (Odds ratio 4.6, Fisher's Exact *P*-value 0.03) between titres $\geq 1:80$ to Pomona and carcasses with multifocal interstitial nephritis in a prevalence survey but not in a small sample size case-control study with low power. White-spot lesions have also been consistently observed in leptospirosis in sheep in many earlier studies (Dozsa and Sahu 1970; Smith and Armstrong 1975; Davidson and Hirsh 1980; McCaughan et al 1980; Ellis et al 1984; Kingscote 1985) and deer (Wilson et al 1998) in contrasted to observations for cattle. Other studies in swine (Jones et al 1987; Boqvist et al 2003) and cattle (Prescott et al 1987; Skilbeck et al 1988; Uzal et al 2002a) found no significant associations but were based on small samples and failure to demonstrate leptospire with immunofluorescence and Warthin-Starry stains in kidney sections. The positive association between WSK lesions and kidney culture results found in our study is consistent with results from studies in pigs in Australia (Peet et al 1983; Mercy et al 1988) and Canada (Baker et al 1989).

It is evident that WSK lesions in young sheep provide some indication of exposure to leptospire, but it is equally apparent that they are by no means fully reliable predictors of serological or culture status, since both sensitivity and positive predictive values are low. Furthermore, some animals, 3.4% and 2.5% respectively, with no WSK have tested positive to serology and culture tests and animals with positive titres and lesions may or may not be shedding leptospire.

Nevertheless, despite low positive predictive values, it would be advisable to continue to condemn WSK for human consumption and to take extra hygienic and other precautionary measure when processing sheep with white spotted kidneys to minimise exposure to live leptospire, because carcasses with white-spot kidneys are associated with higher serological and cultural prevalences.

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