Leptospirosis in humans and pastoral livestock in New Zealand

A thesis presented in partial fulfilment of the requirements for the doctoral degree of Doctor of Philosophy at Massey University

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January 2013

Abstract

This PhD investigated leptospirosis in humans and pastoral livestock in New Zealand (NZ). A longitudinal 'abattoir study', in which blood from workers (*n*=592) from sheep (*n*=4), deer (*n*=2) and beef (*n*=2) slaughtering abattoirs was tested by the microscopic agglutination test (MAT), revealed that 10-31%, 17-19% and 5% of workers respectively, had antibodies against *Leptospira interrogans* sv Pomona (Pomona) and/or *L. borgpetersenii* sv Hardjobovis (Hardjobovis). While the annual infection risk for meat workers of sheep plants was 11.1%, it was 0% in workers processing deer and 1.2% in those processing beef cattle. Sixty workers had a history of probable leptospirosis while working in abattoirs between 1962 and 2010 and three sheep abattoir workers within the one year study period. In sheep abattoirs, new infection with Hardjobovis or Pomona measured by serology was associated with a two-fold higher risk of 'flu-like' illness, and an average of four days absence from work. The average annual risk of experiencing flu-like symptoms due to infection with *Leptospira* measured by serology was 2.7%. The under-ascertainment of officially notified cases with leptospirosis in the last five years was estimated at between 16 and 56 times. Work position was the strongest risk factor for sero-positivity with Pomona and/or Hardjobovis in sheep and deer abattoir workers. The prevalence and new infection risk was highest in workers at the beginning of the slaughter board and the use of personal protective equipment (PPE) appeared not to reduce the risk of sero-positivity or new infection. The risk factor analysis revealed that the infection risk prevailed in the abattoirs and was not evident for non-work related risk factors, such as hunting, home slaughtering and farming.

In a multi-species cross-sectional 'farm study' (*n*=238), 97% of sheep and beef and 76% of deer farms had at least one in 20 animals MAT sero-positive against Hardjobovis and/or Pomona. Overall, 50% of adult sheep, 58% of adult beef and 34% of yearling/adult deer were positive against either serovar. Hardjobovis was more prevalent in all three livestock species than Pomona. The regional prevalence distribution in sheep was different for Hardjobovis and Pomona. Grazing beef with deer reduced the likelihood of positivity against Pomona in beef. Co-grazing with another species did not increase the odds of the within-herd prevalence for deer and sheep of Pomona or Hardjobovis and for beef the within-herd prevalence of Hardjobovis controlling for other farm-level risk factors. The incidence of probable leptospirosis in cattle herds in 2009 was 2.6%, in sheep flocks 0% and in deer herds 1%. Tailing rates of sheep farms were positively correlated with prevalence of Hardjobovis: a 1% increase in prevalence was equivalent to a 0.11 increase in tailing percentages, which is unlikely to be causative since this association lacks biological plausibility. All other reproduction and culling rates of any species were not significantly associated with prevalence.

Acknowledgements

"Ehara taku toa, he taki tahi, he toa taki tini"

"My success should not be bestowed onto me alone, as it was not individual success but success of a collective"

I sincerely thank my four supervisors Cord Heuer, Peter Wilson, Jackie Benschop and Julie Collins-Emerson for guiding me, sharing their knowledge and expertise. Their enthusiasm and open door policy were motivating, and I truly enjoyed our lively discussions. Even though they were rigorous in their scientific approach, there was always time for a smile or a joke. I learned so much from each one of you – thank you!

I especially thank all meat workers and farmers who gave freely of their time, labour, patience and blood. Thanks too, to the veterinarians who did most of the sampling on farms, to the abattoir management who let me conduct the study in their abattoirs, to the staff who took their time to show and explain me procedures and to the nurses and phlebotomists who were willing to get up early in the morning to help me with blood sampling (names not mentioned due to confidentiality reasons).

I am immensely grateful to Sarah Moore, who was an irreplaceable help in data collection and entry, communication of results and a great travel companion. You became a wonderful friend and without your support I could not have done this!

A huge thanks to Neville Haack and Raewynne Pearson for all the lab work, to Saskia Prickaerts and a long list of students who helped processing samples, to Fang Fang, Prakriti Bhattarai, Rayon Gregory, Claire Cayol and Emilie Vallee for the help with interviewing, to Brian O'Leary, Massood Sujau and Simon Verschaffelt for the support to build the database and queries, to Paulina Guzman for the data entry, to Roger Lentle for advice regarding the Massey University Human Ethics Committee application, and to Mark Stevenson, Geoff Jones, Lesley Stringer, Ahmed Rabiee and Sarah Rosanowski for good advice on using Stata commands, to Cristobal Verdugo, Chris Jewel and John Alawneh for statistical advice. To the occupational health physicians John Reekie and John Kerr for advice and support in organizing the abattoir study, to Heather Duckett for helping in sampling logistics, to Christine Cunningham and Wendy Maharey for administrative support, to Bruce White for great support at the library, to Sabine Walser for the help with formatting, and to the NZ Veterinary Pathology (NZVP) for their support in sample processing and storage.

I gratefully acknowledge the support of the Swiss National Science Foundation (SNF) and Rural Woman NZ (RWNZ), without which I could not have undertaken the studies. Further, I am thankful for the financial contributions to the projects from the Alma Baker Trust Fund, the Tertiary Education

Commission (TEC) via the Institute of Veterinary, Animal and Biomedical Sciences, Massey University and the Department of Labour (DoL).

I would like to express my affection and appreciation to all the EpiCentre/Hopkirk people for their friendship, for sharing their thoughts and ways widening my horizon and for shared meals and barbecues during these years. Further, to my climbing buddies for discovering with me the beauty of NZ and the simplicity of life. Huge thanks to Lesley, Marta and Raewynne and her family for sharing their homes, their wonderful friendship and support in many ways.

Further, I would like to thank my previous mentors Joachim Frey, Ulrich Kihm, Dirk Pfeiffer and Hans Matter who have supported me on my professional path.

Finally I want to express my gratitude to my much-loved mother Agathe and father Sundar, my grandmothers Oma and Mimi who have given me love and support, to my siblings Jeshua, Eli and Sarah for existing, and my soul-sister Sabine for constant support on all levels, and to my beloved Sebastian who unexpectedly entered and entirely changed my life.

It was an amazing experience to live in New Zealand - such a precious land full of beautiful landscapes and wonderful space. I will never forget you, Aotearoa!

List of Publications

Dreyfus, A., Verdugo, C., Benschop, J., Collins-Emerson, J., Wilson, P., & Heuer, C. (2011). **Leptospirosis sero-prevalence and associated economic loss in New Zealand livestock.** In Proceedings of the Epidemiology and Animal Health Management Branch of the New Zealand Veterinary Association (pp. 3.12.1-3.12.10). Wellington, New Zealand: VetLearn.

Dreyfus, A., Verdugo, C., Wilson, P., Benschop, J., Collins-Emerson, J., & Heuer, C. (2010).**A survey of clinical leptospirosis in animals and people on mixed-species farms: Preliminary observations.** In Proceedings of a Deer Course for Veterinarians (Cervetec2010) Vol. 27 (pp. 77-80). Queenstown, New Zealand: Deer Branch: New Zealand Veterinary Association.

Dreyfus, A., Benschop, J., Collins-Emerson, J., Wilson, P., & Heuer, C. (2010). **Sero-prevalence of leptospirosis in deer slaughter premise workers in New Zealand.** In Proceedings of a Deer Course for Veterinarians (Cervetec2010) Vol. 27 (pp. 73-76). Queenstown, New Zealand: Deer Branch: New Zealand Veterinary Association.

Benschop, J., Dreyfus, A., Fang, F., Collins-Emerson, J., Wilson, P., & Heuer, C. (2011). **Update on leptospirosis research in New Zealand: The human-animal interface.** In No editor (Ed.), Proceedings of the Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association 40th Annual Conference (pp. 2.24.1-2.24.3). Wellington, New Zealand: VetLearn.

Benschop, J., Dreyfus, A., Fang, F., Collins-Emerson, J., Wilson, P., & Heuer, C. (2011). **Leptospirosis research in New Zealand: The human-animal interface.** In No editor (Ed.), Proceedings of the Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association 40th Annual Conference (pp. 35 pages). Wellington, New Zealand: VetLearn.

Heuer, C., Dreyfus, A., Wilson, P., Benschop, J., Subharat, S., Ayanegui-Alcerreca, Midwinter, A. (2010). **Epidemiology and control of leptospirosis in New Zealand.** In Society for Veterinary Epidemiology and Preventive Medicine (pp. 174-185). Nantes, France: Society for Veterinary Epidemiology and Preventive Medicine.

Heuer, C., Wilson, P., Collins-Emerson, J., Dreyfus, A., Subharat, S., Fang, F., Midwinter, A. (2009). **Massey researchers contribute to international leptospirosis conference.** Vetscript, 22(11), 26-28.

List of Presentations and Posters

Dreyfus, A., Benschop, J., Wilson, P. R., Collins-Emerson, J., & Heuer, C. (2012). **Leptospirosis incidence and risk factors in meat workers in New Zealand.** In book of abstracts of the 13th International Symposium on Veterinary Epidemiology and Economics. Poster 69, page 175. ISVEE, Maastricht, Holland.

Dreyfus, A., Verdugo, C., Benschop, J., Collins-Emerson, J., Wilson, P. and Heuer, C. (2012). **Leptospirosis sero-prevalence in New Zealand livestock and associated risk factors.** In book of abstracts of the 13th International Symposium on Veterinary Epidemiology and Economics. Page 175. ISVEE, Maastricht, Holland.

Dreyfus, A., Wilson, P. R., Benschop, J., Collins-Emerson, J., & Heuer, C. (2011, September 19). **Leptospirosis infection in meat workers in New Zealand- Prevalence, new infection rate and risk factors.** In *VII Reunion de la International Leptospirosis Society*. Merida, Yucatan, Mexico.

Dreyfus, A., Verdugo, C., Benschop, J., Collins-Emerson, J., Wilson, P., & Heuer, C. (2011). **Leptospirosis sero-prevalence and associated economic loss in New Zealand livestock.** Branch New Zealand Veterinary Association annual conference Epidemiology and Animal Health Management Hamilton, New Zealand.

Dreyfus, A., Wilson, P. R., Benschop, J., Collins-Emerson, J., Moore S. & Heuer, C. **Adjusting the leptospirosis sero-prevalence of NZ abattoir workers for sampling bias.** Presented at the Australian College of veterinary scientists Science Week, 1-3 July 2010 in Surfers Paradise, Australia.

Dreyfus, A., Verdugo, C., Wilson, P., Benschop, J., Collins-Emerson, J., & Heuer, C. (2010). **A survey of clinical leptospirosis in animals and people on mixed-species farms: Preliminary observations.** New Zealand Veterinary Association, Deer Branch. Queenstown, New Zealand.

Dreyfus, A., Benschop, J., Collins-Emerson, J., Wilson, P., & Heuer, C. (2010). **Sero-prevalence of leptospirosis in deer slaughter premise workers in New Zealand.** New Zealand Veterinary Association, Deer Branch. Queenstown, New Zealand.

Dreyfus, A. S., Benschop, J., Collins-Emerson, J. M., Wilson, P. R., & Heuer, C. (2009). **Determinants of leptospirosis infection of meat workers in New Zealand.** In 6th Annual Scientific Meeting of International Leptospirosis Society. Gokulam Convention Centre, Cochin, India.

Dreyfus, A., Benschop, J., Collins-Emerson, J., Wilson, P., & Heuer, C. (2009). **Leptospirosis in meat workers: A pilot cohort study.** In NZVA Epi Branch Conference. Palmerston North, New Zealand.

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Preface

"The intuitive mind is a sacred gift, the rational mind a faithful servant, we have created a society that honours the servant and has forgotten the gift"

Albert Einstein

Chapter 1

General Introduction

1. Introduction

Leptospirosis is a zoonotic bacterial disease of increasing prevalence, a worldwide distribution, and with potentially serious consequences for human and animal health (Levett, 2001; Bharti et al., 2003). It is an endemic disease of domestic and wild animals in New Zealand (NZ) and the most important occupationally-acquired zoonotic disease of abattoir workers and farmers. In NZ, serovars are largely identical in terms of serovars seen in livestock and humans, yet most farmed livestock are unvaccinated and people remain occupationally exposed (Thornley et al., 2002).

The scope of this general introduction is to give a short overview of the microbiology, epidemiology, taxonomy and classification, pathology, pathogenesis, immune response, epidemiology, clinical signs, diagnostic methods, treatment and control strategies. Further, given the importance of this disease in the meat industry in NZ, it provides a summary of the history of abattoirs in NZ. Then, it will focus in more detail on the epidemiology of leptospirosis in NZ for both humans and livestock. Extensive literature reviews on leptospirosis in general and in NZ have been published recently (Faine et al., 1999; Levett, 2001; Bharti et al., 2003; Ayanegui-Alcérreca, 2006; Adler and de la Pena Moctezuma, 2010; Subharat, 2010 ; Hartskeerl et al., 2011). Therefore, this review focuses on the topics relevant to this thesis.

2. Leptospirosis Overview

2.1. Microbiology

Leptospirosis is a zoonotic disease occurring in many mammals and is caused by a bacterium of the genus *Leptospira* spp. (Figure 1). Leptospires are motile, obligate aerobic spirochaetes with an optimum growth temperature of 28‑30^{o} C that have characteristics of both Gram-negative and -positive bacteria (Faine et al., 1999; Haake et al., 2000). The composition of the lipopolysaccharide (LPS) is similar to the LPS of Gram-negative bacteria (Vinh et al., 1986). However, it is less endotoxic (Shimizu et al., 1987). They belong to the order Spirochaetales, family Leptospiraceae, genus *Leptospira*. Leptospires are catalase and oxidase positive and their size is about 0.25 x 6.25 µm. The genome is made out of two circular chromosomes. The taxonomy is based on either serological

(*sensu lato*) or molecular classification (*sensu stricto*) (Bharti et al., 2003). Serological taxonomy divides *Leptospira (L.)* into two species *L. interrogans* (pathogenic) and *L. biflexa* (non-pathogenic), into more than fifty serogroups and nearly 300 serovars on the basis of surface antigens. The molecular classification system groups leptospires depending on DNA relatedness in 18 genomospecies being pathogenic, non-pathogenic or opportunistic (Yasuda et al., 1987; Ramadass et al., 1992; Hartskeerl et al., 2011). The serological taxonomy is still more commonly used in epidemiological studies and as well in this thesis.

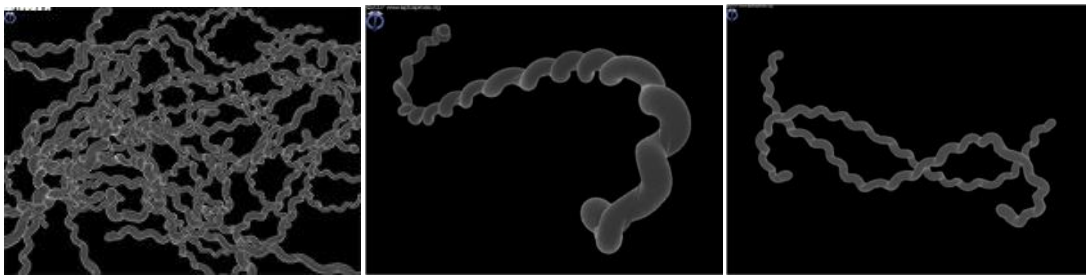

Figure 1: Computer model of *Leptospira interrogans*[1]

2.2. Epidemiology

Leptospirosis is a zoonotic disease of increasing prevalence, a worldwide distribution, and with potentially serious consequences for human and animal health (Levett, 2001; Bharti et al., 2003). Transmission occurs from exposure to urine or aborted tissues of infected animals, either directly or via contact with contaminated water or soil (Hartskeerl et al., 2011). Sometimes *Leptospira* remain in the genital tract and may be transmitted by fluids of the reproductive tract (Ellis et al., 1986). Adler et al. postulate that *Leptospira* may enter through wet skin (Adler and de la Pena Moctezuma, 2010).

Leptospira spp. persist in a humid environment (i.e. alkaline soil and water) for months or years (Miller et al., 1991). High rainfall and flooding are favourable for leptospirosis outbreaks, which occurred in sheep flocks during or after major floods in NZ (Dorjee et al., 2005a).

The infection outcome varies with the grade of adaptation of the serovar type to the infected mammal species and is probably due to direct pathogen effects and (genetically determined) host immune responses (Bharti et al., 2003). Commonly each serovar is adapted to one or more hosts. Hosts are divided into maintenance/reservoir hosts and accidental/dead end hosts. Specific *Leptospira* serovars are well adapted to particular mammals, which are then called maintenance hosts. If pathogen adaptation is weak, the mammal is considered an accidental host. Table 1 illustrates existing *Leptospira* serovars and their corresponding maintenance hosts in New Zealand

[1] Source: www.leptospirosis.org. Accessed July 12, 2012

(NZ) (Marshall and Manktelow, 2002; Ayanegui-Alcerreca et al., 2007). Maintenance hosts become carriers, which shed *Leptospira* intermittently for months or years (Smith et al., 1994; Ayanegui-Alcérreca, 2006).

Humans are considered accidental hosts and there is very little evidence of shedding, therefore human-to-human transmission is regarded negligible (Levett, 2001). Recently, this nomenclature has been questioned, as leptospirosis is a dynamic disease and strains adapt to new hosts with ecological conditions (i.e. climate) changing or with shifts in farming practice (Hartskeerl et al., 2011). Therefore, in this thesis serovars will be referred to as being adapted or non-adapted to a host.

Table 1: Classification of *Leptospira* species endemic in New Zealand and host animals ('maintenance hosts') to which the serovars are adapted to (Marshall and Manktelow, 2002; Ayanegui-Alcerreca et al., 2007; Dorjee et al., 2008)

Serovar	Serogroup	Adapted to
L. borgpetersenii Hardjobovis	Sejroe	Cattle, deer, sheep
L. interrogans Pomona	Pomona	Pig, deer
L. interrogans Copenhageni	Icterohaemorrhagiae	Norway rat
L. borgpetersenii Ballum	Ballum	Black rat, mouse, hedgehog
L. borgpetersenii Balcanica	Sejroe	Possum
L. borgpetersenii Tarassovi	Tarassovi	Pig

Leptospirosis occurs worldwide in mammals. Even though incidence of human leptospirosis is higher in low income countries, infection and disease also exist in industrialised nations. Leptospirosis is generally underreported due to nonspecific symptoms, lack of awareness, challenging diagnostics and poor access to health care (Bharti et al., 2003).

The occurrence of different serovars in a population depends on the prevalence and density of domestic and feral hosts, on environmental factors such as climate and rainfall, vaccination policy, farm management, occupation (farming, meat industry etc.), recreational activities (water sports) and the socio-economic situation (housing, hygiene, rodent control). Rodents are important transmission pathways in urban households with poor hygiene in low income countries (i.e. slums). In moist tropical environments, water (e.g. paddy fields) is an important reservoir for a large variety of serovars (Bharti et al., 2003). In temperate regions, e.g. NZ, infection is due to occupational exposure to domestic carrier animals and recreational activities, such as water sports. Generally in NZ, only few serovars predominate and infection is seasonal, especially with flooding in summer (Thornley et al., 2002).

2.3. Pathogenesis, Clinical Symptoms and Pathology

The mechanisms causing disease are still not entirely understood (Adler et al., 2011). Pathogenic leptospires enter the body through mucous membranes (eyes, mouth, lips and reproductive organs) or skin abrasions, enter the blood stream and lymphatic system and are disseminated through various organs, such as the kidneys, liver, lungs, eyes, brain, muscles and heart (Levett, 2001). Leptospires cause vasculitis leading to damage of endothelial membranes and necrosis of organs. Acute infection induces an interstitial nephritis, whereas chronic carriers have very little to no renal pathology (Bharti et al., 2003). Superficial kidney lesions ('white spots') were strongly associated ($p < 0.0001$) with sero-prevalence against *L. interrogans* serovar Pomona (Pomona) or *L. borgpetersenii* serovar Hardjobovis (Hardjobovis) in slaughter lambs in a NZ abattoir (Dorjee et al., 2005a). Host-adapted serovars colonize the proximal renal tubules of the kidneys and are shed in the urine. The persistence of *Leptospira* spp. in kidneys may be enhanced by the fact that the renal tubule is an immunoprivileged site. Experiments in rats have shown that *Leptospira* down-regulate the expression of proteins that are recognized by the humoral immune response (Ko et al., 2009).

The clinical manifestation depends on the serovar, virulence and endemicity of a strain, infectious dose, age and immune status of the host. Clinical signs in animals are usually inapparent or mild when infected by host-adapted serovars. Infection with non-host-adapted serovars may lead to mild to severe icterohaemorrhagic disease with fever, dull appearance, pyrexia, agalactia, hematuria, haemolytic anaemia, jaundice, haemoglobinuria and/or renal failure. Acute forms may manifest with abortion storms (cattle), agalactia in ewes, cows and sows and mortality in lambs, calves, piglets and weaner deer (Ellis, 1994; Dorjee et al., 2005a). Infections with host-adapted serovars have either no ill effects or are sub-clinical and/or chronic and may cause poor reproductive performance (Dhaliwal et al., 1996b), reduced milk production (Dhaliwal et al., 1996a), stillbirth, weak offspring and reduced growth as shown in deer (Ayanegui-Alcerreca et al., 2007; Subharat et al., 2012a).

In humans, infection with *Leptospira* spp. varies from being sub-clinical (asymptomatic), to a mild to a highly acute disease, depending on the infecting serovar, age, health and immunological competence (Adler and de la Pena Moctezuma, 2010). Leptospirosis can occur in two phases: the first phase (1 week) is characterized by bacteraemia with fever and the second phase by icterohaemorrhagia with jaundice, renal failure, hepatic failure, myocarditis, uveitis and/or pulmonary haemorrhage. This less common second phase is called 'Weil's disease', amongst others, and has a case fatality rate of 5-15%. A mild form with fever and 'flu-like symptoms is more common, a reason for leptospirosis being underreported, as it is often misdiagnosed or the ill do not seek medical attention. Common symptoms associated with acute leptospirosis are fever, myalgia,

vomiting, headache, anorexia, conjunctival suffusion, nausea, sore eyes and prostration (Bharti et al., 2003). The incubation period is 5 to 14 days with the disease lasting 2 to 3 weeks involving a long recovery phase (World Health Organisation, 2003), which includes symptoms such as weakness, tiredness, depression, and even psychosis (Adler and de la Pena Moctezuma, 2010).

2.4. Immunology

Leptospires induce a serovar-specific immune response consisting of a cellular (type 1) and humoral (type 2) mediated immunity. Sero-conversion may occur after 2 - 10 days after onset of disease, dependent on the individual's immunological competence, infecting serovar, animal species and the infective dose. IgM class antibodies usually appear earlier than IgG class antibodies, and remain for months or years at a low detectable titre. IgG antibodies may not be detected at all, or for only a short period, or persist for several years. The antibodies are directed against both non-specific and serovar specific antigens (Faine et al., 1999; World Health Organisation, 2003). The primary immunological response is humoral mediated and the presence of immunoglobulin has shown to be protective in challenge trials (Flint and Liardet, 1980; Palit et al., 1996). However, beef cattle have responded to vaccination with a cellular response (Naiman et al., 2001) and presence of antibodies were not always protective (Adler and de la Pena Moctezuma, 2010). The level of antibody response is influenced by its initial level, the rates of decay, its continued production, the infected species, the age of the host and how well adapted the serovar is to its host (Faine et al., 1999). *Leptospira* can be isolated in urine from hosts that demonstrate a low or no detectable titre (Mackintosh et al., 1980a; Faine et al., 1999). Serovars that are more likely to cause clinical disease like Pomona and Copenhageni, may induce higher titres than Hardjobovis infections (Faine et al., 1999; Ayanegui-Alcérreca, 2006).

The duration of detectable antibodies in humans after natural infection with *Leptospira* varied substantially within and between studies, with sero-positive persons becoming sero-negative between 6 and 60 months after infection. The large range in titre duration is possibly related to re-exposure, infecting serovar and initial titre (Mackintosh et al., 1980b; Blackmore et al., 1984; Romero et al., 1998; Faine et al., 1999; Cumberland et al., 2001).

2.5. Diagnostic Tests

Leptospires can be visualized by dark-field or phase-contrast microscopy of wet preparations with a low sensitivity and specificity (Bharti et al., 2003). Detection methods for leptospires are either direct, identifying *Leptospira* antigen or genomic substances, or indirect, detecting host antibodies. Direct methods are, among others, direct microscopic examination for leptospires, culture, real-time

polymerase chain reaction (Real-Time PCR) and molecular typing, such as DNA hybridisation and multi locus sequence typing (MLST). In humans, the organism can be isolated from blood, urine, cerebrospinal fluid and tissue samples in the first week and from urine in the second and third week of illness. Culture of *Leptospira* spp. is insensitive and slow and hence not widely used as a routine method in diagnostics. However, in research it is widely used (Faine et al., 1999; Bharti et al., 2003). The advantage of the PCR is its sensitivity, even with antibacterial treatment and its capacity of detection at an earlier stage than any indirect method. Unfortunately, in general PCRs only differentiate between pathogenic and non-pathogenic strains, but not between serovars (O'Keefe, 2002). However, a real-time PCR for detection of pathogenic *Leptospira* species based on amplification of DNA gyrase subunit B gene, which had been optimized and evaluated with kidney and urine samples from NZ farmed deer, was able to distinguish Hardjobovis and Pomona from 14 samples and was consistent with MAT results (Subharat et al., 2011b). Hence, in regions with few pathogenic *Leptospira* species and serovars prevalent, PCR may be appropriate to identify *Leptospira* at serovar level.

A quick diagnosis of leptospirosis is crucial on an individual level in order to start treatment in a timely fashion and the specification of the serovar is less important. However, the distinction of the infecting serovar is often important to understand the aetiology of an outbreak in a population and to implement adequate control measures. Research has shown that MLST was useful to characterize leptospiral strains (Heuer et al., 2009) and serovars (Platero, 2009) and might be useful to investigate inter-species transmission in the future.

Indirect tests are more frequently used for diagnostic purposes and are, among others, the Microscopic Agglutination Test (MAT) and enzyme-linked immunosorbent assay (ELISA), both detecting IgG and/or IgM. Pooled sensitivity and specificity of ELISA tests were 0.78 (95% CI 0.77‑0.79) and 0.91 (95% CI 0.91‑0.92) in a meta-analysis for the purpose of detecting *Leptospira* specific antibodies (Signorini et al., 2012). In that study, a convalescent state of disease was significantly associated with higher diagnostic accuracy and IgM ELISA was more accurate, independent of the stage of disease.

The MAT is currently the standard reference test to detect leptospiral antibodies and diagnose acute leptospirosis. It requires expertise, pathogen containment level 2 (PC2) laboratory safety and is time-consuming, as live *Leptospira* culture is used as the antigen. Serial dilutions of test serum are incubated with cultures of specific *Leptospira* serovars (antigen). Antibodies in positive sera agglutinate with antigen and are assessed microscopically. The titre cut-off of 1:48 is recommended to determine exposure to leptospires, but not for clinical disease. To diagnose an acute infection with *Leptospira*, either a MAT titre ≥400 or a fourfold rise in titre between sera taken five to 10 days

apart is recommended. Serology is not very useful to determine carrier state, as bacteriologically proved carriers may be MAT-negative and MAT-positive animals may not be shedding *Leptospira* (Faine et al., 1999).

Fang et al. (2013) investigated the impact of storage time and batching of frozen animal samples on MAT titres against serovars Hardjobovis and Pomona by linear regression. Animal species, supplier, and slaughter line were considered as potential confounders in the analysis. At the Molecular Epidemiology and Public Health Laboratory in NZ, the median of interval between sampling and processing for serovar Hardjobovis was 140 days (minimum, 4; maximum, 156), while the median of interval for serovar Pomona was 105 days (minimum, 4; maximum, 121). No significant association was detected between MAT results (for Hardjobovis or Pomona) and the days of intervals.

The MAT has a reported sensitivity of 91% - 100% and specificity of 94% to 100% for detecting antibodies in reconvalescent blood samples (McBride et al., 2007). However, a recent study re-evaluated data from 1652 patients with suspected leptospirosis tested by culture, MAT, immunofluorescence assay (IFA), lateral flow (LF) and/or PCR targeting the 16S rRNA gene by using Bayesian latent class models and random-effects meta-analysis and concluded that MAT had a sensitivity of 50% (but a specificity of 99%) on the day of admission of patients with acute leptospirosis to the hospital and for some patients after a two week follow-up sample (Limmathurotsakul et al., 2012). Even though part of the insensitivity of the MAT is probably due to the testing of blood in the acute stage, where sensitivity is reported to be between 34 and 78% (McBride et al., 2007), the authors questioned whether MAT should remain the reference test in the future. However, there is a qualitative difference between a test diagnosing clinical disease (for which the authors do not state the case definition and MAT cut-off) and testing sero-positivity in a research context. Unfortunately, the sensitivity and specificity of the MAT has not been validated for the NZ context and it is unknown whether it differs between species (Collins-Emerson, 2013, personal communication). The sensitivity and specificity of the MAT depend among other factors on the prevalent serovars, whether these serovars belong to the same serogroup, whether many cross-reactions may be expected from the prevalent serovars and the chosen titre cut-off defining a positive test result. Further, test results should be interpreted in a context, for example when herd-level prevalence is measured, cross-reactivity with other serovars, which are not adapted to the host, of which the prevalence is measured, become negligible. Moreover, the positive or negative predictive values of a test (the proportion of test resuts that are truly positive, or negative respectively) do not only depend on sensitivity and specificity, but as well on prevalence. If the MAT is used in an environment or risk group with a high prevalence for a given serovar, positive test

results for this serovar are much more likely to be truly positive.

The MAT is not specific for any particular class of antibody, but differentiates between serogroups and serovars (Faine et al., 1999). Levett et al. (2001) however, called into question that the MAT had the ability to differentiate between serovars and declared that the MAT was not even very accurate in distinguishing between serogroups (Levett, 2001). Also Smythe et al. (2009) concluded that the MAT was not serovar specific when conducting a study with the aim to determine whether MAT provided an accurate guide to the infecting serovars of *Leptospira* in Thailand (Smythe et al., 2009). Nevertheless, in NZ serovar attribution is most likely possible by MAT, probably due to restricted serovar diversity. Hathaway (1981) as well as Marshall and Manktelow (2002) have shown that in NZ a restricted number of serovars and serogroups are endemic . Apart from Hardjobovis and *L. borgpetersenii* sv. Balcanica (Balcanica), which have different maintenance host secies, all isolated serovars in NZ belong to different serogroups (Table 1). Therefore, infection of a specific serovar in a host species can be determined by serology in NZ. Several studies have been conducted in NZ in recent years, where serovars determined by serology had been also confirmed by direct methods. For example, MAT serology and serovar isolates had good kappa agreement by DNA sequencing results (Subharat et al., 2011b; Subharat et al., 2012a).

Further, unpublished work conducted at the Molecular Epidemiology and Public Health Laboratory in NZ by Fang et al. (2013) in the frame of a current PhD project, demonstrated good correlation between MAT results and the infecting serovar, based on multi locus sequence typing (MLST of isolates). The same author conducted challenge trials in sheep with serovars Pomona and Hardjobovis. MAT testing of sera from those challenged sheep reproduced appropriately the corresponding serovar (Fang, 2013).

Because of the high endemic levels of the investigated serovars, namely Hardjobovis and Pomona in livestock and humans in this thesis, the positive predictive value of the MAT for these serovars is most likely high and therefore the possibility of misclassification low.

Cross-reactions occur between Hardjobovis and Balcanica, which is host adapted to possums (Faine et al., 1999; O'Keefe, 2002). However, since Balcanica is likely not adapted to livestock, the infection will remain sporadic and should not hamper the interpretation of the test result in a herd setting (Mackintosh et al., 1981).

2.6. Treatment

Usefulness of antimicrobial treatment is controversial; however, penicillin and doxycycline have been studied in randomised controlled intervention trials and are widely used for treatment of leptospirosis in humans. A Cochrane review concluded that “there was insufficient evidence

available to advocate for or against the use of antibiotics in the therapy for leptospirosis. Among survivors who were hospitalised for leptospirosis, use of antibiotics for leptospirosis may have decreased the duration of clinical illness by two to four days, though this result was not statistically significant" (Brett-Major and Coldren, 2012). To be effective, treatment should be started early during the acute stage of illness (Bharti et al., 2003). Supportive treatments such as fluid therapy and dialysis are needed with acute, severe forms.

Animal leptospirosis can be treated with dihydrostreptomycin and oxytetracycline. Dihydrostreptomycin has been shown to be able to eliminate leptospires from animals (Hartskeerl et al., 2011).

2.7. Vaccination and Control

In the animal population leptospirosis is controlled by limiting direct and indirect transmission between susceptible hosts, carriers and the environment. Options for control are detection of the source by identification of the serovar, management of herds by vaccination, buying *Leptospira* spp. free or vaccinated animals and management of pastures by fencing off standing water, effluent control, setting traps, and antimicrobial treatment or culling of diseased animals (Department of Labour and Accident Compensation Corporation, 2001; Hartskeerl et al., 2011).

Whereas leptospirosis originating from serovars carried by rodent and other feral animals is not possible to eradicate by vaccination (Hartskeerl et al., 2011), vaccination could be an option for control if leptospirosis is mainly a problem in livestock, especially if the variety of serovars is limited (as for example in NZ). In a population, where most human leptospirosis cases derive from exposure to livestock, control in livestock will directly influence the incidence in humans (Marshall, 1987). However, the correct choice of the vaccine and timely application is critical for the effectiveness (Benschop et al., 2012).

Nine leptospirosis vaccines for cattle, two for sheep and three for deer are presently registered in NZ, of which all include Hardjobovis and Pomona and some also include serovar *L. interrogans* Copenhageni (Copenhageni), but none serovar *L. borgpetersenii* Ballum (Ballum). The vaccines for cattle and deer are supposed to prevent urinary shedding if animals had not been infected prior to vaccination. However, a cross-sectional study discovered *Leptospira* positive urine by PCR and/or dark-field microscopy in vaccinated dairy cows (Benschop et al., 2012). Whether these cows already had been infected before vaccination is unknown and more research on shedding in vaccinated cows is required. A summary of vaccine efficacy trials with the outcome "urinary shedding" conducted between 1957 and 2012 can be found in a recently published report reviewing vaccines and vaccination policy in NZ. The following summary of these trials is cited from this report (Benschop et

al., 2012): “Published studies are difficult to compare directly, as they include natural and experimental infection challenges and vary in dose of challenge and in the leptospiral serovars used in the vaccine and for challenge. Additionally, there are differences in age at vaccination, interval between vaccination and challenge and method of measuring and quantifying leptospiral shedding. Route of challenge also varies between the studies: intravenous, intraperitoneal, conjunctival, oral and intranasal methods of delivering a challenge dose have all been applied in the studies reported here….. The vaccines administered in the reported trials range from mono- to multivalent preparations and use different adjuvants and strains of the organism, although often in the literature there is little detail on the vaccine preparation itself. Key findings of some of the individual trials that measured efficacy of vaccine to prevent urinary shedding are briefly summarised here” (Benschop et al., 2012).

2.7.1. Vaccination challenge trials in cattle

“Early studies of vaccine efficacy were carried out with preparations containing serovar Pomona. Gillespie and Kenzy (1958) demonstrated that urinary shedding could be prevented in heifers using vaccines containing a killed suspension of serovar Pomona. Twelve heifers were vaccinated at 6-8 months of age and, along with five controls, were experimentally challenged 8 months later with urine containing serovar Pomona from shedder cattle via the conjunctival route and in drinking water. Urine was classified for leptospiral shedding by darkfield examination or by ‘laboratory animal’ inoculation. Shedding was identified in 1/12 vaccinates and 5/5 controls.

Ris and Hamel (1979) assessed a commercial monovalent Pomona vaccine (A) and experimental Pomona vaccines prepared with different adjuvants (B and C) in three groups of four 9-month-old heifers, comparing them to a control group of four heifers. Experimental challenge was by the intramuscular route 47 weeks later. Urinary shedding of leptospires, as assessed by culture, was prevented in all of the vaccinates but detected in all of the controls….

Studies in cattle have similarly examined the efficacy of vaccination with a monovalent preparation of serovar Hardjobovis or Hardjoprajitno to prevent urinary shedding of leptospires. In a US study, Bolin et al. (2001) vaccinated two groups of eight 8-12 month old heifers with two different monovalent Hardjobovis vaccines - a commercially available vaccine (A) and a reference vaccine (B) - keeping a third group of eight heifers as controls. The heifers were experimentally challenged four months later with a US strain of serovar Hardjobovis, by conjunctival instillation or intraperitoneal inoculation. Vaccine A was shown to prevent urinary shedding and renal colonisation in 8/8 heifers. In contrast, all heifers inoculated with vaccine B were urine and tissue positive. The study also showed differences in shedding outcomes between the different routes of leptospiral challenge.

Challenge via the conjunctival route resulted in leptospiruria in 4/4 controls compared to 2/4 controls challenged intraperitoneally. Leptospires were identified in the kidneys of all controls.

More recently, Zuerner et al. (2011) assessed the efficacy of a monovalent Hardjobovis vaccine (Mono1) to prevent urinary shedding in Holstein steers when challenged three months later with serovar Hardjobovis by the conjunctival route. None of the eight vaccinates and 4/4 controls were urine culture positive following challenge. However, the presence of leptospires in urine was also assessed by PCR, which identified 6/8 vaccinates and 4/4 controls as positive. This is one of the few cattle studies to use PCR to identify urinary shedding. Although identifying bacterial DNA does indicate at least transient colonisation of the kidney, the technique cannot distinguish between live and dead bacteria. The relevance of the finding to transmission of infection to other animals or humans is thus unknown.

Efficacy studies of bivalent vaccines containing Hardjobovis and Pomona serovars have similarly demonstrated efficacy of the vaccines in preventing urinary shedding in cattle. The published literature includes studies carried out in New Zealand, such as those of Marshall et al., who examined the efficacy of a serovar Hardjo/Pomona vaccine. The first study (Marshall Rb, 1979) involved nine calves vaccinated at 3-4 months old and given a booster vaccination six weeks later, and ten unvaccinated controls. The calves were exposed, seven months after vaccination, to cattle known to be shedding Hardjo and urine was monitored by culture and dark-ground microscopy over a period of four months. None of the vaccinates and 6/10 of the controls shed Hardjo in urine.

In the second study (Marshall et al., 1982), the efficacy of the Pomona component of the same vaccine was assessed in six-month old heifers, this time using subcutaneous challenge with serovar Pomona at 19 days post-vaccination rather than natural challenge. None of the 11 vaccinates and 8/11 unvaccinated controls yielded positive urine cultures during 32 days of follow-up" (Benschop et al., 2012).

2.7.2. Vaccine efficacy in sheep and deer

"A single sheep study (Marshall et al., 1979) was identified by the systematic literature search. The research was carried out in New Zealand, and involved 19 Romney ewes aged 7-9 months. Nine were vaccinated twice, one month apart, with a bivalent Pomona/Hardjo preparation and 10 remained as untreated controls. Challenge six weeks later, with a bovine isolate of serovar Hardjo, was by the intraperitoneal or the intramuscular route, while infection status was established by culture of kidneys at post-mortem three weeks after challenge. Two vaccinates and all controls were *Leptospira* positive. A notable additional finding from this study was that two of the vaccinates which resisted challenge showed no MAT response to vaccination, and two with titres rising from 24

to 96 between weeks 12 and 13 were culture negative. Although demonstrating the efficacy of vaccine to prevent kidney colonisation, the short timescale of the trial meant the study does not provide any evidence of the duration of vaccine induced immunity in sheep" (Benschop et al., 2012).

"In deer, vaccination has been shown to prevent urinary shedding of leptospires in a natural challenge situation (Subharat et al., 2012a). The study, carried out in five commercial deer herds in 2007, followed on from the research of Ayanegui-Alcerreca (2006), who found vaccination in herds naturally infected with serovars Hardjo and Pomona reduced urinary shedding by 44%. In the 2007 study, 435 three-month old deer were treated with streptomycin and 217 were then inoculated with a bivalent Hardjo and Pomona vaccine while 218 were maintained as unvaccinated controls. Challenge was natural, with trial animals mixed with deer infected with Hardjo on the same farm. Urine from 110 female deer from each trial group was monitored for shedding using culture and PCR, with positive PCR results (8/34) seen only in control animals on two farms six months later. On one farm 1/9 controls were culture positive. Although the proportion of controls in which shedding was detected by culture appears low, sampling of the deer mixed with the trial animals found shedding rates of up to 83%, illustrating that challenge was occurring" (Benschop et al., 2012).

The presence of maternally derived antibodies (MDA) does not necessarily inhibit the efficacy of vaccines. However, this report points out that the best timing of vaccination is when MDA have waned and young animals have not been naturally challenged yet. It recommends that based on current knowledge, young stock in highly infected herds should be vaccinated at an early age (a few weeks) and then receive two boosters in three months intervals. Subsequently annual boostering should be the rule. In vaccinated herds or in a low challenge environment it is recommended to vaccinate young stock before six months of age with boosters given annually (Benschop et al., 2012).

In the human population leptospirosis is controlled by detection and removal of the infection source by treating or culling the carrier animal, implementing rodent control, building rodent proof wells and vaccination of pets and domestic animals, vaccinating humans or applying short term medical prophylaxis in highly exposed persons (Faine et al., 1999; Hartskeerl et al., 2011). In many countries there is no vaccine for humans released on the market. Countries that have used vaccines containing whole killed leptospires in the past are Cuba (Martinez et al., 2004), China (Chen, 1985), Russia, France (Hartskeerl et al., 2011), Vietnam and Japan (Adler and de la Pena Moctezuma, 2010). These vaccines are serovar specific and boosting is required regularly to maintain the protection.

Options to reduce the risk of transmission are wearing personal protective equipment (PPE) (gumboots, aprons, gloves, safety glasses), covering skin lesions with waterproof dressings and washing immediately after urine exposure (Department of Labour and Accident Compensation Corporation, 2001). Awareness is raised by establishing a national notification system, informing

veterinarians, doctors, farmers and meat industry as responsible partners, risk groups (meat workers, water sports, farmers, veterinarians, technicians) and the wider community with brochures, signs and or press releases.

3. The Meat Industry in New Zealand

The highest proportion of notified leptospirosis cases in NZ[2] occur in the meat industry. As meat workers are an important study population in this thesis, an overview of the meat industry, its history in NZ and the meat worker union follows.

After the successful first shipment of frozen carcasses in 1882, the NZ meat industry grew because of a demand for meat from the British market. While in 1882 there were three freezing works in NZ, by 1893 there were 21. In the 1930s many NZ's freezing works belonged to British companies. However, by the end of the 20th century, meat works were mainly owned by NZ farmer cooperatives. In 2006, 362,000 tonnes of sheep meat were exported from NZ and over 370,000 tonnes of beef. In 2009 NZ counted about 80 meat-processing plants with approximately 26,500 employees[3].

The first meat worker union was formed in 1901 to improve wages and working conditions and in 2005 one union covering the whole country was created. As the Maori land-holdings were reduced from 26.8 million to 3.4 million hectares by 1921, Maori increasingly entered the workforce of freezing works and joined the unions in increasing numbers[4]. The meat worker union plays an important role in protecting their members from contracting occupationally acquired diseases and is involved in improving the safety regarding leptospirosis by raising awareness among workers, supporting their members with occupational disease insurance claims (Accident Compensation Corporation, ACC) and being involved in strategic meetings of the meat industry and partner organisations.

Introduction of the chain in 1930 and mechanisation of steps in the slaughter process, increased production. For example, one meat plant in 2009 ran four chains that processed eight sheep per minute[5,6,7]. A natural consequence of increased production is that workers are exposed to

[2] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-2010.
[3] Jane Tolerton. 'Agricultural processing industries - Freezing works, 1880s to the 1970s', Te Ara - the Encyclopedia of New Zealand, accessed September 12, 2012. URL: http://www.TeAra.govt.nz/en/agricultural-processing-industries
[4] http://www.nzmeatworkersunion.co.nz/our_history.html. Accessed September 12, 2012.
[5] Jane Tolerton. 'Agricultural processing industries - Freezing works, 1880s to the 1970s', Te Ara - the Encyclopedia of New Zealand, accessed September 12, 2012. URL: http://www.TeAra.govt.nz/en/agricultural-processing-industries
[6] http://www.nzmeatworkersunion.co.nz/our_history.html. Accessed September 12, 2012

more animals per day and these are potentially shedding *Leptospira*. The average daily number of sheep processed by an eviscerator at one slaughterhouse was calculated to be 225, and by a meat inspector and an offal handler 374 and 1123, respectively (Dorjee et al., 2011). On average, meat workers were calculated to be exposed to 5-25 sheep per day shedding live *Leptospira* spp. (Heuer et al., 2008). Further, on the chain some workers are potentially more exposed than others, as they concentrate on specific tasks, with different exposure risks to animal urine. The meat industry became aware of the threat leptospirosis is posing to its employees and its potential economic effect and introduced regular information days on leptospirosis, strict rules regarding PPE and hygiene in collaboration with occupational health physicians and the Department of Labour and opened its doors to researchers to investigate this debilitating disease.

Figure 2: Comparison of a slaughter house in the early 1930s (left)[8] with a modern one (right)[9]

4. Leptospirosis in New Zealand

4.1. Leptospirosis in Animals

Leptospira are widespread in livestock and leptospirosis is currently the most important occupationally-acquired zoonotic disease in NZ (Thornley et al., 2002). While in many, mainly subtropical countries, numerous animal hosts and *Leptospira* serovars survive in a complex ecological environment, the epidemiology of leptospirosis in NZ is based on a relatively small number of serovars (six) known to be endemic. Table 1 summarizes established hosts and adapted

[7] Cybèle Locke. 'Ngā uniana – Māori and the union movement - Māori rural workers and unions', Te Ara - the Encyclopedia of New Zealand, accessed September 12, 2012. URL: http://www.TeAra.govt.nz/en/nga-uniana-maori-and-the-union-movement

[8] Processing sheep carcasses, Christchurch Meat Company. Webb, Steffano, 1880?-1967: Collection of negatives. Ref: 1/1-019459-G. Permission received from Alexander Turnbull Library, Wellington, New Zealand

[9] Photograph by David Bruce. Permission received from Otago Daily Times

serovars. Hardjobovis is regarded to be host-adapted to deer and beef cattle showing a subclinical infection pattern (Hathaway, 1981; Marshall and Manktelow, 2002). Sheep are considered to be sporadically infected by Hardjobovis (Blackmore et al., 1982). However, the more recently researched occurrence of Hardjobovis in sheep may be an indication of Hardjobovis becoming adapted to sheep (Dorjee et al., 2008), which has been discussed in Gerritson (Gerritsen et al., 1994). Pomona is considered to be adapted to pigs (Bolt I, 1995) and possibly deer (Ayanegui-Alcerreca et al., 2007; Subharat, 2010), and infection in beef and sheep is thought to be sporadic with occasionally high lamb or calf mortality and abortion in cattle (Marshall and Manktelow, 2002; Dorjee et al., 2005a; Dorjee et al., 2008). *L. borgpetersenii* sv Tarassovi is adapted to pigs, *L. borgpetersenii* sv Balcanica (Balcanica) to possums and *L. interrogans* sv Copenhageni (Copenhageni) and *L. borgpetersenii* sv Ballum (Ballum) to rodents (Hathaway, 1981; Marshall and Manktelow, 2002). Animal leptospirosis is not a notifiable disease in NZ. Nevertheless, from several studies we know that the two most frequent serovars in cattle, deer and sheep in NZ are Hardjobovis and Pomona. Six percent of 2,758 lamb carcasses sampled in abattoirs and 44.2% of 95 slaughter lines of sheep were sero-positive against Hardjobovis and/or Pomona (5% and 1%, respectively of individual animals; 33% and 4% respectively of slaughter lines) with a MAT titre of ≥1:48 considered positive (Dorjee et al., 2008). A nationwide survey of 110 deer farms found Hardjobovis alone on 61% (MAT titre cut-off 1:24), Pomona alone on 3.6% (MAT titre cut-off 1:48), and both serovars together on 16.4% of farms (Ayanegui-Alcérreca, 2006). A survey in Hawke's Bay found that 100% of 50 beef herds had titres against Hardjobobis and 64% of 1491 beef cattle had a MAT titre ≥ 1:96 (Matthews et al., 1999).

A prevalence study in healthy dogs found that 14.2% (66/466) of dogs in NZ had antibodies against Hardjobovis (3.5%), Pomona (1.3%) or Copenhageni (9.5%) (MAT titre cut-off 1:100). Hardjobovis was mainly found in rural dogs (4.8%)(O'Keefe et al., 2002) and positive titres to Hardjobovis were associated with breeds of dogs used as farm working dogs (Harland et al., 2012).

In NZ, domestic ruminants are commonly farmed in multi-species pastoral systems, where beef cattle, sheep and/or deer are often grazed on the same pastures (Hoskin, 2007). Most animals are kept extensively all year outside and animal waste is generally not removed from the environment. It can therefore accumulate in run-offs, valley water tanks, rivers and ground water.

The climate is cool to temperate to warm with annual rainfall ranging from 300 mm in semi-arid areas like Central Otago to 8000 mm in wet regions like the Southern Alps. Temperatures generally do not fall below 0^{o} or rise above 30^{o} Celsius[10]. These farming and environmental conditions possibly allow *Leptospira* spp. to reach an endemic status in the absence of a vaccination program covering

[10] http://en.wikipedia.org/wiki/Climate_of_New_Zealand. Accessed August 1, 2012.

all domestic livestock species.

Whereas a large proportion of dairy farmers vaccinate their stock against leptospirosis and the NZ pig industry has introduced compulsory vaccination of pig herds[11], less than 10% of deer, sheep or beef farmers are currently using vaccination, partially due to uncertainty about economic impacts of the disease and the cost-efficiency and efficacy of vaccines for preventing them (Keenan, 2007a; Wilson et al., 2008a).

A negative economic impact of leptospirosis in deer has been suggested. A study in farmed deer in NZ reported that yearling deer with evidence of infection during the growth period were 3.7 kg lighter than those without the evidence of infection. Additionally, weaning rates were reduced by 11% (Ayanegui-Alcerreca et al., 2007). Vaccination of deer is therefore not only a means to reduce human leptospirosis incidence but may also be financially attractive for deer farmers.

The impact on growth and reproduction performance in sheep and beef cattle has not been quantified at industry level in NZ. Sporadic outbreaks of Pomona were reported to cause 5-15% of lamb mortality (Dorjee et al., 2005a). Reduced conception rates (Dhaliwal et al., 1996b) and 6% of fetuses lost (Sanhueza, 2012) were attributed to beef being infected with Hardjobovis or Pomona.

4.2. Leptospirosis in Humans

A recently conducted farmer survey indicated that the incidence of human leptospirosis tended to be lower on farms where livestock was vaccinated (Dreyfus et al., 2010c). Since vaccination of dairy cows commenced in the 1980s, the incidence of notified human leptospirosis cases in the farming industry dropped from 234/100,000 to 90/100,000 (Marshall, 1996; Thornley et al., 2002). Whether this drop in notified cases is causally related to vaccination or to other factors such as higher awareness with behavioural risk reduction (use of PPE, avoiding urine splashes) and changes in milking techniques (i.e. rotary sheds) is an unresolved question. Possibly other factors were important contributors, since the main drop in human cases apparently occurred between 1979 and 1982 before most dairy farmers started with vaccination of their cows in 1983 (Marshall R, 1996). Nevertheless, leptospirosis is still the most important occupationally-acquired zoonotic disease in NZ, where livestock appear to be an important source of human leptospirosis, with farmers and meat workers being at a high risk (Thornley et al., 2002). In NZ, human leptospirosis is notifiable. NZ is classified as having a moderate incidence for the Asia Pacific region (1-10/100'000) (Victoriano et al., 2009). The surveillance system is based on medical practitioners' and laboratory reporting and is recorded by the Institute of Environmental Science and Research (ESR). Most notified human

[11] Point 6 in the "Animal Status Declaration" (ASD) form of the NZ Pork Industry Board: http://www.nzpork.co.nz/Portals/NZPib/Documents/Publications/NZPork%20ASDP%20Download%20FORM.pdf

leptospirosis cases are presently, in order of frequency, caused by Ballum, Hardjobovis, and Pomona. The annual human incidence in the general population declined from 17/100,000 in 1978 to 2.9/100,000 in 1996-98 but increased to 3.8/100,000 in 2002 and 2003. From 2003-2009, the incidence was on average 2.1/100,000 with a small increase in 2008 to 2.8/100,000. In 2008, the risk of contracting clinical leptospirosis among meat workers and farmers was 79 times higher than in the general population. From 2006 to 2010, 427 cases of leptospirosis were notified (86.4% laboratory confirmed), with a rate of 2 cases per 100,000 population. Of those with occupation recorded annually, 52% (range 36-71%) were farmers or farm workers and 30% (range 18 - 48%) abattoir workers or butchers[12]. Leptospirosis can result in severe human illness but is rarely fatal in NZ: during 2003-2005, 207 cases were hospitalised (Vickery et al., 2006). An unknown number of leptospirosis cases may be misdiagnosed as influenza or remain undiagnosed because medical attention is not sought, possibly due to difficulties accessing medical services in rural areas and because of similarity to 'flu' symptoms. Medical practitioners often do not test for leptospirosis because of a general lack of awareness about this disease or due to the absence of specific symptoms. Hence, the officially reported numbers mainly represent severe clinical cases and milder forms are believed to remain under-reported (Thornley et al., 2002). However, the rate of under-reporting is unknown to date.

The severe form of leptospirosis is a debilitating disease with long-term health effects, including psychological problems with a negative impact on families and rural communities (Holt.N, 2010) (personal communication). Apart from the suffering leptospirosis is creating, it also has an economic impact due to high hospital costs because of interventions such as dialysis and intensive patient care and due to absenteeism, creating costs for employers, employees and ACC. Even though the mild form of leptospirosis with 'flu-like' symptoms may not always be as serious from a medical point of view, there may still be an economic impact due to absenteeism of the workforce, which has not been quantified yet. Further, cases with 'flu-like' symptoms will most probably not be accepted as a leptospirosis case by ACC, whose case definition for a confirmed leptospirosis case requires isolation of the bacterium or a MAT titre ≥800 or a four-fold titre increase between two samples taken[13], commonly only found in the severe, acute form (Faine et al., 1999; Levett, 2001).

Historically, the risk of contracting leptospirosis was associated with exposure to pigs and cattle, especially dairy cattle (Blackmore and Schollum, 1982b). A cross-sectional study in dairy farm workers revealed a sero-prevalence (≥1:24) against Hardjobovis and/or Pomona of 34% among milkers and time spent in the milking shed as a risk factor. A case-control study found a positive

[12] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-2010.
[13] http://www.acc.co.nz/for-providers/clinical-best-practice/acc-review/WCMZ003166

association between farms with Hardjobovis sero-positive dairy herds and Hardjobovis sero-positive persons (>1:96) on that farm (Mackintosh et al., 1980b; Mackintosh et al., 1982). A study among 70 pig farmers revealed 31% sero-positive (≥1:24) against Pomona (Schollum and Blackmore, 1982). Studies in multispecies meat abattoirs slaughtering sheep, beef and/or pigs were carried out in the 1980s revealing a sero-prevalence against Pomona, Hardjobovis, and/or Tarassovi of 4.1% in meat workers and of 9.5% in meat inspectors (Blackmore et al., 1979; Blackmore and Schollum, 1982a). In the last decade human leptospirosis cases have been reported from sheep-only abattoirs suggesting that sheep were a possible reservoir for *Leptospira* infection in humans. Research investigating this trend found that 21% of sero-positive, random sampled slaughtered sheep had culture positive kidneys for Hardjobovis or Pomona (Dorjee et al., 2008), meaning 13 of 1,000 sheep potentially shed leptospires, exposing meat workers to 5-25 shedding live *Leptospira* spp. sheep carcasses per day (Heuer et al., 2008). A *Leptospira* spp. sero-prevalence of 9.5% was reported in workers from one abattoir slaughtering sheep with workers on the slaughter board having a 23-85-fold higher risk of being sero-positive than workers in the boning, cutting, chilling or rendering areas (Benschop et al., 2009; Heuer et al., 2010).

Efforts have been made to improve the protection of exposed persons to leptospires under the 'Health and Safety in Employment' act (HSE). Keenan assessed the risk of leptospirosis at the workplace and the department of labour (DOL), the Occupational Safety and Health Service (OSH) and ACC developed guidelines on control of and protection against leptospirosis (Department of Labour and Accident Compensation Corporation, 2001; Keenan, 2007a). These guidelines include recommendations on PPE to be worn by persons who are potentially exposed to animal urine at work, such as plastic aprons or overalls, gumboots, face shields or visors and gloves depending on the exposure situation and 'practicality' (if wearing protective gear does not impede an activity). However, on what evidence these policies had been developed is not visible from the text. In the abattoirs the use of PPE is sometimes limited by its practicality, e.g. for meat workers who have difficulties grasping a part of the carcass firmly with gloves or whose visors steam up and reduce visibility while performing a highly physical job (Study participants, 2010) (personal communication). Meat plants developed their own PPE policy along with penalties for the workers who do not comply. The PPE policies differ depending on the company and species slaughtered. However, apart from commonly used safety gear such as gum boots and aprons, most companies expect workers to wear safety shields or glasses and gloves in the area of removal of guts and organs of the genital-urinary tract. Slaughter plants processing pork only accept vaccinated animals and most dairy farmers protect their workers and themselves by vaccinating dairy cows.

5. Thesis Aim and Structure

The aim of this PhD project was to investigate leptospirosis at the human-animal interface in New Zealand (NZ) in order to understand both the extent to which animals are infected and to which transmission occurs from infected animals to humans. This thesis examined the *Leptospira* spp. sero-prevalence and incidence in meat workers and sero-prevalence in domestic livestock, evaluated risk factors, attempted to raise awareness of the disease and risks, and assessed the impact and economic effects of leptospirosis leading to the development of recommendations for disease control measures on farms and at the meat industry level. To achieve this aim, two cross-sectional and one cohort study were conducted. The chapters are in the form of manuscripts prepared for publication in peer-reviewed journals. Therefore, background information will be repeated and presentation has been influenced by the format of the style used in journals.

Chapter 2 describes a cross-sectional study investigating leptospirosis in farmers and domestic livestock (beef cattle, deer and sheep) in NZ. The aim was to determine the animal- and farm-level sero-prevalence of *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona) in beef, sheep and deer in NZ and to evaluate the association between sero-prevalence against both serovars and risk factors, such as co-grazing or region of the farm. A further objective was to describe the incidence of clinical leptospirosis outbreaks in animals and humans. Results may raise awareness of the disease frequency in these three animal species and identified risk factors may be useful to enhance control of leptospirosis on farm level. The incidence in farmers will give hints about the precision of the reporting system in place. Leptospirosis is currently the most important occupationally-acquired zoonotic disease in NZ. In the absence of a human vaccine, vaccination of livestock would be a reasonable measure to control the disease, given the rather small array of existing serovars in NZ. However, most sheep, beef and deer farmers do not vaccinate, mainly because of unawareness of disease and economic impacts of the infection.

Therefore, we investigated in Chapter 3 the economic loss in NZ livestock from the data of the cross-sectional study already described in Chapter 2. The objective of the study was to get farmer reported estimates of production performance, such as weaning and calving rates. Further, to evaluate the association between sero-prevalence and production performance of deer, sheep and beef cattle controlling for different types of farms (species combination and herd size) and for the North and South Island.

Chapter 4 presents a cross-sectional study investigating leptospirosis in abattoir workers in NZ, who carry the consequences of the burden of disease from the species described in Chapter 2. The objectives of this study were to determine the sero-prevalence of Pomona and Hardjobovis in

abattoir workers processing sheep, beef cattle or deer, to identify risk factors for sero-positivity related to occupational and non-occupational activities and to identify risk factors for probable leptospirosis and/or 'flu-like-illness'. The outcome will enable plant managers to adapt control measures and meat workers to know better how to protect themselves if necessary.

Finally, Chapter 5 describes the cohort study investigating sero-conversion to Pomona and Hardjobovis and risk factors in abattoir workers in NZ. The aims of this study were therefore to determine the annual risk of infection and the incidence of probable clinical leptospirosis. Further, to identify risk factors for new infection related to occupational as well as non-occupational activities and to evaluate the proportion of workers in the population with 'flu-like' disease attributable to new infections with Hardjobovis and/or Pomona and estimate absenteeism. The findings are likely to generate recommendations for the improvement of control measures in abattoirs and help estimate the impact of the disease.

Chapter 6 is a general review and reflective critique of the studies of this thesis. It updates current knowledge and extends to possible control measures. The chapter discusses the study designs, analyses and limitations and suggests how the work could have been improved and proposes useful further research.

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Chapter 2

Leptospira and Leptospirosis in Sheep, Beef and Deer Farms and Farmers in New Zealand

A. Dreyfus, J. Benschop, J. Collins-Emerson, P. Wilson, C. Verdugo, C. Heuer

1. Abstract

The aim of this cross sectional study was to determine the herd/flock and within-herd/flock levels of sero-prevalence of *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona) in beef, sheep and deer in New Zealand (NZ) and to evaluate the association between the within-herd/flock sero-prevalence against both serovars and farm level risk factors. A further aim was to describe the incidence of clinical leptospirosis occurrence in animals and in humans on the study farms.

From 1,940 responses to a postal survey, 238 stratified-randomly selected farms from eight of 16 geographical regions in both the North and South Islands were sampled between June 2009 and July 2010. Twenty blood samples were collected from each species mob (162 sheep flocks, 116 beef herds, 99 deer herds). Farmers were interviewed on-site about clinical leptospirosis in humans and livestock, on demographic livestock data and grazing management. Serum antibodies were measured against Hardjobovis and Pomona using the Microscopic Agglutination Test (MAT). Species-specific associations between sero-prevalence and risk factors within-herd/flock were determined by multivariate logistic regression taking clustering, potential confounders and effect modifiers into account.

Ninety seven percent of sheep flocks and beef herds and 76% of deer herds were positive against Hardjobovis and/or Pomona (≥1 animal sero-positive at titre ≥1:48). Overall, of 3,361 adult sheep, 43% (95% CI 42-45%) were sero-positive against Hardjobovis, 14% (95% CI 13-15%) against Pomona and 50% (95% CI 49-52%) against either serovar. Of 2,308 adult beef, 50% (95% CI 48-52%) were positive against Hardjobovis, 25% (95% CI 23-27%) against Pomona and 58% (95% CI 56-60%) against either serovar. Of 1,992 adult deer, 26% (95% CI 24-28%) were positive against Hardjobovis, 11% (95% CI 9-12%) against Pomona and 34% (95% CI 32-36%) against either serovar. Six respondents reported seven human leptospirosis cases in farmers/farm workers between 2006 and 2009. The incidence of probable human cases in 2009 was 1 in 638 (0.16%, 95% CI 0.0008-1%). The incidence of probable leptospirosis on farms in cattle herds in 2009 was 2.6% (95% CI 0.7-7.9%), in sheep flocks 0% and in deer herds 1% (95% CI 0.05-6.3%).

The following risk factors were statistically significantly (p-value ≤0.05) associated with the within-herd sero-prevalence of Hardjobovis or Pomona.

Beef: Hardjobovis: 'herd size' (OR 1.4, 95% CI 1.1-1.8), 'presence of valley dams' (OR 0.6, 95% CI 0.3-1.0) and 'vaccination of beef cattle' (OR 2.9, 95% CI 1.6-5.3). Pomona: 'Grazing beef cattle without contact to deer' (OR 2.7, 95% CI 1.4-5.2), 'vaccination of beef cattle' (OR 3.22, 95% CI 1.7-5.9), 'presence of valley dams' (OR 0.2, 95% CI 0.1-0.6) and 'presence of a stream' (OR 2.7, 95% CI 1.2-6.1).

Deer: Hardjobovis: 'herd size' (OR 1.6, 95% CI 2.4-9.5). Pomona: 'herd size' (OR 1.8, 95% CI 2.7-16.8) and 'number of dogs present' (OR 0.6, 95% CI 1.0-0.1). If deer herds were sampled in summer they were 4.8 times as likely to be positive compared to being sampled in winter (95% CI 1.5-11.8).

Sheep: Hardjobovis: 'herd size' (OR 1.56, 95% CI 1.26-1.94), 'number of dogs present' (OR 0.75, 95% CI 0.56-0.99) and 'region'. Sheep flocks in Otago and Southland had 1.8 times the odds of being positive, compared to Waikato. All other regions had a lower sero-prevalence with ORs between 0.4 and 0.7 compared to Waikato. Pomona: 'region'. Sheep flocks in Hawke's Bay and East Coast had 2.7 times the odds (95% CI 1.26-5.61) of being positive than those in Waikato.

Sero-prevalence in beef was not associated with co-grazed sheep, and that in deer and sheep was not associated with co-grazing with any other species. Grazing beef with deer reduced the likelihood of sero-positivity against Pomona in beef.

While Hardjobovis and Pomona were highly prevalent in NZ livestock, confirmed clinical disease was rarely reported. Hardjobovis was more prevalent in all three species than Pomona. The generally different sero-prevalence distribution for Hardjobovis and Pomona suggested that the two serovars may have diverse ecologies.

2. Introduction

Leptospirosis is a zoonotic bacterial disease of most mammal species. After a leptospiraemic phase, leptospires colonize and mainly persist in the kidney of carrier animals and are excreted in the urine for several months, sometimes years by carrier animals. Infection with *Leptospira* spp. occurs via skin abrasions or mucous membranes with transmission occurring through exposure to the urine of carrier animals, either directly or via contact with contaminated water or soil (Bharti et al., 2003). Over 20% of sero-positive animals continue to excrete bacteria through urine (Dorjee et al., 2008).

Leptospiral infection is widespread in livestock in New Zealand (NZ) (Marshall and Manktelow, 2002). Prevalent in many, mainly subtropical countries, and in numerous animal hosts, *Leptospira* serovars survive in soil for several weeks (Miller et al., 1991). The epidemiology of leptospirosis in NZ however, is based on a relatively small number of endemic serovars (six from several hundred known worldwide) (Hathaway, 1981; Marshall and Manktelow, 2002). Animal leptospirosis is not a notifiable disease in NZ. The two most frequent serovars in cattle, deer and sheep in NZ are *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona). Various surveys have shown up to 81% of deer herds, 80% of beef cattle herds and 44% of sheep flocks had evidence of infection with these serovars (Hathaway, 1981; Marshall and Manktelow, 2002; Ayanegui-Alcerreca et al., 2007; Dorjee et al., 2008). Hardjobovis is regarded to be host-adapted to deer and beef cattle showing a subclinical infection pattern ((Hathaway, 1981). Reduced conception rates were found by Dhaliwal (1996) and in a case control study, 6% of fetuses lost in beef cattle were sero-positive against Hardjobovis (Dhaliwal et al., 1996b; Sanhueza, 2012). Blackmore et al. (1982) examined serum samples for evidence of leptospiral antibodies from 928 sheep from 45 lines and kidneys from 12 of these lines for evidence of infection with *Leptospira*. While 20% of the sheep had MAT titres of 1:48 or greater to Hardjobovis, Hardjobovis was isolated from the kidneys of three animals in one line. The farm, where these three animals originated from, was visited 18 months later and serum (*n*=291) and urine samples (*n*=95) were collected. The serological Hardjobovis prevalence was 0%, 44% and 84% in lambs, hoggets and ewes, respectively. In none of the urine samples leptospires were demonstrated. Sheep were considered to be sporadically infected by Hardjobovis (Blackmore et al., 1982). Pomona is believed to be adapted to pigs (Bolt I, 1995) and possibly deer (Ayanegui-Alcerreca et al., 2007; Subharat, 2010) and infection in beef and sheep is considered sporadic with occasional high lamb or calf mortality and abortion in cattle (Marshall and Manktelow, 2002; Dorjee et al., 2005a; Dorjee et al., 2008).

A sero-prevalence study in healthy dogs in NZ found that 14.2% (66/466) had antibodies against either Hardjobovis (3.5%), Pomona (1.3%) or *L. interrogans* Copenhageni (9.5%); Hardjobovis was mainly found in rural dogs (O'Keefe et al., 2002).

In NZ, domestic ruminants are commonly farmed in multi-species pastoral systems where beef, sheep and/or deer are often simultaneously grazed on the same pastures (co-grazing). Therefore, it is necessary to address all potential carrier species on farm when researching leptospirosis in NZ.

Whereas a large proportion of NZ dairy farmers vaccinate their stock against leptospirosis and the NZ pig industry has introduced compulsory vaccination of pig herds[14], less than 10% of deer, sheep or beef farmers are currently using vaccination (Keenan, 2007a; Wilson et al., 2008a). Since vaccination of dairy cows commenced in the early 1980s, the incidence of notified human leptospirosis cases in the farming industry dropped from 234/100,000 to 90/100,000 (Marshall, 1996; Thornley et al., 2002).

In NZ, livestock appear to be an important source of human leptospirosis, with farmers and meat workers being most at risk (Thornley et al., 2002). NZ is classified as having a moderate incidence for the Asia Pacific region (1-10/100'000) (Victoriano et al., 2009). Notified human leptospirosis cases are, in order of frequency, caused by *L. borgpetersenii* serovar Ballum (Bal), Hardjobovis, and Pomona. From 2006 to 2010, 427 clinical cases of leptospirosis were notified[15] (86.4% laboratory confirmed), with an average rate of two cases per 100,000 population. Of those with occupation recorded annually, 52% (range 36 -71%) were farmers or farm workers and 30% (range 18 - 48%) abattoir workers or butchers.

No population-based estimates of *Leptospira* sero-prevalence, its risk factors and clinical disease incidence are currently available for sheep, beef or deer in NZ. The objectives of the study were to determine the herd/flock, and within-herd/flock sero-prevalence of the two most commonly diagnosed *Leptospira* serovars, Hardjobovis and Pomona, in beef, sheep and farmed deer in NZ and to evaluate the association between the within-herd/flock sero-prevalence and herd/flock level risk factors. A further aim was to describe the incidence of clinical leptospirosis occurrence in animals and humans on the study farms.

3. Methods

3.1. Study Population and Design

A cross-sectional study of sheep, beef cattle and deer farms was conducted in eight regions in NZ: Waikato, Wairarapa, Hawke's Bay, Manawatu-Wanganui, Taranaki, Marlborough, Canterbury and Southland. Regions were chosen based on differences in weather condition (ecologically different

[14] Point 6 in the "Animal Status Declaration" (ASD) form of the NZ Pork Industry Board: http://www.nzpork.co.nz/Portals/NZPib/Documents/Publications/NZPork%20ASDP%20Download%20FORM.pdf

[15] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-1010.

regions), livestock presence and available contact with the local veterinary practices. Farms were selected from a sampling frame comprising farms that had participated in a country-wide survey, in which questionnaires were sent to 7,998 client farmers of 28 veterinary practices located as above from December 2008 to March 2009. The survey gathered farm-level information on animal demographics (e.g. region, herd size, livestock species farmed ('farm-class'), presence of dogs), on farm management (access to surface water sources ('waterways'), co-grazing of different species (grazing one species simultaneously with another species), leptospirosis vaccination status), on reproduction performance and the occurrence of leptospirosis in humans and deer, sheep and beef in the four years prior to the survey. Further questions targeted Paratuberculosis (Ptb), which was investigated in this study along with leptospirosis (Verdugo et al., 2010). A total of 1,940 (25%) correctly filled questionnaires were returned, representing the sampling frame for the study. In the second step, 300 farms were randomly selected and 238 visited for blood sampling in a stratified-random fashion to obtain an equal representation of farm-class strata, representing deer, sheep and/or beef cattle farms. Reasons for the target sample of 300 farms not being met were: unavailability of animal crushes (n=6), inaccessibility of species mobs (n=17), or losses to follow-up following initial farm enrolment (n=39). For a farm to be classified as a deer, sheep and/or beef farm, a minimum of 40 deer, 400 sheep, and/or 40 beef cattle, respectively, had to be present. This cut-off differentiated approximately between hobby and commercial farming and should make the sample more representative of all farms in NZ. The farms were then allocated to single or multispecies categories in the following seven strata, four for each species: 1) sheep, 2) beef cattle, 3) deer, 4) sheep and beef cattle, 5) beef cattle and deer, 6) sheep and deer, and 7) sheep and beef cattle and deer. However, if a farm was categorized into one of these strata (i.e. sheep only), but farmed another species below the given cut-off (i.e. 10 cattle), these 10 cattle would still be included in the data analysis (for example whether they shared the same pasture with these sheep). The frequencies of farms with different species in the sampling frame was compared with a national livestock statistics database ("AgriBase™") for evidence on the representativeness of the survey farms (Verdugo et al., 2010).

3.2. Sample Size and Power Calculation

The target sample size for farms (*n*=300) was based on a comparison of prevalence between exposed and non-exposed farms where exposure was, for example, co-grazing with another species. To show a prevalence ratio of 2.2 significant at p=0.05 with 80% power and 10% frequency of exposure in the reference group, 292 farms were required.

Twenty adult female breeding animals from each species present on farm were randomly selected. This sample was sufficient to detect an animal prevalence within herds or flocks of 15% with 95% certainty, assuming a MAT sensitivity of 85% and specificity of 99% at the cut-off titre 1:48 and one or more sero-positive animals defining an exposed herd or flock. Under these conditions, the probability of correctly classifying non-exposed herds was 82%.

3.3. Data Collection and Management

Between June 2009 and July 2010, 10 ml blood samples were collected by caudal (beef) or jugular (deer and sheep) venepuncture by veterinarians, from a randomly selected sample of sheep (n=20, mixed-age ewes two-years and older), beef (n=20 adult cows) and deer (n=20, yearlings (12-24 months-of-age), (both sexes), when present on each farm. The selection of animals occurred by rounding up the targeted age group of a pasture mob, running them through a race and sampling the first 20. Blood was couriered in an icepack-cooled container to the Molecular Epidemiology and Public Health Laboratory at Massey University in Palmerston North. After centrifugation of the clotted blood at 3000 rpm for 6 minutes, the serum was aliquoted both into cryovials and microtitre plates and stored between a few days and a half a year at -80º C and -20º C, respectively before being tested. Serum antibodies were measured against Hardjobovis and Pomona using the microscopic agglutination test (MAT) at doubling dilutions from 1:24 to 1:3072 (Faine et al., 1999).
Farmers were interviewed on site by the sampling veterinarians. The questionnaire gathered the same information as the survey described above for 2008-9, and covered the intervening year prior to blood sampling.

3.4. Case Definitions for *Leptospira* Sero-prevalence and Leptospirosis Incidence

A human leptospirosis case was defined as 'probable' if the farmer reported clinical illness that he/she, members of his/her family or farm workers was/were diagnosed as leptospirosis with any serovar by a doctor or laboratory. A 'suspected' human leptospirosis case was defined as when the farmer suspected that he/she, members of his/her family or farm workers had fallen ill with leptospirosis. The incidence of human leptospirosis was calculated by dividing the number of cases by the total number of persons having contact with livestock on the study farms.

An animal was considered sero-positive (positive) when it had a MAT titre of ≥1:48 against either serovar. A herd/flock was defined as positive if at least one of the 20 sampled animals of the species was positive. A probable leptosirosis case in an animal had to be diagnosed by a veterinarian or laboratory and could be any serovar. The respondent was given the following definition for suspected leptospirosis in animals: blood stained urine, often with jaundice or multiple sudden

deaths, but not confirmed by a veterinarian or laboratory. The incidence of leptospirosis occurrence was calculated by dividing the number of farms with leptospirosis cases by the total number of study farms.

3.5. Statistical Analysis

Questionnaire information and serological test results were entered into an Access© database and analyzed using Microsoft Excel©, Stata 10 (StataCorp. LP) or SAS (SAS Institute Inc., Cary, NC, USA). Exploratory data analysis was conducted to find missing observations and outliers by using histograms, 2 x 2 tables and summary measures.

The outcomes of interest were whether farmers, their family and workers had experienced probable or suspected leptospirosis between 2006 and 2009, whether livestock on the farm experienced probable or suspected leptospirosis between 2006 and 2009 and whether sheep, deer or beef were positive against Hardjobovis and/or Pomona (individual and farm level). These outcome variables were explored by descriptive statistics. Confidence intervals for proportions were calculated by the Fleiss method (Fleiss, 1981). The association between within-herd sero-prevalence and herd level risk factors was evaluated by chi-square analysis, separately for each species (sheep, cattle and deer) and serovar (Pomona and Hardjobovis). Associations were analysed in three steps: firstly, by crude comparison of risk factors with outcomes; secondly, by multivariate logistic regression; and thirdly, by extending the final generalized linear model with generalized estimating equations (GEE) (Liang and Zeger, 1986) to control for correlation (clustering) within herds.

3.5.1. Risk factors for within-herd/flock sero-prevalence

We were primarily interested whether grazing of one species simultaneously with another species on the same paddock ('co-grazing') was associated with higher/lower within-herd/flock sero-prevalence against Hardjobovis and or Pomona in the investigated species, for example, whether grazing of sheep together with deer altered the sero-prevalence of Hardjobovis or Pomona in sheep. For this reason, we created six 'co-grazing' variables (three species and two serovars) with each two categories, which indicated whether one species was co-grazed with another species or not. Other risk factors of interest were numbers of dogs present on farm (continuous), type of waterways on farm (valley dams, springs, river or stream), vaccination status of species on farm, farm region (collapsed from eight into five areas), farm size in hectares (continuous) and herd size (continuous or categorical). The categories for herd size were based on the quartiles of the data. Further, the variable 'sampling season' with four categories was investigated: (i) spring (September - November),

(ii) summer (December - February), (iii) autumn (March - May) and (iv) winter (June - August). All exposure variables are listed in tables 3-5.

Associations between risk factors and within-herd/flock sero-prevalence with Pomona and Hardjobovis were tested by univariable binomial logistic regression for sheep (Table 3), beef (Table 4) and deer (Table 5). Exposure variables with a Wald Test/Chi Square p-value ≤0.3 were included in the multivariable binomial logistic regression with sero-status to Pomona and Hardjobovis as the outcome variables. If exposure variables were highly correlated, we only entered one in the multivariable analysis.

A forward selection method was chosen to evaluate exposure and confounding variables, starting with a null model with only an intercept included and then adding one variable at a time. A variable was retained if the Likelihood Ratio Test (LRT) was statistically significant at a p-value ≤0.05 or if their presence changed the crude OR of fitted variables in the model by +/-15%. Multiplicative interaction was tested for the terms: 'co-grazing beef with sheep'*'co-grazing deer with sheep', 'co-grazing beef with deer'*'co-grazing sheep with deer' and 'co-grazing sheep with beef'*'co-grazing deer with beef'. If the LRT was statistically significant (p ≤0.05) the interaction term was retained in the model. Vaccinated herds were not excluded from the analyses, but the effect of vaccination on the outcome was controlled for in the regression model. Finally the model was extended by GEE to control for clustering, by expanding the standard errors (SE) ('robust SE') and increasing the p-values.

We calculated the adjusted sero-prevalence for each species and region with the final GEE extended models as the predicted probability of the coefficient adjusted for the risk factor variables, which remained in the model (Dohoo et al., 2003a).

4. Results

4.1. Descriptive Analysis

Blood sampling occurred on 238 farms from 377 herds/flocks and 7,661 animals. Sampling comprised 116 beef cattle herds (*n*=2,308 cattle), 162 sheep flocks (*n*=3,361 sheep) and 99 were deer herds (*n*=1,992 deer) (Tables 1 & 2). Leptospirosis vaccines were administrated on one sheep farm (0.6%), in 21 beef cattle farms (18%) and five deer farms (5%). The distribution of sampled sheep flocks, beef and deer herds, respectively, by region was: Canterbury (*n*=34, 25, and 44), Otago & Southland (*n*=32, 5, and 14), Wairarapa (*n*=8, 8, and 2), Manawatu-Wanganui & Taranaki (*n*=53, 49, and 19), Hawkes's Bay & East Coast (*n*=32, 26, and 16) and Waikato (*n*=3, 3, and 4). Farmers recalled that 63% (*n*=2126) of sheep were co-grazed with beef and 18% (*n*=597) with deer. Further, that 73%

(*n*=1683) of beef were co-grazed with sheep and 21% (*n*=488) with deer. Twenty-nine percent (*n*=575) of deer were reported to be co-grazed with beef and 57% (*n*=1132) with sheep (Tables 3-5). The average herd/flock sizes were 404.5 (beef cattle), 3683.7 (sheep) and 688 (deer). The frequencies of farms with different species in the sampling frame were representative of farms in NZ when compared with a national livestock statistics database ("AgriBase™", data not shown)

4.2. Clinical Leptospirosis in Humans and Livestock

4.2.1. Humans

Six of 238 farms reported seven human leptospirosis cases in farmers or farm workers. Four cases were probable, one was suspected and two were not specified. Two probable cases occurred in 2006, one probable case in 2008 and four in 2009 (1 probable). One case occurred on a deer farm, one on a sheep and deer farm and five on sheep farms. The two deer farms with human cases had a sero-prevalence against Hardjobovis of 20% and 65% and the sheep flocks between 10% and 100% with a median of 60% against either Hardjobovis or Pomona, with four farms displaying dual infections (Hardjobovis and Pomona). All farms with leptospirosis cases kept between three and 20 dogs. None of the livestock or dogs had been vaccinated against leptospirosis. The number of potentially exposed persons on farms was 638. The incidence of probable clinical leptospirosis for 2009 was therefore 1:638; hence 0.16% (95% CI 0.0008-1%) of exposed people fell ill with probable leptospirosis. The three year cumulative incidence was 4:638 = 0.63% (95% CI 0.2-1.7%).

4.2.2. Livestock

Seven farmers reported ten veterinarian or laboratory confirmed clinical leptospirosis episodes in animals, occurring between May and October, and during 2006 to 2009 in their beef cattle. Three clinical leptospirosis episodes occurred in 2009, hence the herd incidence in cattle in 2009 was 3:116 = 2.6% (95% CI 0.7-7.9%). Seven outbreaks took place in adult cattle and two were in newborn calves. Clinical symptoms were abortions in four, reproductive failure (non-pregnancy) in one, mortality of early born calves in two and jaundice and hemoglobinuria ('red water') in one episode. Six of the seven farmers had vaccinated their cattle against leptospirosis in 2009. Herds with clinical leptospirosis had a sero-prevalence against either serovar of between 40% and 100% with a median of 95%. All farms had dual infections (Hardjobovis and Pomona).

One sheep farmer reported suspected leptospirosis in sheep in 2009, which was not confirmed by a veterinarian or laboratory test. The flock was not vaccinated. Wasting was reported as a clinical sign. However, serology was negative so the incidence of clinical leptospirosis in sheep flocks in 2009 was therefore likely to have been 0%.

One leptospirosis episode was reported in 2008 (suspected) and one in 2009 (probable) in the participating deer farms in adult hinds. Both herds were not vaccinated. The MAT results showed a sero-prevalence against Hardjobovis of 65% and 80%, respectively. The incidence of outbreaks in deer herds in 2009 was 1.01% (95% CI 0.05-6.3%).

4.3. Sero-prevalence and Antibody Titres

4.3.1. Sheep

Of 3,361 sheep, 43% (95% CI 42-45%) were sero-positive against Hardjobovis, 14% (95% CI 13-15%) against Pomona and 50% (95% CI 49-52%) against either serovar (Table 1). Of 162 sheep flocks, 91% (95% CI 86-95%) were sero-positive against Hardjobovis, 74% (95% CI 66-80%) against Pomona and 97% (95% CI 93-99%) against Hardjobovis and/or Pomona (Table 2). Sheep flocks had a median sero-prevalence of 44% for Hardjobovis and 10% for Pomona. If the vaccinated farm was excluded, the between-flock prevalence for Hardjobovis was 91% (95% CI 86-95%), for Pomona 74% (95% CI 66-80%) and either serovar 91% (95% CI 86-95%). Antibody titres ranged from 1:24 to 1:3072 for both serovars, with the highest number of animals showing 1:48 for Pomona and 1:192 for Hardjobovis (Figure 1).

4.3.2. Beef

Of 2,308 beef cattle, 50% (95% CI 48-52%) were sero-positive against Hardjobovis, 25% (95% CI 23-27%) against Pomona and 58% (95% CI 56-60%) against either serovar (Table 1). Of 116 beef herds 92% (95% CI 85-96%) were sero-positive against Hardjobovis, 72% (95% CI 63-80%) against Pomona and 97% (95% CI 92-99%) against Hardjobovis and/or Pomona (Table 2). Beef herds had a median sero-prevalence of 51% for Hardjobovis and 15% for Pomona. If vaccinated herds were excluded, the between-herd prevalence for Hardjobovis was 90% (95% CI 82-95%), for Pomona 68% (95% CI 58-77%) and either serovar 97% (95% CI 90-99%). Antibody titres ranged from 1:24 to 1:3072 for both serovars, with the highest number of animals showing 1:24 for Pomona (which is test negative according to our case definition) and 1:192 for Hardjobovis (Figure 1).

4.3.3. Deer

Of 1,992 deer, 26% (95% CI 24-28%) were sero-positive against Hardjobovis, 11% (95% CI 9-12%) against Pomona and 34% (95% CI 32-36%) against either serovar (Table 1). Of 99 deer farms, 60% (95% CI 49-69%) were sero-positive against Hardjobovis, 49% (95% CI 39-60%) against Pomona and 76% (95% CI 66-84%) against Hardjobovis and/or Pomona (Table 2). If vaccinated herds were excluded, the between-herd prevalence for Hardjobovis was 59% (95% CI 48-69%), for Pomona 47%

(95% CI 37-58%) and either serovar 75% (95% CI 65-83%). Deer herds had a median sero-prevalence of 5% for Hardjobovis and 0% for Pomona. Antibody titres ranged from 1:24 to 1:3072 for both serovars, with the highest number of animals showing 1:24 for Pomona (which is test negative according to our case definition) and 1:96 for Hardjobovis (Figure 1).

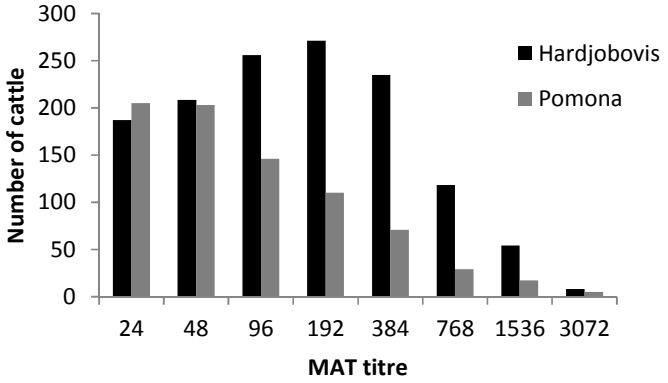
Table 1: Animal-level sero-prevalence (%) and 95% confidence intervals (CI) by species for *Leptospira borgpetersenii* serovar Hardjobovis (Har) and *L. interrogans* serovar Pomona (Pom) with a MAT titre cut-off of 1:48 in adult breeding stock sampled between June 2009 and July 2010. Vaccinated herds were included

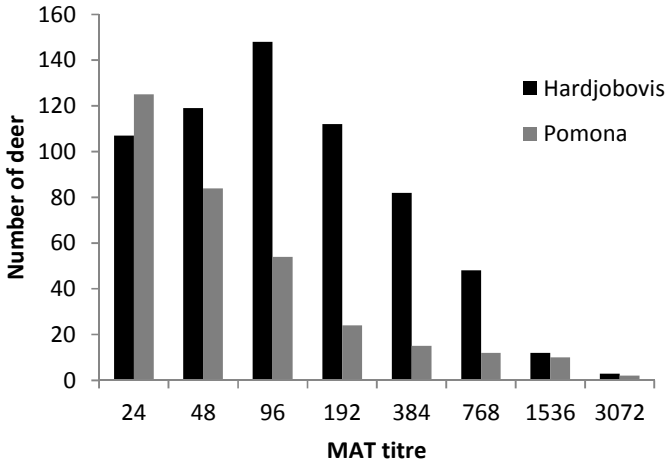
Species	No of animals	Har n (%)	95% CI	Pom n (%)	95% CI	Either Pom or Har n (%)	95% CI
Beef	2,308	1,150 (49.8)	47.8-51.9	581 (25.2)	23.4-27.0	1346 (58.3)	56.3-60.3
Sheep	3,361	1,456 (43.3)	41.6-45.0	471 (14.0)	12.8-15.2	1698 (50.5)	48.8-52.2
Deer	1,992	524 (26.3)	24.4-28.3	201 (11.0)	8.8-11.5	674 (33.8)	31.8-36.0
Tot	7,661						

Table 2: Herd/flock-level sero-sero-prevalence (%) and 95% confidence intervals (CI) by species for *Leptospira borgpetersenii* serovar Hardjobovis (Har) and *L. interrogans* serovar Pomona (Pom) with a MAT titre cut-off of 1:48 in adult breeding stock sampled between June 2009 and July 2010. Vaccinated herds were included

Species	No of herds	Har n (%)	95% CI (%)	Pom n (%)	95% CI (%)	Either Pom or Har n (%)	95% CI (%)
Beef	116	107 (92.2)	85.4-96.2	84 (72.4)	63.2-80.1	113 (97.4)	92.1-99.3
Sheep	162	148 (91.4)	85.7-95.0	120 (74.1)	66.5-80.5	157 (96.9)	92.6-98.9
Deer	99	59 (59.6)	49.2-69.2	49 (49.5)	39.4-59.7	75 (75.8)	65.9-83.6
Tot*	377						

*Several herds were present on the same farm, so the total of study farms was 238





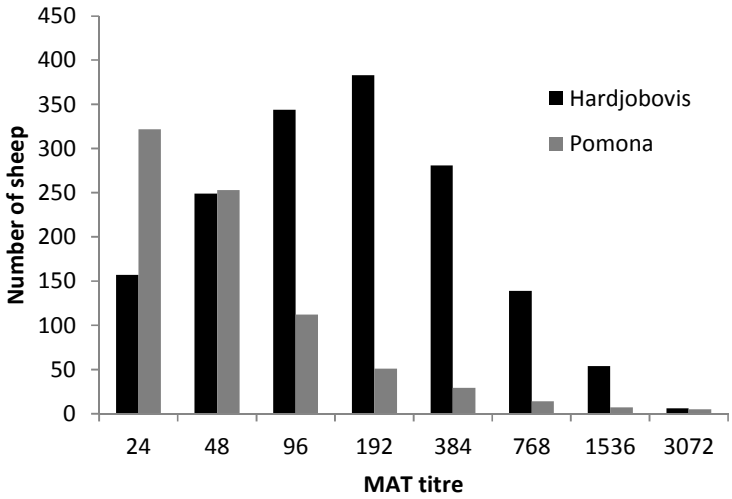


Figure 1: Frequency distribution of positive antibody titres against *Leptospira borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona in beef cattle (top), deer (centre), and sheep (bottom)

4.4. Risk Factors for Sero-prevalence

4.4.1. Sheep

The bivariable associations between farm-level risk factors and the within-flock sero-prevalence against Pomona and Hardjobovis (outcome) for sheep are presented in Table 3. Many of the unadjusted risk factors and several in the logistic regression model were significantly ($p < 0.05$) associated with the outcome (data not shown). However, after adjustment for clustering within-flock, the remaining significant (p-value ≤0.05) risk factors for the outcome 'within-flock sero-prevalence against Hardjobovis' were, 'presence of dams or rivers', 'herd size', 'region' and 'numbers of dogs present'. Sheep flocks of larger size (Odds Ratio (OR) 1.56, 95% CI 1.26-1.94) and those with a smaller number of dogs present on farms were more likely to be positive (OR 0.75, 95% CI 0.56-0.99). The odds of infection differed by region (Chi p-value=0.014). Sheep flocks in Otago and Southland had 1.8 times the odds of being positive, compared to those in Waikato. In all other regions the sero-prevalence was lower with ORs between 0.4 and 0.7 compared to Waikato. The presence of dams or rivers was associated with a decreased OR for sero-prevalence in sheep flocks (OR 0.6, 95% CI 0.4-1.0). Grazing beef or deer together with sheep had no statistically significant effect on sero-prevalence in sheep (Table 6).

For the outcome 'within-flock sero-prevalence against Pomona', the remaining risk factor was 'region' (Chi p-value=0.006). Sheep flocks in Hawke's Bay and East Coast had 2.7 times the odds (95% CI 1.26-5.61) of being positive than those in Waikato. Differences between other regions and Waikato were not statistically significant. Grazing beef or deer with sheep had no statistically significant effect on sero-prevalence in sheep (Table 6).

None of the interaction terms improved the fit of the model.

4.4.2. Beef

The bivariable associations between farm-level risk factors and the within-herd sero-prevalence against Pomona and Hardjobovis for beef herds are presented in Table 4. Many of the unadjusted risk factors were significantly ($p < 0.05$) associated and several in the logistic regression model with the outcome (data not shown). However, after adjustment for clustering within-herd, the remaining significant (p-value ≤0.05) risk factors for the outcome 'within-herd sero-prevalence against Hardjobovis' were, 'herd size', 'presence of dams' and 'vaccination of beef cattle'. Beef herds of larger size were more likely to be positive (OR 1.42, 95% CI 1.12-1.81) and those which were vaccinated more likely to be positive (OR 2.9, 95% CI 1.6-5.3). Farms with dams were less likely to be positive (OR 0.6, 95% CI 0.3-1.0). Grazing of deer or sheep together with beef had no statistically significant effect on sero-prevalence in beef (Table 7).

For the outcome 'within-herd sero-prevalence against Pomona', the remaining risk factors were 'co-grazing beef with deer', 'vaccination of beef cattle', 'presence of dams' and 'presence of stream'. If beef herds were grazed alone and not together with deer, they were 2.7 times more likely to be positive (95% CI 1.44-5.24) (hence co-grazing was associated with a decreased OR) and if beef were vaccinated against leptospirosis, they were more likely to be positive (OR 3.22, 95% CI 1.7-5.9). The presence of dams decreased (OR 0.2, 95% CI 0.1-0.6) and of a stream (OR 2.7, 95% CI 1.2-6.1) increased the odds of beef cattle being positive. Grazing of sheep with beef had no statistically significant effect on sero-prevalence in beef (Table 7).

None of the interaction terms improved the fit of the model

4.4.3. Deer

The bivariable associations between farm level risk factors and the within-herd sero-prevalence against Pomona and Hardjobovis for deer herds are presented in Table 5. Many of the unadjusted risk factors were significantly ($p < 0.05$) associated and several in the logistic regression model with the outcome (data not shown). However, after adjustment for clustering within herd, the remaining significant (p-value ≤ 0.05) risk factors for the outcome 'within-herd sero-prevalence against Hardjobovis' were 'herd size' (OR 1.59, 95% CI 2.39-9.49). Grazing of beef or sheep with deer had no statistically significant effect on sero-sero-prevalence in deer (Table 8).

For the outcome 'within-flock sero-prevalence against Pomona', the remaining significant risk factors (p-value ≤ 0.05) were 'sampling season', 'herd size' and 'number of dogs present'. If deer herds were larger, they were more likely to be positive (OR 1.81, 95% CI 2.73-16.78) and the more dogs present, the less likely deer herds were positive against Pomona (OR 0.59, 95% CI 1.0-0.14). If deer herds were sampled in summer they were 4.8 more likely to being positive compared to being sampled in winter (95% CI 11.81-14.88). Grazing of sheep or beef together with deer had no statistically significant effect on sero-prevalence in deer (Table 8).

None of the interaction terms improved the fit of the model.

Table 3: Frequencies of risk factors (N tot), the distribution of sero-positive sheep against *Leptospira interrogans* sv Pomona (Pom) and *Leptospira borgpetersenii* sv Hardjobovis (Har) by risk factor (N positive, Prevalence (Prev %), 95% confidence interval (CI)) and unconditional associations of risk factors with sero-prevalence for Pom and Har in 162 sheep flocks and 3359 sheep (Odds Ratio (OR), 95% CI, p-value)) blood sampled between June 2009 and July 2010

		Hardjobovis						Pomona					
Risk factor & category	**N tot**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**
Vaccination status													
sheep not vaccinated	3337	1455	43.6	43.6-43.6	ref			470	14.1	14.1-14.1	ref		
sheep vaccinated	22	1	4.5	2.3-6.8	0.1	0.0-0.5	0.006	1	4.5	2.3-6.8	0.3	0.0-2.2	0.228
Flock size													
<1637	829	282	34.0	34.0-34.1	ref			105	12.7	12.6-12.7	ref		
≥1637	844	391	46.3	46.3-46.4	1.7	1.4-2.0	<0.001	111	13.2	13.1-13.2	1.0	0.8-1.4	0.767
≥2744	840	381	45.4	45.3-45.4	1.6	1.3-2.0	<0.001	101	12.0	12.0-12.1	0.9	0.7-1.3	0.690
≥4770	846	402	47.5	47.5-47.6	1.8	1.4-2.1	<0.001	154	18.2	18.1-18.3	1.5	1.2-2.0	0.002
Region													
Canterbury	715	222	31.0	31.0-31.1	ref			64	9.0	8.9-9.0	ref		
Otago & Southland	654	463	70.8	70.7-70.9	5.4	4.3-6.8	<0.001	100	15.3	15.2-15.4	1.8	1.3-2.6	<0.001
Wairarapa	160	60	37.5	37.2-37.8	1.3	0.9-1.9	0.115	10	6.3	5.9-6.6	0.7	0.3-1.3	0.270
Manawatu-Wanganui & Taranaki	1098	428	39.0	38.9-39.0	1.4	1.2-1.7	0.001	157	14.3	14.3-14.3	1.7	1.2-2.3	0.001
Hawkes's Bay & East Coast	669	254	38.0	37.9-38.0	1.4	1.1-1.7	0.007	136	20.3	20.3-20.4	2.6	1.9-3.6	<0.001
Waikato	63	29	46.0	45.2-46.8	1.9	1.1-3.2	0.016	4	6.3	5.6-7.1	0.7	0.2-2.0	0.486
Co-grazing													
Sheep grazing without beef	1233	557	45.2	45.1-45.2	ref			151	12.2	12.2-12.3	ref		
Sheep co-grazing beef	2126	899	42.3	42.3-42.3	0.9	0.8-1.0	0.104	320	15.1	15-15.1	1.3	1.0-1.6	0.024
Sheep grazing without deer	2762	1197	43.3	43.3-43.4	ref			394	14.3	14.2-14.3	ref		
Sheep co-grazing deer	597	259	43.4	43.3-43.5	1.0	0.8-1.2	0.984	77	12.9	12.8-13	0.9	0.7-1.2	0.383

Table 3: (continuation from page 41)

		Hardjobovis						Pomona					
Risk factor & category	**N tot**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**
Waterways													
Springs absent	1457	673	46.2	46.2-46.2	ref			227	15.6	15.5-15.6	ref		
Springs present	1902	783	41.2	41.1-41.2	0.8	0.7-0.9	0.004	244	12.8	12.8-12.9	0.8	0.7-2.0	0.023
Dams absent	1205	662	54.9	54.9-55	ref			152	12.6	12.6-12.7	ref		
Dams present	2154	794	36.9	36.8-36.9	0.5	0.4-0.5	<0.001	319	14.8	14.8-14.8	1.2	1.0-1.5	0.079
Stream absent	804	402	50.0	49.9-50.1	ref			145	18.0	18-18.1	ref		
Stream present	2555	1054	41.3	41.2-41.3	0.7	0.6-0.8	<0.001	326	12.8	12.7-12.8	0.7	0.5-0.8	<0.001
Number of dogs present	cont	-	-	-	1.0	1.0 -1.0	<0.001	-	-	-	1.0	1.0-1.0	0.106
Farm size in hectares	cont	-	-	-	1.0	1.0-1.0	0.21	-	-	-	1.0	1.0-1.0	<0.001
Sampling season													
Spring (Sept-Nov)	768	325	42.3	42.3-42.4	ref			147	19.1	19.1-19.2	ref		
Summer (Dec-Feb)	301	174	57.8	57.6-58	1.9	1.4-2.4	<0.001	36	12.0	11.8-12.1	0.6	0.4-0.8	0.005
Autumn (Mar-May)	300	132	44.0	43.8-44.2	1.1	0.8-1.	0.618	46	15.3	15.2-15.5	0.8	0.5-1.1	0.147
Winter (Jun-Aug)	1990	825	41.5	41.4-41.5	1.0	0.8-1.1	0.681	242	12.2	12.1-12.2	0.6	0.5-0.7	<0.001

Table 4: Frequencies of risk factors (N tot), the distribution of sero-positive beef cattle against *Leptospira interrogans* sv Pomona (Pom) and *Leptospira borgpetersenii* sv Hardjobovis (Har) by risk factor (N positive, Prevalence (Prev %), 95% confidence interval (CI)) and the unconditional association of risk factors with sero-prevalence for Pom and Har in 116 beef herds and 2308 beef cattle (Odds Ratio (OR), 95% CI, p-value)) blood sampled between June 2009 and July 2010

		Hardjobovis						Pomona					
Risk factor & category	**N tot**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**
Vaccination status													
beef not vaccinated	1886	860	45.6	45.6-45.6	ref			370	19.6	19.6-19.6	ref		
beef vaccinated	422	290	68.7	68.6-68.8	2.6	2.1-3.3	<0.001	211	50.0	49.9-50.1	4.1	3.3-5.1	<0.001
Herd size													
<131	550	200	36.4	36.3-36.5	ref			96	17.5	17.4-17.5	ref		
≥131	573	257	44.9	44.8-44.9	1.4	1.1-1.8	0.004	131	22.9	22.8-22.9	1.4	1.0-1.9	0.024
≥285	597	324	54.3	54.2-54.4	2.1	1.6-2.6	<0.001	183	30.7	30.6-30.7	2.1	1.6-2.8	<0.001
≥555	588	369	62.8	62.7-62.8	2.9	2.3-3.7	<0.001	171	29.1	29-29.2	1.9	1.5-2.6	<0.001
Region													
Canterbury	488	201	41.2	41.1-41.3	ref			74	15.2	15.1-15.3	ref		
Otago & Southland	94	56	59.6	59-60.1	2.1	1.3-3.3	0.001	28	29.8	29.3-30.3	2.4	1.4-3.9	0.001
Wairarapa	166	98	59.0	58.7-59.3	2.1	1.4-2.9	<0.001	32	19.3	19-19.6	1.3	0.8-2.1	0.215
Manawatu-Wanganui & Taranaki	980	496	50.6	50.6-50.7	1.5	1.2-1.8	0.001	229	23.4	23.3-23.4	1.7	1.3-2.3	<0.001
Hawkes's Bay & East Coast	520	278	53.5	53.4-53.6	1.6	1.3-2.1	<0.001	182	35.0	34.9-35.1	3.0	2.2-5.0	<0.001
Waikato	60	21	35.0	34.2-35.8	0.8	0.4-1.3	0.358	36	60.0	59.2-60.8	8.4	4.7-14.9	<0.001
Co-grazing													
Beef grazing without sheep	625	304	48.6	48.6-48.7	ref			173	27.7	27.6-27.8	ref		
Beef co-grazing sheep	1683	846	50.3	50.2-50.3	1.1	0.9-1.3	0.487	408	24.2	24.2-24.3	0.8	0.7-1.0	0.091
Beef grazing without deer	1820	942	51.8	51.7-51.8	ref			500	27.5	27.4-27.5	ref		
Beef co-grazing deer	488	208	42.6	42.5-42.7	0.7	0.6-0.8	<0.001	81	16.6	16.5-16.7	0.5	0.4-0.7	<0.001

Table 4: (continuation from page 43)

		Hardjobovis						Pomona					
Risk factor & category	**N tot**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**
Waterways													
Springs absent	978	438	44.8	44.7-44.8	ref			201	20.6	20.5-20.6	ref		
Springs present	1330	712	53.5	53.5-53.6	1.4	1.2-1.7	<0.001	380	28.6	28.5-28.6	1.5	1.3-1.9	<0.001
Dams absent	662	353	53.3	53.2-53.4	ref			214	32.3	32.3-32.4	ref		
Dams present	1646	797	48.4	48.4-48.5	0.8	0.7-1.0	0.033	367	22.3	22.3-22.3	0.6	0.5-0.7	<0.001
Stream absent	464	238	51.3	51.2-51.4	ref			100	21.6	21.4-21.7	ref		
Stream present	1844	912	49.5	49.4-49.5	0.9	0.8-1.1	0.48	481	26.1	26.1-26.1	1.3	1.0-1.6	0.045
Number of dogs present	cont	-	-	-	1.0	1.0-1.0	<0.001	-	-	-	1.0	1.0-1.0	0.749
Farm size in hectares	cont	-	-	-	1.0	1.0-1.0	<0.001	-	-	-	1.0	1.0-1.0	
Sampling season													
Spring (Sept-Nov)	687	308	44.8	44.8-44.9	ref			206	30.0	29.9-30.1	ref		
Summer (Dec-Feb)	226	122	54.0	53.8-54.2	1.4	1.1-1.9	0.017	24	10.6	10.4-10.8	0.3	0.2-0.4	<0.001
Autumn (Mar-May)	264	135	51.1	50.9-51.3	1.3	1.0-1.7	0.081	72	27.3	27.1-27.5	0.9	0.6-1.2	0.41
Winter (Jun-Aug)	1131	585	51.7	51.7-51.8	1.3	1.1-1.6	0.004	279	24.7	24.6-24.7	0.8	0.6-0.9	0.013

Table 5: Frequencies of risk factors (N tot), the distribution of sero-positive deer against *Leptospira interrogans* sv Pomona (Pom) and *Leptospira borgpetersenii* sv Hardjobovis (Har) by risk factor (N positive, Prevalence (Prev %), 95% confidence interval (CI)) and the unconditional association of risk factors with sero-prevalence for Pom and Har in 99 deer herds (Odds Ratio (OR), 95% CI, p-value)) and 1992 deer blood sampled between June 2009 and July 2010

		Hardjobovis						Pomona					
Risk factor & category	**N tot**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**	**N positive**	**Prev %**	**95% CI (%)**	**OR**	**95% CI (%)**	**p-value**
Vaccination status													
Deer not vaccinated	1870	492	26.3	26.3-26.3	ref			165	8.8	8.8-8.9	ref		
Deer vaccinated	122	32	26.2	25.8-26.6	1.0	0.7-1.5	0.984	36	29.5	29.1-29.9	4.3	2.8-6.6	<0.001
Herd size													
<277	483	93	19.3	19.2-19.4	ref						ref		
≥277 – 437	491	99	20.2	20.1-20.3	1.1	0.8-1.4	0.722	56	11.4	11.3-11.5	1.6	1.0-2.5	0.036
≥438 – 749	507	171	33.7	33.6-33.8	2.1	1.6-2.9	<0.001	45	8.9	8.8-9.0	1.2	0.8-1.9	0.415
≥750	511	161	31.5	31.4-31.6	1.9	1.4-2.6	<0.001	64	12.5	12.4-12.6	1.8	1.2-2.7	0.009
Region													
Canterbury	916	280	30.6	30.5-30.6	ref			59	6.4	6.4-6.5	ref		
Otago & Southland	270	103	38.1	38-38.3	1.4	1.1-1.9	0.02	28	10.4	10.2-10.6	1.7	1.0-2.7	0.031
Wairarapa	40	2	5.0	3.8-6.3	0.1	0.0-0.5	0.004	1	2.5	1.3-3.8	0.4	0.0-2.8	0.334
Manawatu-Wanganui & Taranaki	376	82	21.8	21.7-21.9	0.6	0.5-0.8	0.002	80	21.3	21.1-21.4	3.9	2.7-5.6	<0.001
Hawkes's Bay & East Coast	321	54	16.8	16.7-17	0.5	0.3-0.6	<0.001	27	8.4	8.3-8.6	1.3	0.8-2.1	0.234
Waikato	69	3	4.3	3.6-5.1	0.1	0.0-0.3	<0.001	6	8.7	8.0-9.4	1.4	0.6-3.3	0.469
Co-grazing													
Deer grazing without beef	1417	413	29.1	29.1-29.2	ref			142	10.0	10-10.1	ref		
Deer co-grazing beef	575	111	19.3	19.2-19.4	0.6	0.5-0.7	<0.001	59	10.3	10.2-10.3	1.0	0.7-1.4	0.872
Deer grazing without sheep	860	244	28.4	28.3-28.4	ref			63	7.3	7.3-7.4	ref		
Deer co-grazing sheep	1132	280	24.7	24.7-24.8	0.8	0.7-1.0	0.068	138	12.2	12.1-12.2	1.8	1.3-2.4	<0.001

Table 5: (continuation from page 45)

Risk factor & category	N tot	Hardjobovis						Pomona					
		N positive	Prev %	95% CI (%)	OR	95% CI (%)	p-value	N positive	Prev %	95% CI (%)	OR	95% CI (%)	p-value
Waterways													
Springs absent	1025	235	22.9	22.9-23	ref			78	7.6	7.6-7.7	ref		
Springs present	968	289	29.9	29.8-29.9	1.4	1.2-1.7	<0.001	123	12.7	12.7-12.8	1.8	1.3-2.4	<0.001
Dams absent	1032	297	28.8	28.7-28.8	ref			62	6.0	6.0-6.1	ref		
Dams present	960	227	23.6	23.6-23.7	0.8	0.6-0.9	0.009	139	14.5	14.4-14.5	2.6	1.9-3.6	<0.001
Stream absent	682	170	24.9	24.9-25.0	ref			46	6.7	6.7-6.8	ref		
Stream present	1310	354	27.0	27.0-27.1	1.1	0.9-1.4	0.313	155	11.8	11.8-11.9	1.9	1.3-2.6	<0.001
Number of dogs present		-	-	-	1.0	0.9-1.0	<0.001	-	-	-	1.0	0.9-1.0	0.015
Farm size in hectares		-	-	-	1.0	1.0-1.0	0.276	-	-	-	1.0	1.0-1.0	0.298
Sampling season													
Spring (Sept-Nov)	854	225	26.3	26.3-26.4	ref			69	8.1	8.0-8.1	ref		
Summer (Dec-Feb)	102	10	9.8	9.3-10.3	0.3	0.2-0.6	<0.001	17	16.7	16.2-17.2	2.3	1.3-4.0	0.005
Autumn (Mar-May)	158	57	36.1	35.8-36.4	1.6	1.1-2.3	0.013	15	9.5	9.2-9.8	1.2	0.7-2.1	0.554
Winter (Jun-Aug)	878	232	26.4	26.4-26.5	1.0	0.8-1.2	0.971	100	11.4	11.3-11.4	1.5	1.1-2.0	0.021

Table 6: Clustering and confounder adjusted sero-prevalence (Prev %) of *Leptospira interrogans* sv Pomona and *Leptospira borgpetersenii* sv Hardjobovis and associated risk factors in sheep flocks (n=162) blood sampled between June 2009 and July 2010 calculated by multivariable binomial logistic regression using generalized estimating equations

Category		Prev (%)*	95% CI (%)	OR*	95% CI	p-value
Hardjobovis						
Co-grazing	Sheep grazing without beef	47.9	37.3-58.8	ref	-	-
	Sheep co-grazing beef	47.9	40.0-56.0	1.0	0.6-1.6	1.000
	Sheep grazing without deer	46.1	38.3-54.0	ref	-	-
	Sheep co-grazing deer	49.8	39.0-60.6	1.2	0.7-1.4	0.547
Flock size	Continuous	-	-	1.6	1.3-1.9	<0.001
Number of dogs	Continuous	-	-	0.7	0.6-1.0	0.045
Region[1]	Canterbury	33.5	23.8-44.8	0.4	0.1-1.5	0.187
	Otago & Southland	68.2	55.6-78.6	1.8	0.5-6.8	0.401
	Wairarapa	42.1	25.1-61.2	0.6	0.1-2.6	0.498
	Manawatu-Wanganui & Taranaki	43.8	33.8-54.3	0.6	0.2-2.4	0.510
	Hawkes's Bay & East Coast	45.2	33.7-57.1	0.7	0.2-2.5	0.567
	Waikato	54.7	26.7-79.9	ref	-	-
Water-ways	Dams or rivers absent	54.3	43.6-64.6	ref	-	-
	Dams or rivers present	41.6	33.9-49.8	0.6	0.4-1.0	0.036
Pomona						
Co-grazing	Sheep grazing without beef	9.8	6.6-14.2	ref	-	-
	Sheep co-grazing beef	11.9	9.1-15.5	1.2	0.7-2.1	0.419
	Sheep grazing without deer	11.1	8.9-13.7	ref	-	-
	Sheep co-grazing deer	10.6	7.0-15.7	0.9	0.6-1.6	0.833
Flock size	Continuous	-	-	1.2	1.0-1.4	0.092
Region[2]	Canterbury	8.7	5.8-12.8	1.0	0.5-2.2	0.907
	Otago & Southland	15.1	9.3-23.5	1.9	0.9-4.2	0.095
	Wairarapa	5.1	2.3-11.1	0.6	0.2-1.8	0.344
	Manawatu-Wanganui & Taranaki	13.5	9.0-19.8	1.7	0.8-3.8	0.189
	Hawkes's Bay & East Coast	19.5	14.1-26.4	2.7	1.3-5.6	0.011
	Waikato	8.4	4.8-14.3	ref	-	-
Water-ways	Springs absent	12.8	9.5-17.0			-
	Springs present	9.1	6.8-12.1	0.7	0.5-1.0	0.061

*Adjusted by the logistic regression model. The overall chi p-values were [1]0.014 and [2]0.006.

Table 7: Clustering and confounder adjusted sero-prevalence (Prev %) of Leptospira interrogans sv Pomona and Leptospira borgpetersenii sv Hardjobovis and associated risk factors in beef herds (n=116) blood sampled between June 2009 and July 2010 calculated by multivariable binomial logistic regression using generalized estimating equations

Risk factor	Category	Prev (%)*	95% CI (%)	OR*	95% CI	p-value
Hardjobovis						
Co-grazing	Beef grazing without sheep	60.5	47.3-72.3	ref	-	-
	Beef co-grazing sheep	55.6	43.6-67.1	0.8	0.5-1.5	0.499
	Beef grazing without deer	60.9	52.0-69.1	ref	-	-
	Beef co-grazing deer	55.2	39.6-69.8	0.8	0.4-1.4	0.434
Region[1]	Canterbury	56.4	39.9-71.7	2.7	0.6-13.7	0.217
	Otago & Southland	65.4	45.8-80.9	4.0	0.7-21.5	0.105
	Wairarapa	71.8	55.5-83.9	5.4	1.1-26.8	0.039
	Manawatu-Wanganui & Taranaki	62.1	50.2-72.7	3.5	0.7-16.5	0.116
	Hawkes's Bay & East coast	59.4	46.2-71.4	3.1	0.6-15.1	0.160
	Waikato	32.0	9.9-67.0	ref	-	-
Herd size	Continuous	-	-	1.4	1.1-1.8	0.004
Vaccination of beef	Beef not vaccinated	45.0	35.7-54.6	ref	-	-
	Beef vaccinated	70.1	55.6-81.4	2.9	1.6-5.3	0.001
Waterways	Dams absent	64.7	52.3-75.3	ref	-	-
	Dams present	51.2	39.3-62.9	0.6	0.3-1.0	0.038
Pomona						
Co-grazing	Beef grazing without sheep	29.4	18.0-44.3	ref	-	-
	Beef co-grazing sheep	19.9	12.3-30.6	0.6	0.3-1.2	0.130
	Beef grazing without deer	34.8	24.9-46.2	ref	-	-
	Beef co-grazing deer	16.3	8.7-28.2	0.4	0.2-0.7	0.002
Region[2]	Canterbury	14.8	6.4-30.6	0.3	0.0-1.4	0.111
	Otago & Southland	16.7	4.5-46.4	0.3	0.0-2.2	0.242
	Wairarapa	20.5	8.7-41.1	0.4	0.1-2.1	0.271
	Manawatu-Wanganui & Taranaki	23.7	15.0-35.4	0.5	0.1-2.1	0.318
	Hawkes's Bay & East Coast	37.3	24.3-52.6	0.9	0.2-4.2	0.889
	Waikato	39.9	15.3-70.9	ref	-	-
Vaccination of beef	Beef not vaccinated	15.2	9.8-22.8			
	Beef vaccinated	36.6	22.7-53.1	3.2	1.7-5.9	<0.001
Waterways	Dams absent	38.8	24.8-54.8	ref		
	Dams present	14.1	7.7-24.2	0.2	0.1-0.6	0.002
	Springs absent	18.2	10.5-29.9	ref		
	Springs present	31.7	19.9-46.5	2.1	1.0-4.5	0.060
	Stream absent	16.4	8.2-30.2	ref		
	Stream present	34.5	24.5-46.2	2.7	1.2-6.1	0.019

*Adjusted by the logistic regression model. The overall chi p-values were [1]0.49 and [2]0.06.

Table 8: Clustering and confounder adjusted sero-prevalence (Prev %) of *Leptospira interrogans* sv Pomona and *Leptospira borgpetersenii* sv Hardjobovis and associated risk factors in deer herds (n=99) blood sampled between June 2009 and July 2010 calculated by multivariable binomial logistic regression using generalized estimating equations

Risk factor	Category	Prev (%)*	95% CI (%)	OR*	95% CI	p-value
Hardjobovis						
Co-grazing	Deer grazing without sheep	15.1	9.3-23.5	ref	-	-
	Deer co-grazing sheep	9.1	4.9-16.3	0.6	0.2-1.2	0.134
	Deer grazing without beef	13.4	7.7-22.1	ref	-	-
	Deer co-grazing beef	10.3	6.0-17.2	0.7	0.4-1.5	0.419
Region[1]	Canterbury	27.2	17.9-39.1	18.5	2.3-151.6	0.007
	Otago & Southland	26.7	13.3-46.3	18.1	2.1-154.1	0.008
	Wairarapa	3.2	1.5-6.9	1.7	0.2-13.7	0.638
	Manawatu-Wanganui & Taranaki	22.4	11.6-38.9	14.3	1.5-133.7	0.020
	Hawkes's Bay & East coast	17.4	7.2-36.5	10.4	1.0-104.1	0.046
	Waikato	2.0	0.2-13.8	ref	-	-
Water-ways	Springs absent	8.9	4.6-16.6	ref	-	-
	present	15.3	10.0-22.8	1.8	0.9-3.8	0.102
Herd size	Continuous			1.6	1.1-9.5	0.024
Pomona						
Season	Spring[1]	4.2	1.5-11.1	0.6	0.2-2.0	0.414
	Summer[2]	23.2	11.6-41.02	4.2	1.5-11.8	0.007
	Autumn[3]	3.9	1.4-10.4	0.6	0.2-1.7	0.305
	Winter[4]	6.7	3.8-11.8	ref	-	-
Co-grazing	Deer grazing without sheep	8.5	4.9-14.5	ref	-	-
	Deer co-grazing sheep	6.23	3.1-12.2	0.7	0.3-1.6	0.427
	Deer grazing without beef	5.8	2.911.3	ref	-	-
	Deer co-grazing beef	9.2	5.3-15.7	1.7	0.7-3.8	0.238
Region[2]	Canterbury	5.5	2.7-11.0	1.0	0.3-3.3	0.992
	Otago & Southland	6.9	2.3-19.3	1.3	0.3-5.7	0.745
	Wairarapa	3.6	0.5-21.4	0.7	0.1-5.9	0.702
	Manawatu-Wanganui & Taranaki	25.6	12.9-44.4	5.9	1.6-22.7	0.009
	Hawkes's Bay & East Coast	6.8	2.9-15.3	1.3	0.3-4.7	0.730
	Waikato	5.5	2.0-14.2	ref	-	-
Herd size	Continuous			1.8	1.2-2.7	0.005
Numbers of dogs	Continuous			0.6	0.3-1.0	0.048

[1]September, October and November, [2] December, January and February, [3] March, April and May, [4] June, July and August.*Adjusted by the logistic regression model. The overall chi p-values were [1]0.334 and [2]0.322.

Figure 2 illustrates the relationship (fitted line, by the GEE extended logistic regression model) between adjusted prevalence against Hardjobovis in sheep and (i) number of dogs present (above) and (ii) the flock size (below). The adjusted prevalence increased with a decreasing number of dogs on farm (p-value 0.058) and an increasing flock size (p-value <0.001).

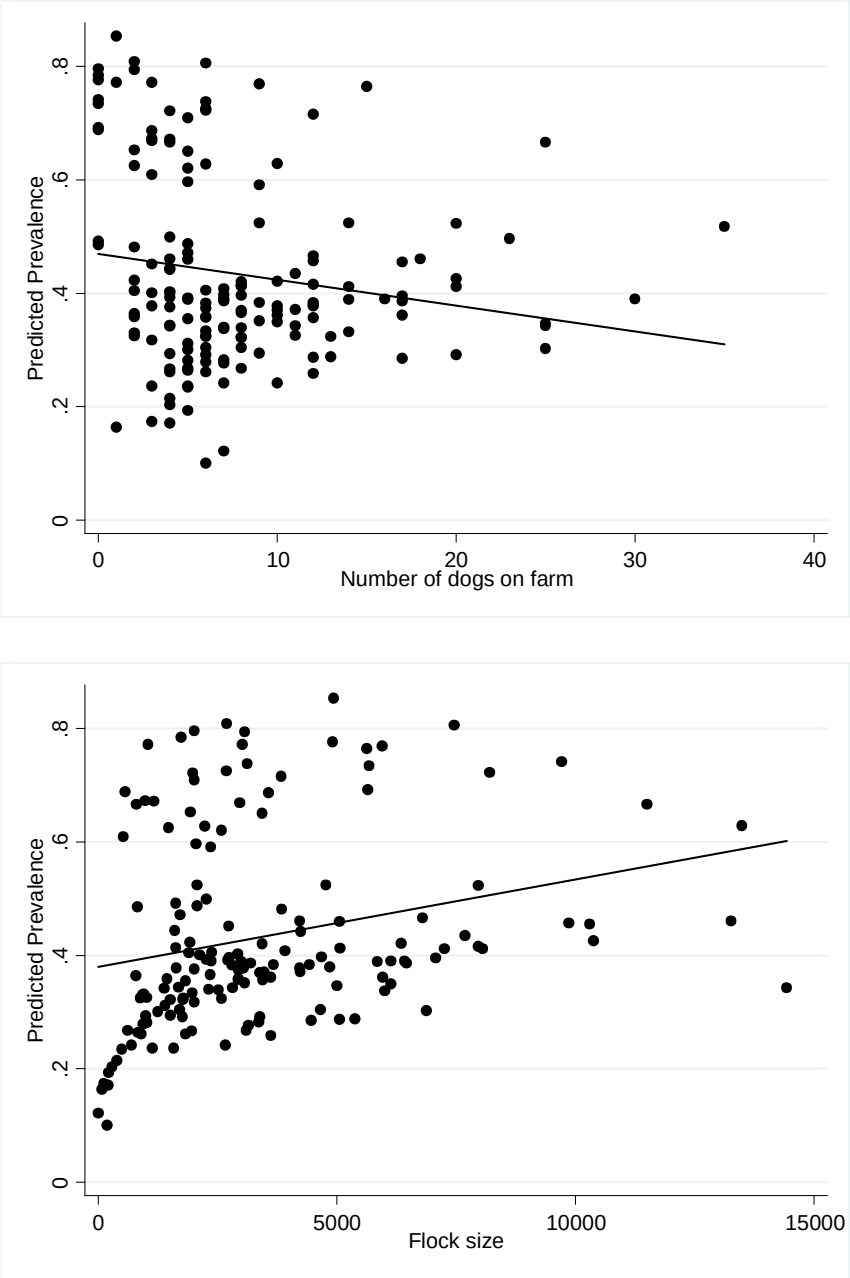


Figure 2: Scatter plots displaying the relationship (by the generalized equations estimator extended logistic regression model adjusted) between sero-prevalence against *Leptospira borgpetersenii* sv Hardjobovis in sheep and (i) number of dogs present (above) and the flock size (below)

5. Discussion

This is the first nationwide study on sero-prevalence in the adult sheep, deer and beef cattle population, confirming earlier periodic species-specific observations of high sero-prevalence of Hardjobovis and Pomona in beef cattle and deer, both at herd and within herd levels. In the absence of national surveillance data, this information contributes to calculating potential disease specific economic loss, planning a vaccination strategy or estimating the potential public health risk for persons exposed to these animal species. Hardjobovis was more prevalent in all three livestock species than Pomona. The sero-prevalence against Hardjobovis (43%) and Pomona (14%) was at a level in sheep consistent with the conclusion that Hardjobovis and possibly Pomona are well adapted to sheep, and that sheep may have evolved to being a so called 'maintenance host'. This conclusion is consistent with observations and statements made by (Dorjee et al., 2008), who isolated *Leptospira* by culture from kidneys of 22% of lamb carcasses sero-positive against Hardjobovis. The conclusion stands in contrast to inferences by Blackmore et al. (1982) where sheep were ruled out as a reservoir host for any of these two serovars (Blackmore et al., 1982).

In our study, the sero-prevalence of Pomona was higher than in former sero-prevalence studies of sheep (0.5-4% of slaughtered lambs; Dorjee et al (2008)), deer (14% in mixed age hinds; Ayanegui-Alcérreca, (2006)) and beef cattle (19% in mixed age cows; Sanhueza (2012)) (Ayanegui-Alcérreca, 2006; Dorjee et al., 2008; Sanhueza, 2012). The higher sero-prevalence in sheep reported here is likely to be partially attributable to the higher age in this study than in the abattoir surveys, and secondly survey data are a snap shot of a dynamic infectious disease process. Ayanegui-Alcérreca (2006) has shown a significant age difference in sero-prevalence in weaner compared to yearling and adult farmed deer (Ayanegui-Alcérreca, 2006). This may also be the case for sheep, as it is biologically plausible: infection occurs after the decay of maternal antibodies and the older the animal, the more likely had a direct and indirect transmission of *Leptospira* occurred. The within-herd/flock sero-prevalence of Pomona seems rather high in sheep (14%) and beef (25%) to be regarded as a sporadic infection, indicating that Pomona may have adapted to sheep and beef in NZ. This statement would be supported if sero-positive animals were also shedding. In an abattoir study (Dorjee et al., 2008), one of six (17%) lambs sero-positive for Pomona were kidney-culture positive for Pomona with the authors concluding that Pomona appeared to be a sporadic infection. However, the evidence for this conclusion was based on only 6 carcasses sero-positive for Pomona and animals were young lambs in slaughter age. In an abattoir study in the Waikato region, urine, kidney and blood was sampled from 399 lambs and 146 beef from six suppliers following a period of heavy rain during warm weather. The shedding rate -determined by positive urine PCR- in sero-

positive (MAT cut-off ≥1:48) sheep was 54.1%, whilst that in sero-negative sheep it was 2.8% and in sero-positive cattle it was 28.2%, whilst that in sero-negative cattle it was 3.0% (Fang, 2013). Further, a cohort study conducted in sheep abattoir workers (*n*=384) from 2010-2011 (Chapter 5), found that 7.3% of sero-negative participants sero-converted against Pomona, which indirectly indicated that sheep were shedding Pomona.

The data was analysed and presented with vaccinated animals included. One could worry that inclusion of vaccinated animals may have increased the sero-prevalence overestimating the true sero-prevalence related to natural exposure. However, it was observed that antibody titres due to vaccination may be lower and wane faster than those from natural infection (Strother, 1974) and therefore should not bias the sero-prevalence in a cross-sectional study. Vaccination was evaluated as a risk factor in the multivariable analysis and was found in beef cattle to increase sero-prevalence. Since vaccinated beef herds were included in the model we controlled for the possible bias of vaccination and we were able to analyse the data of the full study population.

The stated prevalence is the apparent prevalence; hence we did not calculate the true prevalence with the Rogan-Gladen formula (Rogan and Gladen, 1978) to correct for the MAT not being 100% sensitive and specific. The reason for this is that the sensitivity and specificity of the MAT used in the NZ context has not been calculated (Collins-Emerson, personal communication) and the sensitivity of 91% - 100% and specificity of 94% to 100% for detecting antibodies in reconvalescent human blood samples reported by McBride et al. (2007) was estimated in an urban setting with different serovars being prevalent and therefore not deemed reliable enough. Since the lack of calculating the true prevalence is not a source of differential misclassification, conclusions on risk factors should not be influenced.

In sheep, the adjusted sero-prevalence differed by region with the sero-prevalence against Hardjobovis being highest in Otago and Southland with 68% and against Pomona in the Hawkes's Bay and East coast with 19%. Therefore, these regions have the highest risk of infection for persons exposed to sheep (urine), since over 20% of sero-positive lambs are shedders and 13 of 1,000 sheep carcasses processed by abattoirs can be expected to potentially shed leptospires and expose workers (Dorjee et al., 2008; Fang, 2013). Therefore, occupational safety and health personnel may be well advised to strengthen awareness of the leptospirosis risk from sheep, foremost in these high-prevalence regions.

The different spatial distribution of Hardjobovis and Pomona and the lack of evidence for them being present in the same herd or animal suggest that the two serovars behave differently in the same environment. Hence, Hardjobovis and Pomona may have diverse ecologies. A reason for diverse transmission patterns could be the stronger influence of seasonal characteristics (rainfall,

temperature, flooding), the length of survival in soil or host preference of one serovar over the other.

Grazing cattle with deer reduced the likelihood of positivity against Pomona in beef. An explanation could be host specificity of Pomona. The Pomona serovar infecting beef may be more likely to infect deer than cattle (or boost their immune system). If a serovar was more likely to infect deer than beef, co-grazed deer with beef would have a lower stocking density on a given paddock than deer grazed alone and indirect transmission due to contact with contaminated urine would be less probable. Co-grazing with another species did not increase the odds of the within-herd sero-prevalence for deer and sheep of Pomona or Hardjobovis and for beef the within-herd sero-prevalence of Hardjobovis. For deer, the present results are in contrast to those of a longitudinal serological survey in 20 mixed species deer farms (beef and/or sheep) (Subharat et al., 2012b) in which deer herds were more likely to be Hardjobovis or Pomona positive on farms when deer were co-grazing with Hardjobovis or Pomona positive cattle herds, respectively. While a longitudinal study design is preferable to the cross-sectional design, the 19 farms involved in the Subharat (2012) study were all from the Hawke's Bay region, and the outcome measure was sero-prevalence and not incidence. Therefore, whether co-grazing is a risk factor for sero-prevalence remains unresolved. Co-grazing may be mainly a risk factor for introduction *per se* of *Leptospira* into a naïve herd. However, this is an independent research question and could not be tested in this study due to the high endemic levels of *Leptospira,* the cross sectional study design and missing information on animal movement data.

The question as to whether co-farming of other species or co-grazing is a risk factor for *Leptospira spp.* sero-prevalence in cattle or sheep at the individual or herd level, has been raised by several authors. In addition to Subharat (2012), a study in Tanzania found that cattle co-grazed with sheep or goats were more likely to have increased antibody titres against a large number of serovars (Schoonman and Swai, 2010). In Brasil, *L. interrogans* sv Hardjoprajnito (Hardjoprajnito) sero-prevalence in sheep herds was positively associated with the presence of cattle (Genovez et al., 2011). However, the sample only comprised eight farms. Positivity against one of 24 serovars in cow herds in Brazil was positively associated with the presence of deer (although whether farmed or wild was not stated) and positivity against Hardjobovis or Hardjoprajnito with presence of swine (Oliveira et al., 2010). Marques et al. (2010) tested a representative sample of farms with cows against 16 *Leptospira* serovars in Brazil and found, among other risk factors, that presence of sheep, goats and capybaras (endemic rodents) was statistically significantly and positively associated with sero-prevalence (Marques et al., 2010). The presence of sheep and swine was positively associated with positivity against 22 serovars in a Brazilian study in adult cow herds (Castro et al., 2009).

However, apart from the NZ study (Subharat et al., 2012b), the significance of all these study results is questionable as confounding factors and clustering within herds was not taken into account in the analyses. Also, in our study a great number of risk factors were statistically significant before adjusting for clustering that became insignificant after adjustment. A further problem in most of the studies referred to here is that the sero-prevalence was measured against a multitude of serovars, of which each may have different ecological relationships in a host-environment network typical for *Leptospira* spp (Hathaway, 1981).

The association with a reduced OR of valley dams may be due to the reduction of flooding of waterways. There was also a tendency that springs and streams on farms increased sero-prevalence. Higher prevalence related to flooding was also observed by sampling sheep carcasses immediately after a major flood in 2004 and in the subsequent relatively dry year 2005 (Dorjee et al., 2008). Deer herds and sheep flocks had a lower sero-prevalence with a larger number of dogs present. A larger number of dogs may be a proxy for farm location, as in the hill country, where flooding is less likely, more dogs are likely to be used to handle livestock. However, this conclusion is in contrast to the findings of a longitudinal study, where deer herds were more likely to be Hardjobovis positive on farms with hilly topography (Subharat et al., 2012b). Another explanation is a presumably lower stocking density reducing contacts between animals on large farms in extensive grazing areas. Herd size was a risk factor for sero-prevalence; this is a common finding for infectious diseases, as larger herds are more likely to retain shedders and are more frequently engaged in stock trading. Deer sampled in summer were more likely to be positive than those sampled in winter. In NZ, rainfall is usually higher in winter than summer, although summer floods are often observed in small locations and sometimes in entire regions as in the Manawatu in February 2004. The survival of *Leptospira* is enhanced by higher temperatures and humid environments (Levett, 2001). Therefore, access to contaminated and flooded surface water may be a contributory cause for infection (Vermunt et al., 1994; Dorjee et al., 2005a). However, 'sampling season' should not be over interpreted as a 'standalone' risk factor in this study.

All the above conclusions are not strongly evidenced by the data due to the cross-sectional study design and are rather hypotheses about transmission patterns of these two serovars. There is uncertainty about the time sequence, as exposure could have occurred at variable distance from the year where sero-prevalence was measured. Therefore, animals could have been infected, before co-grazing or a new species on farm was introduced.

Even though the ability of the MAT to distinguish between serovars had been questioned (Levett, 2001; Smythe et al., 2009), it is unlikely that this was the case for Hardjobovis and Pomona in this thesis, as the prevalent serovars in NZ belong to different serogroups apart from Hardjobovis

and *Leptospira borgpetersenii* Balcanica (Balcanica) (Hathaway, 1981). Further, several studies have been conducted in NZ in recent years, where serovars determined by serology had been also confirmed by direct methods. For example, MAT serology and serovar isolates had good kappa agreement by DNA sequencing results (Subharat et al., 2011b; Subharat et al., 2012a). Most research on sero-prevalence in animals outside of NZ was based on a MAT cut-off of 1:100. Nevertheless, a cut-off of 1:48 was chosen in order to be able to compare our results to results of former studies in humans and animals conducted in NZ. In humans the titre cut-off of 1:48 is recommended to determine exposure to leptospires, but not for clinical disease (Faine et al., 1999; Shivakumar and Krishnakumar, 2006b). Therefore, a cut-off of 1:48 should be applicable for measuring the prevalence of exposure in NZ, especially given the high endemic levels, which improve the positive predictive value of the MATFrom the serovars prevalent in NZ *Leptospira borgpetersenii* Balcanica may cross react with Hardjobovis (Faine et al., 1999) and may have reduced the MAT specificity to a certain degree. However, whilst Balcanica is most likely to be present in possums (Hathaway, 1981), it may be speculated that it infects livestock incidentally and does not spread within livestock. Since most positive herds contained several positive animals, it is reasonable to assume that their antibodies developed predominantly through exposure to Hardjobovis and not Balcanica, where only a few sporadic cases may be expected.

The power calculation was based on a sample size of 300 farms. We sampled 238 farms due to non-compliance or lack of sampling facilities on-farm. However, at the high endemic level of Hardjobovis and Pomona, 238 farms with 377 herds/flocks was a sufficient sample size to observe an odds ratio of 2.35 with 80% power, hence sufficient for the purpose of finding major risk factors.

The 2009 incidence of reported probable leptospirosis clinical disease reports was 2.6% in cattle herds, 0% in sheep flocks 0%, and 1% in deer herds. Regardless of the potential recall bias of farmers, the data are an indication for the low number of clinical expression of the wide-spread infection with Pomona and Hardjobovis in sheep, deer and beef in NZ. The incidence of probable leptospirosis in humans on farms in 2009 was 1 in 638 or 157 per 100 000 farmers/farm workers. This exceeds the rate of the official public health report[16] of 2009 relating to the risk for the general population of 1.6 per 100 000 of which 60.6% were farmers. However, confidence intervals are extremely wide and therefore the incidence data here should not be over interpreted.

The case definitions of leptospirosis in animals and humans are not totally specific for leptospirosis. However, the questionnaire was designed for lay persons and hence the question had to be designed to facilitate a range of understanding. On the other hand, it is possible that the

[16] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance report 2009.

reported leptospirosis episodes originated from serovars other than Hardjobovis or Pomona and that respondents did not detect sporadic or milder forms of leptospirosis in their herds or themselves, and that the leptospirosis incidence was underestimated. We therefore believe it is likely that the true clinical incidence or leptospirosis in animals is higher than estimated in this study.

6. Conclusion

The sero-prevalence of Hardjobovis and Pomona in NZ sheep, beef cattle and deer indicated that these serovars were prevalent at a high endemic level during the year of sampling, albeit apparently without major clinical losses in livestock. Hardjobovis was more prevalent in all three species than Pomona.

The regional sero-prevalence distribution was different for Hardjobovis and Pomona, suggesting that the two serovars behave differently in the same environment and Hardjobovis and Pomona may have diverse ecologies.

At the high endemic level observed it was difficult to measure an effect of co-grazing on sero-prevalence. Co-grazing with another species did not increase the odds of the within-herd sero-prevalence of Pomona or Hardjobovis for deer and sheep, or Hardjobovis for beef. Grazing beef with deer reduced the likelihood of positivity against Pomona in beef possibly due to host specificity of Pomona. A pathogen preference for either species cannot be inferred from this data.

To appreciate the complexity of the *Leptospira* ecology, multi-factor scenarios may be developed considering farm topography, rainfall, livestock movements, contacts between species including wildlife, and multiple serovars.

7. Acknowledgements

A special thanks to all farmers who took their time to gather their animals and to take part in the interview and to the 28 veterinary practices involved for their time and collaboration.

We gratefully acknowledge the Johne's Disease Research Consortium (JDRC) for permitting survey data and samples collected for Johne's Disease research to be used for this project , the Alma Baker Trust Fund, the Swiss National Science Foundation (SNF) and Rural Woman NZ (RWNZ) for other research costs and PhD candidate support.

We thank NZ Veterinary Pathology (NZVP) for their support in sample processing and storage. A very special thanks to Neville Haack for all the lab work and also to S. Prickaerts, P. Guzman, D. Tapia, R. Pearson, T. Delaune, M. Turlin, L. Stringer, J. Manuel Sanhueza, P. Moono, J. Job, R. Gregory, A. Kurian, G. Anakkaramadathil, S. Jacob, P. Jaros and S. Moore for technical assistance with sample management and analyses.

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Chapter 3

Economic Loss Associated with *Leptospira* Sero-prevalence in New Zealand Sheep, Cattle and Deer

A. Dreyfus, P. Wilson, J. Benschop, C. Verdugo, J. Collins-Emerson and C. Heuer

1. Abstract

Leptospiral infection is widespread in livestock in New Zealand (NZ) and currently the most important occupationally-acquired zoonotic disease in farmers and meat workers. The two most frequent serovars in cattle, deer and sheep in NZ are *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona). The same serovars occur in humans, suggesting a human health risk from animal sources.

The objective of the study was to evaluate the association between within-herd/flock *Leptospira* sero-prevalence and reproduction and culling, controlling for different farming enterprises (species combination and herd size) and for the farm being located in the North or South Island. From a sampling frame of client farms of 28 veterinary practices in eight regions throughout NZ, 238 farms were stratified-randomly selected between June 2009 and July 2010. Blood samples were collected on each farm by veterinarians from a systematic random sample of 20 sheep, 20 beef and 20 deer as present on farm. Farmers were interviewed on site by questionnaire about livestock demographics (e.g. region, herd size, livestock species farmed), vaccination status and production performance. Serum antibodies were measured against Hardjobovis and Pomona using the microscopic agglutination test (MAT). An animal was considered sero-positive when it had a MAT titre of ≥1:48 against either serovar. A herd/flock was considered positive if one or more animals were sero-positive.

Ninety seven percent of sheep and beef and 76% of deer farms had at least one animal sero-positive for Hardjobovis and/or Pomona. On sheep farms, after adjustment for confounding effects a 1% increase in sero-prevalence of Hardjobovis was associated with a 0.11% increase in tailing percentage (p=0.009). All other reproduction and culling percentages in any species were not significantly associated with sero-prevalence of Hardjobovis or Pomona. The cross-sectional study design at the farm level may not have been sufficiently sensitive to detect subclinical production effects; hence longitudinal study designs at the animal-level are suggested as more appropriate for that purpose.

2. Introduction

Leptospiral infection is widespread in livestock in New Zealand (NZ) and the most important occupationally-acquired zoonotic disease in farmers and meat workers (Marshall and Manktelow, 2002; Thornley et al., 2002). After infection, leptospires persist in the kidneys of carrier animals and can be transmitted directly via urine or indirectly through water or soil. *Leptospira* can survive for several months in humid, warm environments, but rapidly perish in dry conditions. While in many, mainly sub-/tropical countries, numerous animal hosts and *Leptospira* serovars survive in a complex ecological environment, the epidemiology of leptospirosis in NZ is based on a relatively small number of serovars known to be endemic (six from several hundred known worldwide) (Hathaway, 1981; Marshall and Manktelow, 2002). Leptospirosis is not a notifiable disease in animals in NZ and there is no publicly funded control program for leptospirosis in livestock.

The two most frequent serovars in cattle, deer and sheep in NZ are *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona). Various surveys have shown up to 81% of deer herds, 80% of beef cattle herds and 44% of sheep flocks had evidence of infection with these serovars (Hathaway, 1981; Marshall and Manktelow, 2002; Ayanegui-Alcerreca et al., 2007; Dorjee et al., 2008). Hardjobovis and Pomona occur in livestock and humans. Whereas previously the risk of *Leptospira* infection was mainly associated with dairy cows and pigs (Blackmore and Schollum, 1982b), more recent research in sheep and deer abattoirs and meat workers has shown that contact with sheep and deer risks exposure to Pomona and Hardjobovis (Benschop et al., 2009; Dorjee et al., 2011) (Chapters 4 and 5). Since vaccination of dairy cows commenced in the 1980s, the incidence of notified human leptospirosis cases in the farming industry dropped from 234/100,000 to 90/100,000 (Thornley et al., 2002). Whereas approximately 90% of dairy farmers vaccinate dairy cattle against leptospirosis, less than 10% of deer, sheep or beef farmers are currently using vaccination (Keenan, 2007b; Wilson et al., 2008b). This may be because of lack of awareness of *Leptospira* infection in these species and due to uncertainty about economic impacts of the disease.

While clinical leptospirosis is known to cause jaundice, haemoglobinuria and kidney disease, this clinical expression of leptospirosis is only seen sporadically in pastoral livestock species in NZ. A recent mail survey (2006-8) of 1940 respondents of 7998 clients of veterinary practices in NZ, reported a three year clinical leptospirosis incidence of 1.2% in sheep flocks (14/1193), 2.1% in beef cattle (22/1061) and 4.7% in deer herds (11/233) (Dreyfus et al., 2010c). Hardjobovis is regarded as host-adapted in deer, beef cattle and possibly sheep, which respond to infection mainly with subclinical disease, whereas the role of Pomona is unclear and infection with Pomona is considered

more sporadic with outbreaks occasionally occurring (Marshall and Manktelow, 2002; Dorjee et al., 2005b).

Most farmers do not perceive leptospirosis to be a production-limiting factor. Even though almost every farmer is aware of the disease in humans, the perceived risk of contracting leptospirosis does not seem to be high enough to trigger protective measures by deer, sheep and beef cattle farmers. Hence only few use vaccination for control. If production effects, such as on weight gain, or increased calving or weaning percentages could be demonstrated, farmers might be more likely to adopt vaccination of their livestock, which would cost them between 1.20 and 2.00 NZD[17] per animal and vaccination dose, as they would get a return for their investment. In the absence of a human vaccine in NZ, this would be desirable from a public health point of view, given the high incidence in farmers and abattoir workers[18]. Subclinical economic effects of leptospirosis in sheep and beef cattle in NZ have not been quantified in longitudinal studies. However, a study in farmed deer in NZ reported that yearling deer with evidence of infection during the growth period to 12 months-of-age were 3.7 kg lighter than those without the evidence of infection. Additionally, weaning rates were reduced by 11% (Ayanegui-Alcerreca et al., 2007). A subsequent study reported a 5.7% mean higher weaning percentage in primiparus hinds attributable to vaccination, if it was established that the deer were challenged from weaning onwards (Subharat et al., 2011c).

Preliminary study results indicate that herds in which calves of dairy cows were first vaccinated against leptospirosis at 3-months of age had a lower probability of having older cows shedding leptospires in urine than those in which vaccination of calves commenced later than 3-months of age (Parramore et al., 2011; Benschop et al., 2012). Hence, correct implementation of the vaccination program is crucial to avoid cows from shedding leptospires.

The objective of this study was to evaluate associations between within herd/flock *Leptospira* sero-prevalence and reproduction performance and culling of deer, sheep and beef cattle controlling for different farming enterprises (species combination and herd size) and for the farm being located in the North or South Island.

[17] Estimates given to the author by product managers of the companies Virbac, Merck and Pfizer New Zealand, June 2013
[18] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-2010

3. Methods

3.1. Study Population and Design

A cross-sectional study of sheep, beef cattle and deer farms was conducted in eight regions in NZ: Waikato, Wairarapa, Hawkes Bay, Manawatu-Wanganui, Taranaki, Marlborough, Canterbury and Southland (Figure 1). Farms were selected from a sampling frame comprising 7,998 client farmers of 28 veterinary practices, which had participated in a country-wide survey from December 2008 to March 2009. The survey gathered farm-level information such as region, herd size, livestock species farmed ('farm-class'), vaccination status and on reproduction performance, by questionnaire. Further questions targeted paratuberculosis (Ptb), which was investigated in this study along with leptospirosis (Verdugo et al., 2010). A total of 1,940 (25%) correctly filled questionnaires were returned, representing the sampling frame for the next step. In the second step, 238 farms were selected for blood sampling in a stratified-random fashion to obtain an equal representation of farm-class strata, representing deer, sheep and/or beef cattle farms. For a farm to be classified as a 'commercial' deer, sheep and/or beef farm, a minimum of 40 deer, 400 sheep, and/or 40 beef cattle, respectively, had to be present on farm. This information was available from the questionnaires of the initial farmer's survey. The farms were then allocated to single or multispecies categories in the following seven strata, four for each species: sheep, beef cattle, deer, sheep and beef cattle, beef cattle and deer, sheep and deer, and sheep and beef cattle and deer. The frequency of farms with different species in the sampling frame was compared with a national livestock statistics database ('AgriBase™') for evidence on the representativeness of the survey farms (Verdugo et al., 2010).

3.2. Sample Size and Power Calculation

The target farm sample size of *n*=300 was based on a comparison of prevalence between exposed and non-exposed farms where exposure was farm type. To show a prevalence ratio of 2.2 significant at p=0.05 with 80% power and 50% frequency of exposure, 292 farms were required.

Twenty adult female breeding animals from each species present on farm were randomly selected. This sample was sufficient to detect an animal prevalence within herds or flocks of 15% with 95% certainty, assuming a MAT sensitivity of 85% and specificity of 99% at the cut-off titre 1:48 and one or more sero-positive animals defining an exposed herd or flock. Under these conditions, the probability of correctly classifying non-exposed herds was 82%.

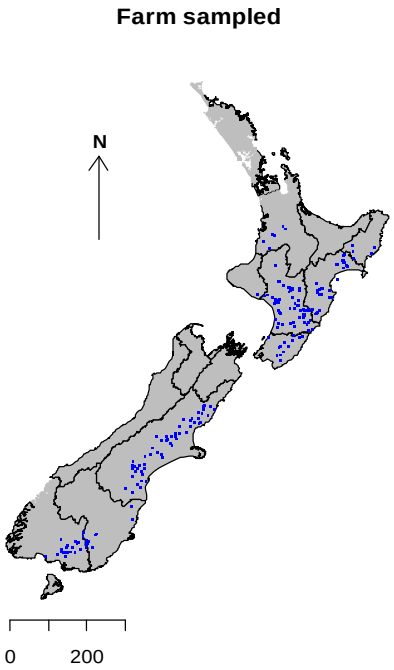

Figure 1: Sampled regions in New Zealand. Map with courtesy from C. Verdugo

3.3. Sample Collection and Data Management

Between June 2009 and July 2010, 10 ml blood samples were collected by caudal (beef) or jugular (deer and sheep) venopuncture by veterinarians, from 20 randomly selected sheep (mixed age ewes two-years and older), beef (mixed age cows) and deer (yearlings, 12-24 months, both sexes), when present on farm. Blood was couriered in an icepack-cooled container to the Molecular Epidemiology and Public Health Laboratory at the Hopkirk Institute, Massey University in Palmerston North. After centrifugation of the clotted blood at 3000 rpm for 6 minutes, the serum was aliquoted both into cryovials and microtitre plates and stored at -80º C and -20º C, respectively. Serum antibodies were measured against Hardjobovis and Pomona using the microscopic agglutination test (MAT) at doubling dilutions from 1:24 to 1:3072 (Faine et al., 1999). An animal was considered sero-positive when it had a MAT titre of ≥1:48 against either serovar. A herd/flock was defined as positive if at least one of the 20 sampled animals of the species was positive.

Farmers were interviewed on site by the sampling veterinarians. The questionnaire gathered the same information as the survey described above for 2008-9, and covered the intervening year prior to blood sampling.

Reproduction percentages were defined as:

Tailing percentage = Number lambs at tailing in 2009 / number pregnant ewes wintered in 2008
Pregnancy percentage = Number cows/hinds diagnosed pregnant in 2009 / number mated in 2009
Calving percentage = Number of cows calved 2009 / number cows pregnant 2008
Culling percentage = Number of animals sold or culled for any reason between 2008 and 09/ number of animals in flock/herd at time of sampling
Weaning percentage = Number weaner deer in 2009 / number hinds at calving 2008

In the questionnaire from 2009 approximately 25-50% of reproduction percentages were missing. Hence when data on reproduction percentages in 2009 were unavailable, data of stated reproduction percentages from the 2008 postal survey were used. Hence the reproduction percentages are composed of information from 2008 and 2009. The reason for missing data may be due to the fact that veterinarians often interviewed farmers in the field where sampling occurred and farmers did not have the necessary documentation on place to answer the question.

3.4. Statistical Analysis

All production percentages were described using means (normally distributed), confidence intervals, medians (skewed distribution) and percentiles. Bar charts were used to display titre distributions, and crude comparisons between sero-prevalence and reproduction outcomes were explored using scatter plots.

We used linear regression to test the hypothesis that the within-herd sero-prevalence for Hardjobovis or Pomona (exposures) was associated with the following herd-level production outcomes: tailing, pregnancy, culling, weaning and calving percentages (Table 1) (basic model). The following risk factors were added to the basic model with a manual forward stepwise approach and retained if their inclusion increased the adjusted R-squared and hence contributed to a better fit of the model: herd size (continuous), location of the farm (categorical: North or South Island), year when production parameter was reported (2008 or 2009), vaccination status (for beef; categorical) and species present on farm. The variable 'species present on farm' had four categories and varied by species (sheep: category 1: sheep; category 2: sheep and beef; category 3: sheep and deer; category 4: all three species; deer: category 1: deer; category 2: deer and beef; category 3: sheep and deer; category 4: all three species; beef: category 1: beef; category 2: sheep and beef; category 3: beef and deer; category 4: all three species). The pregnancy percentage was analyzed in beef cattle for three age groups, namely 15 months, 27 months old and >27 months ('mixed age') and in

deer for two age groups, 18 months old and >24 months ('mixed age'). Hence, for the pregnancy percentage, age group was also included as a covariate in the regression model. Interaction between sero-prevalence and age was tested. To control for correlation due to repeated measures in herds, herd was treated as a random effect in a mixed model when testing pregnancy percentage as an outcome. To see whether the assumptions for linear regression were met and to detect influential outliers, the outcome variables and residuals were checked for normality and residuals for homoscedasticity.

In order to see whether higher antibody titres had a stronger effect on reproduction percentages, we conducted a sensitivity analysis by running the linear model for different titre cut-offs (1:48, 1:192, 1:384 and 1:768). Vaccinated herds were not excluded from the analyses, but the effect of vaccination on the outcome was controlled for in the regression model for beef. For deer herds the association between vaccination and sero-prevalence was not statistically significant and for sheep the association was not tested, since only one farm had vaccinated animals.

4. Results

Blood sampling occurred on 238 farms from 377 herds/flocks and 7,661 animals. Sampling comprised 116 beef cattle herds (*n*=2,308 cattle), 162 sheep flocks (*n*=3,361 sheep) and 99 were deer herds (*n*=1,992 deer). Ninety seven percent of sheep and beef and 76% of deer farms had at least one in 20 animals sero-positive against Hardjobovis and/or Pomona. Overall, 50% of adult sheep, 58% of adult beef and 34% of adult/yearling deer were positive against either serovar (Figure 2). Hardjobovis was more prevalent in all three livestock species than Pomona. The within- and between herd sero-prevalences and titre distributions by serovar are described in chapter 2.

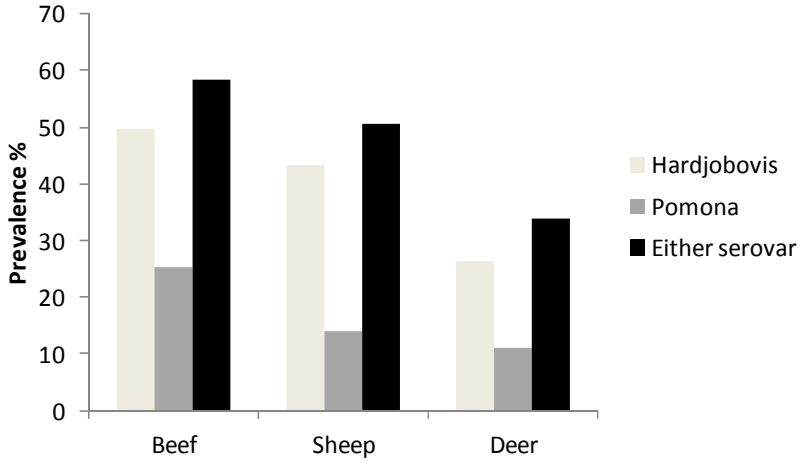

Figure 2: Distribution of the within-herd/flock sero-prevalence for *Leptospira interrogans* sv Pomona and *L. borgpetersenii* sv Hardjobovis by serovar and livestock species

4.1. Production Effects

Data are presented in Table 1. In sheep flocks, the mean tailing percentage was 130% (95% CI 78-178%) and the median culling percentage 15% (95% CI 6-20%). For beef cattle, the median age-specific pregnancy percentages were 90% for 15-month-old (95% CI 83-95%), 93% for 27 month-old, (95% CI 85-100%) and 91% for mixed age cows, 95% CI 86-95%), the median calving percentage was 92% (95% CI 87-96%) and the median culling percentage was 12% (95% CI 7-18%). In deer, the median pregnancy percentage was 89% in rising-2-year-old (95% CI 79-96%) and 95% in mixed age hinds (95% CI 92-98%), the median culling percentage (combined ages) was 10% (95% CI 5-18%) and the mean weaning percentage (combined ages) 87% (95% CI 46-98%).

Table 1: Reported reproduction percentages by species and age group (for pregnancy percentage), as a mean[1] when normally distributed, otherwise as a median[2] of all sampled herds or flocks

Reproduction parameter	Definition	Species	Number of herds/flocks	Mean[1] (%), (95% CI)/ Median[2] (%), (25th & 75th Percentile)
Tailing %	No. lambs at tailing in 2009[3]/no. pregnant ewes wintered in 2008	Sheep	159	130 (78,178)[1]
Pregnancy %	No. cows/hinds scanned pregnant in 2009[3]/no. mated in 2009	Beef[4]		
		≤15 months	49	90 (83, 95)[2]
		≤27 months	73	93 (85, 100)[2]
		>27 months	104	91 (86, 95)[2]
		Deer[4]		
		≤24 months	67	89 (79, 96)[2]
		>24 months	80	95 (92,98)[2]
Calving %	No. calved 2009[3]/no. pregnant 2008	Beef	109	92 (87, 96)[2]
Culling %	No. sold or culled/no. of animals in flock/herd at time of sampling	Sheep	153	15 (6, 20)[2]
		Beef	103	12 (7, 18)[2]
		Deer	81	10 (5, 18)[2]
Weaning %	No. weaners in 2009[3]/no. hinds at calving 2008	Deer	86	87 (46, 98)[1]

[3]Depending on the reproduction parameter between 25 and 50% of percentages were based on data from 2008; [4]the observations of pregnancy percentages exceed the total number of herds, as one herd may include animals from different age groups.

Once controlled for species combination on farm, island, reporting year of the tailing percentage, sero-prevalence of Pomona and herd size, the tailing percentage in sheep flocks was positively correlated with sero-prevalence of Hardjobovis, thus for 1% change in sero-prevalence of Hardjobovis, the tailing percentage increased by 0.11% (p-value 0.001) (Table 2). Choosing antibody titre cut-offs of 1:192, 1:384 and 1:768 for a sheep to be regarded sero-positive did not have a great influence on the association between sero-positivity and tailing percentage, which changed by 0.11% (p-value 0.004), 0.9% (p-value 0.2) and 0.11% (p-value 0.3) at each titre cut-off, respectively. Sero-prevalence of Pomona (exposure) and reproduction percentages (outcomes) did not reveal statistically significant associations in linear regression. Assumptions of linear regression were tested and not violated.

The predicted tailing percentage of sheep flocks increased with within-flock sero-prevalence of Hardjobovis (Figure 3). Flocks with ≤25% animals sero-positive had a mean predicted tailing percentage of 127% (median 127%), flocks with between 25-75% had a mean predicted tailing percentage of 128% (median 128%), and flocks with more than 75% had a tailing percentage of 135% (median 137%).

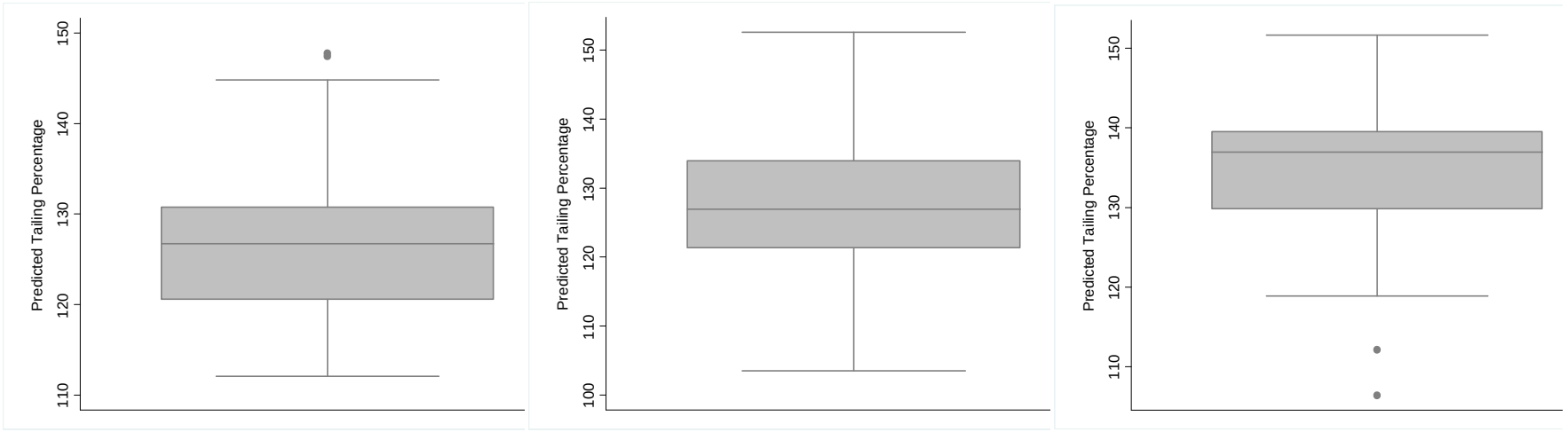

Figure 3: Box plots of predicted tailing percentages (y-axis) of sheep flocks by low (left), medium (middle) and high (right) within-flock sero-prevalence of *Leptospira borgpetersenii* serovar Hardjobovis, where low is ≤25%, medium is 25-75% and high is >75% of sheep being positive

Vaccinated beef herds were 2.6 times (95% CI 2.1-3.3, p<0.001) as likely to be sero-positive as were non-vaccinated beef herds, hence vaccination status was included as a confounding factor in the regression model analyzing reproduction parameters of beef cattle. None of the reproduction percentages in beef cattle and deer were significantly associated with the sero-prevalence of Hardjobovis.

Table 2: Results of a linear regression model of the effect of within-flock sero-prevalence of *Leptospira borgpetersenii* sv Hardjobovis (Har) and other risk factors on tailing percentage in sheep flocks (*n*=148)

Effect on tailing percentage	Level	Coefficient	Standard Error	95% Confidence Interval	P-value
Intercept		141.4	4.9	131.7 - 151.0	<0.001
Prevalence Har	%	0.11	0.04	0.0 - 0.2	0.011
Prevalence Pomona	%	0.06	0.08	-0.1 - 0.2	0.453
Species on farm	Sheep	ref	-	-	-
	Sheep & beef	-1.23	4.15	-9.4 - 6.9	0.768
	Sheep & deer	-0.08	5.13	-10.1 – 10.0	0.987
	Sheep, beef & deer	4.93	4.40	-3.7 - 13.5	0.264
Farm location	North Island	-9.6	2.8	-15.0 - 4.2	0.001
	South Island	ref	-	-	-
Flock size	per 100 sheep	-0.11	0.04	-0.0 - 0.0	0.002
Year of tailing percentage	2008	-11.96	2.76	-17.4 - 6.5	<0.001
	2009	ref	-	-	-

5. Discussion

While the study showed a positive association between the sero-prevalence for Hardjobovis and tailing rate in sheep, no relationships were observed between leptospiral seroprevalence and reproductive outcomes in beef cattle and deer, or on culling rate in any species.

In NZ livestock farming, tailing and weaning percentage are an indicator for fertility in sheep and deer since farmers mostly count their lambs at tailing and deer calves at weaning. Counts at calving/lambing are not available as dams and offspring are usually set stocked[19] for two-three months where farmers cannot make head counts. Thus, weaning rates are affected by fetal loss, stillbirth and pre-weaning mortality.

In beef herds we used calving percentage, which is mainly affected by fetal loss, hence likely to be more specific for estimating the impact of leptospirosis. A disadvantage of calving percentage

[19] Set stocking is the opposite to pasture rotation, i.e. keeping animals on the same pasture block for several months at a relative low stocking density.

may be the difficulty in comparing the results with NZ data in the literature, where often the ratio of 'number weaned divided by number mated' was used, because NZ farmers mainly count the number of calves at weaning or marking. Both parameters are potentially inaccurate if deaths and adult animals culled between mating and weaning are not taken into account. Regardless, trends should be still comparable, even though the absolute measures have different denominators.

The information about reproductive performance of deer, sheep and beef cattle at farm level was based on a questionnaire and farmers probably provided crude estimates subject to non-differential recall bias. However, when compared to NZ figures, percentages of tailing in sheep, weaning in deer and pregnancies in deer were quite representative. In our study, the mean tailing percentage in sheep flocks was 130%, which is similar to an overall NZ average of 127% in 2009 as shown in the NZ Agricultural Statistics[20]. The median pregnancy percentages in 15-month and 27-month-old beef cattle were 90% and 93% in our study, similar to 90% and 91%, respectively, reported by Mc Fadden (McFadden et al., 2005). However, others have reported lower pregnancy percentages of 72-86% for 15-month-old heifers (Hickson, unpublished data from 2006)

In a longitudinal observational NZ study of 15 red deer farms, where about 2,700 hinds were individually monitored for reproductive success, pregnancy rates at scanning of adult hinds (≥24 months) within mating mobs were generally over 90% and weaning percentages of yearling and adult hinds were 84.1 and 91.6%, respectively (Audige et al., 1999). The reproduction percentages of deer in our study are comparable, with a mean pregnancy rate of 89% (24 months) and 95% (≥24 months) and a mean weaning percentage of 87%, both ages combined.

The hypothesis that reproduction percentage would be negatively associated with *Leptospira* sero-prevalence was not confirmed by the data of this study, in any species. By contrast, the tailing percentage of sheep was positively associated with sero-prevalence to Hardjobovis. No other reproduction percentages were statistically significantly associated with *Leptospira* sero-prevalence.

Reproduction parameters were a mix of data from 2008 and 2009 (see methods), contributing to inaccuracy due to bias by year, since the measured sero-prevalence did not necessarily relate to those reproduction parameters of the previous year. For this reason, we included a variable in the linear regression model, which indicated from which year (2008 or 2009) the farmer reported production parameter was used. This should have largely controlled for the bias. However, the measured sero-prevalence may not relate directly to the given annual production cycle, but titres could be a legacy from previous year/s, as antibody titres are recorded to last months to years (Faine et al., 1999). In most herds most likely animals were present with antibodies from exposure

[20] http://www.stats.govt.nz/browse_for_stats/industry_sectors/agriculture-horticulture-forestry/AgriculturalProduction_final_HOTPJun11final.aspx

to *Leptospira* occurring in years prior to sampling. Hence, results may still be confounded due to the cross-sectional study design, which does not take time sequence into account.

The inverse association between tailing percentage and sero-prevalence to Hardjobovis is not likely to be causal. Both could have the same antecedent cause. Tailing percentages could be higher in more intensive systems with strip grazing where the leptospirosis infection pressure may be higher, for example, due to more level ground for surface water. Conversely, production outputs may be poorer in hilly areas where there is less surface water and lower stocking density, and therefore the infection pressure is likely to be lower. Therefore, we suggest including topography, stocking density and rainfall in a statistical analysis of future studies.

We did not test the blood samples for other in NZ present infectious diseases causing infertility, such as Bovine Virus Diarrhea virus (BVDv) or *Neospora* (Thobokwe G, 2004; Howe et al., 2007). This could have introduced confounding bias if the occurrence of the infectious agents were associated with *Leptospira* prevalence in a host population. A study investigating BVDv using ELISA, and *Leptospira* spp. using MAT as causes of abortion on NZ dairy farms with a history of abortion associated with *Neospora* caninum found multiple infectious etiologies at herd-level and in individual cows (Weston et al., 2012a). Weston et al. (2012) did not find associations between *Leptospira* spp., BVDv or *Neospora* caninum (Weston et al., 2012b). However, a study investigating interactions between BVDv and *Neospora* caninum in a South Waikato cow herd found that the proportion aborting was considerably higher among cows that were sero-positive to both BVDv and *Neospora* than to cows that were sero-negative to either or both of these infections. The authors concluded that these results provided evidence of an interaction between these agents in causing abortion in the investigated herd (Williamson et al., 2005). The same authors conducted a literature review on studies researching an association between abortive agents and found that some did and others did not find an association. Hence research results are contradictory. If there was a positive relationship between different abortive agents, a stronger negative effect on reproduction parameters would be expected. However, since this study found no or a positive effect of sero-prevalence on reproduction, no impact on study results is expected.

It was observed that clinical symptoms due to *Leptospira* exposure in the human population were reduced in an endemic environment (Bharti et al., 2003). Reasons may be explained by the concept of 'endemic stability', which "is a widely used term in the epidemiology of ticks and tick-borne diseases. It is generally accepted to refer to a state of a host tick pathogen interaction in which there is a high level of challenge of calves by infected ticks, absence of clinical disease in calves despite infection, and a high level of immunity in adult cattle with consequent low incidence of clinical disease" (Jonsson et al., 2012). The concept of endemic stability has been transferred

from the veterinary field into public health (Coleman et al., 2001). The authors "postulate that endemic stability requires only that (1) the probability, or severity, of clinical disease after infection increases with age, and (2) after one infection, the probability that subsequent infections result in disease is reduced". The epidemiology of leptospirosis does not show requirement 1, but most likely requirement 2. Nevertheless, maybe the concept may be as well applicable to leptospirosis. Therefore, it is possible that because of the endemic state of Hardjobovis and Pomona on many of the study farms, even subclinical symptoms with an impact on fertility were rare. The endemic state in sero-positive herds is maintained by the fact that without disease control a certain percentage of animals continue to be shedding *Leptospira*, for months or years (Miller et al., 1991) (re-)infecting naïve animals, which again become shedders. Therefore, animals in a sero-positive herd are probably constantly exposed and re-infected. The high within-herd/flock sero-prevalence of Hardjobovis and/or Pomona of 50% in adult sheep, 58% in adult beef and 34% in adult/yearling deer described in this and chapter 2 can only be maintained when there are frequent re-infections, because titre duration is limited (Faine et al., 1999). Whether sero-conversion is more likely to lead to (sub-)clinical symptoms with an effect on fertility than infection in the presence of an antibody titre (anamnestic response), is unknown. If this was the case, it could be another reason for not having been able to measure a productive effect in this study, given the high within-herd prevalence. However, a cohort study in meat workers (Chapter 5) revealed that sero-converting against Pomona and/or Hardjobovis compared to experiencing an anamnestic response did not lead to more persons experiencing clinical symptoms Therefore, it is possible that an anamnestic response may also have a subtle production effect, but perhaps too subtle to be measured with farm-average production data.

The power calculation was based on a sample size of 392 farms. Nevertheless, we sampled 238 farms due to non-compliance or lack of sampling facilities on-farm. Since most herds were sero-positive, we could not investigate the difference between infected and non-infected herds *per se*. Thus our study probably had limited power as production differences between flocks/herds might have been too subtle to be significant at the available study size. Further, finding subclinical effects in adult animals as sampled in this study is probably less likely, than in yearlings or younger animals, as infectious disease will affect adult less than younger animals because of the herd immunity effect.

The cross-sectional study design introduced an uncertainty about the time sequence: infection indicated by sero-prevalence might not have occurred in the year before the two seasons upon which the production data were based. A longitudinal animal based study design is therefore recommended, using either markers for infection over the production cycle to stratify outcome

from infected and non-infected animals within a herd as adopted by Ayanegui-Alcerreca (2006) or vaccination as used by Subharat et al. (2011) in farmed deer (Ayanegui-Alcérreca, 2006; Subharat et al., 2011c). Currently, there are no data available about weaning or growth effects of leptospirosis in beef cattle or sheep in NZ. In beef cattle a case-control study conducted in NZ in 2010 (Sanhueza, 2012) found an association between Hardjobovis and Pomona sero-prevalence and increased risk of foetal loss, estimating that 5% and 4% of foetal losses were attributable to Hardjobovis and Pomona, respectively. A case-control study on the association between the prevalence of contagious reproductive pathogens and beef cow fertility in NZ published by Beef&Lamb[21] NZ did not find an association between pregnancy percentages of beef herds and the sero-prevalence of Hardjobovis or Pomona (Heuer et al., 2007).

Overseas, the role of Hardjobovis and Pomona as causative agents of infertility in cattle and sheep has been contradictory in the literature. A study in Ontario, Canada (Prescott et al., 1988) estimated that 6.1% (34/553) of aborted foetuses in cattle examined between 1985-1987 using immunofluorescence, were associated with serovar Hardjobovis or Hardjoprajitno. In Ireland 50% of investigated abortions in cattle were associated with the presence of serovar Hardjobovis or Hardjoprajitno (Ellis et al., 1985). However, the authors did not discuss the likelihood of abortions being caused by other infectious agents, which had not been tested for. Maybe *Leptospira* was present but not the cause? The sero-prevalence of Hardjobovis or Hardjoprajitno in the Canadian study was between 8% (dairy herds) and 44% (beef cattle herds). In contrast, (Chappel et al., 1989) did not find an association between Hardjobovis and abortions in dairy cattle in Victoria, Australia. Those authors cultured kidneys and other organ material from 195 aborted foetuses and used an immunofluorescent staining technique on tissues of 49 foetuses to detect leptospires. Thirty-two percent of the aborting cows had MAT titres ≥64 against Hardjobovis and/or Pomona; the percentage of MAT positive non-aborting cows was not mentioned. Therefore, inferences to be drawn from the study are limited.

In Northern Ireland approximately 17% of 872 aborted lambs examined between 1981-1987 were positive for leptospires on fluorescent antibody examination, with the infecting serovar mainly being Hardjobovis or Hardjoprajnito and a smaller number associated with Pomona and other serovars determined by culture (Ellis, 1994). The author did not mention a comparison of sero-prevalence of antibodies against leptospires between aborting and non-aborting animals, making inferences difficult.

[21]http://beeflambnz.com/Documents/Farm/Management%20of%20beef%20cattle%20for%20high%20fertility%20-%20Part%202.pdf. Accessed October 13, 2012.

The overall pregnancy rate of 673 cows in five dairy herds in the United Kingdom was 28.5% higher in sero-negative cows than of cows with MAT titres ≥1:100 against Hardjobovis (P<0.001) (Dhaliwal et al., 1996b). Another cross-sectional study conducted in 16 herds and 384 cows in the Brazilian State of Pihau (Guitian et al., 1999) assessed the association between the sero-prevalence of various *Leptospira* serovars and reproductive failure by univariable analysis, combining abortion, retention of placenta, 'weak calf syndrome' and pregnancy failure in the first cycle in one outcome. The study found a statistically significant association between Hardjobovis sero-prevalence and reproductive failure (OR 9.3). Blood samples were tested by MAT, but a definition of the titre cut-off for a sero-positive cow was not found in the article. On one farm in Brazil pregnancy and calving percentages did not differ between sero-positive and -negative beef cows (Fava et al., 2004). None of the studies took potential confounders, such as herd size, topography, flooding or correlation within herd into account, thus neither considered possible common antecedent causes nor adjusted for an underestimation of the variance. Findings from these studies may therefore not be conclusive.

In a clinical trial, calving percentages (determined by the surrogate measure of lactation failure) increased significantly from 81 to 88% when a Hardjobovis vaccine in beef cattle was used (Holroyd and Smith, 1976). The same author also reported higher weaning percentages in vaccinated than non-vaccinated cows (Holroyd, 1980).

The above literature review does not lead to a clear conclusion regarding an association between *Leptospira* sero-prevalence and fertility in beef and sheep, despite the range of the study designs used. It is possible that the same serovar differed in terms of virulence and infectivity and species for different regions and countries and in order to find those differences molecular techniques would have had to be applied. It is also possible that different breeds react differently to infection and/or that different serovars have different effects on reproduction. None of the cross-sectional studies controlled for confounding factors, such as herd size, rainfall etc., which may have biased the results. Clinical symptoms with an impact on fertility due to *Leptospira* fluctuate between years, as they are likely to be influenced by climate and the endemicity in herds or regions. With high endemicity production loss may likely be lower.

Despite the limitations, our study should have revealed production effects if they existed at a large magnitude. Hence, production effects in NZ in the year of sampling were either small or non-existent. The detection of subtle differences requires a longitudinal study at the individual animal level, e.g. sampling at the beginning and end of the production season and measuring the within-herd association of production performance with sero-conversion of individual animals. An alternative is to use a vaccination approach as adopted by Subharat et al. (Subharat et al., 2011c) in which animals were cleared of infection with antimicrobials, and half vaccinated, and after

development of immunity, exposed to natural infection by introduction to known infected herds. The study ideally would include information on topography, climate, rainfall patterns, breed, prevalence of other infectious agents, nutrition, pre- and postpartum management, housing etc. Further, the study should not only measure the difference in production effects between sero-positive and sero-negative animals, but also between those sero-converting and those experiencing an anamnestic response. Moreover, one should also try to investigate differences in production effects between animals changing/increasing their sero-status and coming from negative, low, medium or high sero-prevalent herds.

6. Conclusion

Negative associations between pregnancy, culling, weaning and calving percentages and within-herd/flock sero-prevalence of Hardjobovis or Pomona were not identified in this study. Higher tailing percentages in sheep flocks with relatively high sero-prevalence for Hardjobovis are unlikely to be causative since this association lacks biological plausibility. The cross-sectional study design investigating effects at the farm level may not have been adequate to detect subtle subclinical effects. Various longitudinal study designs at the animal level are suggested as a more appropriate means for evaluating production effects of leptospiral infection.

7. Acknowledgements

A special thanks to all farmers who took their time to gather their animals and to take part in the interview and to the 28 veterinary practices involved for their time and collaboration.

We gratefully acknowledge the Johne's Disease Research Consortium (JDRC) for permitting survey data and samples collected for Johne's Disease research to be used for this project , the Alma Baker Trust Fund, the Swiss National Science Foundation (SNF) and Rural Woman NZ (RWNZ) for other research costs and PhD candidate support.

We thank NZ Veterinary Pathology (NZVP) for their support in sample processing and storage. A very special thanks to Neville Haack for all the lab work and also to A. Riddler, S. Prickaerts, P. Guzman, D. Tapia, R. Pearson, T. Delaune, M. Turlin, L. Stringer, J. Manuel Sanhueza, P. Moono, J. Job, R. Gregory, A. Kurian, G. Anakkaramadathil, S. Jacob, P. Jaros and S. Moore for technical assistance with sample management and analyses.

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Chapter 4

Sero-prevalence and Risk Factors for Leptospirosis in Abattoir Workers in New Zealand

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1. Abstract

Leptospirosis is an endemic bacterial disease of domestic and wild animals in New Zealand and the most important occupationally-acquired zoonotic disease of abattoir workers and farmers. Serovars are largely identical in terms of serovars seen in livestock and humans, yet most farmed livestock are unvaccinated and people remain occupationally exposed. The objectives of this study were to determine the sero-prevalence (prevalence) of leptospiral antibodies in abattoir workers and to identify risk factors for exposure to *Leptospira*.

We conducted a cross-sectional study in eight abattoirs, four slaughtering sheep, two cattle and two deer. Sera were collected from 567 abattoir workers volunteering in 2009-10 and tested by the Microscopic Agglutination Test (MAT) with a titre cut point of 1:48 for *Leptospira interrogans* sv Pomona (Pomona) and *Leptospira borgpetersenii* sv Hardjobovis (Hardjobovis). Information on risk factors including personal data, workplace, lifestyle, clinical history and potential confounding variables were recorded by questionnaire. Association between prevalence and risk factors, most importantly work position, were determined by species specific multivariable analysis, taking potential confounders and effect modifiers into account.

Sixty two (10.9%, 95% confidence interval CI 8.2-17.2%) workers had *Leptospira* antibody titres ≥1:48, against Hardjobovis or/and Pomona, 29 against Pomona (5%, 95% CI 3-7) and 45 against Hardjobovis (8%, 95% CI 6-10). Workers from the four sheep abattoirs had an average prevalence of leptospiral titres (Hardjobovis or Pomona) of 10, 11, 12 and 31%, from the two deer abattoirs 17 and 19% and the two beef abattoirs 5 and 5%. Prevalence was significantly higher in workers in sheep and deer than in cattle plants. Antibodies were more frequently found against serovar Hardjobovis (8.1%) than Pomona (5.1%), similar to the serovar distribution reported from livestock. About 11% of workers reported to have experienced probable leptospirosis during a median period of five years prior to the study. Prevalence was not associated with worker's recall of influenza-like-illness during the preceding 36 months. The strongest risk factor for workers in sheep and deer abattoirs being sero-positive was work position. For participants from sheep abattoirs, prevalence

was highest at the beginning of the slaughter board (stunning and hide removal) (30%; OR 10.4, 95% CI 2.8-38.8, p <0.001), followed by activities involving the removal of high risk material (guts, bladder, and kidneys) (17%; OR 8.2, 95% CI 2.1-32.7, p=0.003), working in the offal/pet food area (11%; OR 6.5, 95% CI 1.4-29.8, p=0.017), and working in the boning room or office (2%; reference group). Wearing personal protective equipment, such as gloves, facemasks, safety/normal glasses or a balaclava was not protective against infection. Non work related risk factors, such as home slaughtering, farming or hunting were not significantly associated with sero-prevalence in this study.

2. Introduction

Leptospirosis is a zoonotic bacterial disease of most mammal species. After a leptospiraemic phase, leptospires colonise and mainly persist in the kidney of carrier animals and are excreted in the urine for several months, sometimes years in carrier animals. Infection with *Leptospira* spp. occurs via skin abrasions or mucous membranes with transmission occurring through exposure to the urine of carrier animals, either directly or via contact with contaminated water or soil. Human-to-human transmission is considered to be very rare (Bharti et al., 2003).

Leptospirosis is widespread in livestock in New Zealand (NZ). While in many, mainly subtropical countries numerous animal hosts and *Leptospira* serovars survive in a complex ecological environment, the epidemiology of leptospirosis in NZ is based on a relatively small number of six serovars known to be endemic (from several hundred known worldwide). The two most frequent serovars in cattle, deer and sheep in NZ are *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona) (Marshall and Manktelow, 2002; Ayanegui-Alcerreca et al., 2007). Sixty percent of NZ deer herds, 92% of beef cattle herds, and 91% of sheep flocks are sero-positive against Hardjobovis and/or Pomona (Dreyfus et al., 2011). In NZ, livestock appear to be an important source of human leptospirosis, with farmers and meat workers being at a high risk (Thornley et al., 2002). Studies revealed that 62% of farmed deer (Ayanegui-Alcerreca et al., 2010a) and 48.5% of 1966 sheep sampled in abattoirs were sero-positive against Hardjobovis and/or Pomona (Dorjee et al., 2008). The researchers estimated a daily exposure for each abattoir worker to 5-9 deer and 5-26 lamb carcasses that were kidney culture positive for *Leptospira*, hence presented potentially infectious exposure (Dorjee et al., 2011).

NZ is ranked with a high incidence of notified human cases among temperate developed countries (Thornley et al., 2002) and medium for the Asia Pacific region (Victoriano et al., 2009). Leptospirosis can result in severe human illness but is rarely fatal in NZ. During 2003-2005, 207 cases were hospitalised in NZ (Vickery et al., 2006). Notified human leptospirosis cases mainly represent severe clinical cases and milder forms remain under-reported (Thornley et al., 2002) and are, in order of frequency, caused by *Leptospira borgpetersenii* serovar Ballum, Hardjobovis, and Pomona. From 2006 to 2010, 427 cases of leptospirosis were notified (86.4% laboratory confirmed), with a rate of 2 cases per 100 000 population. Of those with occupation recorded annually, 52% (range 36-71%) were farmers or farm workers and 30% (range 18-48%) abattoir workers or butchers[22].

Studies in multispecies abattoirs processing sheep, beef and/or pigs were carried out in the

[22] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-2010

1980s revealing a sero-prevalence (prevalence) against Pomona, Hardjobovis, and/or *Leptospira borgpetersenii* serovar Tarassovi of 4.1% in meat workers and of 9.5% in meat inspectors (Blackmore et al., 1979; Blackmore and Schollum, 1982a). More recently, a leptospirosis prevalence of 9.5% workers from one abattoir slaughtering sheep was reported with workers on the slaughter board having a 23-85-fold higher risk of being sero-positive than workers in the boning, cutting, chilling or rendering areas (Benschop et al., 2009; Heuer et al., 2010). Those findings prompted the researchers and the meat industry to assess the risk of *Leptospira* exposure in other sheep and as well beef and deer abattoirs.

The objectives of this study were to determine the prevalence of *Leptospira* in abattoir workers processing sheep, beef cattle or deer, to identify risk factors for sero-positivity related to occupational and non-occupational activities and to identify risk factors for probable leptospirosis and/or 'flu-like-illness'.

3. Methods

3.1. Study Design, Data Collection and Management

All procedures were approved by the Massey University Human Ethics Committee in 2009 (Southern A, application 09/08). Eight purposively selected abattoirs: four processing sheep, two beef and two deer, agreed to participate in a cross-sectional prevalence study on leptospirosis in meat workers. Two abattoirs were located in the South Island and six in the North Island of NZ. Abattoir managers and supervisors, health and safety personnel, meat union representatives and workers were provided with information in meetings about the study objectives and procedures. Participation was voluntary and not based on random sampling.

Between November 2009 and March 2010, blood was collected from voluntarily participating meat workers by certified phlebotomists, and trained researchers conducted interviews. All workers at the selected plants were informed about the research purpose prior to the study and about results after completion. Study participants were informed of their test result by personal mail which, if positive, advised consultation with either an occupational physician or medical practitioner. Information on work and non-work related risk factors including work positions for the last season, past work positions (for three former seasons), years worked in an abattoir, number of months working in the last and three previous slaughter seasons, personal protective measures (e.g. safety glasses, gloves) worn in the current and previous work positions, lifestyle (hunting, farming, home slaughtering, outdoor activities in the last three years) and personal data such as age, gender,

type of residence and ethnicity were recorded by questionnaire. Further, workers were asked whether they had been diagnosed with leptospirosis during their lifetime, whether they had had 'flu-like' symptoms over the past three years, how many days they were absent from work and whether they had received compensation. Details about exposure variables (=risk factors) are listed in tables 3-8.

Ten ml of blood was collected with BD Vacutainer® Plus tubes, coated with silicone and micronised silica particles to accelerate clotting, stored between 4º and 10º C in a mobile fridge, and couriered within 24 hours in an icepack cooled Biocontainer© to the Molecular Epidemiology and Public Health Laboratory at Massey University in Palmerston North, NZ. After centrifugation of the clotted blood at 3000 rpm for 6 minutes, the serum aliquots were transferred to duplicate cryovials and microtitre plates and stored at -80º C.

3.2. Serological Testing

The microscopic agglutination test (MAT) was used to measure serum antibodies against Pomona and Hardjobovis at doubling dilutions from 1:24 to 1:1536 as described previously (Faine et al., 1999). The MAT was always performed by the same trained laboratory technician. The MAT is the standard reference test for *Leptospira* with a reported sensitivity of 91% to 100% and specificity of 94% to 100% (McBride et al., 2007).

3.3. Case Definitions

A *sero-positive case* was a participant with a titre of ≥1:48 against Pomona and/or Hardjobovis.

A *probable leptospirosis case* had been diagnosed with leptospirosis of any serovar, by a health professional, at any point in time before the study period, on the basis of clinical symptoms with or without confirmation by laboratory test.

A *case of 'flu-like-illness'* was an 'influenza-like' ('flu-like') illness in the three years before the blood sample, including one of the following symptoms: fever, headache, sweating, sore eyes, severe debility or sore muscles. Fever was not further defined, as participants were not able to remember the degree in Celsius. Workers were explained that the symptoms had to be severe enough that they felt like going home and rest.

3.4. Sample Size and Power Calculation

To detect an Odds ratio (OR) of 2.5 with 80% power, a type I error of 5%, a prevalence of 9% in the exposed group, and an exposed to non-exposed ratio of 1/3, the required sample size was 280 study

participants. To analyse the results for all abattoirs together, the sample size was doubled to take clustering within abattoir into account. Hence a total required study size was 560 workers (McDermott et al., 1994).

3.5. Data Entry and Validation

Questionnaire information and serological test results were entered into an Access© database and analyzed using Microsoft Excel©, Stata 10 (StataCorp. LP) or SAS (SAS Institute Inc., Cary, NC, USA). Correct data entry was validated by randomly choosing 5% of the questions for each abattoir and comparing them with manual questionnaire entries.

3.6. Data Analysis

Exploratory data analysis was conducted to find missing observations and outliers by using histograms, 2x2 tables and summary measures. To determine bivariate correlations among exposure factors, potential confounders and MAT results, we conducted chi-square tests for categorical and Pearson correlation for continuous variables.

The outcomes of interest were whether workers were sero-positive against Hardjobovis, Pomona or either serovar, and whether they had experienced 'probable leptospirosis' or 'flu-like-illness'. These outcome variables are shown as abattoir specific prevalence. The association between prevalence and risk factors was evaluated by chi-square analysis, separately for each slaughter species (sheep, cattle, and deer). The frequency, sero-status and time away from work of probable leptospirosis cases and whether they received compensation from the workers' occupational disease insurance (Accident Compensation Corporation, ACC) were described.

Associations were analysed in two steps, firstly, by crude comparison of risk factors with outcomes, and secondly, by multivariate logistic regression.

3.6.1. Categories of work position and Personal Protective Equipment

Questions on work position were detailed, as this was likely the main potential risk factor for infection. Workers reported 153 work tasks, many of them being synonyms or overlapping between work positions. Depending on the abattoir and species slaughtered, some staff performed a wide range of activities in the abattoir, whereas others were occupied with a single task. In order to understand the risk of infection in different positions, we assigned work tasks to different work position categories with a similar exposure to urine or to organs of the urinary tract. With the aim to

maximise statistical power, exposure groups with less than eight workers were merged with adjacent exposure groups.

Since the slaughter procedure is specific for each species, the work position categories were different for the three species. For sheep abattoirs, work positions were categorised into four (Table 3), for deer into two (Table 5) and for beef cattle into four categories (Table 7). Figure 1 illustrates the work position categories for sheep: the reference (category 0) workers (blue) were from the 'boning' room (where the carcass is cut into pieces), the 'chillers', 'freezers', 'blood processing' or from the office; category 1 (green) workers were from the 'offal'/'casing'/'pet food' rooms (where organs were handled) and hide processors, cleaners, renderers and engineers; category 2 (purple) included persons working in the middle and end of the slaughter board (where animals were opened, organs removed and carcasses were inspected); category 3 (red) were persons working in the yards (where animals were washed and waiting for slaughter) and at the beginning of the slaughter board (where animals were stunned, bled and hides were removed). The slaughter board contains workers from categories 2 and 3, but excludes workers from the 'yards'.

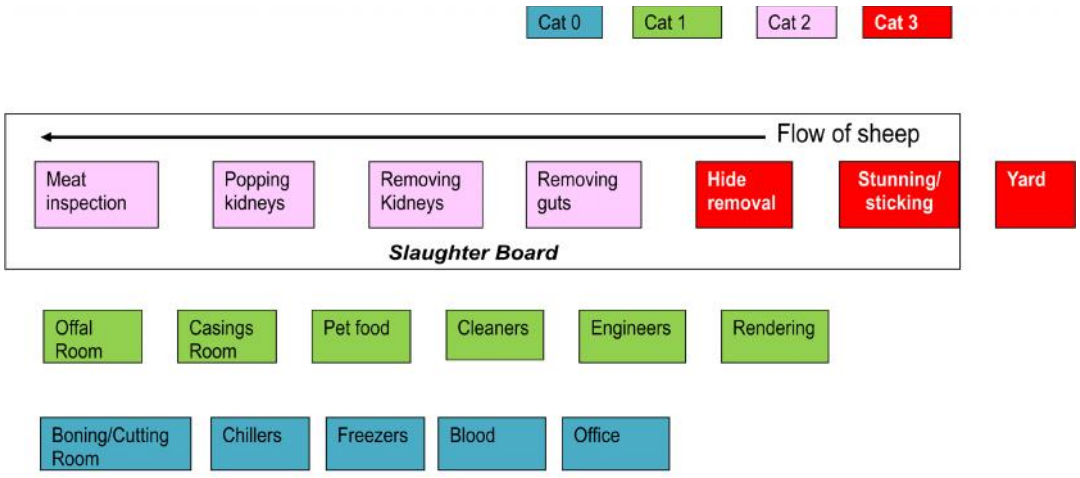

Figure 1: Schematic description of the various workplaces of workers in sheep abattoirs by category (colours) used in multivariable analysis

Work position categories in beef abattoirs also consisted of four categories, but differed from the composition of work tasks in sheep abattoirs. Category 3 included the yards, all workers on the slaughter board and meat inspectors, and category 2, workers in the offal/casings/pet food room. In category 1 were persons working in maintenance, cleaning or in the 'plasma room' where blood is processed. Category 0 was the same as in sheep abattoirs.

Since most workers from deer abattoirs performed multiple tasks at the slaughter board, they could only be attributed to two work position categories: category 1 included workers at the slaughter

board, in the yards and in the offal room and category 0 workers in boning or chilling rooms, and in the office, as in sheep and beef abattoirs.

In the interview, study participants were asked about the type and frequency of Personal Protective Equipment (PPE) worn for every task at the meat plant. Type was defined as 'facemasks', 'safety or normal glasses', 'gloves on one or two hands' and 'balaclava or beard mask'. Frequency categories were 'always' (1), 'often' (2), 'sometimes' (3) or 'never' (4). For increased power, exposure types were also analysed as two categories by merging categories 1 and 2, and 3 and 4.

3.6.2. Risk factors for sero-positivity

Associations between exposure variables and sero-status of workers to either serovar were tested by univariable logistic regression or Fishers exact test (if an exposure/sero-status subset included less than five workers) for sheep (Tables 3 & 4), deer (Tables 5 & 6) and beef (Tables 7 & 8) abattoir workers. Exposure variables with a Wald test/Chi square p-value ≤0.3 were included in the multivariable logistic regression with sero-status to either serovar as the dependent variable (Table 9).

Due to differences in slaughter procedures and worker positions, three multivariable logistic regression models were developed: one for each species. A forward selection method was chosen to evaluate exposure and confounding variables, starting with a null model with only an intercept included and then adding one variable at a time. A variable was allowed to enter if the Likelihood Ratio Test (LRT) was statistically significant at a p-value ≤0.20 and retained at p ≤0.05 or if their presence changed the crude OR of work position and/or fitted variables in the model by ±15% to account for bias.

Multiplicative interaction was first evaluated with Maentel Haenszel (MH) analysis and in the final model again by multivariable logistic regression. If the MH or the LRT were statistically significant (p ≤0.05) the interaction term was retained in the model. The following interaction terms were tested: 'gender*wearing gloves', 'work position*gender', 'work position*wearing gloves', 'work position*wearing safety/normal glasses', 'wearing gloves*abattoir' and 'wearing goggles or glasses*abattoir'.

3.6.3. Model diagnostics

To test the assumption of linearity for the continuous exposure variables, we plotted the Log odds of being sero-positive against quartiles of the continuous variables 'age', 'time worked at the current abattoir' and 'time worked in the meat industry'. If the assumption was violated the quartiles were maintained. The Hosmer-Lemeshow statistic was used to test the distributional

assumption and the Pseudo R-square to evaluate the overall model fit. Influential covariate patterns and leverage were examined using histograms of 'Pearson Residuals', 'Hat matrix', 'Cook's distance' and 'DFBeta' (Hosmer and Lemeshow, 2000).

4. Results

4.1. Data Entry Validation

Thirty-two randomly chosen questionnaires from 567 (5%) were examined for data entry errors. Each questionnaire contained at least 170 questions. We found 6 errors, equivalent to a data entry error rate of (6/170 x 32) = 0.001% which was deemed acceptable.

4.2. Participants, Slaughter Plants and Study Population

A total of 567 workers were interviewed and blood sampled. The number of participants by plant ranged from 21-112 (Table 2) and participation proportions by plant ranged from 11-61% (Table 1). The larger the workforce of an abattoir, the smaller was the participation rate. We estimated that about 30-50% of the work force was present at the recruitment meetings. On average, participants worked 9.9 months in the slaughter season preceding the interview (*n*=528), with 20 (3.8%) having worked 3 or fewer months. In 2006 there were 24,093 people employed in the meat industry (Meat Industry Association figures). The total work force at the eight study abattoirs (*n*=2,661) represented approximately 11% of all workers in the meat industry in NZ assuming employment remained about constant.

Table 1: Number of workers, proportion recruited for the study, the species and total number processed and the regional origin of animals slaughtered in participating slaughter plants

Abattoir	total number of workers	Study recruits (%)	Species	Number of animals processed per year	Regions of animal origin
Sheep 1	889	12	Lamb, mutton, bobby calves	1,797809	Hawke's Bay, Waikato, Wairarapa, Bay of Plenty, Northland
Sheep 2	378	26	Lamb, mutton, goats	600,469	Gisborne, Hawke's Bay, Waikato, Bay of Plenty
Sheep 3	300	11	Lamb	780,000	Central Hawke's Bay, East Coast, Wairarapa, Manawatu
Sheep 4	180	51	Lamb, mutton, bobby calves, goats	488,546	Wanganui, Manawatu, Taranaki, other
Deer 1	41	51	Venison	24,222	Canterbury
Deer 2	59	61	Farmed & feral[a] venison	41,055	South Waitaki River to Rakaia, North Canterbury
Beef 1	486	15	Beef cattle, dairy cows	93,837	East Coast, West Coast, Waikato, Bay of Plenty, Northland
Beef 2	328	34	Beef cattle	159,347	Taranaki, Waikato, Manawatu, Hawke's Bay

[a]Feral venison is integrated in the slaughter line after the stunning box. At this stage, the carcass has been opened and intestines and the urinary bladder have been removed, hence urine exposure is reduced.

4.3. Study Outcomes

4.3.1. Sero-prevalence and antibody titres

Sixty two study participants (10.9%, 95% CI 8.5-13.9%) were sero-positive against either Hardjobovis or Pomona, of whom 10 (5.4%, 95% CI 2.7-10.0%) were from beef (*n*=185 workers), 10 (17.5%, 95% CI 9.1-30.3%) from deer (*n*=57 workers) and 42 (12.9%, 95% CI 9.6-17.2%) from sheep abattoirs (*n*=325 workers). The prevalence against Pomona and/or Hardjobovis in workers from the four sheep abattoirs ranged from 10-31%, from the two deer abattoirs 17 and 19% and was 5% in the two beef abattoirs. Twenty three sheep abattoir workers (7.1%) had antibodies against Pomona, 28 (8.6%) against Hardjobovis and 9 (2.8%) against both serovars. Three deer abattoir workers (5.3%) had antibodies against Pomona, eight (14%) against Hardjobovis and one (1.8%) against both serovars. Three (1.6%) beef abattoir workers had antibodies against Pomona, nine (4.9%) against Hardjobovis and two (1.1%) against both serovars (Table 2).

Table 2: Sero-prevalence (%) and 95% confidence intervals (CI) of workers of eight abattoirs processing sheep, deer or beef with antibodies to *Leptospira interrogans* serovar Pomona (Pom), *Leptospira borgpetersenii* serovar Hardjobovis (Har) and to either serovar

		Prevalence (%)					
Abattoir	**N Workers**	**Pom**	**95% CI**	**Har**	**95% CI**	**Either**	**95% CI**
Sheep1	104	5	2-11	10	5-17	12	7-19
Sheep2	97	8	4-16	4	2-10	11	6-19
Sheep3	32	16	7-32	28	15-46	31	18-49
Sheep4	92	5	2-12	7	3-14	10	5-18
Deer1	21	5	1-27	19	7-41	19	7-41
Deer2	36	6	11-20	11	4-26	17	8-32
Beef1	73	3	1-10	4	1-12	5	2-14
Beef2	112	1	0-6	5	2-11	5	2-11
Total	567	5	3-7	8	6-10	11	8-14

Reciprocal antibody titres against Pomona ranged from 24-768 in sheep and deer abattoir workers and from 24-48 in beef abattoir workers. Against Hardjobovis, titres ranged from 24-768 in sheep and beef abattoir workers and from 24-1536 in deer abattoir workers (Figure 2).

In three sheep, one deer and two beef abattoirs the prevalence of Hardjobovis titres in meat workers was higher than Pomona ($p < 0.05$), and in one deer and one sheep plant there was no statistically significant difference between Hardjobovis and Pomona prevalence ($p > 0.05$).

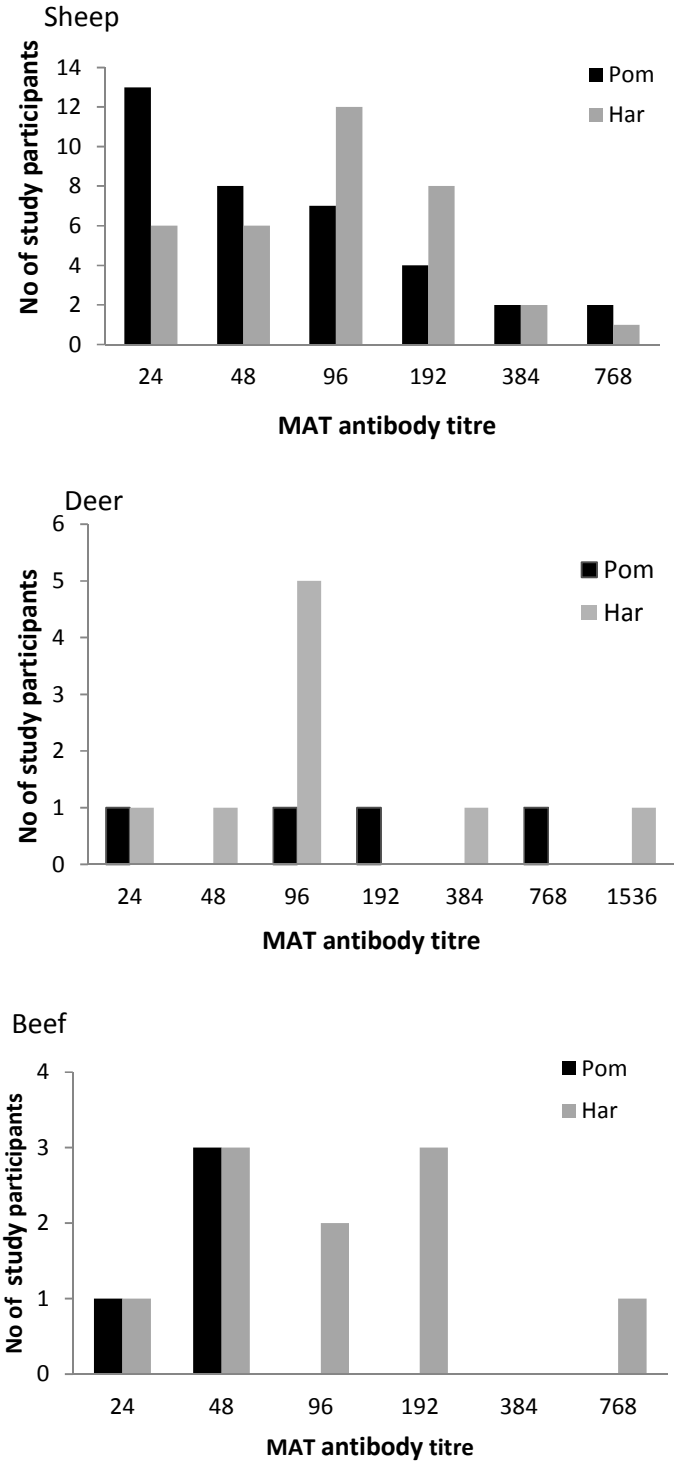

Figure 2: Frequency histogram showing the number of sero-positive study participants at each MAT titre to serovars *Leptospira interrogans* sv Pomona (Pom, black) and *Leptospira borgpetersenii* sv Hardjobovis (Har, grey) in sheep (top), deer (middle) and beef abattoirs (bottom)

4.3.2. Disease and risk factors for disease

Sixty workers had a history of probable leptospirosis while working in abattoirs between 1962 and 2010, of whom 27 were still sero-positive in this study. Probable leptospirosis occurred in sero-positives between 1980 and 2008 and in sero-negatives between 1984 and 2008. Two reported having had probable leptospirosis twice. Forty (12%) probable leptospirosis cases were in sheep abattoir, five (9%) in deer abattoir and 15 (8%) in beef abattoir workers. Twenty remembered the number of days being ill and away from work with the mean being 14.8 days (range 0-60 days). The question whether worker received occupational disease insurance compensation (Accident Compensation Corporation, ACC) was answered by 49 affected workers and 21 (43%) had received compensation. Sheep plant workers who reported being ill with probable leptospirosis were 10.3 (95% CI 4.85-21.88), deer plant workers 31 (95% CI 2.92-321.7) and beef plant workers 49 (95% CI 10.58-224.29) times as likely to be sero-positive, respectively, as were workers without experiencing leptospirosis ($p < 0.0001$).

The continuous variable 'years worked in the meat industry' (OR 1.003; 95% CI 0.99-1.01; $p < 0.01$) and 'work position' was positively associated with previous clinical disease in sheep abattoir workers (offal/pet food room: OR 5.3; 95% CI 1.4-19.7; removing kidneys/meat inspection: OR 10.9; 95% CI 3.4-34.9; stunning/removing pelts: OR 7.6; 95% CI 2.5-23.8; all $p < 0.01$). Gender, age and ethnicity were not significantly associated with disease. In beef plants 'age' (OR 1.9; 95% C 1-1.2; $p < 0.01$) and 'work position' (Offal/pet food room: dropped by model. Removing kidneys/meat inspection: OR 4.9; 95% CI 3.4-34.9; $p=0.07$. Stunning/removing pelts: OR 5.7; 95% CI 1.4-23.3; $p=0.01$) were positively associated with probable leptospirosis.

There was no statistically significant association between having had a *'flu-like-illness'* and sero-status for neither serovar and species.

4.3.3. The use of personal protective equipment

The frequency of wearing PPE differed by species and abattoir. Whereas in sheep abattoirs 67% overall (range 42-78% between abattoirs) of slaughter board, offal room and yard workers reported to (always or often) wear gloves on both hands, 17% did in deer (7-33%) and 80% (77-84%) in beef abattoirs.

Seventy-one percent (range 11-93% between abattoirs) of sheep, 43% (28-67%) of deer and 38% (28-51%) of beef slaughter board, offal room and yard workers reported to (always or often) wear safety/normal glasses and 12%, 9% and 4%, respectively, to wear facemasks. On the slaughter board itself the requirement for wearing gloves and glasses varied between abattoirs. While 95% of workers in the stunning area of one sheep plant (no. four) reported wearing glasses or facemasks,

7% did in another sheep plant (no. three). There was, however, no significant difference in wearing glasses or facemasks in areas where kidneys were removed and offal was handled.

4.3.4. Risk factors for sero-prevalence in sheep plants

The crude associations between exposure variables and sero-positivity against Pomona and/or Hardjobovis for sheep abattoir workers are presented in tables 2 and 3. Compared with the workers working in the office, boning room or chillers (reference group), meat workers working in the offal room (OR=6), removing kidneys (OR=10) or stunning/pelting (OR=17) had a higher odds of positivity. Being male (OR=6.4), working in sheep plant 3 (OR=3.5), having had probable leptospirosis (OR=10.3), always or often wearing a facemask (OR=2.8), always wearing safety glasses (OR=2.5) increased the odds of positivity in meat workers. Moreover, the longer they worked at the current plant, the more likely they were positive against *Leptospira*.

The variables remaining in the final logistic regression model were 'work position', 'gender', 'years worked in the meat plant' and 'meat plant' (Table 9). Compared with the workers working in the office, boning room or chillers (reference group), meat workers working in the offal room had 6.5 (95% CI 1.4-29.8, p=0.017) times the odds of being sero-positive against Pomona and/or Hardjobovis, and workers at the middle and end of the slaughter floor had 8.2 times the odds (95% CI 2.1-32.7, p=0.003) and those at the beginning of the slaughter floor 10.4 times the odds (95% CI 2.8-38.8, p <0.001). Men were 3.1 (95% CI 0.8-11.7, p=0.09) times as likely to be sero-positive as women once controlled for the effect of work position. For every year working in the meat plant the odds of being sero-positive increased 1.08-fold, suggesting an eight percent increase in risk for each year of working in the plant (p=0.01). Compared to working in sheep plant 1, working in sheep plants 2, 3 and 4 increased the odds of sero-positivity by 4.5 (p=0.02), 6.3 (p=0.004) and 2.1 (p=0.2) respectively. The variables 'gender' and 'wearing safety/normal glasses' were confounding variables as they reduced the adjusted OR for sero-positivity of work position category 3 (stunning/pelting) by more than 15%. However, 'gender' was marginally significantly associated with sero-status (p=0.09) whereas 'wearing safety/normal glasses' was not (p=0.7). None of the tested interactions were significant. Maybe females were more careful not to get splashed with urine when working in the stunning/pelting area.

Table 3: Frequencies of work-related exposure variables and their unconditional association with sero-prevalence of *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in sheep plant workers (n=325) blood sampled and interviewed from January - April 2010

Variable	Category	% Workers (n)	Sero-Prevalence %	Crude OR	95% CI	P-value
Work position	Boning, chillers, office	42.5 (138)	2.2	Ref		<0.001[2]
	Offal, pet food	13.5 (44)	11.4	5.8	1.3-25.2	0.02
	Gut & kidney removal, meat inspection	17.5 (57)	17.5	9.6	2.5-36.3	0.001
	Yards, stunning, pelting	26.5 (86)	27.9	17.4	5.0-60.0	<0.001
Wear gloves on both hands	never	29.2 (95)	13.7	Ref		0.99[2]
	sometimes	12 (39)	12.8	0.9	0.3-2.8	0.894
	often	2.8 (9)	11.1	0.8	0.1-6.8	0.829
	always	56 (182)	12.6	0.9	0.4-1.9	0.806
Wear safety glasses	never	37.9 (123)	7.3	Ref		0.11[2]
	sometimes	12.9 (42)	16.7	2.5	0.9-7.3	0.085
	often	6.2 (20)	15.0	2.2	0.5-9.1	0.261
	always	43.1 (140)	16.4	2.5	1.1-5.6	0.028
Wear a facemask	never or sometimes	93.2 (303)	11.9	Ref		
	often or always	6.8 (22)	27.3	2.8	1.0-7.6	0.04
Wear a balaclava	never or sometimes	69.5 (226)	11.1	Ref		
	often or always	30.5 (99)	17.2	1.7	0.9-3.2	0.1
Months worked at current plant	≤60	34.2 (111)	6.3	Ref		0.04[2]
	>60 - 108	20.9 (68)	13.2	2.3	0.8-6.4	0.122
	> 108-192	20.9 (68)	16.2	2.9	1.0-7.8	0.039
	>192	24 (78)	19.2	3.5	1.4-9.1	0.009
Months worked in meat industry[1]	≤60	28.3 (73)	6.8	Ref		0.25[2]
	>60 - 123	22.9 (59)	16.9	2.8	0.9-8.6	0.078
	> 123-288	27.5 (71)	15.5	2.5	0.8-7.6	0.108
	>288	21.3 (55)	14.5	2.3	0.7-7.5	0.162
Smoking at work	No	71.7 (231)	13.9	Ref		
	Yes	28.3 (91)	11.0	0.8	0.4-1.6	0.493
Abattoir	Sheep plant 1	32 (104)	11.5	Ref		0.04[2]
	Sheep plant 2	29.9 (97)	11.3	1.0	0.4-2.3	0.965
	Sheep plant 3	9.9 (32)	31.3	3.5	1.3-9.1	0.011
	Sheep plant 4	28.3 (92)	9.8	0.8	0.3-2.1	0.692

[1]n=258; [2]p-value of the LRT for all exposure categories combined.

Table 4: Frequencies of clinical, demographic and non-work related exposure variables and their unconditional association with sero-prevalence of *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in sheep plant workers (n=325) blood sampled and interviewed from January - April 2010

Variable	Category	% Workers (n)	Sero-Prevalence %	Crude OR	95% CI	P-value
Probable leptospirosis[1]	No	87.7 (285)	8.1	Ref		
	Yes	12.3 (40)	47.5	10.3	4.8-21.9	<0.001
Had flu-like-illness	No	63.4 (206)	13.1	Ref		
	Yes	36.6 (119)	12.6	0.9	0.5-1.9	0.88
Gender	Female	29.5 (96)	3.1	Ref		
	Male	70.5 (229)	17.0	6.4	1.9-21.1	0.003
Age	≤ 37	25.5 (83)	14.5	Ref		0.5[5]
	>37, ≤48	28 (91)	8.8	0.6	0.2-1.5	0.246
	>48, ≤55	22.2 (72)	12.5	0.8	0.3-2.1	0.723
	>55	24.3 (79)	16.5	1.2	0.5-2.7	0.725
Hunting any Species[2]	No	83.1 (270)	13.3	Ref		
	Yes	16.9 (55)	10.9	0.8	0.3-2.0	0.626
Hunting pigs, deer or feral goats	No	85.9 (279)	13.3	Ref		
	Yes	14.2 (46)	10.9	0.8	0.3-2.1	0.655
Farming[3]	No	84.6 (275)	13.8	Ref		
	Yes	15.4 (50)	8.0	0.5	0.2-1.6	0.26
Ethnicity	NZ European	33.9 (110)	8.2	Ref		0.19[5]
	NZ Maori	57.5 (187)	15.5	2.0	0.9-4.5	0.072
	Other	8.6 (28)	14.3	1.9	0.5-6.6	0.33
Slaughtering at home[4]	No	69.5 (226)	10.6	Ref		
	Yes	30.5 (99)	18.2	1.9	1.0-3.6	0.06

[1]was not included in the multivariable model, as it was an intermediate variable between exposure and antibody level; [2]pigs, deer, goats, birds, rodents, possums; [3]pigs, goats, sheep, beef cattle, alpaca or deer; [4]sheep, goats, pigs, beef or deer; [5]p-value of the LRT for all exposure categories combined.

4.3.5. Risk factors for sero-prevalence in deer abattoirs

Tables 4 and 5 list crude associations between exposure variables and sero-status for Pomona and/or Hardjobovis in deer abattoir workers. Work position, the use of PPE and having had probable leptospirosis were risk factors for being sero-positive (OR >1; p ≤0.05).

Table 5: Frequencies of work related exposure variables and their unconditional association with sero-prevalence of *Leptospira interrogans* sv Pomona (Pom) and/or *Leptospira borgpetersenii* sv Hardjobovis (Har) in deer plant workers (n=57) blood sampled and interviewed in November 2009

Variable	Category	% Workers (n)	Sero-Prevalence %	Crude OR	95% CI	P-value
Work position	Boning, chillers, office	59.7 (34)	2.9	Ref		
	Offal, pet food, gut & kidney removal, yards, stunning, pelting	40.4 (23)	39.1	21.2	2.4-183.7	0.006
Wear gloves on both hands	never	73.7 (42)	21.9			0.65[2]
	sometimes	5.3 (3)	10.0	0.4	0.0-3.7	0.416
	often		0.0	[c]		
	always	21.1 (12)	15.4	0.6	0.1-3.6	0.624
Wear safety glasses	never	73.7 (42)	7.1			0.004[2]
	sometimes	5.3 (3)	33.3	6.5	0.4-94.1	0.17
	often		0.0			
	always	21.1 (12)	50.0	13.0	2.5-66.4	0.002
Wear a facemask	never or sometimes	96.5 (55)	16.4	ref		
	often or always	3.5 (2)	50.0	5.1	0.3-89.5	0.264
Wear a balaclava	never or sometimes	91.2 (52)	17.3	ref		
	often or always	8.8 (5)	20.0	1.2	0.1-12.0	0.88
Months worked at current plant	≤60 months	50 (13)	15.4	[3]		
	>60 - 108 months	15.4 (4)	0.0			
	> 108-192 months	34.6 (9)	33.3			
	>192 months		0.0			
Months worked in meat industry[1]	≤60 months	21.1 (12)	0.0	[3]		
	>60 - 123 months	26.3 (15)	33.3			
	> 123-288 months	24.6 (14)	14.3			
	>288 months	28.1 (16)	18.8			
Smoking at work	No	58.9 (33)	18.2	ref		
	Yes	41.1 (23)	17.4	0.9	0.2-3.8	0.939
Abattoir	Deer plant1	36.8 (21)	19.0	Ref		0.9[2]
	Deer plant2	63.2 (36)	16.7	0.8	0.2-3.4	0.82

[1]n=258; [2]p-value of the LRT for all exposure categories combined;. [3]model did not run, due to data sparsity issues.

Table 6: Frequencies of clinical, demographic and non-work related exposure variables and their unconditional association with sero-prevalence of *Leptospira interrogans* sv Pomona (Pom) and/or *Leptospira borgpetersenii* sv Hardjobovis (Har) in deer plant workers (n=57) blood sampled and interviewed in November 2009

Variable	Category	% Workers (n)	Sero-Prevalence %	Crude OR	95% CI	P-value
Probable leptospirosis[1]	No	91.2 (52)	11.5	Ref		
	Yes	8.8 (5)	80	30.7	2.9-321.8	0.004
Had flu-like-illness	No	38.6 (22)	22.7	ref		
	Yes	61.4 (35)	14.3	0.7	0.1-2.2	0.42
Gender	Female	15.8 (9)	0.0	Ref		
	Male	84.2 (48)	20.8	[6]		
Age	<32	26.3 (15)	20.0	Ref		0.93[5]
	32-42	24.6 (14)	14.3	0.7	0.1-4.7	0.685
	43-47	24.6 (14)	21.4	1.1	0.2-6.6	0.924
	>47	24.6 (14)	14.3	0.7	0.9-4.7	0.685
Hunting any species [2]	No	63.2 (36)	13.9	Ref		
	Yes	36.8 (21)	23.8	1.9	0.5-7.7	0.347
Hunting pigs, deer or feral goats	No	70.2 (40)	12.5	Ref		
	Yes	29.8 (17)	29.4	2.9	0.7-11.8	0.135
Farming[3]	No	80.7 (46)	17.4	Ref		
	Yes	19.3 (11)	18.2	1.1	0.2-5.8	0.951
Ethnicity	NZ European	87.7 (50)	18.0	Ref		
	NZ Maori	7 (4)	25.0	0.8[7]	0.1-7.1	0.809
	Other	5.3 (3)	0.0			
Slaughtering at home[4]	No	64.9 (37)	18.9	Ref		
	Yes	35.1 (20)	15.0	0.8	0.2-3.3	0.711

[1]Not included in the multivariable model, as it was an intermediate variable between exposure and antibody level, hence not a confounder;[2]pigs, deer, goats, birds, rodents, possums; [3]pigs, goats, sheep, beef cattle, alpaca or deer; [4]sheep, goats, pigs, beef & or deer; [5]p-value of the LRT for all exposure categories combined; [6]the category group 'female' had no *Leptospira* sero-positive observations; [7]ethnicity: category 2 and 3 were collapsed into category 2.

After adjusting for PPE (facemask or safety/normal glasses) in the multivariable logistic regression analysis, study participants working at the slaughter board or offal room were 12.7 (95% CI 1.33-120.6, p=0.027) times as likely to be sero-positive against Pomona and/or Hardjobovis than participants working in the office, boning room or chillers. Sero-prevalence for workers wearing PPE was 4.24 (95% CI 0.79-22.82, p=0.093) as high as those who did not wear PPE, a marginally significant finding (Table 9). The sample size of 57 deer abattoir workers had limited statistical power for the risk factor analysis. It was therefore difficult to achieve adequate statistical precision for these exposure factors.

Including PPE ('wearing a facemask or safety/normal glasses') in the multivariable model,

reduced the crude OR of the high-risk work position from 21.1 to an adjusted OR of 12.7. This is presumably because workers in high-risk work positions wore more often facemasks or safety glasses than workers in the boning room or office (43.5% vs 5.9%, p-value 0.001).

Since no female participant was sero-positive, a model including gender and work position failed to converge due to only 9 female workers in the two participating deer plants. None of the tested interactions were significant.

4.3.6. Risk factors for sero-prevalence in beef abattoirs

Tables 6 and 7 show unadjusted associations between exposure variables and sero-status for Pomona and/or Hardjobovis in beef abattoir workers. The odds of being sero-positive increased linearly with age ($p < 0.05$) and having had probable leptospirosis was positively associated with being sero-positive (<0.001). However, none of the work positions was associated with an increased or decreased risk of being sero-positive.

Table 7: Frequencies of work related exposure variables and their unconditional association with sero-prevalence of *Leptospira interrogans* sv Pomona (Pom) and/or *Leptospira borgpetersenii* sv Hardjobovis (Har) in beef plant workers (n=185) blood sampled and interviewed from January - April 2010

Variable	Category	% Workers (n)	Sero-Prevalence %	Crude OR	95% CI	P-value
Work position	Boning, chillers, office	44.9 (83)	3.6	Ref		0.77[3]
	Maintenance	5.4 (10)	10	3.0	0.3-31.6	0.368
	Offal, pet food	11.4 (21)	9.5	2.8	0.4-18.0	0.276
	Yards, stunning, pelting, gut & kidney removal, meat inspection	38.4 (71)	5.6	1.6	0.3-7.4	0.552
Wear gloves on both hands[1]	never	20.1 (37)	2.7	Ref		0.25[3]
	sometimes	13 (24)	0.0	[4]		
	often	2.7 (5)	0.0	[4]		
	always	64.1 (118)	7.6	2.8	0.4-24.3	0.309
Wear safety glasses	never	66 (122)	5.7	Ref		0.47[3]
	sometimes	4.3 (8)	0.0	[4]		
	often	2.7 (5)	20.0	4.1	0.4-41.8	0.233
	always	27 (50)	4.0	0.7	0.1-3.4	0.644
Wear a facemask	never or sometimes	97.8 (181)	5.0	Ref		
	often or always	2.2 (4)	25.0	6.4	0.6-67.5	0.124
Wear a balaclava[1]	never or sometimes	92.4 (171)	5.8	[4]		
	often or always	7.6 (14)	0.0			
Months worked at current plant	<61 months	27 (50)	2.0	Ref		0.12[3]
	61 - 108 months	24.3 (45)	0.0	[4]		
	109-192 months	21.6 (40)	12.5	7.0		0.082
	>192 months	27 (50)	8.0	4.3	0.5-39.5	0.202
Months worked in meat industry[2]	<61 months	22.7 (42)	2.4	Ref		0.19[3]
	61 - 123 months	26.5 (49)	0.0	[4]		
	1243288 months	22.2 (41)	12.2	5.7	0.6-51.0	0.12
	>288 months	28.7 (53)	7.5	3.3	0.4-31.1	0.288
Smoking[2]	No	73.4 (127)	7.1	Ref		
	Yes	26.6 (46)	2.2	0.3	0.0-2.4	0.248
Abattoir	Beef plant1	39.5 (73)	5.5	Ref		
	Beef plant2	60.5 (112)	5.4	1.0	0.3-3.6	0.971

[1]n=184; [2]n=173; [3]p-value of the LRT for all exposure categories combined; [4]model did not converge, category dropped, due to sparse data.

Table 8: Frequencies of clinical, demographic and non-work related exposure variables and their unconditional association with sero-prevalence of *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in beef plant workers (n=185) blood sampled and interviewed from January - April 2010

Variable	Category	% Workers (n)	Sero-Prevalence %	Crude OR	95% CI	P-value
Probable leptospirosis[1]	No	91.9 (170)	1.8	Ref		
	Yes	8.1 (15)	46.7	48.7	10.6-224.3	<0.001
Had flu-like-illness	No	49.7 (92)	7.6	Ref		
	Yes	50.3 (93)	3.2	0.4	0.1-1.6	0.2
Gender	Female	24.3 (45)	0.0	[7, 8]		0.06[6]
	Male	75.7 (140)	7.1			
Age	≤34	25.4 (47)	0.0	[7,8]		0.1[6]
	>34, ≤48	26.5 (49)	4.1			
	>48, ≤56	24.3 (45)	6.7			
	>56	23.8 (44)	11.4			
Hunting all species [2]	No	86 (159)	5.0	Ref		
	Yes	14.1 (26)	7.7	1.6	0.3-7.9	0.581
Hunting pigs, deer or feral goats	No	88.1 (163)	5.5	Ref		
	Yes	11.9 (22)	4.5	0.8	0.1-6.8	0.85
Farming[3]	No	84.3 (156)	5.8	Ref		
	Yes	15.7 (29)	3.4	0.6k	0.1-4.8	0.616
Ethnicity	NZ European	41.1 (76)	5.3	Ref		0.07[6]
	NZ Maori	44.9 (83)	2.4	0.4	0.1-2.5	0.357
	Other	14.1 (26)	15.4	3.3	0.8-14.2	0.113
Slaughtering at home[4]	No	72.4 (134)	6.0	Ref		
	Yes	27.6 (51)	3.9	0.6	0.1-3.1	0.585

[1]was not included in the multivariable model, as it was an intermediate variable between exposure and antibody level;[2]pigs, deer, goats, birds, rodents, possums; [3]pigs, goats, sheep, beef cattle, alpaca & or deer; [4]sheep, goats, pigs, beef & or deer, [5]is an intermediate variable; [6]p-value of the LRT for all exposure categories combined; [7]the category groups 'female' and 'age group 1' had no *Leptospira* sero-positive observations; [8]model did not run, due to data sparsity issues.

In the multivariable model, aging by one year increased the odds of being sero-positive 1.09-fold, suggesting a 9% increase in risk for each year of age (p=0.02). As in the deer model, none of the tested female workers in the beef abattoirs were sero-positive despite 45 female participants, thus a model with gender failed to converge. Work position and abattoir were not significantly associated with sero-positivity (Table 9).

Diagnostics of the final multivariable models indicated a sufficient fit of the data for all three species. Even though outliers were identified, they did not impact on the inferences from the analysis.

Table 9: Joint multivariable analysis of data from all plants: significant effects on sero-prevalence of *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in abattoir workers processing sheep (n=325), deer (n=56) and beef (n=185) (November 2009 – April 2010)

Species	Variable	Category	Adjusted OR	95% CI	P-value
Sheep	Work position	Boning, chillers, office	Ref		
		Offal, pet food	6.5	1.4-29.8	0.017
		Gut removal, pulling kidneys	8.2	2.1-32.7	0.003
		Yards, stunning, pelting	10.4	2.8-38.8	<0.001
	Gender	Female	Ref		
		Male	3.1	0.8-11.7	0.089
	Years worked at meat plant	(continuous)	1.1	1.0-1.1	0.011
	Meat plant	Sheep 1	Ref		
		Sheep 2	4.5	1.2-16.3	0.022
		Sheep 3	6.3	1.8-22.4	0.004
		Sheep 4	2.1	0.7-6.3	0.201
Deer	Work position	Boning, Chillers, Office	Ref		
		Offal, pet food, gut removal, pulling kidneys, yards, stunning, pelting	12.7	1.3-120.6	0.027
	Wear facemask, or safety glasses	Never or sometimes	Ref		
		Often or always	4.3	0.8-22.8	0.093
Beef	Work position	Boning, chillers, office	Ref		
		Maintenance	2.0	0.3-23.4	0.59
		Offal, pet food	3.1	0.5-20.6	0.25
		Yards, stunning, pelting, gut, kidney removal & meat inspection	2.2	0.5-10.8	0.32
	Age (years)	continuous	1.1	1.0-1.2	0.02

The log likelihood values and p-values resulting from comparing the nested with the final model were for the sheep model -99.5; ($p < 0.001$), for the deer model -18.4 ($p = 0.08$) and beef model -34.3 ($p = 0.006$). The nested model included work position as single exposure variable and the Log likelihood of these nested models was -107.4 (sheep), -19.9 (deer) and -38.1 (beef).

5. Discussion

5.1. Sero-prevalence

We observed differences in prevalence between abattoirs and between slaughter species. Across all work positions, the prevalence of workers from the four sheep abattoirs ranged from 10-31%, from the two deer abattoirs from 17-19% and the prevalence in the two beef abattoirs was 5%. The finding of a high prevalence in sheep plant workers contrasts the statement in a study, where sheep were not regarded as an exposure source for people (Hathaway, 1981). Therefore, the epidemiological importance of sheep may have changed in NZ.

A possible reason for the difference between sheep and beef abattoir worker prevalences is in variable slaughter procedures and species peculiarities. During interviews, participants reported that sheep often urinate spontaneously when stunned, whereas this was rarely observed in beef cattle. Exposure is likely to increase due to stunned sheep touching down on the platform where urine of former sheep accumulates. In addition, sheep and deer plants process more animals per time unit than beef plants. Therefore, sheep plant workers may be more exposed than beef plant workers. Moreover, most dairy cows that are part of the slaughtered stock were vaccinated, which is expected to reduce shedding (Marshall et al., 1982; Bolin and Alt, 2001). Additionally, sheep and deer may have higher rates of shedding than beef cattle. In an abattoir study in the Waikato region, urine, kidney and blood was sampled from 399 lambs and 146 beef from six suppliers following a period of heavy rain during warm weather. The shedding rate -determined by positive urine PCR- in sero-positive (MAT cut-off ≥1:48) sheep was 54.1%, whilst that in sero-negative sheep it was 2.8% and in sero-positive cattle it was 28.2%, whilst that in sero-negative cattle was 3.0% (Fang et al., 2010). However, the study is not representative for NZ, due to the regional concentration of suppliers. Another speculative reason could be variability in pathogenicity within serovar strains infecting sheep and cattle.

The prevalence difference between sheep abattoirs is probably not associated with different procurement areas, as recently conducted cross sectional study in livestock revealed similar within-flock prevalences for the Hawkes Bay and Taranaki region of approximately 38% (Chapter 2). Age at slaughter is an important predictor of animal shedding (Dorjee et al., 2005b). Thus, if a plant received a higher proportion of older animals for slaughter, its employees were more exposed. Moreover, the sampling of sheep plants occurred over several months (January-April), with the prevalences consequently being subject to seasonal fluctuation.

While there was a negative correlation between compliance in wearing gloves and glasses or facemasks and prevalence in slaughter floor workers of sheep plants, in deer and beef plant workers there was no such correlation (hence plants with a higher prevalence did not have a lower compliance in wearing PPE). That some plants achieved better compliance with PPE policy than others may therefore not have accounted for prevalence differences across plants since the multivariable model suggested that wearing PPE tended to increase the risk of being sero-positive rather than decrease it.

The study may have an overrepresentation of persons from the ‘slaughter’ area compared to the general workforce because of the voluntary nature of sampling, possibly due to a higher risk perception and clinical leptospirosis history in that group. Further, because of voluntary sampling, the proportion of sampled workers from different working areas may differ between plants. As a

consequence, the crude overall prevalences may not be comparable between plants without appropriate adjustment by standardisation for demographic factors of factory workers (Dreyfus et al., 2010b). However, prevalences within work position categories and the OR from the multivariable logistic regression analysis are not affected by this sampling bias.

We sampled workers from four sheep plants, belonging to two different companies from two regions in the North Island with approximately 17% of the total sheep meat processing industry population. The two deer plants were located in one region and belonged to two companies. We probably included less than 10 percent of all deer and beef meat workers in NZ in our study population[23]. The two beef plants were located in two regions, both belonging to the same company. The selection of all plants was not random, but based on contacts of co-authors to the meat industry. While sheep and beef plants mainly slaughtered cattle from the North Island, deer plants received their stock from the South Island. Exposure of meat workers occurred hence only to animals from a certain part of NZ. The external validity for sheep plants is better compared to beef and deer plants, given the number and the regional distribution of plants and the ratio of study population/total of plant workers. However, given the non-random sampling procedure of plants and workers, representativeness of the general meat worker population can not be guaranteed. Nevertheless, the size (number of workers) of the plants and the slaughter procedures are representative of the meat industry in NZ.

Even though the ability of the MAT to distinguish between serovars had been questioned (Levett, 2001; Smythe et al., 2009), it is unlikely that this was the case for Hardjobovis and Pomona in this thesis, as the prevalent serovars in NZ belong to different serogroups apart from Hardjobovis and *Leptospira borgpetersenii* Balcanica (Balcanica) (Hathaway, 1981). Further, several studies have been conducted in NZ in recent years, where serovars determined by serology had been also confirmed by direct methods. For example, MAT serology and serovar isolates had good kappa agreement by DNA sequencing results (Subharat et al., 2011b; Subharat et al., 2012a).

In this study we measured prevalence of exposure not clinical disease and therefore chose a lower MAT titre cut-off (1:48), than studies intending to detect clinical leptospirosis (1:≥100). Even though Faine et al. (1999) and Shivakumar et al. (2006a) suggested a titre cut-off of 1:50 to test exposure to *Leptospira* spp., they did not specify the sensitivity and specificity of the MAT for the given cut-off. In the literature search, we could not find any specification of sensitivity and specificity of the MAT to estimate prevalence of exposure for a given cut-off. In a study evaluating the MAT sensitivity and specificity of acute (MAT cut-off 1:100) and convalescent (MAT cut-off not mentioned) sera in an urban setting in Brazil (McBride et al., 2007), the MAT testing of convalescent

[23] Statistics on frequency of meat workers by slaughter species were not available

sera had a sensitivity of 91% - 100% and specificity of 94% to 100%. If we assumed that the MAT in our study had a 91% sensitivity and 94% specificity, the tested prevalence in meat plants was likely under-estimated. For example, the average prevalence in the four sheep plants with 325 workers was 16%. With 91% sensitivity and 94% specificity, the true prevalence would have been 20% (Rogan and Gladen, 1978), hence our study outcome was a conservative estimate. However, since we used a MAT titre cut-off of 1:48 and tested for the serovars Hardjobovis and Pomona, which are less likely to be encountered in a urban setting, where Copenhageni is predominant (McBride et al., 2007), it is possible that the sensitivity and specificity of the MAT in NZ are not the same as in Brazil.

Cross-sectional studies on *Leptospira* spp prevalence in meat workers have been conducted in several countries. Apart from Babamahmoodi et al. in Iran, all researchers used MAT for testing. The prevalence in butchers in India was 30% (Sharma et al., 2006), in abattoir workers in Nigeria 29.5% (MAT cut-off 1:100) (Ezeh et al., 1988), in Tanzania approximately 18% (MAT cut-off 1:160) (Schoonman and Swai, 2009), in Brazil 4% (MAT cut-off 1:100) (Goncalves et al., 2006) and Iran 4% (Babamahmoodi et al., 2009) and in all these countries *Leptospira* was regarded to be an occupational hazard. In Vojvodina, the average prevalence of the meat workers was 2.3% and, similar to our findings, it was determined that workers at the slaughter board had the highest risk of being sero-positive (6%) compared to workers of other areas of the plant (Bacic et al., 1994). In NZ studies on leptospirosis prevalence in meat workers were conducted in multispecies abattoirs slaughtering sheep, beef and sometimes pigs in the 1980s, revealing a prevalence between 0-2.7% against Hardjobovis and 0.8-8.9% against Pomona (MAT titre cut-off 1:24), where Pomona was believed by the authors to be largely derived from processing pigs (Blackmore et al., 1979; Blackmore and Schollum, 1982a). A recently conducted study in a sheep slaughtering plant revealed in 242 meat workers a prevalence of 9.5% against Hardjobovis or Pomona (MAT titre cut-off 1:24) (Benschop et al., 2009; Heuer et al., 2010). In our study, workers from the four sheep abattoirs had an average prevalence of leptospiral titres (Hardjobovis or Pomona) of 10, 11, 12 and 31%, from the two deer abattoirs 17 and 19% and the two beef abattoirs 5 and 5%. A precise comparison between the older Blackmore et al. studies having tested workers from multispecies plants and this abattoir study is difficult because we tested workers from single species plants, where *Leptospira* prevalence can be associated with exposure to one species. Nevertheless, the prevalence in sheep and cattle meat workers seems to have increased, especially since we used a higher MAT cut-off of 1:48 to define a sero-positive test result.

In six of eight meat plants serovar Hardjobovis was more prevalent than Pomona in meat workers ($p < 0.05$). This serovar distribution of Hardjobovis and Pomona is as well described in slaughter lambs and deer (Wilson et al., 1998; Dorjee et al., 2005b; Ayanegui-Alcerreca et al.,

2010a). However, the higher prevalence of Hardjobovis in meat workers may be due to longer antibody titre duration of Hardjobovis and not because of a higher infection risk (Chapter 5).

Many cross sectional studies in an occupational setting suffer from the 'healthy worker effect', meaning that the ill people are not participating in the study because they stayed at home, leading to an underestimation of the effect. This study was not affected as we were measuring prevalence and not clinical cases of leptospirosis, of which the severe cases are reasonably rare and the duration of absence of mild cases quite short (Chapter 5).

5.2. Probable Leptospirosis

A previous experience of probable leptospirosis was strongly predictive for the presence of antibody. It is a common finding that antibodies from clinical leptospirosis persist for many years (Blackmore and Schollum, 1982a; Blackmore et al., 1984). However, that these workers were serologically positive up to 20-30 years after the clinical episode may be attributable to continued high exposure and multiple re-infection rather than antibody persistence *per se*. A relevant question at this point is, how many new infections remain clinically dormant, how often disease occurs at what degree of severity, and how long antibodies persist. Data on incidence of *Leptospira* infection and disease are presented in the thesis Chapter 5 where these questions are further discussed.

Despite 43.6% (247/567) workers reporting 'flu-like illness' during the past 36 months, there was no statistically significant association between 'flu-like illness' and leptospirosis sero-positivity. This would suggest that almost all infections with *Leptospira* were asymptomatic. However, the time period of 36 months in which 'flu-like illness' occurred was most likely too long to measure an association between 'flu-like illness' and positivity.

5.3. Risk Factors for Sero-positivity

This study demonstrated that work position was the strongest risk factor for sero-positivity with Pomona and/or Hardjobovis in sheep and deer abattoir workers.

The higher prevalence in workers at the beginning of the slaughter board and the gradual reduction along the slaughter line in sheep plants is consistent with a study conducted two years earlier in one of the sheep plants of this study (Heuer et al., 2010). Urine splashing due to stunning and subsequent contamination of pelts and carcasses are thought to be causes for infection, which may be difficult to control while handling carcasses. The prevalence of workers half way down the slaughter board may be attributable to exposure to *Leptospira* from organs of the genital-urinary tract. Evisceration may therefore pose another risk of infection, either when organs are removed

from the carcass, processed or inspected. However, even though exposure to shedding may even be higher at evisceration and offal handling than at the beginning of the slaughter line (Dorjee et al., 2011). The time and place of exposure is more predictable in these positions and workers can clean hands more frequently than at the physically challenging and injury prone positions at the head of the slaughter board. Persons in the other processing areas (boning room, chillers) or in the office had little or no exposure to urine and were therefore less likely to get infected. Controlling leptospirosis in livestock aside, control measures targeting the most high risk components of the abattoir process would be likely to have the greatest protective impact. Hence, measures to contain urine during stunning and urinary tract tissues should be investigated. The removal of the platform sheep land on after stunning, may be a useful control measure, as each sheep landing on the platform might get contaminated with urine from former sheep, increasing the risk of spreading contaminated urine further down the line.In order to understand the risk of infection in different work positions, we assigned work tasks to different work position categories with a similar exposure to urine or to organs of the urinary tract. However, this was not always possible, as some participants worked in more than one position with different levels of exposure risk. Such workers were allocated to the exposure category, where they spent most of their working time. A few workers (*n*=18) who spent an equal amount of time in different exposure categories were randomly allocated to one of those categories. This may have introduced a certain degree of non-differential misclassification bias leading to an overestimation of prevalence in low risk work positions.

Thirty-six percent of 439 meat workers reported that wearing PPE was annoying. The use of PPE appeared not to reduce the risk of sero-positivity, as PPE in the multivariable model did not show any evidence for protective effects and in deer workers it even had a marginally significant positive OR (p=0.08). This may have biological plausible reasons which would warrant further investigation. For example, workers may wipe their eyes to remove the sweat more often with their contaminated hands when wearing glasses or facemasks. Moreover, meat workers informed us that they often had to lift up their safety glasses or masks to remove sweat and fog, and restore visibility.

When responding to questions about wearing PPE, participants might have been biased by the employment policy enforcing the use of PPE, despite a clear statement that interviews were confidential. This could have led to an overstatement of wearing PPE by participants in high-risk work positions, thus reducing the possibility of determining a protective effect of PPE in the analysis. Similarly, if study participants who had experienced clinical leptospirosis and were therefore sero-positive became more compliant with wearing PPE, the observed risk associated with PPE might have been overestimated.

Nevertheless, the finding that the PPE may not be protective warrants further investigation.

Using PPE for most tasks is not comfortable, and if workers are mandated to wear protective gear, it seems reasonable to expect a benefit from doing so. However, if PPE is not protective, other means of protection may have to be considered, for example vaccination of farmed livestock. No registered vaccine is currently available for humans in NZ. Since vaccination of dairy cows commenced in the 1980s, the incidence of notified human leptospirosis cases in the farming industry decreased from 234 annual cases per 100,000 to 90 per 100,000 (Marshall, 1996; Marshall and Manktelow, 2002; Thornley et al., 2002). Whereas a large proportion of dairy farmers are known to vaccinate their stock against leptospirosis and the NZ pig industry introduced compulsory vaccination of pig herds[24], less than 10% of deer, sheep or beef farmers are currently using vaccination (Keenan, 2007a; Wilson et al., 2008a). Vaccination also has the potential to protect farmers and farm workers, veterinarians and veterinarian technicians, shearers, truck drivers, artificial insemination technicians and home butchers who are also at risk of infection (Marshall et al., 1979; Mackintosh et al., 1980a; Allen et al., 1982; Bolin and Alt, 2001; Subharat et al., 2012a).

In our analysis, male workers of sheep plants were more likely to be sero-positive than females. This association was not confounded by age, hunting or home slaughter and there was no difference in the frequency of wearing PPE in exposed work position categories between men and women. Females were as likely to get exposed to urine as men within the work position categories with high urine exposure, as there was no gender difference in tasks performed. Moreover, there was no interaction between gender and work position. Therefore, the evidence suggested that the prevalence difference between males and females was independent of their work position. For deer and beef plants the gender effect could not be investigated, due to lack of females in high risk work position categories.

Studies of dairy farm workers and pig farmers showed similar findings in relation to gender, with males being more likely to be sero-positive than females (Mackintosh et al., 1980b; Schollum and Blackmore, 1982). The authors argued that woman might take more care with personal hygiene. The study in dairy farmers controlled for wearing gloves or working part-time, whereas the one in pigs did not control for confounding factors. Whether, the gender effect was due to unknown confounding factors or females working more carefully, avoiding exposure or due to a true gender-specific susceptibility or immune response remains subject to speculation.

The identification of worker age as a risk factor in beef plants could not be explained with increased exposure over time as the variable 'time worked in the industry' was not statistically significant in the model. A possible reason could be changes in the immune system with age.

[24] Point 6 in the "Animal Status Declaration" (ASD) form of the NZ Pork Industry Board: http://www.nzpork.co.nz/Portals/NZPib/Documents/Publications/NZPork%20ASDP%20Download%20FORM.pdf

Categories of age were not associated with work position categories. Therefore, age did not confound the relationship between work position and prevalence.

Other exposure factors, such as home slaughtering, farming or hunting or smoking were all unrelated to prevalence in the multivariable analysis, regardless of species processed. This partially contrasts the findings of a previous study in one sheep plant, where home slaughtering was found to be a risk factor (Heuer et al., 2010). Our study had sufficient statistical power to identify strong risk factors (OR>2.5) and we studied four instead of one sheep plant and therefore believe that the results of our study have more power and are more representative.

A sero-positive case was a study participant with a titre of ≥1:48 against Pomona and/or Hardjobovis. The reason for not distinguishing between serovars was that, in NZ, beef, sheep and deer were known to be most frequently infected with either or both serovars, and there is no evidence that human exposure factors would be different for the two serovars (Hathaway, 1981; Wilson et al., 1998; Subharat et al., 2011c). Further, compiling both serovars together in one outcome increased the power of the study. We ran the multivariable analysis for Pomona and Hardjobovis prevalence separately and were assured that risk factors for the two serovars did not differ. The titre cut-off of 1:48 was recommended to determine exposure to leptospires, but not for clinical disease (Faine et al., 1999; Shivakumar and Krishnakumar, 2006b).

6. Conclusion

This study demonstrated that sheep and deer abattoir workers were at substantial risk of being sero-positive with Pomona and/or Hardjobovis with workers from the four sheep abattoirs having an average prevalence of leptospiral titres (Hardjobovis or Pomona) of 10, 11, 12 and 31%, from the two deer abattoirs of 17 and 19%. In workers, antibodies were more frequently found against serovar Hardjobovis (61%) than Pomona (39%), and this was similar to the serovar difference reported from livestock. The strongest risk factor for sero-prevalence of workers in sheep and deer abattoirs was work position. For participants from sheep plants, prevalence was highest at the beginning of the slaughter board, lower in those working where activities involved the removal of high risk material (guts, bladder, and kidneys), lower in those in the offal/pet food area, and lowest in those in the boning room or office. This finding warrants research into changes in the procedures in the stunning and pelting area in sheep abattoirs.

The data suggested that wearing personal protective equipment such as gloves, facemasks, safety/normal glasses or a balaclava did not protect against infection. Hence this study raised questions about best practice use of PPE. Non work-related risk factors, such as home slaughtering,

farming or hunting were not significantly associated with prevalence in this study. About 11% of workers reported to have experienced probable leptospirosis during a median period of five years prior to the study, confirming the public health significance of this disease. The incidence of mild or severe clinical leptospirosis in abattoir workers and the economic impact remains unknown and warrants clarification by further research.

7. Acknowledgements

Funding was provided by Rural Woman NZ, the Department of Labour, the NZ Tertiary Education Commission (TEC) via the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, and the Swiss National Science Foundation (SNSF).

We are very grateful to study participants, abattoir managers, nurses and phlebotomists without whom the study would have been impossible to conduct. Thanks also to Sarah Moore, who was an irreplaceable help in data collection and entry, communication of results and a great travel companion.

We thank occupational health physicians Drs. J. Reekie and J. Kerr for advice and support, Heather Duckett for helping in sampling logistics, Christine Cunningham and Wendy Maharey for administrative support, Brian O'Leary, Masood Sujau and Simon Verschaffelt for help with building the database. Fang Fang, Prakriti Bhattarai, Rayon Gregory, Claire Cayol and Emilie Vallee for interviewing workers, Neville Haack and Rae Pearson for MAT testing and Roger Lentle for advice for the Massey University Human Ethics Committee application.

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Chapter 5

New Infection with *Leptospira* and Associated Risk Factors in Meat Workers in New Zealand

A. Dreyfus, J. Benschop, J. Collins-Emerson, P. Wilson, C. Heuer

1. Abstract

Leptospirosis is an endemic bacterial disease of sheep, beef cattle and deer in New Zealand and currently the most important occupational zoonotic disease in meat workers and farmers. While vaccination of dairy cattle and pigs is widely adopted, other livestock are rarely vaccinated. A previous study in meat workers revealed a sero-prevalence against *L. interrogans* sv Pomona (Pomona) and/or *L. borgpetersenii* sv Hardjobovis (Hardjobovis) of 10-31% in workers at sheep (*n*=4 abattoirs), 17-19% at deer (*n*=2) and 5% at beef abattoirs (*n*=2). The objectives of this study were to determine the annual rate of new infections with *Leptospira* by serological evidence in meat workers and the proportion of new infection leading to clinical disease, to evaluate risk factors for infection, and to determine the proportion of workers in the population with 'flu-like' disease attributable to new infections with Hardjobovis and/or Pomona.

We conducted a cohort study in eight abattoirs slaughtering sheep (n=4; one abattoir twice in subsequent years), cattle (*n*=2) and deer (*n*=2). Sera were collected twice from 592 participants between 50 and 61 weeks apart, and tested by the Microscopic Agglutination Test for Pomona and Hardjobovis. Information on potential risk factors including personal data, workplace, lifestyle and clinical history were recorded and analysed by multivariable logistic regression.

Forty-nine (8.3%, 95% CI 6.2-10.9) participants either sero-converted or had at least a 2-fold increased serological titre against Pomona and/or Hardjobovis within 365 days. While in sheep meat workers, the annual infection risk was 11.1% (95% CI 8.5-14.8), in deer meat workers it was 0% and in those processing beef cattle, 1.2% (95% CI 0.2-4.6). The estimated titre duration of antibodies against Pomona was 10 months and against Hardjobovis 29 months. Risk factors for new infection (determined by serological evidence) in sheep abattoirs were: worker position, abattoir and time worked in the meat industry. The new infection risk was highest at the beginning of the slaughter line (stunning and hide removal) (RR 7.5, 95% CI 2.5-22.4), followed by positions on the line involving the removal of high risk material (guts, bladder, and kidneys) (RR 5.2, 95% CI 1.7-16.0). Risk was lower in the offal/pet food area (RR 4.1, 95% CI 1.0-16.4), and lowest in the boning room or

office. Wearing personal protective gear was not shown to reduce the risk of new infection, when adjusted for work position. The under-ascertainment of officially notified cases with leptospirosis in the last five years was estimated at between 16 and 56 times.

Three workers reported a diagnosis of probable clinical leptospirosis within the study period. In sheep abattoirs, new infection with Hardjobovis or Pomona was associated with a 2-fold higher risk of 'flu-like' illness (47% of infected vs. 24% of non-infected workers), and an average of 4 days absence from work. The average annual risk of experiencing flu-like symptoms due to infection with *Leptospira* was 2.7% and increased with the number of years that workers had been working in the abattoir.

This study has demonstrated ongoing exposure to leptospirosis in meat workers, risk factors for challenge, and the illness and sick leave rates associated with leptospirosis.

2. Introduction

Leptospirosis is a zoonotic bacterial disease affecting most mammalian species. After a leptospiraemic phase, leptospires colonize and persist mainly in the kidney, and are excreted in the urine for sometimes a lifetime (Bharti et al., 2003). Infection with *Leptospira* spp. occurs via skin abrasions or mucous membranes after exposure to the urine of carrier animals, either directly or via contact with contaminated water or soil. Human-to-human transmission is considered to be very rare (Bharti et al., 2003).

Leptospirosis is widespread in livestock in New Zealand (NZ). Up to 76% of adult deer herds, 97% of adult beef cattle herds, and 97% of adult sheep flocks are sero-positive against Hardjobovis and/or Pomona (Dreyfus et al., 2011). The two most frequent serovars in cattle, deer and sheep in NZ are *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona) (Marshall and Manktelow, 2002; Ayanegui-Alcerreca et al., 2007).

In NZ, livestock appear to be an important source of human leptospirosis, with farmers and meat workers being at a high risk (Thornley et al., 2002). A study revealed that a total of 158/2758 (5.7%, 95% CI 4.2–6.7) lambs sampled in an abattoir were positive against Hardjobovis and/or Pomona with substantial differences between study periods. The carcass prevalence in positive lines was 115/565 (20.4%, 95%CI 17.2–23.9) during the first and 43/633 (6.8%, 95% CI 5.1–9.0) during the second study period (Dorjee et al., 2011). The latter authors estimated that, on an average working day, an abattoir worker was exposed to 5-26 lamb carcasses that were kidney culture positive for *Leptospira*, hence potentially infectious.

Whereas almost all dairy farmers vaccinate their stock against leptospirosis and the NZ pig industry has introduced compulsory vaccination of pig herds[25], less than 10% of deer, sheep or beef farmers are currently using vaccination (Keenan, 2007a; Wilson et al., 2008a).

NZ is classified as having a moderate incidence of human leptospirosis in the Asia Pacific region (1-10/100'000) (Victoriano et al., 2009). From 2006 to 2010, 427 clinical cases of leptospirosis were notified (86.4% laboratory confirmed), an average annual rate of two cases per 100,000 total population. Of those with occupation recorded (91%), 52% (range 36 -71% annually) were farmers or farm workers and 30% (range 18 - 48% annually) abattoir workers or butchers. The risk among meat workers and farmers of contracting leptospirosis was hence higher than in the general population. *Leptospira* species and serovars were recorded for 67% of cases on average, of which 41% tested positive against Hardjobovis, 24% against Ballum (Ballum), 19% against Pomona and 16% against other serovars. Since 2008 however, *Leptospira borgpetersenii* serovar Ballum was

[25] Point 6 in the "Animal Status Declaration" (ASD) form of the NZ Pork Industry Board: http://www.nzpork.co.nz/Portals/NZPib/Documents/Publications/NZPork%20ASDP%20Download%20FORM.pdf

consistently the serovar most frequently determined from notified human cases (The Institute of Environmental Science and Research (ESR), 2006-2010).

Leptospirosis can result in severe human illness but is rarely fatal in NZ. During 2003-2005, 207 cases were hospitalised (Vickery et al., 2006). An unknown number of leptospirosis cases may be misdiagnosed as influenza or remain undiagnosed because medical attention is not sought, possibly due to difficulties accessing medical services in rural areas and because of under-diagnosis due to similarity to 'flu' symptoms. Further, medical practitioners often do not consider or test for leptospirosis because of a general lack of awareness about this disease or due to the absence of specific symptoms. Hence, the officially reported numbers mainly represent severe clinical cases, and milder forms are believed to remain under-reported (Thornley et al., 2002). However, the rate of under-reporting is unknown to date.

In the last four decades, three cross-sectional studies investigated *Leptospira* sero-prevalence in meat workers in NZ (Blackmore et al., 1979; Blackmore and Schollum, 1982a; Benschop et al., 2009) estimating sero-prevalences against Pomona, Hardjobovis, and/or *Leptospira borgpetersenii* serovar Tarassovi of between 4.1% and 9.5%. In an abattoir processing sheep, workers on the slaughter board had a 23-85-fold higher risk of being sero-positive than workers in the boning, cutting, chilling or rendering areas (Heuer et al., 2010). However, no longitudinal study on *Leptospira* incidence in NZ in general and in abattoirs specifically has been conducted; hence the true rate of new infections and their association with mild or severe clinical leptospirosis in any occupational group and the potential economic impact remain unknown.

The objectives of this study were therefore to determine the annual risk of infection and the associated incidence of probable or suspected clinical leptospirosis, and to identify risk factors for new infection related to occupational as well as non-occupational activities in meat workers. The findings were likely to generate recommendations for the improvement of control measures in abattoirs.

3. Methods

3.1. Study Design, Data Collection and Management

We conducted a cohort study among meat workers from eight purposively selected abattoirs comprising four sheep (one ('sheep 1') studied twice), two beef and two deer abattoirs in NZ. The two deer abattoirs were located in the South Island and the sheep and beef abattoirs were in the North Island. Abattoir managers, health and safety personnel, meat union representatives and

workers were provided in meetings with information about the study objectives and sampling procedure. Participation was, of necessity, voluntary rather than based on random sampling. To estimate the proportion of new infection with *Leptospira* by serological evidence, sample and data collection occurred twice, at intervals ranging from 50 – 61 weeks. Participating meat workers were blood sampled by certified phlebotomists and interviewed at each blood sampling by trained researchers using a questionnaire. Participants were informed about their MAT test result after each sampling. The first blood sample was used to establish the antibody titre status against Pomona and Hardjobovis and the second determined whether or not a worker was exposed to *leptospira* during the study period, as described below. Study participants of 'sheep abattoir 1' were sampled the first time between February and April 2008 and the second time in April 2009 (see table 4). All abattoirs, comprising four sheep (including abattoir 1), two deer and two beef abattoirs were sampled initially in November 2009 - March 2010, and again in November 2010 - May 2011. The cumulative incidence was adjusted to 365 days for each abattoir assuming that the risk for infection was constant. A participation proportion was calculated as the ratio of the study population to the entire workforce of an abattoir. All procedures were approved by the Massey University Human Ethics Committee in 2008 and 2009 (Southern A, application 05/123 and 09/08).

3.1.1. Sample size estimation

To detect a relative risk (RR) of 2.5 for new infections, a prevalence of a risk factor of 10% in the unexposed population, to achieve 80% power and 95% confidence, 280 meat workers had to be sampled twice. The number was doubled to consider a design effect due to sampling at several abattoirs (Dohoo et al., 2003b).

3.1.2. Serological testing

Ten ml of blood were collected into Beckton Dickenson Vacutainer® Plus tubes, coated with silicone and micronized silica particles to accelerate clotting, stored between 4º and 10º C in a mobile fridge, and couriered within 24 hours in an icepack cooled Biocontainer© to the Molecular Epidemiology and Public Health Laboratory at Massey University in Palmerston North, NZ. After centrifugation at 3000 rpm for six minutes, the serum was aliquoted into duplicate cryovials and microtitre plates and stored at -80º C.

The microscopic agglutination test (MAT) was used to measure serum antibodies against Pomona and Hardjobovis at doubling dilutions from 1:24 to 1:1536 as described previously (Faine et al., 1999). The MAT was always performed by the same trained laboratory technician. A titre cut-off of 1:48 was used to declare that a worker was previously exposed to leptospires (Faine et al., 1999;

Shivakumar and Krishnakumar, 2006b).

3.1.3. Study population and case definitions

The study population comprises all workers who were sampled at least twice. Some workers (*n*=57) in one sheep abattoir (abattoir 'sheep 1') were sampled over two study periods, hence their infection rates were measured twice (up to four blood samples per participant). All workers who were sero-positive at the beginning of the sampling period were retained in the study population, as they remained at risk of getting infected with another *Leptospira* serovar or re-exposed, the latter being called an 'anamnestic response'.

Cumulative Incidence: a worker who either sero-converted or who had an anamnestic response against Pomona and/or Hardjobovis between the first and second sample was defined as newly infected and contributed to incidence. Seroconversion occurred where a worker who was sero-negative had a MAT titre change to 1:48 or with a MAT titre of 1:24 at first sampling, had a two-fold increase in titre at second sampling, hence changed to a MAT titre of ≥1:96. If an initial MAT titre of ≥1:48 increased at least two-fold between the first and second sampling, the worker had an anamnestic response*,* for example a titre change from 1:48 to 1:192. The transitions are illustrated in Figure 1. The incidence of workers reporting flu-like illness between sampling dates was compared between seroconverting and anamnestic response groups to provide evidence for the assumption that both definitions equally indicated a new infection.

Probable clinical leptospirosis was determined as a worker reporting having been diagnosed with leptospirosis of any serovar by a health professional between the two sampling times, on the basis of clinical symptoms with or without confirmation by laboratory test.

Possible clinical leptospirosis was determined as a worker reporting to have had 'flu-like' illness and having sero-converted or showed an anamnestic response between the two sampling times but without confirmation by a health professional, and not being in the above category.

'Flu-like' illness was defined as an event of illness associated with fever, headache, arthralgia, myalgia, lethargy, nausea/vomiting and/or photo-sensitivity and includes the above two categories. Workers were explained that the symptoms had to be severe enough that they felt like going home and rest.

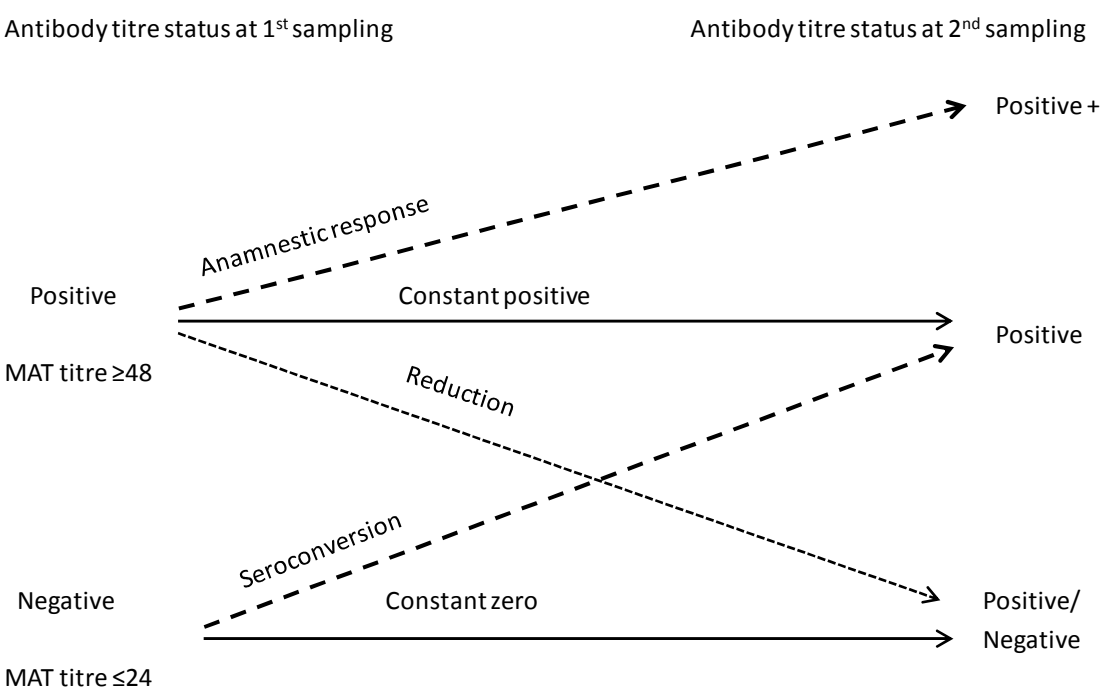

Figure 1: Illustration of the transition of serum antibody titres against *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis between first and second samplings.

3.2. Data Analysis

Questionnaire information and serological test results were entered into an Access© database and analyzed using Microsoft Excel©, Stata 10 (©StataCorp. LP) or SAS (SAS Institute Inc., Cary, NC, USA). Accuracy of data entry was validated by randomly selecting 5% of the questionnaires from each abattoir and comparing them with manual questionnaire entries.

Exploratory data analysis was conducted to find missing observations and outliers using histograms, 2x2 tables and summary measures. To determine correlations among risk factors, potential confounders and MAT results, we conducted chi-square tests for categorical variables and Pearson correlation for continuous variables.

3.2.1. Outcome and risk factors

The four outcomes of interest were (i) a 'new infection' with Hardjobovis and/or Pomona (by sero-conversion or anamnestic response), (ii) an episode of 'probable clinical leptospirosis', or (iii) 'probable clinical leptospirosis' between samplings and (iv) whether a worker experienced 'flu-like' symptoms. The latter outcome (iv) includes the outcomes (ii) and (iii).

Information on work- and non-work-related potential risk factors included current work positions for the current season and past work positions (for three former seasons). In order to

understand the risk of infection in different work positions in sheep abattoirs, we allocated work position into four categories based on possible exposure to urine or to organs of the urinary tract: Category 1 (reference category) workers were from the 'boning' room (where the carcass is cut into pieces), the 'chillers', 'freezers', 'blood processing' and office; Category 2 workers were from the 'offal'/'casing'/'pet food' rooms (where organs were handled) and the hide processors, cleaners, renderers and engineers; Category 3 included workers in the middle and end of the slaughter board (where animals were opened, organs removed and carcasses were inspected); Category 4 were workers in the yards (where animals were washed and waiting for slaughter) and at the beginning of the slaughter board (where animals were stunned, bled and hides were removed). The slaughter board contained workers from Categories 3 and 4, but excluded workers from the 'yards'.

Workers were asked about the type and frequency of personal protective equipment (PPE) worn for every task in the abattoir. Types were defined as 'facemasks', 'safety or normal glasses', 'gloves on one or two hands' and 'balaclava or beard mask'. Frequency categories were 'always' (1), 'often' (2), 'sometimes' (3) or 'never' (4). For increased statistical power, PPE were also analysed as two categories by merging PPE type categories 1 and 2, and 3 and 4. Of further interest were years worked in an abattoir, number of months worked in the study and three previous seasons, lifestyle (hunting, farming, home slaughtering, outdoor activities in the study year and the last three years) and personal data such as age, gender, type of residence and ethnicity. Workers were asked whether they had been diagnosed with leptospirosis or whether they had experienced 'flu-like' symptoms in the study year, how many days they were absent from work and whether they had received financial compensation from the worker occupational disease insurance (Accident Compensation Corporation, ACC) . The risk factor variables were described by worker frequency and sheep (Tables 5 & 6), deer and beef (Tables 1 & 2) abattoirs.

3.2.2. New infection risk and titre duration

The abattoir-specific cumulative annualised incidence or risk of infection (%) given by serological evidence was calculated as number of new infections with Hardjobovis and/or Pomona divided by the sum of days between samplings of all participating workers and multiplied by 365. Confidence intervals were calculated by the Fleiss method (Fleiss, 1981). For sheep abattoir workers, the new infection risk was shown for each exposure variable described in Tables 5 and 6.

The duration of the antibody titre (D) over the threshold of 1:48 following infection of sheep abattoir workers was derived from the relationship between the mean sero-prevalence at first sampling (P) and the serological mean study period incidence (I) for the serovars Pomona or Hardjobovis ($_i$) as described in Dohoo et al. (Dohoo et al., 2010). Hence, the duration of the antibody

titre is the average time for a sheep meat worker between having a MAT titre higher than 1:48 to returning to a titre below 1:48 following a typical infection episode.

$$D_i = \frac{P_i}{(1 - P_i) \cdot I_i}$$

3.2.3. Risk factors for new infection with *Leptospira* in sheep abattoir workers

Crude associations between the risk of infection with Hardjobovis and/or Pomona and putative risk factors or confounding factors listed in Tables 5 and 6 were calculated for sheep abattoir workers by univariable and multivariable logistic regression. A forward selection method with a Wald/Chi square test p-value of 0.3 as an entry criterion was chosen to evaluate risk factors and confounding variables in the multivariable model, starting with a null model with only an intercept included and then adding one risk factor at a time. A risk factor was retained if the Likelihood Ratio Test (LRT) was statistically significant at a p-value ≤0.05 or if its presence changed the crude coefficient of work position and/or fitted risk factor variables in the model by ±15%.

Interaction between risk factors was tested by multivariable logistic regression. If the LRT was statistically significant (p ≤0.05) the interaction term was retained in the model. The tested interaction terms were 'gender*wearing gloves', 'work position*gender', 'work position*wearing gloves', 'work position*wearing safety/normal glasses', 'wearing gloves*abattoir' and 'wearing safety/normal glasses*abattoir'.

Because the time at risk was known and each participant had been followed through the risk period, relative risks (RR) were used for the interpretation of risk factors. The method for calculation is described by McNutt et al. (McNutt et al., 2003).

Since 57 persons from abattoir 'Sheep 1' participated twice in the study, over-dispersion was estimated to decide whether adjustment for clustering due to repeated measurements was required in the analysis. Over-dispersion was declared present if the ratio between the residual Pearson Chi-square and residual degrees of freedom was greater than 1.5 (McDermott and Schukken, 1994).

To see whether any new infection with Pomona was associated with pig hunting or farming, we individually addressed this question in a logistic regression model.

We tested the assumption of linearity for the continuous risk factor variables. The Hosmer-Lemeshow statistic was used to test the distributional assumption and the Pseudo R-square was used to evaluate the overall model fit. Influential covariate patterns and leverage were examined using described methods (Hosmer and Lemeshow, 2000).

3.2.4. Illness and population impact

The incidence of probable clinical leptospirosis cases was calculated. The frequency, serological status, time away from work and whether they received compensation were described. To see whether *Leptospira* antibody titres were higher for workers with 'flu-like' symptoms, compared to those without, we performed the Wilcoxon rank-sum (Mann-Whitney) test.

The association between putative risk factors and having 'flu-like' symptoms, including workers with possible and probable leptospirosis, was analysed by multivariable logistic regression. The proportion of illness cases that could be attributed to a *Leptospira* infection (Population Attributable Fraction, PAF) among all workers was calculated by dividing the absolute difference between the risk in the total population and the risk in the unexposed group by the risk in the total population (Brady, 1998). The average annual risk of experiencing flu-like symptoms due to infection with *Leptospira* in sheep abattoirs was estimated by multiplying the PAF by the total risk of having flu-like symptoms in the total study population. The contribution of work position to the proportion of cases with new infection with Hardjobovis and/or Pomona in the exposed and total sheep abattoir worker population was estimated by calculating the PAF (Table 8). For this question, worker position was divided into two categories: 'workers from the slaughter board, yards and offal' and 'workers from other positions'.

The incidences of probable and possible clinical leptospirosis cases and the PAF were extrapolated to the total sheep abattoir worker population to estimate the impact of leptospirosis on the sheep abattoir work force. For the estimation of the degree of under-ascertainment of officially notified leptospirosis cases, we compared the proportion of notified leptospirosis cases from the meat industry (n ~ 25,000 workers) between 2005 - 2010[26] (between 14 and 42 cases per year), with the proportion of possible and probable leptospirosis cases in the sheep abattoir worker study population.

The economic human health impact was calculated and described as the number of days away from work due to probable or possible leptospirosis.

4. Results

Sixty-one randomly chosen questionnaires from 1148 were evaluated for data entry errors. Each questionnaire contained at least 70 questions. We found 11 entry errors, hence the error rate was

[26] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2005-2010

11/(70x61) = 0.002%. Thus, an estimated 99.8% entries were correct, and this was deemed acceptable.

The participation proportion in the first sampling was on average 32% of all workers with a range of 11-61% between abattoir. At the first blood sampling 809 workers participated but 211 (27%) were lost to follow-up i.e. the second sample, resulting in a final study population of 592 workers. For 173 workers we were given a comment for their loss to follow-up: 54 withdrew from the study (mainly for fear of pain at sampling), one died, one was on maternity leave, two were not released from their work position during sampling, 67 had already left work for the day and were unavailable, 29 had left employment at the abattoir or were laid off for the season, and 20 were absent for unknown reasons. Fifty-seven workers in abattoir Sheep 1 participated over both years and were hence sampled four times.

The number of workers by abattoir ranged from 21-135 (sheep), 58-100 (beef) and 18-32 (deer) with a total of 384 sheep, 50 deer and 158 beef abattoir workers participating (Table 4). The sero-prevalence against Hardjobovis and/or Pomona measured at the first sampling was on average 13% in sheep, 17.5% in deer and 5% in beef abattoir workers.

The frequency of workers wearing PPE differed by abattoir species and abattoir ($p \leq 0.001$). Whereas in sheep abattoirs on average 70% (range 47-81% between abattoirs) of slaughter board, yards and offal room workers reported wearing gloves on both hands (always or often), 4% (range 0-8% between abattoirs) of workers did in deer and 88% (range 84-94% between abattoirs) in beef abattoirs. Seventy-three percent of sheep (range 10-88% between abattoirs), 52% of deer (range 31-80% between abattoirs) and 29% (range 20-43% between abattoirs) of beef slaughter board, yards and offal room workers reported wearing safety/normal glasses and 26%, 9% and 0%, respectively, wore facemasks (always or often). There were also statistically significant differences for specific work positions in sheep abattoirs ($p \leq 0.004$): while 89% of persons working in the stunning area of sheep abattoir 1 reported wearing glasses, 12% did in sheep abattoir 3; whereas 89% of persons working in the stunning area of sheep abattoir 2 reported wearing gloves, 25% did in abattoir 3.

The distribution of workers by exposure variable categories is presented in Tables 1 and 2 for deer and beef and in Tables 5 and 6 for sheep abattoirs.

Table 1: Frequency (% and no.) of workers in each work related risk factor variable category in deer and beef abattoir workers

Risk factor	Deer Category	% Workers (n)	Beef Category	% Workers (n)
Work position	1. Boning, chillers, office	54.0 (27)	1. Boning, chillers, office	45.6 (72)
	2-4. Offal, pet food, gut removal, meat inspection, pulling kidneys, yards, stunning, hide removal	46.0 (23)	2. Maintenance, engineers	5.1 (8)
	-	-	3. Offal, pet food	10.8 (17)
	-	-	4. Pulling kidneys, meat inspection, yards, stunning, hide removal	38.6 (61)
Abattoir	Deer1	36.0 (18)	Beef1	36.7 (58)
	Deer2	64.0 (32)	Beef2	63.3 (100)
Wear gloves on both hands	never	68.0 (34)	never	27.9 (44)
	sometimes	14.0 (7)	sometimes	5.1 (8)
	often	4.0 (2)	often	5.1 (8)
	always	14.0 (7)	always	62.0 (98)
Wear goggles or glasses	never	60.0 (30)	never	63.9 (101)
	sometimes	8.0 (4)	sometimes	10.1 (16)
	often	2.0 (1)	often	1.3 (2)
	always	30.0 (15)	always	24.7 (39)
Wear a facemask	never or sometimes	96.0 (48)	never or sometimes	99.4 (157)
	often or always	4.0 (2)	often or always	0.6 (1)
Wear a balaclava	never or sometimes	88.0 (44)	never or sometimes	93.7 (148)
	often or always	12.0 (6)	often or always	6.3 (10)
Months worked at current abattoir	≤72	17.2 (66)	≤72	27.9 (44)
	>72 - 108	12.2 (47)	>72 - 126	22.2 (35)
	> 108-168	16.4 (63)	> 126-252	26.0 (41)
	>168	54.2 (208)	>252	24.1 (38)
Months worked in meat industry	≤96	28.1 (108)	≤72	30.4 (48)
	>96- 144	21.9 (84)	>72- 144	21.5 (34)
	> 144-300	26.3 (101)	> 144-348	24.1 (38)
	>300	23.7 (91)	>348	24.1 (38)
Smoking at abattoir	No	68.0 (34)	No	77.2 (122)
	Yes	32.0 (16)	Yes	22.8 (36)
Urine splashed in face	No	72.0 (36)	No	77.2 (122)
	Yes	24.0 (12)	Yes	18.4 (29)
	Don't know/maybe	4.0 (2)	Don't know/maybe	4.4 (7)

Table 2: Frequency (% and no.) of workers with clinical leptospirosis, demographic and non-work related risk factor variables in deer and beef abattoir workers

Risk factor	Deer Category	% Workers (n)	Beef Category	% Workers (n)
Probable clinical leptospirosis	No	100.0 (50)	No	100.0 (158)
	Yes	0.0 (0)	Yes	0.0 (0)
Had flu-like-illness	No	68.0 (34)	No	77.9 (123)
	Yes	32.0 (16)	Yes	22.2 (35)
Possible leptospirosis	No	50.0 (100)	No	100.0 (158)
	Yes	0.0 (0)	Yes	0.0 (0)
Gender	Female	14.0 (7)	Female	22.2 (35)
	Male	86.0 (43)	Male	77.9 (123)
Age	≤32	26.0 (13)	≤ 36	25.3 (40)
	>32, ≤42	24.0 (12)	>36, ≤49	24.7 (39)
	>42, ≤48	28.0 (14)	>49, ≤58	29.8 (47)
	>48	22.0 (11)	>58	20.3 (32)
Hunting pigs, deer or feral goats	No	82.0 (41)	No	94.3 (149)
	Yes	18.0 (9)	Yes	5.7 (9)
Farming[1]	No	82.0 (41)	No	86.1 (136)
	Yes	18.0 (9)	Yes	13.9 (22)
Ethnicity	NZ European	88.0 (44)	NZ European	43.0 (68)
	NZ Maori	6.0 (3)	NZ Maori	43.7 (69)
	Other	6.0 (3)	Other	13.3 (21)
Slaughtering at home[2]	No	72.0 (36)	No	81.0 (128)
	Yes	28.0 (14)	Yes	19.0 (30)

[1]pigs, goats, sheep, beef cattle, alpaca or deer; [2]sheep, goats, pigs, beef or deer

4.1.1. Antibody titres, new infection and titre duration

Table 3 shows the proportion of workers in each category of antibody titre changes from first to second sampling against Hardjobovis, Pomona or both. Forty seroconversions and 12 anamnestic responses against either Pomona and/or Hardjobovis were observed in 49 workers i.e. 49 new infections during the study period. Three workers sero-converted or had an anamnestic response against both serovars. The titres against Hardjobovis and Pomona ranged for both serovars from 1:24 - 1:768, with a median of 1:96 for the positive titres (1:24-1:768).

Forty-seven of 49 newly infected workers were from sheep abattoirs and two from beef abattoirs. A higher proportion of workers developed antibodies against Pomona than against Hardjobovis (9.4 vs. 3.6%, p=0.02).

Table 3: Proportion of workers from each abattoir type that had each category of antibody titre changes against *Leptospira interrogans* sv Pomona (Pom) and/or *Leptospira borgpetersenii* sv Hardjobovis (Har) or against either of these two serovars between first and second sampling

Plant (# participants)	Antibody titre change	Har %* (n)	Pom %* (n)	Har&/orPom %[1] (n)
Sheep (N=384)	Anamnestic	1.0 (4)	2.1 (8)	3.1 (12)
	Seroconversion	2.6 (10)	7.3 (28)	9.6 (37)
	Constant (Zero)	89.3 (343)	85.7 (329)	95.3 (366)
	Constant (Pos)	4.2 (16)	4.7 (18)	7.8 (30)
	Reduction	2.9 (11)	0.3 (1)	3.1 (12)
Deer (N=50)	Anamnestic	0.0 (0)	0.0 (0)	0.0 (0)
	Seroconversion	0.0 (0)	0.0 (0)	0.0 (0)
	Constant (Zero)	84.0 (42)	94.0 (47)	98.0 (49)
	Constant (Pos)	12.0 (6)	2.0 (1)	14.0 (7)
	Reduction	4.0 (2)	4.0 (2)	8.0 (4)
Beef (N=158)	Anamnestic	0.0 (0)	0.0 (0)	0.0 (0)
	Seroconversion	0.6 (1)	0.6 (1)	1.3 (2)
	Constant (Zero)	93.7 (148)	97.5 (154)	98.7 (156)
	Constant (Pos)	0.6 (1)	1.3 (2)	1.9 (3)
	Reduction	5.1 (8)	0.6 (1)	5.7 (9)

[1]Calculated as a proportion of N (species specific). The 'Har&/orPom' column does not have to sum the 'Har' and 'Pom' columns. It does sum up in the 'Har&/orPom' column if an event occurs for one or the other serovar, however, if the event occurs for both serovars , it will only be counted once in the 'Har&/orPom' column.

The annual abattoir-specific infection risk (cumulative incidence, %) with Pomona and/or Hardjobovis was on average 7.7% (range 0.0-16.4%). While in sheep abattoir workers, the annual infection risk was 11.1% (95% CI 8.5-14.8, range 8.4-16.4%), in deer abattoir workers it was 0.0% and in beef abattoir workers 1.2% (95% CI 0.2-4.6, range 1-1.5%) (Table 4).

Table 4: Plant-specific annual infection risk (or cumulative incidence) (%) with *Leptospira interrogans* sv Pomona (Pom) or *Leptospira borgpetersenii* sv Hardjobovis (Har) by abattoir

Plant	No. of workers	Har	95% CI	Pom	95% CI	Har &/or Pom	95% CI
Sheep1	82	3.1	0.8-9.5	8.3	3.9-16.1	11.5	6.2-19.8
Sheep1[1]	135	6.4	4.5-15.7	3.9	1.6-8.5	8.4	4.7-14.1
Sheep2	68	0.0	0.1-6.2	16.4	9.2-27.2	16.4	7.6-22.9
Sheep3	21	4.2	0.2-22.8	8.4	1.5-28.1	12.6	3.3-32.9
Sheep4	78	0.0	0.1-6.1	10.7	5.1-20.6	10.7	5.1-20.6
Deer1	18	0.0	0.5-21.6	0.0	0.5-21.6	0.0	0.5-21.6
Deer2	32	0.0	0.3-13.3	0.0	0.3-13.3	0.0	0.3-13.3
Beef1	58	1.5	0.1-9.3	0.0	0.1-6.9	1.5	0.1-9.3
Beef2	100	0.0	0.1-4.5	1.0	0.1-6.1	1.0	0.1-6.1
Total	592	2.3	1.4-4.0	5.8	4.2-8.0	7.7	5.8-10.1

[1]Abattoir 1 took part in the study in two consecutive years (2008-09 and 2009-10); however apart from 57 persons, the study population was composed of different persons.

The average titre duration of antibodies against Pomona was 10 and against Hardjobovis was 29 months. This means, for example, that on average a sheep plant worker will have a MAT titre against Pomona above 1:48 for 10 months following a typical infection episode with Pomona.

4.1.2. Risk factors for serological evidence for new infection with *Leptospira* in sheep abattoirs

Because of low/no numbers of newly or re-infected workers in the beef and deer abattoirs, associations between exposure variables and new infection were only analysed for workers at sheep abattoirs. Tables 5 and 6 present new infection rates of workers at sheep abattoirs by serovar and exposure categories. Unconditional analysis rendered the following variables to be significantly and positively associated with the risk of a new infection ($p < 0.05$): 'work position' and 'months worked in the meat industry'.

Table 5: Frequencies of work related risk factors and their unconditional association with new infection with *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in sheep abattoir workers (*n*=384)

Risk factor	Category	% Workers (n)	New infection %	Crude RR	95% CI	P-value
Work position	1.Boning, chillers, office	37 (142)	2.8	Ref		
	2.Offal, pet food	11.5 (44)	9.1	3.2	(0.8-12.9)	0.098
	3.Gut & kidney removal, meat inspection	22.9 (88)	14.8	5.2	(1.7-16.1)	0.004
	4.Yards, stunning, hide removal	28.7 (110)	23.6	8.4	(2.9-24)	<0.01
Wear gloves on both hands	never	27.3 (105)	9.5	Ref		
	sometimes	7.6 (29)	3.4	0.4	(0.0-2.8)	0.333
	often	4.7 (18)	22.2	2.3	(0.7-7.4)	0.152
	always	60.4 (232)	13.8	1.4	(0.7-2.9)	0.307
Wear goggles/ glasses	never	36.5 (140)	8.6	Ref		
	sometimes	6.8 (26)	7.7	0.9	(0.2-4.0)	0.887
	often	3.4 (13)	7.7	0.9	(0.1-6.9)	0.917
	always	53.4 (205)	15.6	1.8	(0.9-3.5)	0.077
Wear a facemask	never or sometimes	83.3 (320)	12.2	Ref	(0.0-0.0)	
	often or always	16.7 (64)	12.5	1.0	(0.5-2.2)	0.948
Wear a balaclava	never or sometimes	73.4 (282)	11.7	Ref		
	often or always	26.6 (102)	13.7	1.2	(0.6-2.2)	0.617
Months worked at current abattoir[1]	≤72	17.2 (66)	9.1	Ref		
	>72 - 120	16.4 (63)	17.5	1.9	(0.7-5.2)	0.198
	> 120-216	13.8 (53)	11.3	1.2	(0.4-3.9)	0.704
	>216	52.6 (202)	11.9	1.3	(0.5-3.2)	0.558
Months worked in meat industry	≤84	28.1 (108)	5.6	Ref		
	>84 - 198	21.9 (84)	14.3	2.6	(1.0-6.9)	0.059
	> 198-324	26.3 (101)	7.9	1.4	(0.5-4.1)	0.511
	>324	23.7 (91)	23.1	4.2	(1.7-10.3)	0.002
Smoking at work	No	71.6 (275)	12.4	Ref	[3]	
	Yes	28.4 (109)	11.9			
Urine splashed in face[2] (n 345)	No	73.3 (253)	13.4	Ref		
	Yes	25.2 (87)	11.5	0.9	(0.4-1.7)	0.664
	Don't know/maybe	1.5 (5)	20.0	1.5	(0.2-10.9)	0.695
Abattoir	Sheep 1 [4]	21.4 (82)	13.4	Ref		
	Sheep 1	35.2 (135)	9.6	0.7	(0.3-1.6)	0.418
	Sheep 2	17.7 (68)	17.6	1.3	(0.6-3.0)	0.511
	Sheep 3	5.5 (21)	14.3	1.1	(0.3-3.8)	0.923
	Sheep 4	20.3 (78)	10.3	0.8	(0.3-1.9)	0.563

[1]n=242; [2]n=242; [3]model did not run; [4]Abattoir Sheep 1 took part in the study in two consecutive years (2008-09 and 2009-10), however apart from 57 persons, the study population was composed of different persons.

Table 6: Frequencies of clinical, demographic and non-work related risk factors and their unconditional association with new infection with *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in sheep abattoir workers (*n*=384)

Risk factor	Category	% Workers (n)	New infection %	Crude RR	95% CI	P-value
Probable clinical Leptospirosis[1]	No	99.0 (380)	11.9	-	-	-
	Yes	1.0 (3)	50.0			
Had flu-like-illness[2]	No	73.4 (279)	9.3	-	-	-
	Yes	26.6 (101)	20.8			
Possible leptosirosis[3]	No	94.3 (22)	0.0	-	-	-
	Yes	5.7 (362)	100.0			
Gender	Female	33.3 (128)	7.8	Ref		
	Male	66.7 (256)	14.5	1.9	(0.9-3.7)	0.084
Age	≤40	25.8 (99)	10.1	Ref		
	>40, ≤50	25.0 (96)	9.4	0.9	(0.4-2.3)	0.871
	>50, ≤57.5	24.2 (93)	16.1	1.6	(0.7-3.6)	0.252
	>57.5	25.0 (96)	13.5	1.3	(0.6-3.1)	0.486
Hunting pigs, deer or feral goats	No	92.5 (355)	12.1	Ref		
	Yes	7.6 (29)	13.8	1.1	(0.4-3.2)	0.804
Farming[4]	No	83.9 (322)	12.7	Ref		
	Yes	16.2 (62)	9.7	0.8	(0.3-1.8)	0.53
Ethnicity	NZ European	42.7 (164)	9.1	Ref		
	NZ Maori	49.2 (189)	14.8	1.6	(0.9-3.0)	0.132
	Other	8.1 (31)	12.9	1.4	(0.5-4.3)	0.541
Slaughtering at home[5]	No	83.3 (320)	12.5	Ref		
	Yes	16.7 (64)	10.9	0.9	(0.4-2.0)	0.744

[1]was not included in the logistic regression model, as it was an intermediate variable between exposure and antibody level, n=383; [2]n=380; [3]was not included in the logistic regression model, as it includes the outcome; [4]pigs, goats, sheep, beef cattle, alpaca or deer; [5]sheep, goats, pigs, beef or deer.

Risk factors significant in the final LR model were 'work position', 'time worked in the meat industry' and 'Abattoir 2' (Table 7). While being highly significant, the dichotomous variable 'probable leptospirosis' was not included in the multivariable model, as it could have been caused by exposure and likely to have caused an increase in serum-antibody, hence not an independent risk for a new infection. Compared to the workers in the office, boning room or chillers (Category 1, reference group), workers in the offal room (Category 2) had 4 (p=0.05) times the risk, workers at the end and middle of the slaughter floor (Category 3) had 5 times the risk (p=0.004) and workers at the beginning of the slaughter floor (Category 4) 7.5 times the risk (p<0.001) of getting newly infected with Pomona and/or Hardjobovis, once controlled for the effect of time worked in the meat industry and working in Abattoir 2. Participants who worked more than 72 months were at 3 (p=0.03) times the risk of becoming sero-positive than persons working up to 72 months in the meat industry once controlled for the effect of work position and Abattoir 2. Persons working in Abattoir

2 had twice the risk of infection, compared to the other abattoirs (p=0.05). None of the tested confounders or interactions were significant. Further, the over-dispersion factor was <1, hence a variance adjustment for repeated sampling of the same worker in two subsequent years was not required.

The model diagnostics indicated a sufficient fit of the data. Even though one outlier was identified, its removal did not change any of the model coefficients by more than 15% and hence, did not impact on the inferences from the analysis.

Table 7: Multivariable logistic regression model: significant risk factors and statistical parameters for new infection with *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis in abattoir workers processing sheep (*n*=384)

Risk factor	Category	RR	95% CI	P-value
Work position	Boning, chillers, office	Ref		
	Offal, pet food	4.1	(1.0-16.4)	0.048
	Gut removal, meat inspection, pulling kidneys	5.2	(1.7-16.0)	0.004
	Yards, stunning, pelting	7.5	(2.5-22.4)	<0.001
Months worked in meat industry	≤72	Ref		
	>72 - 180	3.0	(1.1-7.9)	0.032
	> 180-324	1.3	(0.4-3.9)	0.643
	>324	3.0	(1.1-7.9)	0.026
Abattoir	1 (C1),3,4,1(C2)[1]	Ref		
	2	2.0	(1.0-3.9)	0.046

The Log likelihood of the nested model was -127.2; [1]sheep abattoir 1 (C1) is the same as sheep abattoir 1 (C2), but sampled in a different year with 57 of 135 participants being resampled.

4.1.3. Illness and population impact

Working on the slaughter board, yards or in the offal rooms (categories 1-3) increased the annual risk of new infection by serological evidence 6.3-fold (95% CI 2.3-17.2, p≤0.001). This was equivalent to 77% new infections in the total study population being attributable to working on the slaughter board, in yards or offal rooms (PAF) (Table 8).

The annual risk of probable clinical leptospirosis was 0.78% (3/384, 95% CI 0.20-2.46%) with all cases occurring in two different sheep plants in the second study period. The three probable clinical leptospirosis cases constituted 6.3% (95% CI 1.6-18.6) of all new infections in sheep abattoir workers. Two of those sero-converted from negative and 1:48 to 1:192 against Pomona. The third, who neither sero-converted nor 'boosted', had a positive titre of 1:192 against Pomona in both sampling times and a decaying titre from 1:96 to 1:48 against Hardjobovis. All three were males, between 43-67 years old and worked in sheep abattoirs in the area where the pelt is cut open (beginning of the slaughter board), guts removal or offal room area. They reported being constantly

exposed to organs of the urinary tract or to urine, and found the protective gear to be unpleasant. One person commented that gloves were getting contaminated with urine and that glasses hurt behind the ears, another complained about difficult breathing using the facemask, and the third that facemasks were too hot. Two reported to wearing gloves and one a facemask (always or often). For one person there was no information on PPE available. They reported having been 0, 3 and 84 days, respectively, away from work due to leptospirosis. All three reported not having received ACC compensation.

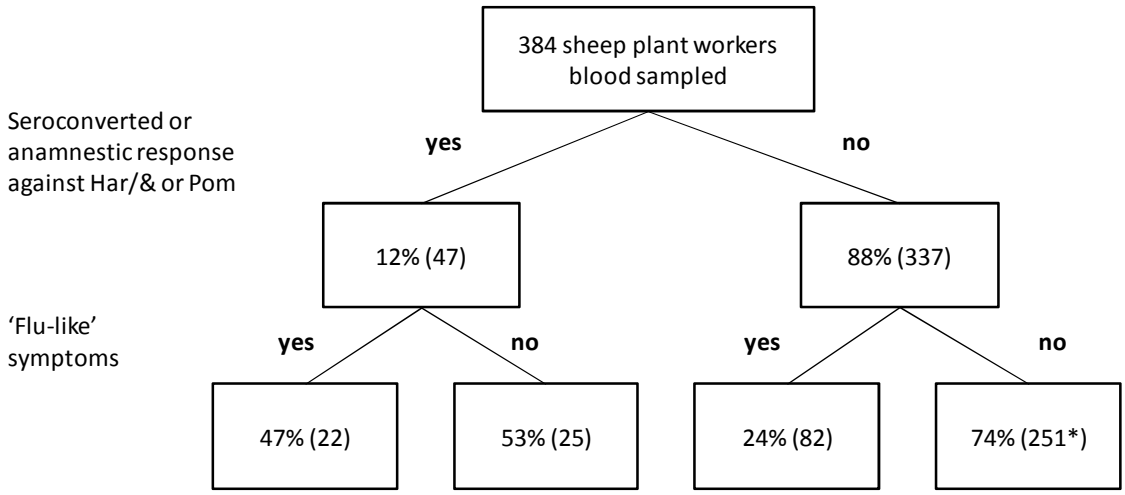

Figure 2: Occurrence of serological response to *Leptospira interrogans* sv Pomona (Pom) and/or *Leptospira borgpetersenii* sv Hardjobovis (Har) and of 'flu-like' symptoms among 384 sheep abattoir workers, including probable and possible clinical leptospirosis cases.*Four non-infected workers had missing data on 'flu-like' symptoms

Since information on 'flu-like' symptoms was missing for four persons, only data from 380 of 384 sheep abattoir workers could be used in the analysis. A total of 104/380 (27.4%, 95% CI 23.0-32.2) sheep abattoir workers including 22/47 (46.8%, 95% CI 32.4-61.8) with serological evidence of new infections and 82/333 (24.6%, 95% CI 20.2-29.7) without, reported to have had 'flu-like' symptoms during the one year study period. Four workers who did not seroconvert had missing data regarding 'flu-like' symptoms (Figure 2). Workers with 'flu-like' symptoms had significantly higher titres against Pomona than those without 'flu-like' symptoms (p=0.02). Hardjobovis titres of workers with 'flu-like' symptoms did not differ from those without 'flu-like' symptoms. Table 8 summarizes data of *Leptospira* infection related to the incidence and proportion of 'flu-like' illness in the total sheep abattoir study population. New infections with *Leptospira* increased the risk of illness with 'flu-like' symptoms 1.9-fold (95% CI 1.3-2.7, p=0.007). Assuming causality, in those who experienced new

infection, 10% (PAF) of 'flu-like' cases were attributable to new infection with Pomona and/or Hardjobovis. The average annual risk of a worker, over all workplaces, experiencing 'flu-like' symptoms due to infection with *Leptospira* was 2.7% (95% CI 0.9-4.8%).

Table 8: The risk, relative risk and population attributable fraction (PAF) of sheep abattoir workers of having 'flu-like' illness when newly infected with *Leptospira interrogans* sv Pomona and/or *Leptospira borgpetersenii* sv Hardjobovis and the risk, relative risk and PAF of having a new infection when working at the slaughter board, yards or offal room (one category) or in other positions

Outcome	Risk factor - Category	Risk	RR (95% CI)	PAF (95% CI)	p-value
Flu-like symptoms	New infection				
	-Yes	0.47	1.9 (1.3-2.7)	0.10 (0.02-0.16)	0.007
	-No	0.25			
New infection	Working				
	-At slaughter board, in yards or offal room	0.18	6.3 (2.3-17.2)	0.77 (0.42-0.90)	<0.001
	-In other positions	0.03			

The under-ascertainment of officially notified cases of leptospirosis was estimated at between 16 and 56 times based on data reported in the past five years[27]. However, this rate includes persons with the mild form of leptospirosis with flu-like symptoms.

The total number of days absent from work due to 'flu-like' symptoms of 104 sheep abattoir workers between the two sampling times was 427 days and for the 22 workers who experienced a new infection and 'flu-like' symptoms it was a total of 80 days. The average time away from work per newly infected worker was 4.4 days (95% CI 2.7-6.1).

5. Discussion

The novel information in this study arises from combining serological data with personal illness episodes to result in pathogen attributable disease incidence. We estimated to what extent abattoir workers, who were subjected to a seemingly high level of exposure to sheep carcasses shedding *Leptospira* (Dorjee et al., 2011), acquired serological evidence of infection and developed clinical disease consistent with leptospirosis. The economic impact of this disease was quantified by inquiry as days absent from work in the preceding 12-months period. In sheep abattoirs, 12% of the workforce showed serological evidence of a new infection with Hardjobovis or Pomona in one calendar year. About 77% of infections were silent (non-clinical) whereas 23% infected workers reported signs of leptospirosis: 47% of infected vs. 24% of non-infected workers experienced 'flu-

[27] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2005-2010

like' symptoms and 2.7-6.1 days absence from work. Extrapolated to the total workforce at NZ sheep plants of approximately 10'000, this means approximately 276 workers are getting ill with leptospirosis every year due to working at an abattoir and about 1,200 total work-days lost. The participants should represent roughly the NZ meat worker population, as on average about 45% of the total number of workers in the participating abattoirs worked on the slaughter board and 44% in the boning, chilling area or offices (hence high and low risk areas were equally distributed). Persons working in the offal or pet food area or cleaners and engineers, hence in the middle risk areas, were underrepresented (11%) though (data notshown) (Dreyfus et al., 2010b).

To accept a leptospirosis case for compensation, the NZ national health insurance scheme (Accident Compensation Corporation, ACC) requires that a patient demonstrates a MAT titre ≥800 or a four-fold titre increase between two subsequent serological tests. Given a median titre of 192 (Pomona) and 96 (Hardjobovis) of workers who sero-converted or had an anamnestic response and had 'flu-like' illness, it seems likely that some of the infected and ill patients fail to comply with this condition claiming compensation, especially, if they were treated with antimicrobials, which may impede a sufficient titre increase after infection necessary to satisfy the ACC regulation (Levett, 2001). However, this is speculation, as the timing of sampling was at variable distance from the worker experiencing clinical disease, hence the data can not be translated into a diagnostic context and more data is needed during the illness and recovery phase for workers with 'flu-like' symptoms for accurate inferences about the degree of under-fulfilment of the ACC criteria.

Further, this study demonstrated a higher rate of illness than inferred from official surveillance statistics in NZ: the rate of clinical illness due to leptospirosis that was not diagnosed and reported was estimated to be 16-56 times higher than the official rate of notified cases. However, it is likely that not all 'flu-like' illness cases were actually leptospirosis cases, even though they were sero-positive, as the clinical signs in the case definition for probable leptospirosis cases were non-specific. Therefore, some 'flu-like' illness cases may genuinely have been due to influenza or another pathogen entirely. Further, probable leptospirosis cases included participants who were entirely classified by clinical and not laboratory diagnosis, leading to potential non-differential misclassification (by the doctor). Also, since our results on clinical leptospirosis relied on reporting of the study participants and were not verified by clinical or laboratory reports, there may have also occurred recall bias. Nevertheless, even if the 16-56 times higher clinical illness rate in the study was slightly overestimated due to the limitations mentioned above, the notified case rates may still underestimate the true rate of leptospirosis in the population due to a large number of undiagnosed illness episodes of moderate severity.

The data revealed differences in new infection risks between slaughter species and between

abattoirs. Workers in abattoirs processing sheep had a substantially higher annual risk of infection (11.1%) than workers processing deer (0.0%) or cattle (1.2%). A possible reason for the higher incidence in sheep abattoirs, despite similar infection rates among sheep and beef (Dreyfus et al., 2011), is that sheep abattoirs process more animals per day than cattle abattoirs and the different slaughter procedure. During interviews, participants reported that sheep urinate spontaneously when stunned, whereas cattle do not. Therefore, sheep abattoir workers may be more exposed to *Leptospira* than beef abattoir workers, especially when stunned sheep drop onto a platform contaminated with pools of urine from other sheep. Another speculative reason could be the variability in pathogenicity for humans within serovar strains infecting sheep and cattle.

Even though deer abattoir workers had a 17% sero-prevalence at the beginning of the study, the annual risk of infection during this study was 0%. Finding a high prevalence in the absence of measurable incidence suggests that workers were positive through continuous exposure and repeated infections whereas negative workers were much less exposed and therefore unlikely to get infected. This problem is enhanced by the small sample size (n=50), with 23 persons working in exposed positions (slaughter, offal), of which seven were already sero-positive at the beginning of the study. In order to have at least one sero-converting worker with 90% probability among the exposed, the annual risk of infection had to be at least nine percent when sampling and testing 23 workers with a test sensitivity of 90% and specificity of 99% (Cameron and Baldock, 1998). Hence, the true incidence in plants processing deer might have been up to 9% in highly exposed workers.

It should be noted that 'infection' has been inferred from serological evidence as this study did not attempt to measure leptospires in blood or urine, or "the entry, development or multiplication of the agent" as infection was defined by Last (Last, 2001). However, we believe serology to be a reasonable approximation because bacterial challenge is required to produce an immune response in the absence of vaccination, and an immune response was significantly associated with clinical disease.

Even though the ability of the MAT to distinguish between serovars had been questioned (Levett, 2001; Smythe et al., 2009), it is unlikely that this was the case for Hardjobovis and Pomona in this thesis, as these two belong to different serogroups. Hardjobovis and *Leptospira borgpetersenii* Balcanica (Balcanica) belong to the same serogroup, however given the sporadic nature of Balcanica in cattle, misclassification is not expected (Hathaway, 1981). Several studies have been conducted in NZ in recent years, where serovars determined by serology had been also confirmed by direct methods. For example, MAT serology and serovar isolates had good kappa agreement by DNA sequencing results (Subharat et al., 2011b; Subharat et al., 2012a).

In this study we measured prevalence of exposure not clinical disease and therefore we chose

a lower MAT titre cut-off (1:48) for a sero-positive case, than studies intending to detect clinical leptospirosis (1:≥100). Faine et al. (1999) and Shivakumar et al. (2006) suggested a titre cut-off of 1:50 to test exposure to *Leptospira* spp.. Similarly, since we did not measure clinical disease but exposure to *Leptospira*, a two-fold titre increase was deemed high enough to measure re-exposure (anamnestic response), still accounting for imprecise test reading by allowing a a two-fold titre increase.

The relative risk (RR) for a person to have 'flu-like' symptoms was similar in the anamnestic response and sero-conversion groups with a RR of 1.5 (p-value 0.26) and 1.8 (p-value 0.008), respectively, compared to persons without serological evidence of new infection. Commonly it is believed that a booster of the humoral immune system, which is measured by an anamnestic response, will extend the period of immunity, during which a person does not develop clinical symptoms. The data, however, suggests that repeated exposure may also lead to a new illness episode, albeit statistically insignificant.

The average titre duration of antibodies against Pomona was estimated to be 10 and against Hardjobovis 29 months, demonstrating that antibodies may persist longer than a year in an infected person. This is useful information for infectious disease modelling and to calculate incidence from more readily-available prevalence data. Thai et al. (Thai et al., 2008) show that in apparently healthy school children in an area in Vietnam with endemic leptospirosis, antibody titres can persist for longer than a year, as 61% of study participants had antibodies against any possible *Leptospira interrogans* serovar two years after first sampling. Both study methods are limited as there is no control for reinfection. Antibody titre persistence is strongly variable and depend on host and pathogen factors, such as immunity, silent or clinical infection, antibody titre, age of the host, infectious dose, serovar and serovar virulence (Lupidi et al., 1991; Cumberland et al., 2001).

The annual *leptospira* infection risk across the study population was 5.8% for Pomona and 2.3% for Hardjobovis, despite the fact that Hardjobovis was more sero-prevalent in workers at the beginning of the study, and also in the source animals, sheep, deer and beef (Dreyfus et al., 2011). In contrast, an earlier analysis of notified leptospirosis data found that the annual number of cases due to Pomona decreased from 62 in 1990 to 26 in 1996, while cases due to Hardjobovis increased from 23 to 30 (Thornley et al., 2002). Speculative reasons for the higher incidence of Pomona than Hardjobovis may be the difference in duration of antibody persistence, host specific susceptibility, a higher amount of shedding from Pomona infected carcasses, a difference in exposure between farmers and abattoir workers, or different trends in 1990/96 to 2008/09. Since Pomona is typically found in pigs (Bolt I, 1995), pig hunting or farming was tested as a risk factor for 'new infection with Pomona' in the multivariable model, but was not statistically significant.

Three workers sero-converted or had an anamnestic response against both serovars. Hence of all new infections, 1.5% was of dual nature. In the literature review no cohort study was found, which indicated concurrent infections with different *Leptospira* serovars. From our data it seems likely that dual infections with Leptospira serovars are rather rare. This may be due to the fact that serovars might be associated with different infectivity and/or pathogenicity in different hosts (e.g. Pomona vs Hardjobovis in cattle).

The study demonstrated that work position was the strongest risk factor for new infection with Pomona and/or Hardjobovis in sheep abattoir workers. The higher risk of infection at the beginning of the slaughter board and the gradual reduction along the slaughter line in sheep abattoirs is consistent with the sero-prevalence data from this study described in thesis Chapter 4 and with a study conducted two years earlier in one of the sheep abattoirs of this study (Heuer et al., 2010). Urine splashing due to stunning and subsequently contamination of pelts and carcasses are thought to be causes for infection, which may be difficult to control while handling carcasses. The risk of new infection of workers half way down the slaughter board may be attributable to exposure to *Leptospira* from organs of the genital-urinary tract. Evisceration may therefore pose another risk of infection, either when organs are removed from the carcass, processed or inspected. However, the time and place of exposure is better predictable in these positions than at the physically challenging positions at the head of the slaughter board. Persons in the other processing areas (boning room, chillers) or in the office had little or no exposure to urine and were therefore much less likely to get infected.

We did not have a control group outside of the workplace. Therefore, the background rate of sero-conversion can not be subtracted and the proportion of infections attributable to meat worker occupation cannot be derived. The incidence of 2.8% in workers from category 1 (office, boning room, chillers) is still higher than the incidence in the general population (2 per 100`000) ref ESR and therefore, can not be used as substitute.

The use of PPE appeared not to reduce the risk of infection, as PPE in the multivariable model did not show any evidence for protective effects. Biologically plausible reasons would be that workers wearing glasses may wipe their eyes to remove sweat with contaminated hands and letting the water run down into the gloves, thereby softening the skin weakening the dermal infection barrier. Adler et al (2010) postulate that *Leptospira* may enter through wet skin, (Adler and de la Pena Moctezuma, 2010) which had been denied before by Faine et al. (1999).

The finding that the PPE may not be protective warrants further investigation. Using PPE is for most tasks not practical and if workers are mandated to wear protective gear, it seems reasonable that there is a benefit of protection. If PPE is not sufficiently protective, other means of protection

should be considered, for example vaccination of farmed livestock.

No vaccine is currently available for humans in NZ. Since vaccination of dairy cows commenced in the 1980s, the incidence of notified human leptospirosis cases in the farming industry decreased from 234/100,000 to 90/100,000 (Marshall, 1996; Marshall and Manktelow, 2002; Thornley et al., 2002). Vaccination may also have the potential to protect farmers and farm workers, veterinarians and vet technicians, shearers, truck drivers of animal transporters, artificial insemination technicians and home butchers who are also at risk of infection (Marshall et al., 1979; Mackintosh et al., 1980a; Allen et al., 1982; Bolin and Alt, 2001; Subharat et al., 2012a).

Non-work related exposure such as age, hunting, farming, home slaughter or smoking were all unrelated to the risk of infection of workers at sheep abattoirs, indicating that occupational exposure was a stronger determinant of risk than non-work exposure. These findings were confirmed in the study on sero-prevalence and risk factors (thesis chapter 4), but contrast the findings of Heuer et al. (Heuer et al., 2010), where home slaughtering was found to be a risk factor for sero-prevalence.

6. Conclusion

This study demonstrated that workers in sheep abattoirs were at substantial risk of new infection with Pomona and/or Hardjobovis within a single slaughter season. It further showed that newly infected workers from sheep abattoirs had a two-fold higher risk of 'flu-like' illness with 2.8% of the workforce being absent from work for four days on average within a single slaughter season due to leptospirosis.

The rate of illness due to leptospirosis in the sheep abattoir study population was about 16-56-times higher than the official rate of notified leptospirosis cases. Sheep abattoir workers were at a higher risk of new infection than deer and beef plant workers, with deer abattoir workers possibly being reinfected frequently, thus working in an environment with a high endemic equilibrium. Sheep abattoir workers were at highest risk of new infection working at the beginning of the slaughter board, followed by activities involving the removal of the intestines, bladder and kidneys and the offal/pet food area. Home slaughtering, farming or hunting were not significantly associated with new infection. Wearing PPE, such as gloves, facemasks, safety/normal glasses or a balaclava was not protective against new infection. Hence this study raises questions regarding the best practice of PPE, which warrants more research attention. Other means of protection should be considered, for example changes in slaughter procedure or vaccination of farmed livestock.

7. Acknowledgements

Funding was provided by the Tertiary Education Commission (TEC) via the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Rural Woman NZ (RW NZ), the Department of Labour (DoL) and the Swiss National Science Foundation (SNSF).

We are very grateful to study participants, managers and health and safety workers of the participating abattoirs, nurses and phlebotomists, without whom the study would have been impossible (names confidential), and to Sarah Moore, who was an irreplaceable help in data collection and entry, communication of results and a great travel companion.

We thank the occupational health physicians J. Reekie & J. Kerr for advice and support, Heather Duckett for helping in sampling logistics, Christine Cunningham and Wendy Maharey for constant administrative support, Brian O'Leary, Masood Sujau and Simon Verschaffelt for help with building the database, Fang Fang, Prakriti Bhattarai, Rayon Gregory, Claire Cayol and Emilie Vallee for interviewing, Neville Haack and Rae Pearson for MAT testing Roger Lentle for advice for the Massey University Human Ethics Committee application and Lesley Stringer and Sarah Rosanowski for good discussions on using Stata commands.

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Chapter 6

General Discussion

1. Introduction

The studies presented in this thesis were designed to estimate the *Leptospira* spp. sero-prevalence and incidence in meat workers and sero-prevalence in beef cattle, deer and sheep; to find risk factors for prevalent and incident infections; to demonstrate the impact and economic effects of leptospirosis to livestock farming and abattoirs, and to inform disease control measures at farm and meat processing levels. The work focused on *Leptospira borgpetersenii* serovar Hardjobovis (Hardjobovis) and *Leptospira interrogans* serovar Pomona (Pomona) since they are the two most common *Leptospira* serovars found in farmed deer, beef cattle and sheep and in notified cases of disease in meat workers in New Zealand (NZ). In order to achieve these aims, two major studies were conducted: a cross-sectional study on 238 farms ('farm study'), in which beef, sheep and/or deer herds/flocks were blood sampled and farmers interviewed by questionnaire (Chapters 2-3); and a longitudinal study including 592 meat workers working in eight abattoirs slaughtering beef cattle, sheep or deer ('abattoir study'). Each meat worker was blood sampled and interviewed twice at a one-year interval (Chapters 4-5). The individual epidemiological studies were presented as papers prepared for submission to refereed scientific journals. The discussion section of each chapter only focuses on the results and study design of the specific chapter. Therefore, this chapter discusses the research findings in an overall context, reflects critically on study design and methodology (if not already done in the individual chapters) and suggests areas for future research.

2. Research Findings in Context

2.1. Leptospirosis in animals

The 'farm study' (Chapter 2) is the first study in NZ to show a nationwide sero-prevalence in a -for NZ representative- adult sheep and beef cattle and yearling/adult deer population. Formerly, sheep were considered to be sporadically infected by Hardjobovis (Blackmore et al., 1982), and infection with Pomona in beef and sheep was thought to be sporadic (Marshall and Manktelow, 2002; Dorjee et al., 2005a; Dorjee et al., 2008). Blackmore et al. (1982) examined serum samples for evidence of leptospiral antibodies from 928 sheep from 45 lines and kidneys from 12 of these lines for evidence

of infection with *Leptospira*. While 20% of the sheep had MAT titres of 1:48 or greater to Hardjobovis, Hardjobovis was isolated from the kidneys of three animals in one line. The farm, where these three animals originated from, was visited 18 months later and serum (*n*=291) and urine samples (*n*=95) were collected. The serological Hardjobovis prevalence was 0%, 44% and 84% in lambs, hoggets and ewes, respectively. In none of the urine samples leptospires were demonstrated. This 'farm study' found high within- and between-herd/flock sero-prevalences for Hardjobovis and Pomona in sheep, beef cattle and deer with 97% of sheep and beef and 76% of deer farms having at least one in 20 animals sero-positive against Hardjobovis and/or Pomona and with 50% of adult sheep, 58% of adult beef and 34% of yearling deer being positive against either serovar. These results support the proposition that Hardjobovis and Pomona are well adapted to beef, sheep and deer in NZ. An indirect indication that sheep are shedding Pomona is given in the 'abattoir study' (Chapter 5), where 7.3% of sero-negative sheep abattoir workers sero-converted against Pomona within one year. Even though the prevalence is high, a low incidence of farmer-reported probable clinical leptospirosis of 2.6%, in cattle herds in 2009, 0% in sheep flocks and 1% in deer herds was observed (Chapter 2). However, outbreaks with clinical disease with Pomona tend to occur in the course of seasonal floods such as reported for sheep flocks after the large Manawatu flood in February 2004 (Dorjee et al., 2005a).

Reasons for such a high within- and between herd sero-prevalence of Hardjobovis and Pomona in NZ may be the combination of several factors: leptospirosis has been prevalent for several decades (Marshall and Manktelow, 2002) without being controlled by vaccination in sheep, beef cattle and most of the deer population. The *Leptosira* serovars hence may have had time to adapt to current climatic conditions and spread within its carrier animal population. The winter temperatures usually do not drop below zero in NZ and rainfall is high in many regions presenting good environmental conditions for *Leptospira* survival in the environment (Levett, 2001). Moreover, NZ is home to a large livestock population, which is kept outside all year, with urine contaminating pastures and streams. Further, the strip grazing and rotational gazing systems often employed, lead to high livestock density, which may promote direct transmission and high urine contamination of pastures. These farming and environmental conditions possibly allow *Leptospira* spp. to reach an endemic status in the absence of a vaccination program covering all domestic livestock species. Co-grazing -a common NZ farm practice- did not seem to be a risk factor for *Leptospira* sero-prevalence in this thesis (Chapter 2).

It is known that *L. borgpetersenii* sv Balcanica (Balcanica) is adapted to possums and *L. interrogans* sv Copenhageni (Copenhageni) and *L. borgpetersenii* sv Ballum (Ballum) to rats, mice and hedgehogs (Hathaway, 1981; Marshall and Manktelow, 2002). However, the frequency of

infection and prevalence of *Leptospira* in these and other feral animals is currently unknown. Leptospirosis notification data from 1990 to 2008 revealed Ballum to emerge as an important cause of human disease (Thornley et al., 2002; Paine et al., 2010). In 2010 Ballum was the most frequently notified serovar in human cases (ESR., 2010).

A lower North Island study demonstrated 11.3% of deer to be sero-positive against Copenhageni and 15% against Tarassovi (Wilson et al., 1998) and a prevalence study in healthy dogs found that 14.2% of dogs in NZ had antibodies against Hardjobovis (3.5%), Pomona (1.3%) or Copenhageni (9.5%) (O'Keefe et al., 2002). Whether Ballum, Tarassovi, Balcanica and Copenhageni infect sheep and cattle is currently unknown and further research on the frequency of these serovars in humans, livestock and wildlife is warranted (see section 4.2).

The reason for not calculating the true prevalence from the apparent prevalence and test sensitivity and specificity is mentioned under 3.1 in this chapter.

2.2. Human Leptospirosis

The incidence of officially notified leptospirosis cases in recent years has been approximately 2/100,000 in NZ. However, this rather low number is generated by using the whole NZ population as the reference population (the denominator) which is rarely exposed to the bacteria. It would seem sensible to look at the incidence in risk groups, as these comprise the people who mostly get exposed and potentially fall ill. In 2008, the risk of contracting clinical leptospirosis among meat workers and farmers was 79 times higher than in the general population[28]. Most notified human leptospirosis cases in NZ are, in order of frequency, caused by Ballum, Hardjobovis, and Pomona. From 2006 to 2010, 427 cases of leptospirosis were notified (86.4% laboratory confirmed). Of those 427 with occupation recorded annually, 52% (range 36-71%) were farmers or farm workers and 30% (range 18 - 48%) abattoir workers or butchers. Other risk groups are forestry workers or persons with recreational activities in the water[29].

A hitherto unknown number of leptospirosis cases may be misdiagnosed as influenza or another febrile disease or remain undiagnosed because medical attention is not sought, possibly due to difficulties accessing medical services in rural areas and because of similarity to 'flu' symptoms. Medical practitioners often do not test for leptospirosis because of a general lack of awareness about this disease or due to the absence of specific symptoms. Hence, the officially

[28]The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-2010.

[29] The Institute of Environmental Science and Research (ESR), Porirua, New Zealand: Notifiable and other diseases in New Zealand. Annual Surveillance reports 2006-2010.

reported numbers mainly represent severe clinical cases and milder forms are believed to remain under-ascertained (Thornley et al., 2002).

Another possible reason for a high under-ascertainment rate may be the use of the current diagnostic methods. The MAT is currently the standard reference test to detect leptospiral antibodies and diagnose acute leptospirosis. However, a recent study re-evaluated data from 1652 patients with suspected leptospirosis tested by several direct and indirect methods by using Bayesian latent class models and random-effects meta-analysis. The authors concluded that the MAT had a sensitivity of 50% and a specificity of 99% for detecting antibody on the day of admission of patients with acute leptospirosis to the hospital and for some patients after a two week follow-up visit (Limmathurotsakul et al., 2012). Even though part of the insensitivity of the MAT in the study could have resulted from blood testing in the acute stage (when the second sample was unavailable), the authors questioned whether MAT should remain the reference test in the future. However, it is important to be aware that there is a difference between a test used for diagnosing clinical disease (for which the authors did not state the case definition and MAT cut-off) and a test to determine sero-status (exposure) in a research context, as done in this thesis. The Enzyme-linked immunosorbent assay (ELISA) is routinely used as a screening test for leptospirosis by most NZ laboratories. Positive samples are then MAT tested. The pooled sensitivity and specificity of the ELISA in a recently conducted meta-analysis were 0.779 (95% CI 0.770–0.789) and 0.913 (95% CI 0.908–0.917), respectively (Signorini et al., 2012). Hence, the rather low sensitivity of the screening test (ELISA) and potentially the confirmatory test (MAT) in the acute phase may be another reason for underreporting.

The 'abattoir study' did not only reveal a high sero-prevalence in meat workers in sheep and deer abattoirs (Chapter 4) and a high infection rate in meat workers of sheep abattoirs (Chapter 5), but it also showed that a fair number developed clinical disease consistent with leptospirosis.

The risk of falling ill with leptospirosis during a worker's life in an abattoir is high: sixty of 567 workers reported that they had experienced serious clinical leptospirosis while working in abattoirs at any time in the past, recalling cases from as early as 1962. While the annual risk of probable clinical leptospirosis was 0.78% (3/384, 95% CI 0.20-2.46%) with all cases occurring in sheep plants in the second study period, the average annual risk of a sheep abattoir worker, over all workplaces, experiencing 'flu-like' symptoms due to infection with *Leptospira* was 2.7% (95% CI 0.9-4.8%).

Even though most of the 2.7% may not develop serious symptoms, the flu-like form of leptospirosis still has an economic impact, measured by the estimated average time away from work per newly infected worker, which was 4.4 days. Most of the newly infected persons in the 'abattoir study' did not have sufficiently high MAT titres to make an insurance claim for

occupational disease insurance (through the Accident Compensation Corporation, ACC). Therefore, most of the loss of income due to absenteeism is either carried by the abattoir or the employee, depending on how many 'sick days' are included in the work contract.

The 'abattoir study' demonstrated a higher rate of illness than inferred from official surveillance statistics in NZ: the rate of clinical illness due to leptospirosis that was not diagnosed and reported was estimated to be 16-56 times higher than the official rate of notified cases. These numbers include persons with 'flu-like' symptoms due to leptospirosis, which are less likely to be recognized compared to the severe form of leptospirosis. It is possible that the under-ascertainment in other exposed groups, such as farmers is even higher, because most meat plants have a nurse in place, who can take blood samples in case of a suspected case of leptospirosis. Further, awareness about the risk of leptospirosis has been raised in meat plants in recent years by informing employees at the beginning of the season about the risks by the occupational health representative.

During the interviews I had the opportunity to talk to many persons who had fallen ill with leptospirosis. Several reported that since their illness they never regained full strength and that it was the worst thing that had happened to them in their entire life. My personal impression from those reports was that leptospirosis is, for some people, a very debilitating disease with a strong impact on the personal life and that it can be a burden for the family and even the community.

Gower, (2012) described similarly the economic, social, health and emotional costs of leptospirosis, affecting the individual, the family unit, the extended family, and friends, and the whole social and business community (Gower, 2012). This is why 'Rural Woman NZ' (RWNZ) – the national organisation of female farmers – has been involved in awareness raising and in funding leptospirosis research during the 1980s and again since 2007.

In view of the findings among abattoir workers, establishing the *Leptospira* sero-prevalence, incidence and disease risks in other occupations at risk, such as farmers, veterinarians, shearers, artificial insemination technicians or stock-truck drivers should be given a research priority in the near future (see section 4.3). Nevertheless, since exposure to animals and urine are not the same in different occupational environments, it would be imprudent to extrapolate infection rates from meat workers to these other risk groups. Even among meat workers, the prevalence and incidence varied widely between slaughtered species (beef cattle, sheep and deer), even though the sero-prevalence was almost equally high among all three livestock species (Chapter 2). Meat workers are exposed to more animals at higher frequency, and are in closer contact with urine than other occupations. However, veterinarians and artificial insemination technicians in NZ may have similar close contact with animal urine and even more from older animals, which are more likely to shed leptospires. They may therefore also have a high risk of contracting leptospirosis.

With a recent rise in the incidence of notified cases due to serovar Ballum in humans in NZ, it is recommended to investigate epidemiological changes of Ballum (see section 4.2), which may be either linked to humans getting in contact with rodent urine or, less likely, to livestock transmitting Ballum. Livestock may get infected through contaminated animal feed or environment (water puddles), becoming the source of Ballum for human cases. However, whether Ballum is adapting to livestock is unknown. If livestock was shedding Ballum, then meat workers exposed to livestock would likely get infected. Therefore, a subset of blood samples from meat workers of deer abattoirs for Ballum was tested, as deer workers had a relative high sero-prevalence against Hardjobovis and/or Pomona (17-19%) and would therefore be sero-positive for Ballum if deer would be shedding Ballum. However, none of the 57 workers were sero-positive for Ballum. With a MAT specificity of 100% and sensitivity of 90%, the result suggests with 95% confidence that the prevalence of serovar Ballum among deer meat workers was not higher than 5.5%, thus clearly lower than Hardjobovis or Pomona. Consequently, slaughter deer, may not be an important occupational hazard for meat workers with respect to infection with serovar Ballum. Similarly, none of the confirmed human leptospirosis cases working in the meat industry in the Waikato region in 2012, tested positive for Ballum (Cowie and Bell, 2012).

The reason for not calculating the true prevalence from the apparent prevalence and test sensitivity and specificity is mentioned under 3.1 in this chapter.

2.3. Animal to Human Transmission of *Leptospira* spp

A post-outbreak abattoir study, where 21% of sero-positive, randomly sampled sheep had culture positive kidneys for Hardjobovis and/or Pomona, demonstrated that 13 of 1,000 sheep potentially shed leptospires, exposing meat workers to 5-25 shedding live *Leptospira* spp. animals per day. However, the shedding rate most likely depends on age and environmental factors, as the sero-prevalence in slaughter lambs was much lower than in adults (Dorjee et al., 2008, 2011) and outbreaks occurred in sheep flocks following seasonal floods as reported after the large Manawatu flood in February 2004 (Dorjee et al., 2005a). In an abattoir study in the Waikato region, urine, kidney and blood was sampled from 399 lambs and 146 beef from six suppliers following a period of heavy rain during warm weather. The shedding rate -determined by positive urine PCR- in sero-positive (MAT cut-off ≥1:48) sheep was 54.1%, whilst that in sero-negative sheep it was 2.8% and in sero-positive cattle it was 28.2%, whilst that in sero-negative cattle it was 3.0% (Fang, 2013).

Given the exposure of meat workers to shedding sheep carcasses (Dorjee et al., 2011), we decided to investigate in this PhD the actual *Leptospira* transmission from slaughtered stock to meat

workers by estimating how many meat workers get infected and how many of the infected would develop signs of illness.

We therefore implemented a longitudinal study in meat workers of abattoirs processing deer, sheep or beef cattle (Chapters 4-5). The sero-prevalence differed by abattoir and slaughtered species in meat workers: 10-31% of workers at sheep abattoirs, 17-19% at deer and 5% at beef abattoirs were sero-positive to Hardjobovis and/or Pomona. Workers in abattoirs processing sheep had a substantially higher annual risk of infection (incidence, 11.1%) than workers processing deer (0.0%) or cattle (1.2%), demonstrating that abattoir workers are not only exposed to *Leptospira* (as previously reported by Dorjee et al. 2011) but that they also get infected (comment about the incidence in deer workers follows).

A possible reason for the higher prevalence and incidence in sheep abattoirs, despite similar infection rates among sheep and beef (Chapter 2), is that sheep abattoirs process more animals per day than cattle abattoirs and the different slaughter procedure. The average daily number of sheep processed by an eviscerator at one slaughter house was calculated to be 225, and by a meat inspector and an offal handler 374 and 1123, respectively (Dorjee et al., 2011). During interviews, participants reported that sheep urinate spontaneously when stunned, whereas cattle don't. Therefore, sheep abattoir workers may be more exposed to *Leptospira* than beef abattoir workers, especially when stunned sheep drop onto a platform contaminated with pools of urine from other sheep. Additionally, sheep and deer may have higher rates of shedding than beef cattle, which was the case for sheep in the above described Waikato study (Fang, 2013). However, this Waikato study is not representative for NZ, due to the regional concentration of suppliers. Another speculative reason for sheep meat workers having a higher infection risk than cattle meat workers could be the variability in pathogenicity for humans within serovar strains infecting sheep and cattle.

Even though deer abattoir workers had a 17% sero-prevalence at the beginning of the study (as reported in Chapter 4), the annual risk of infection during this study was 0% (Chapter 5). This is most likely a type II error due to the small study size (*n*=50). Based on sample size, the upper limit of a 95% confidence interval of a zero percent incidence in abattoirs processing deer is nine percent (Chapter 5). Alternatively, the incidence can be estimated by the formula (Dohoo et al., 2010).

$$I_i = \frac{P_i}{(D_i - D_i * P_i)}$$

In this formula the mean study period incidence (I) for the serovars Pomona or Hardjobovis ($_i$) can be derived from the relationship between the mean sero-prevalence (P) and the duration of the antibody titre (D) over the threshold of 1:48 following infection of abattoir workers.

The duration of the antibody titre against Pomona was 0.8 years and against Hardjobovis 2.4 years. Information on sero-prevalence in deer meat workers is shown in Chapter 4 and was 15% against Hardjobovis and 5.5% against Pomona. With the above formula, the study period incidence in deer meat workers results in 7.3% for Hardjobovis and in 7.2% for Pomona. However, it should be taken into account that the titre duration presented in Chapter 5 had been calculated from incidence data (using the same formula) under conditions where meat workers might have been re-exposed to *Leptospira* more than once between serial samplings and prevalence was variable between years. Further, the data on titre duration was taken from sheep meat workers, which may be different in deer workers if Hardjobovis and Pomona strains infecting deer had a different pathogenicity in humans than strains infecting sheep. These conditions might have biased the calculated incidence of deer workers. Nevertheless, if only prevalence data are available, the above described approach is a convenient approach to estimating incidence. It may also be used to estimate incidence from sero-prevalence data of other risk groups, such as veterinarians or farmers, which hopefully will be available in the future (see sections 4.3 and 4.4).

The 'abattoir study' demonstrated that work position was the strongest risk factor for sero-positivity with Pomona and/or Hardjobovis in sheep and deer abattoir workers and for new infection in sheep abattoir workers. Highly exposed persons working on the slaughter board and in offal/pet food rooms had an average sero-prevalence of 22.7% in sheep, 39.1% in deer and 7.5% in beef abattoirs, confirming results of Dorjee et al. who postulated a work position related risk of exposure to *Leptospira* shedding sheep (Dorjee et al., 2011). The prevalence and new infection risk was highest in workers at the beginning of the slaughter board and was gradually reduced along the slaughter line in sheep abattoir workers. Urination due to stunning and the subsequent contamination of pelts and carcasses are thought to be likely exposures, and these may be difficult to control while handling carcasses. Whether infection occurs mainly through air droplets or contact with contaminated surfaces, like urine soaked fleece is unknown and should be the subject of further research (see section 4.5).

This suggests that at the beginning of the slaughter line more prevention or protection is needed. This protection might not be attainable by wearing more personal protective equipment (PPE) of the kind which had been used in the abattoirs participating in the 'abattoir study'. The use of PPE appeared not to reduce the risk of infection, as PPE in the multivariable model did not show any evidence for protective effects. In deer workers wearing glasses even resulted in a positive odds

ratio (OR) for sero-prevalence, albeit not statistically significant (p-value 0.08). Biologically plausible reasons would be that workers wearing glasses may wipe their eyes to remove sweat with contaminated hands and with respect to wearing gloves, letting the water run down into the gloves or build-up of sweat, thereby softening the skin weakening the dermal infection barrier. Adler et al. postulate that *Leptospira* may enter through wet skin (Adler and de la Pena Moctezuma, 2010), which had been denied before by Faine et al (Faine et al., 1999). The finding that the PPE may not be as protective as thought should be further investigated (see section 4.6). Using PPE for most tasks is not comfortable and if workers are mandated to wear protective gear, it seems reasonable they should get some benefit from doing so.

Working position was the main risk factor for sero-prevalence or new infection and home slaughtering, farming or hunting were unrelated to sero-prevalence or new infection in the multivariable analysis, regardless of species processed. Therefore, infection of meat workers with *Leptospira* mainly occurred within the abattoirs, leaving the responsibility to reduce the risk of infection in the hands of the abattoir management/shareholders/stakeholders. This finding may have an implication on the 'political process' of leptospirosis control in abattoirs.

The annual leptospirosis infection risk across the study population was 5.8% for Pomona and 2.3% for Hardjobovis, despite the fact that Hardjobovis was more sero-prevalent in workers at the beginning of the study (Chapter 4), and also in the source animals, namely sheep, deer and beef (Chapter 2). In contrast, an earlier analysis of notified leptospirosis data found that the annual number of cases due to Pomona decreased from 62 in 1990 to 26 in 1996, while cases due to Hardjobovis increased from 23 to 30 (Thornley et al., 2002). Speculative reasons for the higher incidence of Pomona than Hardjobovis in our study, may be the difference in duration of antibody persistence (Chapter 5), host specific susceptibility, a stronger persistence of the *L. interrogans* strain in the environment (Adler et al., 2011), a higher amount of shedding from Pomona infected carcasses, a difference in exposure between farmers and abattoir workers, or different trends in 1990/96 to 2008/09.

2.4. Leptospirosis Control

Control measures, which are recommended in the literature include -apart from vaccination of herds- the buying of *Leptospira* spp. free or vaccinated animals (including bulls for breeding purposes) and management of pastures by fencing off standing water, implementing effluent control and setting traps for wildlife (Department of Labour and Accident Compensation Corporation, 2001; Hartskeerl et al., 2011). These are possibly good measures to prevent infection. However, once a herd is infected with a host adapted serovar, the above mentioned measures are

not likely to strongly reduce *Leptospira* infection, given the re-infection cycle due to the presence of shedding and susceptible animals when antibodies wane. Because of the high sero-prevalence in sheep flocks, deer and beef cattle herds in NZ (Chapter 2) a correctly implemented vaccination programme is most likely the only way to reduce *Leptospira* infection in livestock in NZ.

Since vaccination of dairy cows commenced in the 1980s, the incidence of notified human leptospirosis cases in the farming industry dropped from 234/100,000 to 90/100,000 (Marshall, 1996; Thornley et al., 2002). Whether this drop in notified cases is causally related to vaccination and/or to other factors such as higher awareness with behavioural risk reduction (use of PPE, avoiding urine splashes) and changes in milking techniques (i.e. rotary sheds) is still open to discussion. Possibly other factors were at least as important contributors to the decreasing incidence, since the main drop in human cases apparently occurred between 1979 and 1982 before most dairy farmers started with vaccination of their cows in 1983 (Marshall R, 1996). Nevertheless, NZ studies showed the reduction of shedding of *Leptospira* in vaccinated deer, consequently reducing the exposure to *Leptospira*. One study showed a reduction in the proportion shedding by 44% after one vaccination course in the face of existing infection and on-going challenge (Ayanegui-Alcérreca, 2006). Another study demonstrated vaccination of non-infected animals to be 100% effective in preventing shedding after natural exposure (Subharat et al., 2012a).

In absence of a human leptospirosis vaccine, with a limited number of serovars present and with livestock being an important source of human infection (Hathaway, 1981; Thornley et al., 2002), vaccination of livestock seems the logical choice to control leptospirosis in NZ, despite the knowledge gaps.

In a longitudinal study in NZ, co-grazing with infected sheep and/or cattle was positively associated with deer herd serological status to both serovars Hardjobovis and Pomona (Subharat et al., 2012b). Therefore, in Chapter 2 the hypothesis was tested that grazing of different livestock on the same paddock (co-grazing) was associated with a higher sero-prevalence in one of the co-grazed species. However, the 'farm study' found no such effect or even the opposite effect: the prevalence in beef cattle was even higher in beef-only farms than in sheep/beef farms. For other species combinations grazing contact between species was not associated with sero-prevalence. Information on topography and rainfall patterns was missing in the model and the study was limited by its cross-sectional design, where the time sequence of exposure and infection is not guaranteed. Therefore, there was a hesitation to recommend control measures based on these results. Also the literature review on this topic in Chapter 2 revealed that studies were contradictory (some found co-farming (farming several livestock species on the same farm) or co-grazing a risk factor for sero-prevalence, others did not) and most studies had limitations in study design and analysis. Therefore,

the thesis does not make recommendations on control measures to reduce the risk of *Leptospira* infection on the level of farm management, such as grazing management.

2.4.1. Leptospirosis vaccination in livestock

The current reasons why farmers vaccinate their animals against leptospirosis is human safety, protection of animals from disease outbreaks and protection of the business from litigation under the Occupational Safety and Health legislation in the event of workers contracting leptospirosis. For deer farmers, additional benefits demonstrated are increased growth and weaning percentages when the infection pressure is high. Those effects are currently under investigation at Massey in sheep and beef cattle. With regards to the pork industry, farmers are required to vaccinate their pigs in order for them to be accepted for slaughter by abattoirs.

Massey University recently released a report on behalf of the New Zealand Veterinary Association (NZVA) describing best-practice protocols for vaccination of livestock for protecting humans against leptospirosis with the current state of knowledge, pointing out areas of uncertainty (Benschop et al., 2012). The following gaps in the current knowledge were identified:

- The impact of maternally derived antibodies (MDA) on vaccination response and immunity in offspring in relation to timing of first vaccination in cattle and sheep;
- The optimal age at first vaccination and duration of immunity under commercial farming conditions;
- The frequency and the quantity of shedding in vaccinated herds or flocks;

A further open question is the impact of long-term vaccination in herds: does vaccination eliminate infection or does it only reduce the proportion infected and shedding and/or the number of organisms shed by shedding animals, thereby reducing the potential infectious dose? Further, how important are farm management factors, such as co-grazing or access to contaminated waterways in a vaccinated herd on the successful elimination of shedding? The report authors recommend a study to bridge the knowledge gaps (see section 4.7).

Based on current knowledge, young stock in highly infected herds should be vaccinated at an early age (from one-two months of age) with one booster 4-6 weeks later and again at the time of the whole-herd annual booster. Subsequently, annual boosters of growing and adult stock should be the rule. In vaccinated herds or in a low challenge environment, it is recommended to vaccinate young stock latest by six months of age followed by a booster 4-6 weeks later, with whole-herd annual boosters (Benschop et al., 2012).

It is known that clinical symptoms due to *Leptospira* exposure in humans are reduced or non-existent in an endemic environment (Bharti et al., 2003). Reasons may be explained by the concept

of ‘endemic stability’, which “is a widely used term in the epidemiology of ticks and tick-borne diseases. It is generally accepted to refer to a state of a host tick pathogen interaction in which there is a high level of challenge of calves by infected ticks, absence of clinical disease in calves despite infection, and a high level of immunity in adult cattle with consequent low incidence of clinical disease” (Jonsson et al., 2012). The concept of endemic stability has been transferred from the veterinary field into public health (Coleman et al., 2001) and an analogy may exist for leptospirosis in animal populations. For example, it is possible that the endemic state on many of the study farms caused a large proportion of stock to have acquired a certain level of immunity, thus reducing any measurable impact on overall weaning rates (Chapter 3), while subtle effects in susceptible animals may prevail. Vaccinating may impose a risk in itself, as if it was discontinued at some point, animals would return to become susceptible. In such circumstances, clinical symptoms or sub-clinical effects on productivity could surface as a consequence of an insufficiently protected herd when non-vaccinated and infected animals were introduced into the herd. Hence, if a vaccination program is not implemented correctly and continuously, it may have an outcome contrary to the desired effect. Therefore, while the economic benefit accrued from production improvement such as demonstrated in deer, from vaccination would be desirable for farmers, from my point of view human safety probably remains the more justifiable argument for the use of vaccination. However, given the reluctance of farmers to vaccinate their livestock despite their generally good knowledge of the health risks from leptospirosis (Dreyfus et al., 2010c), finding and communicating a financial return from vaccination together with practical and evidence based guidelines on vaccination use may have the strongest impact on a pro-vaccination policy change.

In order for veterinarians or farmers to estimate the economic benefits of a vaccination programme on sheep and beef farms, data on the reproductive performance and growth rates in a high and low challenge environment needs to be available. A cost benefit analysis of a vaccination programme may reveal the necessary level of productivity improvement and implementation time for the programme to justify vaccination on economic grounds.

Further, modelling the outcome of various financing strategies could be useful. It could compare the strategies farmers vs. government covering the costs fully or each partially for vaccination, modelling for each strategy the impact on meat price, competitiveness on the global market and tax revenues, taking the potential increase in production due to vaccination into account.

2.4.2. Leptospirosis control in abattoirs

Abattoirs may consider the policy that non-vaccinated sheep, cattle and deer are not accepted for slaughter, as is the case in the pig abattoir industry or to partially or fully subsidise vaccination of livestock from client farmers, given the possible limited protection coming from PPE and the costs from absenteeism due to infection with *Leptospira* (Chapter 6). Further options for control of leptospirosis in abattoirs may include changes in slaughter procedure (see section 4.5).

3. Reflective Critique of Study Methodologies

3.1. Use of the Microscopic Agglutination Test

Levett et al. called into question that the MAT had the ability to differentiate between serovars and declared that the MAT was not even very accurate in distinguishing between serogroups (Levett, 2001). Also Smythe et al. concluded that the MAT was not serovar specific when conducting a study with the aim to determine whether MAT provided an accurate guide to the infecting serovars of *Leptospira* in Thailand (Smythe et al., 2009). Nevertheless, in NZ serovar attribution is most likely possible by MAT, probably due to restricted serovar diversity. Hathaway as well as Marshall and Manktelow have shown that in NZ a restricted number of serovars and serogroups are endemic (Hathaway, 1981; Marshall and Manktelow, 2002). Apart from Hardjobovis and *L. borgpetersenii* sv. Balcanica (Balcanica), which have different maintenance host species, all isolated serovars in NZ belong to different serogroups (Table 1). Therefore, infection of a specific serovar in a host species can be determined by serology in NZ.

From the serovars prevalent in NZ *Leptospira borgpetersenii* Balcanica may cross react with Hardjobovis (Faine et al., 1999) and may have reduced the MAT specificity to a certain degree in the 'farm study'. However, whilst Balcanica is most likely to be present in possums (Hathaway, 1981), it infects livestock sporadically and appears not to spread within livestock. Since most positive herds contained several positive animals, it is reasonable to assume that their antibodies developed predominantly through exposure to Hardjobovis and not Balcanica, where only a few sporadic cases would be expected.

Several studies have been conducted in NZ in recent years, where serovars determined by serology had been also confirmed by direct methods. For example, MAT serology and serovar isolates had good kappa agreement by DNA sequencing results (Subharat et al., 2011b; Subharat et al., 2012a).

Further, unpublished work conducted at the Molecular Epidemiology and Public Health

Laboratory in NZ (the testing site for all serum samples in this thesis) by Fang et al. in the frame of a current PhD project, demonstrated good correlation between MAT results and the infecting serovar, based on multi locus sequence typing (MLST) of isolates. The same author conducted challenge trials in sheep with serovars Pomona and Hardjobovis. MAT testing of sera from those challenged sheep reproduced appropriately the corresponding serovar (Fang, 2013).

In the frame of a study conducted by Ayanegui-Alcerreca et al. MAT results from the Molecular Epidemiology and Public Health Laboratory in NZ had been validated against MAT results of the WHO *leptospira* reference laboratory in Brisbane, resulting in a kappa of 0.81 for Hardjobovis and 1.0 for Pomona at the herd level (Ayanegui-Alcerreca et al., 2010b).

Pomona and Hardjobovis do not belong to the same serogroup (Table 1), so even if we were wrong in our assumption regarding the capacity of the MAT distinguishing serovars, the conclusions in this thesis regarding serovar specificity are still valid. The stated prevalence in this thesis is the apparent prevalence; hence we did not calculate the true prevalence with the Rogan-Gladen formula to correct for the MAT not being 100% sensitive and specific (Rogan and Gladen, 1978). The reason for this is that the sensitivity and specificity of the MAT used in the NZ context has not been calculated (Collins-Emerson, personal communication) and the sensitivity of 91% - 100% and specificity of 94% to 100% for detecting antibodies in reconvalescent human blood samples reported by McBride et al. (2007) was estimated in an urban setting with different serovars being prevalent (McBride et al., 2007) and therefore not deemed reliable enough. Since the lack of calculating the true prevalence is not a source of differential misclassification, conclusions on risk factors should not be influenced.

Because of the high endemic levels of Hardjobovis and Pomona in livestock and humans in this thesis, the positive predictive value of the MAT for these serovars is supposedly high and therefore the possibility of misclassification low.

The prevalence or incidence of a specific *Leptospira* serovar depends on the chosen MAT titre cut-off to define a sample test positive. Most research on sero-prevalence in animals outside of NZ was based on a MAT cut-off of 1:100. Nevertheless, a cut-off of 1:48 was chosen in order to be able to compare our results to results of former studies in humans and animals conducted in NZ. In humans the titre cut-off of 1:48 is recommended to determine exposure to leptospires, but not for clinical disease (Faine et al., 1999; Shivakumar and Krishnakumar, 2006b). Therefore, a cut-off of 1:48 should be applicable for measuring the prevalence of exposure in NZ. Given the few serovars, belonging to different serogroups prevalent in NZ, the problem of crossrectivity should be small.

Fang et al. modelled the association between sero-positivity and risk factors in meat workers of one sheep abattoir for different MAT cut-offs. While the percentage of sero-positive meat

workers changed by choosing a MAT titre cut-off of 1:96 by approximately 40%, the conclusions on risk factors did not (Fang et al., 2009).

3.2. Production Effects in Livestock

The impact of leptospirosis on growth and reproduction performance in sheep and beef cattle has not been quantified at industry level in NZ. Six percent of beef cattle fetuses lost (Sanhueza, 2012) were associated with Hardjobovis prevalence. If it was definitely shown that infection with Hardjobovis and/or Pomona had effects on reproduction and growth in sheep and beef cattle and this knowledge was transferred to farmers together with a clear vaccination strategy, farmers would probably be more motivated to vaccinate their livestock. This was the motivation to investigate whether there was an association between sero-prevalence and production outcomes in data from farms of the cross-sectional 'farm study' (Chapter 3). The hypothesis tested was that leptospiral infection reduced reproductive performance in sheep, deer and cattle. The hypothesis was supported by earlier findings that infection with leptospires in NZ deer reduced growth and weaning rates (Ayanegui-Alcérreca, 2006; Subharat et al., 2011c). Deer were included in this analysis to possibly provide yet further evidence in this species. The hypothesis of reproduction percentages being negatively associated with *Leptospira* sero-prevalence was not confirmed by the data of this study in all three species, with data for deer contrasting with previous research, albeit using different methodologies (Ayanegui-Alcérreca, 2006; Subharat et al., 2011c). The detection of subtle differences of production effects, if they existed, probably was not possible with a cross-sectional study design and requires a longitudinal study investigating changes at animal level, e.g. sampling at the beginning and end of the production season and associate animal growth and reproduction performance with sero-conversion. This kind of study is currently conducted by Emilie Vallée, PhD student at Massey University. The results could be used to conduct a cost-benefit analysis for different vaccination strategies to inform decision makers at farm and industry levels about the return on investments.

3.3. *Leptospira* Infection Risk in Abattoir Workers

One of the aims of the meat worker studies was to evaluate the relative risk of infection for different work positions in the abattoirs. A limitation in this was that many workers fulfilled several tasks on a daily basis and also changed work position within the slaughter season. This occurred across all species but in particular workers from venison abattoirs were multi-tasking the most. Therefore, work positions were described in broad categories including work tasks with a similar

exposure to urine or to organs of the urinary tract. Since the slaughter procedure is specific for each species, the work position categories were different for the three species. For sheep abattoirs, work positions were categorised into four, for deer into two and for beef cattle into four categories (see Chapter 4 for details). Most workers fulfilled a range of tasks which were sequential and could therefore fit into one category. Some however, worked in completely different areas. To address this variability workers were allocated to the activity they performed predominantly (≥60%) and this activity was then fitted into one of the work position categories. About 3.5% (20/592) of workers could not be allocated to any category, as they spent the same amount of time in the different areas. They were randomly distributed to one category. This approach might have introduced a certain degree of non-differential misclassification bias, and under- or overestimated the odds of sero-positivity (Chapter 4) or the relative risk for new infection (Chapter 5). However, it is believed such a bias was small if present at all and should not have had a strong influence on the inferences about the relative risk of work positions.

A similar approach was adopted to the description of a worker's PPE in relation to his/her work position. For example, if a participant in a sheep abattoir worked 70% 'removing kidneys` 'wearing glasses` and 30% at the beginning of the slaughter board without wearing safety glasses, the employee would be designated to 'removing kidneys` and to be 'wearing glasses`. Information on the 30% working in other exposed areas without glasses would not be taken into account in the analysis. Again, this could have biased inferences about the odds associated with the use of PPE.

Moreover, when responding to questions about wearing PPE, participants might have been biased by not admitting to non-compliance of the employment policy enforcing the use of PPE, despite a clear statement that interviews were confidential. This could have led to an overstatement of wearing PPE by participants in high-risk work positions, thus reducing the possibility of determining a protective effect of PPE in the analysis through a differential misclassification bias.

PPE was not a protective factor in the cross-sectional study. Since a cross sectional study design does not take a temporal relationship between exposure and outcome into account, it is possible that study participants who experienced clinical leptospirosis and consequently were sero-positive became more careful and consequently wore PPE after a disease episode, biasing the OR of PPE upwards. The cohort study, however, where a temporal relationship was given, did not render PPE to be protective either. Therefore, it was inferred that there was no clear evidence for PPE being protective. However, it would be premature to present clear recommendations about the use of PPE. We therefore encourage further research to accurately determine the effect of PPE (see section 4.6).

Participation in the 'abattoir study' was 'voluntary' and the proportion of participants from high risk areas was higher in the study than in the entire work force. Consequently, the abattoir specific sero-prevalence was overestimated due to sampling bias, which varied by abattoir. This bias was addressed in a parallel analysis to this thesis. Bias was adjusted by direct standardisation method (Dreyfus et al., 2010a), which will be published independently from the thesis because of time issues. Since this sampling bias did not have an influence on the ORs of work position, it does not impede the conclusions of this thesis.

The infection risk may have been slightly underestimated because workers were sensitized to the leptospirosis risk after the first interview. Workers may have changed their behaviour and became more careful and started avoiding urine while handling carcasses. I was aware of this potential bias when talking about leptospirosis with the study participants, but did not want to decline to inform them because of ethical reasons. In general, they knew about the risks, which was why they participated in the study in the first place.

4. Areas for Future Research

4.1. Prevalence of Ballum in Meat Workers

MAT testing of the sheep and beef meat worker blood samples, which were collected during the 'abattoir study' and stored in a serum bank, is proposed as a first step in the exploration of the epidemiology of Ballum. If the meat worker blood samples test *Leptospira* Ballum antibodies negative, it is more likely that humans get infected by contact with rodent urine and not through livestock.

4.2. Prevalence of Ballum in Sheep and Beef Cattle

MAT testing of the sheep and beef samples of the 'farm study' stored in a serum bank for Ballum is recommended. Deer have been screened before (Subharat et al., 2011a). This would give an indication of whether these species are infected.

4.3. Prevalence of Ballum and other *Leptospira* Serovars in Farmers, Livestock, Dogs and Wildlife

However, for more certainty and a more holistic approach, it would be useful to conduct an observational study on farms, where humans, livestock, dogs and trapped rodents are tested for Ballum, Hardjobovis, Pomona, Balcanica, Copenhageni and other *Leptospira* species in order to identify carriers and know the prevalence. It would be of advantage to not only collect blood samples, but also urine samples from humans, livestock and dogs and to examine the urine of serologically positive animals for shedding. Further, multi locus sequence typing (MLST) or other more discriminatory methods could be used to investigate cross-infection between species (see also 4.5). Knowledge of the epidemiology of Ballum and Balcanica is important, as these may have to be included in modified vaccines for animals.

4.4. *Leptospira* Prevalence in other Professional Groups

It is recommended to MAT test blood samples and interview veterinarians, shearers and other exposed professionals for establishing prevalence and to determine risk factors for infection.

4.5. Strain-specific Susceptibility

The literature search did not reveal any articles describing research on whether there were 'sub-strains' within the serovar Hardjobovis (or within Pomona), that are more likely to infect one animal

species than another. Since the 'farm study' did not reveal 'co-grazing' as a risk factor for the within-herd sero-prevalence of the co-grazed animal species, it would be useful to see whether there is a strain-specific susceptibility in beef cattle, sheep and deer for a 'sub-strain' of the serovar Hardjobovis (or Pomona). This could possibly be tested by investigating molecular differences in the serovar Hardjobovis (or Pomona) isolated from culture and polymerase chain reaction (PCR) positive sheep, beef and deer with multi locus sequence typing (MLST). The finding of a 'species barrier' due to different susceptibility of livestock to sub-strains of serovar Hardjobovis (or Pomona) would influence the vaccination policy on mixed species farms.

4.6. Investigation of *Leptospira* Contamination on the Slaughter Board

In section 2, several hypotheses were developed regarding the difference in sero-prevalence and incidence in meat workers slaughtering different animal species. Based on those hypotheses, it is recommended to test whether:

- sheep and deer urinate, and beef do not urinate spontaneously when stunned, by observation of animals during slaughter or interviewing workers in the stunning area;
- the platform, where sheep drop on to after been stunned is (i) contaminated by urine by taking samples of the platform during the slaughter process and test for the existence of urine and (ii) test urine samples with molecular methods (e.g. PCR) for *Leptospira* (these may be too contaminated to perform a PCR though). If the platform is contaminated with urine, it is recommended to introduce a slaughter procedure without a platform. In this case sheep pelts would still be contaminated with urine due to the spontaneous urinating, although not by urine of multiple animals. Introduction of washing of the carcasses after stunning (this may be impractical due to excess bleeding) or automating of removal of pelts would be further options to reduce the exposure to urine;
- *Leptospira* transmission could occur by aerolized liquid ('air droplets') by testing air droplets for *Leptospira* in different areas around the slaughter board. If air droplets contained an infective dose of *Leptospira*, this could be another reason for glasses or facemasks not to be sufficiently protective. Further, if air droplets proved to play a role in transmission, tackling the problem of leptospirosis 'at the stable' and not shortly 'before the table' is recommended, since air droplets are a logical consequence of wet objects being moved around quickly, which is unavoidable during the slaughter procedure and it is probably not practicable for meat workers to wear biocontainment suits.

4.7. Investigation of *Leptospira* Contamination of PPE and Reassessment of PPE Policies

PPE did not have a protective effect in the multivariable analysis that tested the association between sero-prevalence or new infection in meat workers and PPE. Given certain limitations of the data analysis (see section 5), only a few recommendations on PPE use in the future are made. However, it is highly recommended that the current PPE legislation and policy is reassessed by using evidence-based approach and this informs further development of more useful PPE. The reassessment procedure should ideally have a participatory approach including meat workers or at least representatives for input regarding 'practicality'. Recommended actions:

- Taking samples from the inside of worn gloves at various work stations at the slaughter line and testing them with molecular methods (e.g. PCR) for *Leptospira* contamination. If contamination existed, it is recommended to compare the contamination of the inside of gloves between high and low risk work positions (as described in Chapters 4 and 5);
- If there were a difference in contamination of the inside of gloves between high and low risk positions, there would be some evidence that the commonly used gloves were a risk factor for sero-prevalence and new infection. In that case, a next step could be the development of gloves, which prevent water from running inside. Maybe by using gloves, which reach over elbows?

4.8. Field Trials of Leptospirosis Vaccination Programme

Based on the identified knowledge gaps on vaccination use in livestock (Benschop et al., 2012), the authors recommended large scale field trials of vaccine efficacy in herds and flocks, comparing vaccinated with unvaccinated dams in conjunction with vaccinating offspring at various ages (1, 3, 6 months) in endemic herds/flocks. It is recommended to implement a longitudinal study over several years using numerous whole herds or flocks as study population, in which one group of animals are vaccinated and another group are non-vaccinated in an environment where all animals are equally exposed to natural challenge. In order to examine the impact of vaccination and the influence of a long-term programme on shedding, serial urine sampling of sentinels is recommended. This will answer the question whether vaccination reduces the exposure to *Leptospira*. It would also provide data on how long animals shed for after implementation of a vaccination programme and therefore the time to 'full' protection.

4.9. Field Trials of Production Loss due to Leptospirosis in Livestock in New Zealand

Chapter 3 analysed the production loss in livestock associated with *Leptospira* sero-prevalence and did not find significant effects. Despite the limitations mentioned in Chapters 3 and 6, the farm study should have revealed production effects if they existed at a large magnitude. Hence, production effects in NZ in the year of sampling were either small or non-existent. To detect subtle differences in production effects due to leptospiral infection, a longitudinal study design at the individual animal level, e.g. sampling at the beginning and end of the production season and measuring the within-herd association of production performance with sero-conversion of individual animals is recommended. Potential production effects include growth rates, weaning rates, pregnancy rates and mortality rates. An alternative is to use a vaccination approach as adopted by Subharat et al. (Subharat et al., 2011c) in which animals were cleared of infection with antimicrobials, and half vaccinated, and after development of immunity, exposed to natural infection by introduction to known infected herds. A study ideally would control for, respectively include information on topography, adjacent waterways, climate, rainfall patterns, breed, prevalence of other infectious agents, nutrition, pre- and postpartum management, housing etc.

4.10. Analysis of Cost-Effectiveness of Leptospirosis Control in the New Zealand Livestock Sector

With data on production loss and efficacy and costs of leptospirosis vaccination, the cost-effectiveness of leptospirosis control in the livestock sector can be analysed. The cost per animal and vaccination dose in NZ lies between 1.20 and 2.00 NZD[30]. A cost-effectiveness analysis will enable farmers to decide on whether to start vaccinating their livestock against leptospirosis. Whether vaccination proves to be cost-effective is also relevant for decision makers in the public health sector, as it influences the dynamics in the discussion on the distribution of the financial contribution to the vaccination programme. Why should farmers fully carry the costs if a larger part of society benefits from the investment?

4.11. Estimation of the Burden of Leptospirosis in the New Zealand Public Health Sector

The estimation of the burden of leptospirosis includes the quantification of morbidity, all disabling complications as well as mortality in a single summary measure called Disability-adjusted life year (DALY). The DALY measure combines the years of life lost due to premature death (YLL) and the years lived with disability (YLD) from a disease or condition, for varying degrees of severity, making

[30] Estimates given to the author by product managers of the companies Virbac, Merck and Pfizer New Zealand, June 2013

time itself the common metric for death and disability. One DALY is a health gap measure, equating to one year of healthy life lost (Mathers et al., 2001). Data for the burden estimation ideally stems from studies, such as described in Chapters 4 and 5, as well as from surveillance data. In order to quantify the disease burden from surveillance data, the development of tools and methods to fit models, which account for missing data are necessary. In times of limited and stronger competition for financial resources, it becomes crucial for public health experts and policy-makers to quantify the impact of diseases enabling the prioritization of surveillance and intervention activities and inform resource allocation decisions in the field of veterinary and public health. Hence, this type of study would enable a more subjective discussion on the priority of leptospirosis control in New Zealand. However, it requires that other diseases are quantified the same way.

Even if the burden of leptospirosis for society as a whole was estimated to be low, it might be high if analysed just for specific risk groups, such as meat workers or farmers (hence if the denominator is generated from the meat worker or farmer population and not the entire population of NZ). Whether certain risk groups should carry almost the entire burden of a disease by delivering a service to society (producing and processing meat) might be another approach in discussing the importance of a disease.

4.12. Estimating the Costs of Leptospirosis for Society and the Individual

Estimating the cost of leptospirosis for the individual and society would inform the development of a strategy for control of leptospirosis, thus would be relevant for policy formulation about approaches and funding of disease control. Some of the costs are borne by the individual or the business (time away from work, which is not covered by ACC, legal claims, vaccination costs in the absence of a public funded control program) and some by the nation (treatment or hospital costs, ACC claims, vaccination costs in case of a public funded control programme). This section elaborates on what information is already available and what is still missing.

A Department of Labour (DOL) report on leptospirosis (Keenan, 2007a) estimated the public health costs of leptospirosis in 2007. It mentioned difficulties due to lack of knowledge of the true leptospirosis incidence because of under-ascertainment in the official surveillance system and due to the variability of required time to recover from leptospirosis. Keenan concluded that a leptospirosis case in the meat industry would lead to a loss of 6000 NZ dollars (NZD) due to absenteeism, but he did not differentiate between the costs going to the abattoir, ACC and the meat worker falling ill. Further, he estimated 1500 NZD for medical costs, of which the latter seems rather low in case of hospitalisation and intensive care.

The results shown in Chapter 5 can fill some of the indicated knowledge gaps at least for the

cases occurring in the sheep meat industry. The average annual risk of a sheep abattoir worker experiencing 'flu-like' and more severe symptoms due to infection with *Leptospira* was 2.7% (95% CI 0.9-4.8%). Hence, with an estimated 10,000 sheep abattoir workers employed, approximately 270 new leptospirosis cases occur annually in this industry sector. With information on incidence risks in other potentially affected occupations it would be possible to estimate the total costs.

The average time away from work for severe cases has been estimated to be around 6 weeks (Keenan, 2007a) and for the milder form approximately on average 4 days (Chapter 5). Most of the newly infected persons in the 'abattoir study' did not have sufficiently high MAT titres to make an insurance claim with the occupational disease insurance (Accident Compensation Corporation, ACC). Therefore, most of the loss of income due to absenteeism is either carried by the abattoir or the employee, depending on how many 'sick days' are included in the work contract. The costs of hiring extra labour because of missing employees should be calculated as well.

A small number of leptospirosis cases remain disabled and have to give up their physically demanding jobs, suffering consequently from financial difficulties. These were not mentioned by Keenan, but would be included in a burden of disease study (see 4.11). Further, the emotional costs, originating for example from families getting separated or having to move to another city due to the effect of a family member having contracted leptospirosis (Gower, 2012), were not taken into account either.

The DOL report does not take legal consequences from employees falling ill with leptospirosis into account. However, it could contribute to the economic burden, especially after the 'abattoir study' showed that new infection by serological evidence was associated with work position and not with exposure from non-work related activities, such as hunting, farming or slaughtering at home, placing the responsibility to protect the employees into the hands of the abattoirs. The legal consequences were one of the incentives for the dairy industry to start vaccinating in the 1980s (Marshall, 1996).

4.13. Diagnostic Tests and ACC Case Definition

During interviews in the 'abattoir study' the question whether meat workers received occupational disease insurance compensation (ACC) was answered by 49 of 60 by leptospirosis affected workers and 21 (43%) had received compensation (Chapter 4). The percentage of ESR confirmed leptospirosis cases occurring between 1997 and 2005, which were accepted by ACC ranged between 5 and 64% (Keenan, 2007a). Keenan did not explain why such a low percentage of claims notified to ESR came through as claims to ACC and pointed this question out as an area for further research. With the current case definition only a part of the cases are captured and several persons falling ill

with leptospirosis do not receive compensation. Therefore, the clarification of the ACC claims acceptance criteria[31] (including diagnostic tests and cut-offs used) and procedures is recommended. The identification of useful tests or development of diagnostic tests is suggested to diagnose acute leptospirosis in a timely and accurate fashion. The Health Research Council (HRC) is funding a study at Massey University (Fang, 2013), with one of the aims being the identification of the best diagnostic test or combination of tests for diagnosing acute cases of leptospirosis. This should lead to rapid diagnosis and prompt appropriate treatment. To evaluate a diagnostic test, the estimation of its positive and negative predictive value is helpful[32]. With a high negative predictive value, less leptospirosis cases are missed, which is desirable from an ethical point of view. However, for reducing costs (not for the patient), a high positive predictive value would be preferable. A way to increase the negative predictive value of a diagnostic method is to increase the sensitivity. Currently, an ELISA screening test precedes testing by MAT. The accuracy of ELISAs was reviewed by Signorini et al. (2012) with sensitivity 78% and specificity 91% derived from a meta-analysis without, however, providing details about the gold standard for 'true leptospirosis' (Signorini et al., 2012).

If the MAT were used as the reference test to diagnose acute leptospirosis in the future, maybe the case definition for ACC should be more serovar specific, as host-adapted serovars, such as Hardjobovis, may induce a milder immune response than others (Faine et al., 1999), possibly requiring a lower titre cut-point to define a positive test result. Serovar identification is not particularly important for the individual, for getting diagnosed, receive treatment and compensation. Therefore, molecular tests, such as a PCR of blood could be considered as additional reference test, being more sensitive in the acute phase of the disease. A molecular test result does not have the time lag of the MAT, where several weeks are required to evaluate a raise in titre by serial testing, which may be impeded by antimicrobial treatment. Molecular tests are most useful in the acute phase of illness, when bacteraemia is usually present. However, a new ACC case definition including results of molecular tests would have the advantage of a rapid and sensitive diagnosis of leptospirosis. The most appropriate time frame in which clinical illness may be diagnosed by PCR would have to be determined by further research.

It would be advisable to validate the sensitivity and specificity of the MAT for different titre cut-offs and serovars in the NZ context. The MAT should be ideally evaluated with sera from animals, which had been infected with *Leptospira* (culture positive after experimental or natural challenge) and with sera from *Leptospira* negative animals (specific pathogen free (SPF) kept

[31]http://www.acc.co.nz/for-providers/clinical-best-practice/acc-review/WCMZ003166: a confirmed leptospirosis case requires isolation of the bacterium or a MAT titre ≥800 or a four-fold titre increase between two samples taken

[32]"The predictive value of a positive test is the probability that given a positive test, the animal (or human) actually has the disease. The predictive value of a negative test is the probability that given a negative test, the animal (or human) does not have the disease" Dohoo, I., Wayne, M., Stryhn, H., 2003c. Veterinary Epidemiology Research. AVC Inc..

animals or PCR test negative animals).

5. Conclusion

The 'farm study' in this thesis is the first, for NZ sheep, beef cattle and deer farms, representative study that demonstrates that not only Hardjobovis but also Pomona is endemic in sheep, deer and beef cattle in NZ. Hence, it challenges the previously held notion that sheep are not maintenance hosts for Hardjobovis and Pomona. The thesis provided epidemiological evidence that exposure to sheep and deer in abattoirs leads to infection of meat workers with Hardjobovis and Pomona, especially of those working at the beginning of the slaughter board. Moreover, it demonstrated that meat workers did not only get infected, but also fell ill with leptospirosis at a higher rate than officially notified. The odds of silent vs. clinical infections were 4:1. About 3% of workers experienced leptospirosis with 'flu-like' symptoms in the endemic environment every year. The infection risk was not associated with non-work related risk factors, such as hunting, home slaughtering and farming, identifying the work-place as the source of infection. In our studies PPE did not have the expected protective effect. However, this finding needs additional research because of issues inherent with observational studies. It is recommended to vaccinate livestock against leptospirosis in NZ in order to protect exposed humans from infection. However, further research and information campaigns regarding the correct use and the most effective vaccination schedule(s) are essential to prevent vaccinated animals from shedding *Leptospira*. The epidemiology of serovar Ballum in humans, livestock, dogs, possums and rodents and the prevalence of Hardjobovis, Pomona, Ballum in risk groups, such as farmers, veterinarians and shearers are important areas for future work.

"Leptospirosis not only impacts the person infected, but impacts their families and communities through months of physical recovery and loss of work and income".

Noeline Holt of RWNZ (Harvey, 2010)

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„Kaua e rangiruatia te hā o te hoe; e kore tō tātou waka e ū ki uta“

“Do not lift the paddle out of unison or our canoe will never reach the shore”

Appendix A

Questionnaire Abattoir Study

Study on Leptospirosis among Abattoir Workers

Participant Questionnaire

The research team appreciates your involvement in this study of leptospirosis and is committed to privacy of all personal information.

The information from this questionnaire will help us to assess the risk of contracting leptospirosis in meat plants and to develop control strategies.

Personal information included in the questionnaire will be treated in confidence and will not be published or disclosed to any third parties (for example your employer) by the research team in a manner that would allow identification of participants.

Date of interview: ____/____/2011 Interviewer's name: _____

Meat plant: _____

Participant identification

Name & Sir name	
Did you fill in a consent and confidentiality form?	Yes No Please get both forms filled in now
Postal address	
Contact phone number	
Type of location	Rural Lifestyle* Urban
Date of Birth	(day/month/year) _____/_____/_____
Gender	Male Female
With which ethnic affiliation do you identify with?	NZ-Maori Pacific Islander NZ-European Asian Other _____

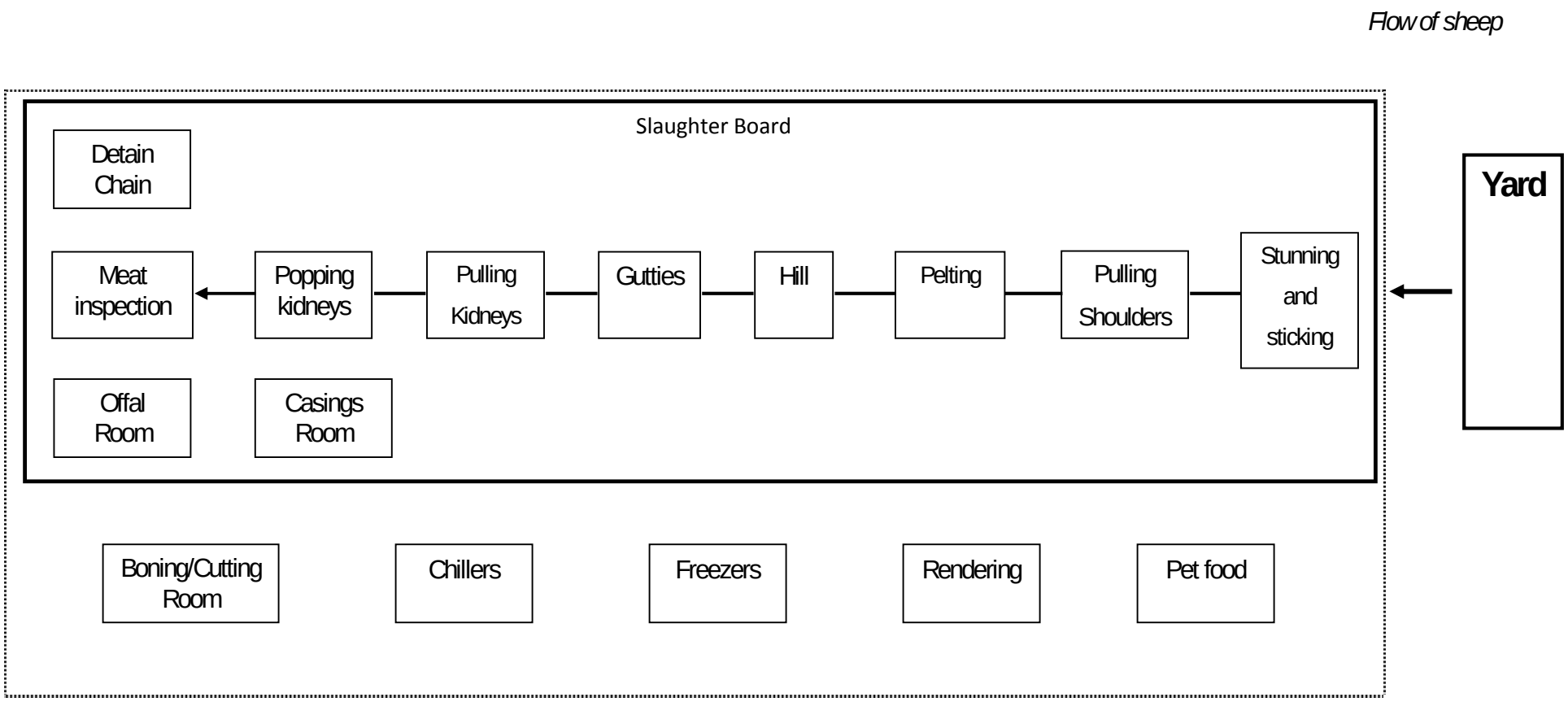
*e.g. living on the outskirts of town on a property with > 1 acre of land

Exposure at work

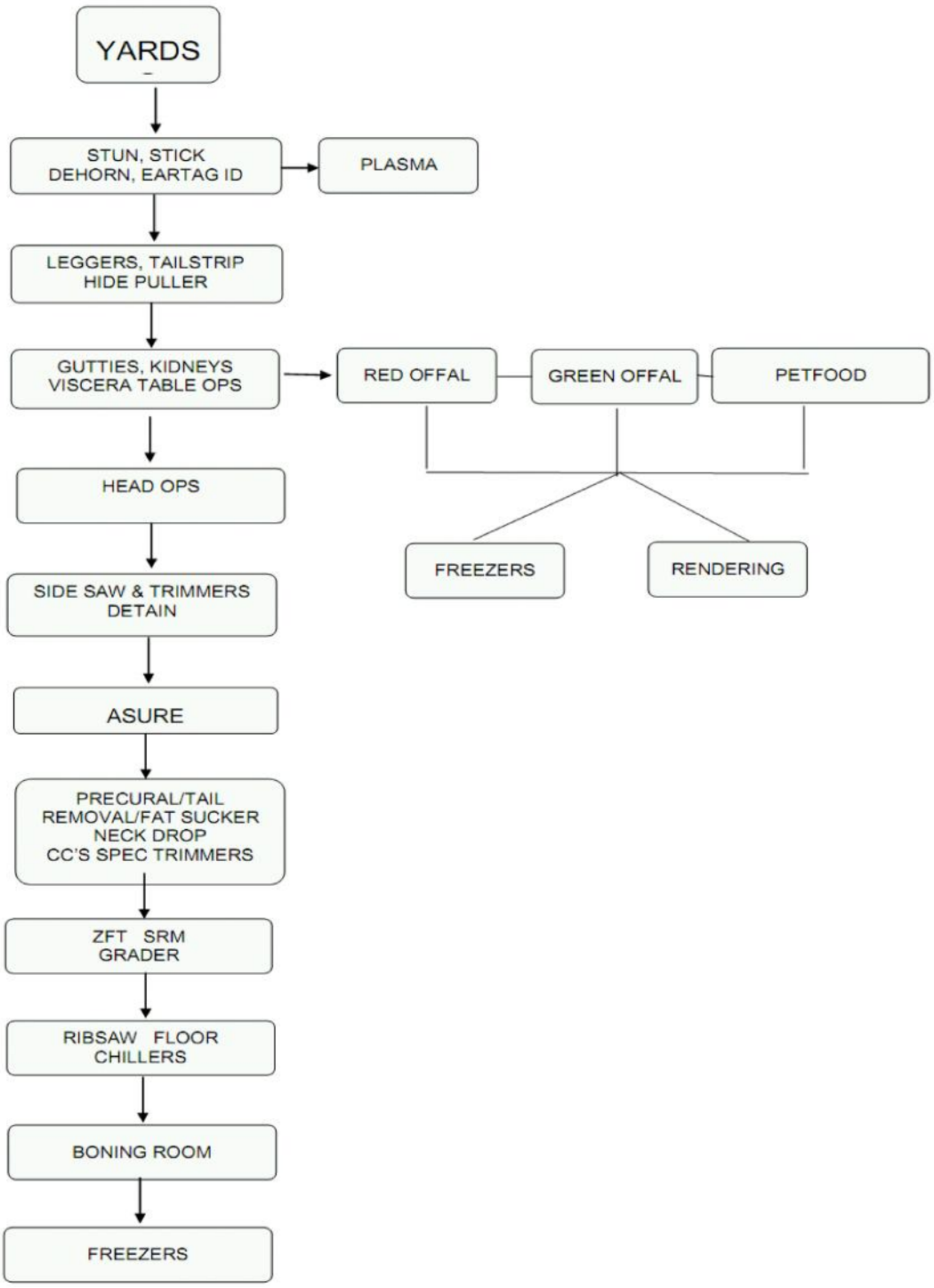
2.1 Current info: your work at the meat plant

For how long have you been working for this meat plant (years)?	_____ years
How many months worked from ___2010 - __ 2011? (Have you had a longer break than 6 weeks)	_____months
Did this plant have a seasonal break?	Yes No
If yes, date work started this season	(day/month/year) _____/_____/_____
Which animal species are you exposed to at work (only ask if multispecies plant)?	
Worked for another meat plant from ___2010 to ___ 2011? If yes, where and species slaughtered/processed?	
Do you believe Leptospirosis presents a serious health risk at your work?	No Yes Maybe Don't know
Job description	
In your daily routine, how often do you get in contact with urine, kidneys, urine bladder or with urine contaminated surfaces?	
Do you recall having animal urine land on your eyes or mouth while in your current job?	No Yes Don't know
Work position/ Job title	What proportion of your time do you spend in the following locations in the plant? Refer to drawing of plant and put a cross where you usually work. If you usually change your work position, indicate all positions and estimate the percentage of time you spend at each position. Interviewer: assign a number to each position.

Work position at the meat plant (Sheep)



Work position at the meat plant (Beef)



Work position at the meat plant (deer)

Location	Process Steps	Tick if part of work	Percentage of work time
Yards	Receive and pen		
	Ante-mortem inspection		
	Wash		
	Stun		
Slaughter Floor	Shackle		
	Stimulate		
	Stick		
	Remove front leg sinew and velvet stubs in season		
	Apply clip to weasand & rod		
	Head work-up		
	Y Cut		
	Belly rip-down, udder, pizzle		
	Clear anus, tail off		
	Tendon and hock removal		
	Remove hide (re-invert to rail) and remove head		
	Evisceration stand: Ring, open belly, eviscerate, trim, popping kidneys		
	Post Mortem Inspection - Inspect head, offal, carcass / retain and re-inspection		
	Carcass dressing check		
	Grading		
	Load carcass to chiller		
Carcass Chillers	Chill carcass		
Boning/Cutting Room			
Special purposes offal	Liver, heart, kidney		
Special purposes Asia	Pizzle, tail, sinew, tongue		
Special purposes blood			
Offal Room			
Freezers			
Pet Food			
Office			
Other			

In your daily routine, how often are you using the following protective clothing (PPE) in each work position?	**Face mask with visor down**: Position 1:_____ always often sometimes never Position 2:_____ always often sometimes never Position 3:_____ always often sometimes never **Goggles/glasses:** Position 1:_____ always often sometimes never Position 2:_____ always often sometimes never Position 3:_____ always often sometimes never **Balaclava (over face):** Position 1:_____ always often sometimes never Position 2:_____ always often sometimes never Position 3:_____ always often sometimes never

Work position/ Job title, continue from last page	**Gloves (any sort):** Position 1:_____both hands one hand always often sometimes never Position 2:_____ both hands one hand always often sometimes never Position 3:_____ both hands one hand always often sometimes never
Do you believe Personal Protective Equipment (PPE) will protect you from contracting Leptospirosis?	No Yes Don't know Don't wear any
Do you find wearing PPE inconvenient?	No Yes Don't wear any
Do you smoke at work breaks?	No Yes

Abattoir work history:

For how long have you been working in an abattoir (years)?	
Have you been working mainly in the meat industry?	Yes No

We want to quantify work place exposure for the last 3 years

Year	**Plant location/ name**	**How many months worked**	**Full time?**	**Species exposure (sheep = S, beef = B, bobbies = BC, deer = D)**	**Positions/ Job titles**
2009/10 (last season)			Yes No		
2008/9 (season before that)			Yes No		
2007/8			Yes No		
Have you worked for an abattoir or butcher before 2007?			Yes No		
If yes, since when?					
If yes, to which animal species were you exposed to?					

2.3 Other regular work history - *not abattoir:*

Over the last 3 years, have you had any other regular work besides your work in an abattoir?

NO Skip to non-work exposures 3.1

YES Complete table below

Type of work	How many hours per week?	Approximately for how long have you done this (years)?	When did you last do this work?
Forestry			
Livestock/ Farming			
Species	Beef cattle Dairy cattle Sheep Goats Deer Pigs		
Horticulture/ cropping/ orchard			
Other _____			

Non-work exposures

Regular contact with live animals at home, friend's or family's house
Over the last 3 years, have you had regular (daily or weekly) contact with animals outside work?

NO Skip to **Wildlife table 3.2**

YES Complete table below

Animal type	**No of animals**	**Animals vaccinated against Leptospirosis?**
Beef cattle		No Don't know Yes
Dairy cattle		No Don't know Yes
Sheep		No Don't know Yes
Goats		No Don't know Yes
Deer		No Don't know Yes
Pigs		No Don't know Yes
Dogs		No Don't know Yes
Cats		No Don't know Yes
Other_____		No Don't know Yes

Wildlife

Over the last 3 years, have you often * seen rats, mice, possums, rabbits or hedge hogs at home (house, garden, surrounding fields)?	Yes No Don't know
Do you set traps or poison for these animals at home?	Yes No Don't know

*often: *more than or once a week*

Home slaughter
Did you home slaughter or have you helped with home slaughtering any animals in the past 3 years?

NO Skip to **Hunting/Trapping Table 3.4**

YES Complete table below

Animal type	How many per year?	How often per year?	When was the last time?
Cattle			
Sheep			
Goats			
Deer			
Pigs			

Hunting/Trapping exposures
Have you been hunting in the last 3 years?

NO Skip to **Other Outdoors Table 3.5**.

YES Complete table below

Animals hunted	For how long have you been doing it (years)?	How many shot or trapped in an average year?	When shot or trapped an animal last time?
Deer			
Wild pig			
Small game *			
Goats			
Other_____			

* e.g. ducks, other birds, possums, rabbits, hares…

Other Outdoor exposures
Over the last 3 years, have you done outdoor activities where you were exposed to fresh water?

NO **Skip to Flooding 3.6**

YES Complete table below

Outdoor activities *fresh* water	**For how long have you been doing it (years)?**	**How often per year?**	**When was the last time?**	**Region?**
Camping beside lakes/rivers				
Water sports in lakes/rivers e.g. swimming, boating, windsurfing, endurance events				
Fresh water fishing				
Did you do any of these activities overseas?	No Yes If yes, specify country(ies) _____			

Flooding
Over the last 3 years has your land been flooded (for several days leaving a water puddle (at least 5m by 10m, 10 cm deep)?

NO **Skip to Previous Illness 4.**

YES When the last time?_____

Previous illness

Have you ever been diagnosed with Leptospirosis?

YES Complete **Lepto Table 4.1**

NO / Don't know Skip to **Other illness Table 4.2**

Leptospirosis

Approximate date:	_____	
How was it diagnosed (test)?	Self diagnosed GP Blood test	
Do you know the serovar and/or titre?	Serovar: _____ Titre:	No
How many days were you off-work or seriously ill?	_____ days	
Please describe the symptoms? *		
Was it treated?	Yes No Don't know/remember Antibiotic treatment If yes, how many days:	
Received ACC compensation?	Yes No Don't know/remember	

* e.g. fever, headache, sore muscles or bones, sore eyes, sweating, severe general debility

Other Illness

Have you had any flu-like symptoms in the last 3 years (*excl. injury*)?	Yes No Approx date _____		
Have you been off work due to this illness?	# days_____		No
Did you ask for professional help?	GP Nurse Other_____		No
Were any blood tests done or samples collected?	Yes No NA		
Was it treated?	Yes , diagnosis of	No Do not remember	
Did you have any of the following symptoms?	Fever Headache Sore muscles Sore eyes Sweating Severe debility		
Was it treated?	Yes No Don't know/remember Antibiotic treatment If yes, how many days:		

“This is the end of the questionnaire. The research team appreciates your involvement in this study of leptospirosis and is committed to privacy of all personal information..

The lab will check your blood for previous exposure for Lepto and we will notify you of the result by mail as soon as possible.”

Appendix B

Questionnaire Farm Study

MASSEY UNIVERSITY

ivabs

Institute of Veterinary, Animal & Biomedical Sciences

JOHNESDISEASE RESEARCH CONSORTIUM

Research into Johne's Disease and Leptospirosis in New Zealand

Farm Health and Production Follow up Survey

"Help to control Johne's disease and Leptospirosis, and reduce the risk of Leptospirosis among farmers, their families and workers"

Rural Women New Zealand

DEER INDUSTRY NEW ZEALAND

MEAT & WOOL NEW ZEALAND

Research into Johne's Disease and Leptospirosis in New Zealand

Farm Health and Production Follow-up Survey

Introduction

Thank you for taking your time to answer the questions of this questionnaire, which is very similar to the survey you filled out in 2008. We are aware that your time is valuable and have reduced the number of questions, but still need up-to-date information on livestock management and disease data. Together with the results of the blood and faecal samples this up-to-date information will help us to find risk factors for disease outbreaks on farm and make recommendations for disease control.

1. Contact details

Please fill in what is different from the provided printed page or is missing:

1.1 Property name: _____

1.2 AHB (Animal Health Board) number: _____Dairy No.:_____

Agribase number: _____

1.3 Contact person: _____

The contact person is (please circle): *Owner / Manager / Both*

1.4 Veterinary or vet-practice commonly used (name): _____

1.5 Postal address: _____

1.6 Farm address (if different):_____

1.7 District: _____ Region: _____

1.8 Phone (home): _____ Phone (business, if different): _____

1.9 Fax: _____ Mobile: _____

1.10 E-mail: _____

2. Livestock

2.1 Please check on printed page whether **farm type** and the number of **livestock wintered** is the same this year. Otherwise please change directly in the print out provided.

3. Leptospirosis, Johne's disease and farm environment

3.1 Has Leptospirosis been diagnosed or suspected in yourself, family members or farm workers in 2009?

☐ YES ☐ NO

If YES, please tick and complete the appropriate boxes in the table below:

Leptospirosis cases	Number of cases	Doctor or laboratory confirmed	Suspected* (but not confirmed)	Year
You personally				
Family members				
Workers				

**Signs of Leptospirosis are severe flu-like, but without nasal discharge or cough. They include fever, headache, sensitivity against light, chills, muscle aches, vomiting, and may include dark urine, red eyes, abdominal pain, diarrhoea, and/or a rash.*

3.2 Have your farm or pet dogs been vaccinated against Leptospirosis in 2009?

☐ YES ☐ NO ☐ Don't know

3.3 How many dogs were on this property 2009 (including farm and pet)? _____

3.4 Has the sampled mob grazed on pastures with access to standing water (i.e. a pond) in the last 12 months?

☐ YES ☐ NO ☐ Don't know

3.5 Describe any unusual environmental conditions experienced by your herd from **January to December 2008** (eg: drought, flood)

☐ No unusual conditions observed OR _____

3.6 Please indicate the abundance of rodents or wildlife species on or immediately surrounding your fenced area

	Abundant	Seen occasionally	Seen rarely	None
Rodents				
Possums				
Hedgehogs				
Rabbits				
Feral sheep/goats/deer				

3.7 Do you set traps or distribute poison?

☐ YES ☐ NO

☐

3.8 Do you spread manure/animal faeces on your paddocks?

☐ YES (Source of faeces: _____) ☐ NO

3.9 Indicate the source(s) of water to your animals in 2008 (*Please tick all that apply*)

☐ Troughs ☐ Dams ☐ Wallows ☐ Natural Springs
☐ Stream ☐ River ☐ Irrigation ditches ☐ Other: _____

3.10 Indicate any livestock species upstream of each water source in **2008 (***Please tick all that apply)*

Water source	**Species upstream in 2008**											
	No livestock species upstream	**Deer**		**Sheep**		**Dairy Cattle**		**Beef Cattle**		**Other**	
		own	other	own	other	own	other	own	other	own	other
Troughs											
Dams											
Natural Springs											
Stream/River											
Irrigation											
Other											

3.11 Please indicate livestock grazed by all neighbours in direct contact (ie: across fenceline) with your livestock paddocks and describe the type of boundary fencing in place (*Please tick all that apply)*

Neighbour(s)	**Species**					**Fencing type**	
	Deer	Sheep	Dairy cattle	Beef cattle	Other	Single	Double
1							
2							
3							
4							
5							

4. Grazing pasture management

4.1 Has your grazing pasture management changed since last year?

☐ YES ☐ NO

4.2 **If YES** and different livestock species have shared pasture, either by co-grazing (same paddock, same time) or by alternately grazing (same paddock, different time), please complete the table below, including an estimated time frame (see examples).

	Grazing pattern	Deer	Sheep	Beef cattle	Dairy
Example 1 Deer	**Co-grazed**		Deer yearlings with MA ewes for 2 months		
	Alternately grazed			Deer yearlings follow cows after 2 month	
Example 2 Beef cattle	**Co-grazed**		Beef calves with hoggets for 7 weeks		
	Alternately grazed	Beef yearlings follow adult hinds after 6 weeks			
Deer	**Co-grazed**				
	Alternately grazed				
Sheep	**Co-grazed**				
	Alternately grazed				
Beef cattle	**Co-grazed**				
	Alternately grazed				
Dairy Cattle	**Co-grazed**				
	Alternately grazed				

The next sections relate to each species: please complete as appropriate.

5 Deer (if you do not have deer, go to section 6)

5.1 Please enter percentages for reproduction and culling.

Percentage of reproduction and culling	2008*	2009
Weaning % (= Weaners in 2009 (08) / hinds at calving 2008 (07))		
Pregnancy % in R2YO hinds (= Number R2YO hinds scanned pregnant in 2009 (08) / number R2YO hinds mated in 2009 (08))		
Pregnancy % in MA hinds (= Number MA hinds scanned pregnant in 2009 (08) / number MA hinds mated in 2009 (08))		
Culling % (= Number sold or culled / number of animals in flock at time of sampling)		

*if 2009 not available yet

5.2 Please enter for each age class the number of **deer that died on farm** (any reason) in the last 12 months (first row) and give an estimate of mortality % (second row, see detail under the table below).

	Weaners*	Yearlings**	Adults***	
			Hind	Stags
Number dead				
Estimated mortality %				

* Number dead weaners/number weaned.
** Number dead yearlings/number present at 12 months of age
*** Number dead adults/number of adults present 12 months ago

5.3 Mob size: what is the approximate number of animals in the mob, which is blood and faecal sampled today?_____

5.4 Have any of your deer **been diagnosed with Johne's disease** by your veterinarian and/or through laboratory testing of blood/faeces/tissue in 2009? Yes / No

5.5 **If YES, or you have suspected cases of Johne's disease**, please list your observations in the table below. *Suspected = you believed it was JD based on illthrift, often with chronic scouring that does not respond to treatment, leading to emaciation and death, but not confirmed by veterinarian or laboratory.*

Month(s)	Year (2005-8 only)	Age range	Number of cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example: June	2005	0.5-1 yrs	7	No ☐	Vet ✓	Lab ✓
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐

5.6 Have any of your deer **been diagnosed with Leptospirosis** by your veterinarian and/or through laboratory testing of blood in 2009?

☐ YES ☐ NO

5.7 **If YES, or if you had suspected cases of Leptospirosis**, please list your observations in the table below: *Suspected = you believed it was Leptospirosis based on blood stained urine (red water), multiple sudden deaths, often with jaundice, but not confirmed by veterinarian or laboratory.*

Month(s)	Age range	Number of cases	Briefly describe the cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example June	< 0.5 yrs	7	7 weaners found dead, no signs of disease seen prior	No ☐	Vet ✓	Lab ✓
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐

5.8 Were any deer vaccinated against **Leptospirosis** in 2009?

☐ YES ☐ NO

If "YES", please provide information about the vaccination protocol.

Deer class		Vaccine name (if known)*	Number of times vaccinated	If more than once, give time interval
Example:		Leptavoid® 3	2	4-6 weeks.
Weaners	**Hinds**			
	Stags			
Yearlings	**Hinds**			
	Stags			
Adults	**Hinds**			
	Stags			

*Note: The 7 in 1 vaccine includes Leptospirosis.

6 Sheep (If you do not have sheep, go to section 7).

6.1 Have you observed or suspected any ewes' abortion since mating? Yes / No
If **Yes**, how many aborted? _____ Diagnosis?_____
Vet confirmation Yes / No

6.2 Please enter percentages for reproduction and culling.

Percentage of reproduction and culling	**2008***	**2009**
Tailing % (= lambs at tailing in 2009 (8) / number pregnant ewes wintered in 2008 (07))		
Pregnancy % (= Number scanned ewes pregnant in 2009 (08) / number ewes mated in 2009 (08))		
Culling % (= Number sold or culled / number of animals in flock at time of sampling)		

*if 2009 not available yet

6.3 Please enter for each age class the number of **sheep that died on farm** (any reason, first row)) in the last 12 months and give an estimate of mortality % (second row, see detail under the table below).

	Lambs*	**Hogget****	**2-Tooth****	**Mixed age****
Number dead or lost				
Estimated mortality %				

**Number dead lambs (on farm)/ number born in the last season.*
***Number of dead in the last 12 months / number present 12 months ago.*

6.4 Mob size: what is the approximate number of animals in the mob, which is blood and faecal sampled today? _____

6.5 Have any of your sheep **been diagnosed with Johne's disease** by your veterinarian and/or through laboratory testing of blood/faeces/tissue in 2009? Yes / No

6.6 **If YES, or you have suspected cases of Johne's disease**, please list your observations in the table: *Suspected = you believe it was JD based on severe wasting in animals that does not respond to treatment, sometimes wool break/poor fleece, ending in death and/or a small, distinct "tail" to the mob, but not confirmed by veterinarian or laboratory.*

Month(s)	**Year (2005-8 only)**	**Age range**	**Number of cases**	**Confirmed by Veterinarian or Lab.-Test? (tick as many apply)**		
Example: July	2007	3-4 yrs	3	No ☐	Vet ✓	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐

6.7 Have any of your sheep **been diagnosed with Leptospirosis** by your veterinarian and/or through laboratory testing of blood in 2009? Yes / No

6.8 **If YES or you have <u>suspected cases of Leptospirosis</u>**, please list your observations in the table: *<u>Suspected</u> = you believed it was Leptospirosis based on blood stained urine (red water) or multiple sudden deaths often with jaundice but not confirmed by veterinarian or laboratory.*

Month(s)	Age range	Number of cases	Briefly describe the cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example October	2-3 yrs	20	Ewes presented abortion and red water, 10 ewes died.	No ☐	Vet ✓	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐

6.9 Were any sheep vaccinated against **Leptospirosis** in 2009? Yes / No
If "YES", please provide information about the vaccination protocol.

Sheep class	Vaccine name (if known)*	Number of times vaccinated	If more than once, give time interval?
Example:	Leptavoid® 2	2	4 to 6 weeks.
Lambs			
Hoggets			
Ewe 2-tooths			
Ewes (Mixed age)			
Rams			

*Note: The 7 in 1 vaccine includes Leptospirosis.

7 Beef cattle (if you do not have beef cattle, go to section 8)

7.1 Have you observed or suspected any heifer/cow aborting? Yes ☐/ No ☐
If **Yes**, how many aborted? _____ Diagnosis? _____
Vet confirmation Yes ☐/ No ☐

7.2 Please enter percentages for reproduction and culling.

Percentage of reproduction and culling	**2008***	**2009**
Calving % across age groups (= no.calved 2009 (08) / no.pregnant 2008 (07)		
Pregnancy % in 15mo heifers (= Number 15mo heifers scanned pregnant in 2009 (08) / number 15mo heifers mated in 2009 (08))		
Pregnancy % in 27mo heifers (= Number 27mo heifers scanned pregnant in 2009 (08) / number 27mo heifers mated in 2009 (08))		
Pregnancy % in MA cows (= Number MA cows scanned pregnant in 2009 (08) / number MA cows mated in 2009 (08))		
Culling % (= Number sold or culled / number of animals in flock at time of sampling)		

*if 2009 not available yet

7.3 Please enter for each age class the number of **beef cattle that died on farm** (any reason, first row) in the last 12 months and give an estimate of mortality % (second row, see detail under the table below).

	Calves	**15 month heifers***	**27 months heifers***	**Mixed age***	
				Cows	**Bulls**
Number dead /lost					
Estimated mortality %					

**It is number dead (on farm)/number present 12 months ago.*

7.4 What was the number of calves marked from this and last year's calving season?
_____ Not available yet ☐

7.5 Mob size: what is the approximate number of animals in the mob, which is blood and faecal sampled today? _____

7.6 Have any of your beef cattle **been diagnosed with Johne's disease** by your veterinarian and/or through laboratory testing of blood/faeces/tissue in 2009? Yes ☐ / No ☐

7.7 **If YES, or you have suspected cases of Johne's disease**, please list your observations below: *Suspected = you believe it was JD based on chronic diarrhoea and weight loss that does not respond to treatment, sometimes a 'bottle jaw and not confirmed by veterinarian or laboratory.*

Month(s)	**Year (2005-08 only)**	**Age range**	**Number of cases**	**Confirmed by Veterinarian or Lab.-Test? (tick as many apply)**		
Example: October	2006	3-4 yrs	2	No ✓	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐
				No ☐	Vet ☐	Lab ☐

7.8 Have any of your beef cattle **been diagnosed with Leptospirosis** by your veterinarian and/or through laboratory testing of blood in 2009? Yes / No

7.9 **If YES or you have suspected cases of Leptospirosis**, please list your observations below:
Suspected = you believed it was Leptospirosis based on blood stained urine (red water) or multiple sudden deaths often with jaundice but not confirmed by veterinarian or laboratory.

Month(s)	Year (2005-8 only)	Age range	Number of cases	Briefly describe the cases	Confirmed by Veterinarian or Lab.-Test? (tick as many apply)		
Example June	2007	0.5-1 yr	5	Red water was observed in five yearlings	No ☐	Vet ✓	Lab ☐
					No ☐	Vet ☐	Lab ☐
					No ☐	Vet ☐	Lab ☐
					No ☐	Vet ☐	Lab ☐
					No ☐	Vet ☐	Lab ☐

7.10 Were any beef cattle vaccinated against **Leptospirosis** in 2008? Yes / No
If "YES", please provide information about the vaccination protocol.

Beef cattle class	Vaccine name (if known)	Number of times vaccinated	If more than once, give time interval?
Example	7-in-1	1	NA
Calves			
Yearlings			
Cows			
Bulls/steers			

8 The information provided **in this questionnaire** was based on (just tick one):

- ☐ Written records of farm data
- ☐ Memory
- ☐ Mostly memory + a few recorded data
- ☐ Mostly recorded data + memory

Thank you for your time!

Comments (Especially about this survey, JD or Leptospirosis):

Confidentiality

All information will be held strictly confidential by the researchers. No information will be released that is in any way identifiable to individual farms, owners or personnel.