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# **Epidemiology, diagnosis and vaccination control of leptospirosis in farmed deer in New Zealand**

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## Abstract

Leptospirosis is a bacterial zoonotic disease of global importance. It is caused by infection with pathogenic *Leptospira* species. Leptospirosis encompasses a wide spectrum of clinical or subclinical disease in both humans and animals. In New Zealand (NZ), leptospirosis is considered to be the most important occupational zoonosis. Livestock farming plays an important role as a major occupational risk factor for human leptospirosis and farmed deer is one of the contributing factors.

Commercial farming of deer began in NZ in the early 1970s. It remains the world's largest and most advanced deer farming industry. Leptospirosis in farmed deer can cause illness and possibly sub-clinical production losses. Farmed deer also play an important role in the transmission of leptospirosis by shedding the organisms in their urine, putting both other animals and humans at risk. *Leptospira* serovars Hardjobovis and Pomona are the most commonly found serovars in this stock group. The first substantial case report of leptospirosis in farmed deer was in the 1980s but it was not until 2006 that a substantial epidemiological study of this disease in farmed deer was reported. The purpose of this research was to improve and extend current knowledge on the epidemiology of leptospirosis on mixed-species deer farms, to develop and validate a novel molecular diagnostic tool and to enhance understanding of control measures and their outcomes by means of vaccination.

A pilot longitudinal seroprevalence survey of leptospirosis on mixed-species deer farms was conducted. Results from this study revealed that leptospiral infection averaged 70% in all species on mixed-species farms in the lower North Island of NZ. Co-grazing with infected sheep and/or cattle was positively associated with deer herd serological status to both serovars Hardjobovis and Pomona which suggests the possibility of inter-species transmission. Results from this study justify further investigation of leptospirosis on mixed-species farm at the national level.

A collaborative study between Massey University and the WHO/FAO/OIE reference laboratory for leptospirosis in Brisbane to investigate for exotic serovars in farmed deer revealed seropositivity to Arborea which has never been found before in NZ. Attempts to isolate Arborea from kidney samples of farmed deer were unsuccessful and require further investigation.

Real-time PCR assay was developed and validated against culture as the gold standard for use on deer kidney tissue and urine as a research and diagnostic tool for determining infection, carrier and shedding status of deer. This research revealed that the real-time PCR assay was highly sensitive (sensitivity: 85% for kidney and 96.7% for urine) and specific (specificity: 99.2% for kidney and 100% for urine). It is a useful tool for the rapid and cost-effective detection of pathogenic leptospires in clinical samples. It can also be used to quantify the concentration of leptospires from clinical samples and identify the likely infecting serovar in NZ when adjunct with a DNA sequencing technique.

Vaccination control for leptospirosis has proven to be efficacious and likely to be cost-effective. Present research has determined the effect of a commercial bivalent leptospiral vaccine (Leptavoid-2, Intervet/Schering-Plough Animal Health Limited, NZ) on leptospiral shedding, growth and reproduction of farmed deer under NZ pastoral conditions. The study was designed to simulate an infection-free herd scenario followed by exposure to natural challenge. Results have shown the potential of vaccine to improve mean weight gain (up to 6.5 kg) and weaning rate (average 6.9%) in infected herds and prevent urinary shedding after natural challenge with Hardjobovis. It also provides the first evidence of adverse subclinical effects on deer production by Hardjobovis alone.

A pilot study to investigate the presence and localisation of pathogenic *Leptospira* in the uterus and foetus of female deer revealed evidence of a foetal infection using real-time PCR. This finding suggests a possible explanation for effects of leptospiral infection on NZ farmed deer reproduction. However, further study is required to justify this proposition.

This research has contributed significantly to understanding of epidemiology of leptospirosis in NZ farmed deer, providing objective data to assist producers in decision-making on leptospirosis control. Furthermore, this study has made available a valuable diagnostic resource for future leptospirosis studies, and has provided direction for future research into leptospirosis on farmed deer and mixed-species farms.

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## **Declaration**

Each chapter in this thesis is set out as a paper in the style and format required of the journal to which it is in the process of being submitted to at the date of submitting this thesis. As a result, there is some repetition, particularly in the methods, and there are inconsistencies with the style and format between the chapters. Contributions to the research have been made by co-authors indicated in each chapter. However, my input was the greatest as I designed the study, undertook all the fieldwork and laboratory work, analysed the data and wrote the manuscripts.

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## **Publications**

**Heuer C, Dreyfus A, Wilson PR, Benschop J, Subharat S, Ayanegui-Alcérreca MA, Fang F, Collins-Emerson JM, Midwinter AC.** Epidemiology and control of leptospirosis in New Zealand. *Proceeding of the SVEPM conference*, 2010 in press

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**Wilson PR, Heuer C, Subharat S, Ayanegui-Alcérreca MA, Collins-Emerson JM.** Leptospirosis on deer farms: To vaccinate or not? *Proceeding of the Deer Branch of the NZVA* 26, 89-94, 2009

**Subharat S, Wilson PR, Heuer C, Collins-Emerson JM.** Leptospirosis: A Massey University research update. *Proceeding of the Deer Branch of the NZVA* 25, 115-20, 2008

**Heuer C, Wilson PR, Benschop J, Subharat S, Collins-Emerson JM, Midwinter A.** Leptospirosis - A rising public health hazard? *Proceeding of the combined meeting of the Food Safety, Animal Welfare & Biosecurity and Epidemiology & Animal Health Management Branches of the NZVA* 273, 101-6, 2008

**Subharat S, Wilson PR, Heuer C, Collins-Emerson JM.** Seroprevalence of *Leptospira* serovars Hardjovis and Pomona on mixed-species farms in the lower North Island of New Zealand. *Proceeding of the Deer Branch of the NZVA* 24, 87-91, 2007

## Presentations

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**Subharat S, Platero E, Collins-Emerson JM\*, Benschop J, Fang F, Wilson PR, Heuer C.**

Evaluation of molecular tools for diagnosis and epidemiological studies of leptospirosis in New Zealand. *The 6th annual scientific meeting of International Leptospirosis Society (ILS), Cochin, India, 2009*

**Wilson PR\*, Subharat S, Ayanegui-Alcérreca MA, Heuer C, Collins-Emerson JM.**

Reduction of weight gain in farmed red deer due to leptospiral infection. *The 6th annual scientific meeting of International Leptospirosis Society (ILS), Cochin, India, 2009*

**Subharat S\*, Wilson PR, Heuer C, Collins-Emerson JM.** Leptospirosis: the facts. *The*

*Deer Industry Technical Conference, Christchurch, New Zealand, 2009*

**Subharat S\*, Wilson PR, Heuer C, Collins-Emerson JM.** Leptospirosis: A Massey

University research update. *The Deer Branch NZVA Conference, Christchurch, New Zealand, 2008*

**Wilson PR\*, Ayanegui-Alcérreca MA, Subharat S, Collins-Emerson JM, Smythe S,**

**Dohnt M, Symonds M, Heuer C, Midwinter AM, Castillo-Alcala F, Mackintosh, CG.** Leptospirosis and *Leptospira* serovar on deer farms in New Zealand. *The 5th annual scientific meeting of International Leptospirosis Society (ILS), Quito, Ecuador, 2007*

**Subharat S\*, Wilson PR, Heuer C, Collins-Emerson JM.** Leptospirosis on mixed-species

farm. *The Deer Branch NZVA Conference, Palmerston North, New Zealand, 2007*

**\* Speaker**



# **Chapter 1**

## **Literature Review**

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## 1.1 Introduction

Leptospirosis is considered to be a serious zoonotic disease of increasing prevalence and worldwide distribution (Bharti et al., 2003, Levett, 2001). Awareness of the disease has increased internationally over the past decade for both developing and industrialized countries. Leptospirosis is caused by infection with pathogenic leptospires: helical shaped motile spirochetes which belong to the family *Leptospiraceae*, genus *Leptospira*. Globally, it is claimed to affect millions of people annually and cause substantial domestic livestock loss. It is widely accepted that the incidence of leptospirosis is under-reported due to lack of clinical suspicion and barriers to diagnostic capacity. Case findings and reporting in both human and veterinary medicine have been limited and biased (Cachay & Vinetz, 2005).

Traditionally, leptospires are classified into two species: *L. interrogans*, comprising all pathogenic strains; and *L. biflexa*, containing the saprophytic strains isolated from the environment. Based on a surface antigenic classification system, there have been over 200 serovars grouped into 23 serogroups of *Leptospira* that have been isolated and described in various parts of the world (Bharti et al., 2003) proving that these organisms are ubiquitous, diverse and complex. Distribution of leptospirosis is largely dependent on the presence of local carrier animals and environmental conditions that enable leptospires to be maintained outside the hosts (Torten & Marshall, 1994).

Leptospirosis is considered to be the most important occupational zoonosis in developed countries (Holk et al., 2000, Jansen et al., 2005). In New Zealand, the epidemiology of leptospirosis follows the pattern that occurs in temperate regions involving relatively few serovars, and dominated by exposure to domestic livestock. Livestock farming plays an important role as a major occupational risk factor for human leptospirosis in New Zealand (Thornley et al., 2002) unlike in other countries where the primary exposure to humans is from vectors such as rodents and wildlife. The major economic impact of leptospirosis in New Zealand involves livestock industries. There are costs associated with prevention for both humans and animals, medical and veterinary care and loss in animal production and human productive working time.

This review describes the history of leptospirosis, taxonomy and classification, epidemiology, pathogenesis, immune response, clinical signs and pathology. Current information on diagnostics and typing are discussed along with animal production issues, treatment and vaccination. In particular the epidemiology of leptospirosis for both humans and New Zealand livestock, with particular reference to farmed deer, is reviewed. In this review, the term “leptospirosis” is defined as both clinical and subclinical disease while “infection” refers to the invasion and multiplication of leptospires in host tissues, manifest either clinically or subclinically.

## 1.2 History of leptospirosis

In humans, a disease resembling leptospirosis was recognized as an occupational hazard of rice harvesting in ancient China, and the Japanese name “Akiyami” or autumn fever still persists (Levett, 2001). It was identified elsewhere more than one century ago as a disease causing severe icterus and renal failure in humans. Initially, it was named “Weil’s disease” after Adolf Weil who first reported it in 1886 (Weil, 1886). Twenty years later, a spirochete in a patient with Weil’s disease that died from “yellow fever” was isolated and named “*Spirocheta interrogans*” as the organism with its hook ends resembles a question mark (Stimson, 1907). This same spirochete was later isolated independently in both Japan and Germany and named as “*Spirocheta icterohaemorrhagiae*” (Hubener & Reiter, 1915, Inada et al., 1916, Uhlenhuth & Fromme, 1915). Two years later, this organism was shown to differ from other spirochetes and reclassified into a new genus called “*Leptospira*” (Noguchi, 1917). Rats were initially considered to be the primary source of this organism (Ido, 1917) but animals now known to be involved in human occupational exposure include livestock, rodents, pets and wild animals. The importance of occupation as a risk factor for leptospirosis was first described in soldiers (Johnston et al., 1983), miners, sewer workers and rice planters (Padre et al., 1988) all of whom work in wet conditions (Faine et al., 1999).

In animals leptospirosis was identified as a clinical entity in 1850, approximately 30 years before Adolf Weil described the disease in humans. In 1898, an epidemic in dogs was recorded in Stuttgart, Germany, but its cause was not confirmed until the aetiology of Weil’s disease was recognized (Torten & Marshall, 1994). The importance of leptospirosis in domesticated animals was increasingly realized in the 1920s when dogs were found to be infected by *L. icterohaemorrhagiae* (Alston & Broom, 1958). During the 1950s to 1960s, the significance of domesticated animals other than dogs in leptospirosis was highlighted by the development of vaccines for veterinary uses. Accumulated evidence showed that leptospires could infect all known mammals and possibly some lower vertebrates such as reptiles, amphibians, fish and birds (Torten & Marshall, 1994, Farr, 1995). In the 1970s and 1980s the ubiquitous distribution of Hardjo infections in cattle and humans who handled them was recognized. Redefinition of the occupational risks to humans, clarification of the means of transmission and improved diagnostic methods were the main advances in those periods. Basic understanding of the microbiology of leptospires and host immune responses provided an improvement in risk management of leptospirosis in animals (Faine et al., 1999).

## 1.3 The organism

Leptospires are spirochetes, a group of bacteria that developed in early bacterial evolution. They belong to the order Spirochaetales family *Leptospiraceae* which includes three genera, namely *Leptospira*, *Leptonema* and *Turneria* (Torten & Marshall, 1994). Their cells have pointed ends with either one or both sides bent into a hook (Figure 1.1). Leptospires are actively motile, exhibiting both translational and non-translational movement (Berg et al.,

1978, Cox & Twigg, 1974). They have a typical double membrane structure similar to other spirochetes, in which the cytoplasmic membrane and peptidoglycan cell wall are closely associated and are overlain by an outer membrane (Haake, 2000). Leptospiral lipopolysaccharide (LPS) has a composition similar to other gram-negative bacteria (Vinh et al., 1986) but with less endotoxic activity (Shimizu et al., 1987).

Leptospire are about 0.25 x 6.25µm and pass through a 0.45µm filter. For direct visualization, dark-field or phase-contrast microscopy of wet preparations is required (Bharti et al., 2003). Leptospire are catalase and oxidase positive. The genome of *Leptospira* consists of two circular chromosomes and is large compared with that of other spirochetes such as *Treponema* or *Borrelia*. This may contribute to its ability to live in various environments (Bharti et al., 2003).

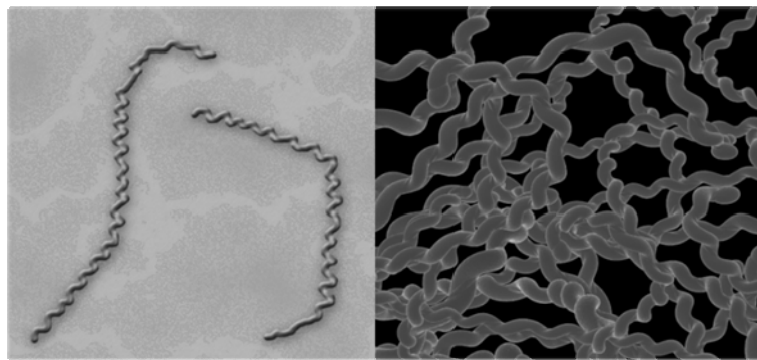


Figure 1.1 Leptospire under electron microscope and computerized graphic model  
(adapted from [www.leptospirosis.org](http://www.leptospirosis.org))

## 1.4 Isolation

Leptospire are obligate aerobes with an optimal growth temperature of 28 to 30°C. They grow in media enriched with vitamins (B2 and B12), long-chain fatty acids, and ammonium salts (Ellinghausen & McCullough, 1965). Long-chain fatty acids are utilized as the sole carbon source and are metabolized by  $\beta$ -oxidation. Isolation of leptospire is time-consuming but with pure subculture into liquid medium they will usually grow in 10 to 14 days. The culture should be retained for at least four months before being discarded as negative. The optimal pH for growth and survival of leptospire is between 7.2 to 7.6 (Faine et al., 1999).

Various kinds of media for leptospire including liquid (EMJH, Korthof, Fletcher and Stuart), semi-solid (media with 0.2% agar) and solid (media with 1% agar) have been developed. Liquid media are necessary for culture and typing. Semisolid media are generally used for isolating strains and for the maintenance of stock cultures, whereas solid media are used for isolating leptospire from contaminated sources, separating mixed culture of leptospire and for detecting haemolysin production (Roth et al., 1961, Stamm & Charon, 1979). Media are made selective by adding several antibiotics, the most common being 5-

fluorouracil and neomycin sulphate (Bharti et al., 2003). Culture from clinical samples is usually a definitive diagnosis of leptospiral infection but its drawback is that it is time-consuming, labour-intensive and easy to contaminate (Ellis, 1986).

## 1.5 Taxonomy and classification

Up to the late 1970s, the genus *Leptospira* comprised two species, *Leptospira interrogans* and *Leptospira biflexa*. *L. interrogans* contained 23 serogroups whose strains were either parasitic or pathogenic to humans or animals. *L. biflexa* contained 28 serogroups whose strains were usually found in fresh surface water or moist soil and were rarely isolated from man or animals (Kmety & Dikken, 1993, Johnson & Faine, 1984). A new family *Leptospiraceae*, which comprised the genera *Leptospira* and *Leptonema*, was proposed at that time (Hovind-Hougen, 1979). In 1981, an additional species, *Leptospira parva* was proposed (Hovind-Hougen et al., 1981). However, according to analysis of G+C content and 16s rRNA gene sequencing showing its extreme heterogeneity, it has been decided that it is sufficiently different from the genus *Leptospira* or *Leptonema* to be ascribed a new genus in the family *Leptospiraceae* called “*Turneriella*” (Figure 1.2) (Levett et al., 2005a).

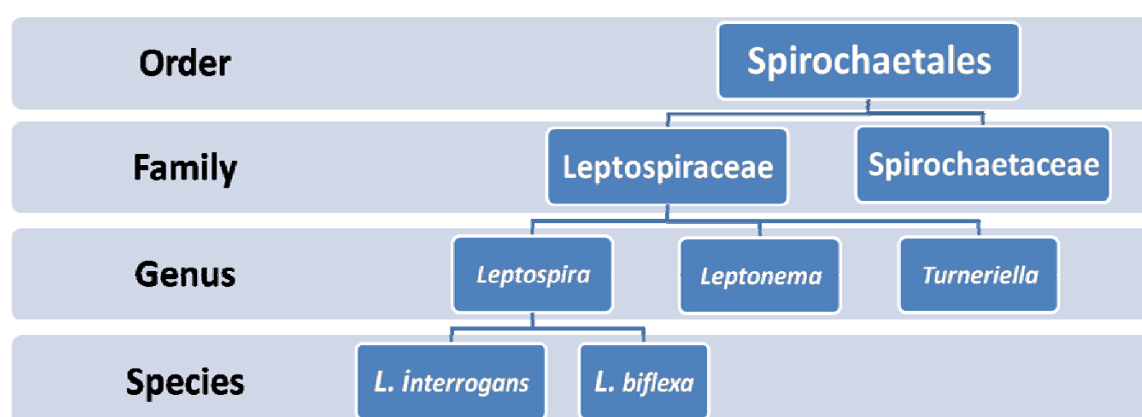


Figure 1.2 Taxonomy of *Leptospira sensu lato*

Currently, the reclassification of leptospires on genotypic grounds is proven to be correct and provides a strong foundation for future classification. However, the genomospecies of *Leptospira* do not correspond to the previous serological classification because pathogenic and non-pathogenic *Leptospira* serovars occur within the same genomospecies. Furthermore, molecular classification is complicated for the clinical microbiologist. Until simple DNA-based methods are developed and validated, serological classification methods need to be retained.

### 1.5.1 Serological classification

A serological taxonomy system (*sensu lato*) is used to divide *Leptospira* into serovars on the basis of surface antigen patterns. Both *L. interrogans* and *L. biflexa* are divided into numerous serovars defined by the Cross Agglutination Absorption Test (CAAT) with homologous antigen. If more than 10% of the homologous titer remains in at least one of the antisera after repeating absorption, two strains are said to belong to different serovars (Dikken & Kmety, 1978). Serovars that are antigenically related are grouped into serogroups (Kmety & Dikken, 1993). While serogroups have no taxonomic standing, they have proved useful for epidemiological understanding (Bharti et al., 2003). There have been over 200 serovars grouped into 23 serogroups of pathogenic *Leptospira* that have been isolated and described (Levett, 2001, Bharti et al., 2003).

### 1.5.2 Genotypic classification

The current classification of *Leptospira* genomospecies are summarized in Table 1.1. Genomic species of leptospires are determined by the group of *Leptospira* whose DNA show 70% or more homology at the optimal re-association temperature of 55°C, or 60% or more homology at a stringent re-association temperature of 70°C and in which the related DNA contains fewer than 5% of unpaired bases (Yasuda et al., 1987). The genotypic classification of leptospires is supported by Multi-locus Enzyme Electrophoresis (MLEE) data (Letocart et al., 1999). DNA hybridization studies have also confirmed the taxonomic status of the monospecific genus *Leptonema* (Brenner et al., 1999). According to the genetic taxonomy of *Leptospira* (*sensu stricto*), DNA-DNA hybridization techniques have classified the genus of *Leptospira* into 18 genomospecies divided into pathogenic, non-pathogenic and opportunistic pathogenic *Leptospira*. Pathogenic *Leptospira* include *L. interrogans*, *L. alexanderi*, *L. kirschneri*, *L. meyeri*, *L. borgpetersenii*, *L. weilii*, *L. noguchii*, *L. santarosai*, *L. genomospecies 1*, *L. genomospecies 4* and *L. genomospecies 5*. Non-pathogenic *Leptospira* include *L. biflexa*, *L. wolbachii* and *L. genomospecies 3*. Opportunistic pathogenic *Leptospira* include *L. fainei*, *L. inadai*, *L. broomii*, and *L. licerasiae* (Levett et al., 2006, Brenner et al., 1999, Matthias et al., 2008). The DNA sequence of the entire genome of *Leptospira* has been recently established (Nascimento et al., 2004, Bulach et al., 2006, Ren et al., 2003) and this has opened a new era of molecular diagnosis using PCR-based methods (Merien et al., 2005, Branger et al., 2005a) and genotyping of leptospires (Majed et al., 2005, Slack et al., 2005).

## 1.6 Epidemiology

Leptospirosis is a worldwide zoonosis. The transmission of infection to humans is usually either by direct or indirect contact with urine of infected animals. The core determinants of transmission are the presence of carriers, suitable environment for survival of leptospires and interaction between humans, animals and the environment (Figure 1.3) (Sehgal, 2006). After leptospires are excreted in urine, they may survive for weeks or months in optimal conditions. The disease is maintained in the environment by persistent renal colonization and urinary

shedding from maintenance hosts. Human infections may be acquired through occupational, recreational or non-vocational exposure. The extent to which infection is transmitted depends on many factors such as climate, population density and the degree of contact between maintenance and incidental hosts (Levett, 2001).

Table 1.1 Classification of *Leptospira* genomospecies

| Genomospecies             | Serovar             | Serogroup           | Pathogenicity |
|---------------------------|---------------------|---------------------|---------------|
| <i>L. interrogans</i>     | Australis           | Australis           | yes           |
|                           | Bratislava          | Australis           | yes           |
|                           | Bataviae            | Bataviae            | yes           |
|                           | Canicola            | Canicola            | yes           |
|                           | Copenhageni         | Icterohaemorrhagiae | yes           |
|                           | Hardjoprajitno      | Sejroe              | yes           |
|                           | Hebdomadis          | Hebdomadis          | yes           |
|                           | Icterohaemorrhagiae | Icterohaemorrhagiae | yes           |
|                           | Lai                 | Icterohaemorrhagiae | yes           |
|                           | Pomona              | Pomona              | yes           |
|                           | Pyrogenase          | Pyrogenase          | yes           |
| <i>L. alexanderi</i>      | Manhao3             | Manhao              | yes           |
| <i>L. kirschneri</i>      | Bim                 | Autumnalis          | yes           |
|                           | Cynopteri           | Cynopteri           | yes           |
|                           | Grippotyphosa       | Grippotyphosa       | yes           |
|                           | Mozdok              | Pomona              | yes           |
|                           | Panama              | Panama              | yes           |
| <i>L. meyeri</i>          | Semaranga           | Semaranga           | yes           |
| <i>L. borgpetersenii</i>  | Arborea             | Ballum              | yes           |
|                           | Ballum              | Ballum              | yes           |
|                           | Castellonis         | Ballum              | yes           |
|                           | Hardjobovis         | Sejroe              | yes           |
|                           | Javanica            | Javanica            | yes           |
|                           | Sejroe              | Sejroe              | yes           |
|                           | Tarassovi           | Tarassovi           | yes           |
| <i>L. weilii</i>          | Celledoni           | Celledoni           | yes           |
| <i>L. noguchii</i>        | Fortbragg           | Autumnalis          | yes           |
| <i>L. santarosai</i>      | Brasiliensis        | Bataviae            | yes           |
|                           | Georgia             | Mini                | yes           |
| <i>L. genomospecies 1</i> | Pingchang           | Ranarum             | yes           |
| <i>L. genomospecies 4</i> | Hualin              | Icterohaemorrhagiae | yes           |
| <i>L. genomospecies 5</i> | Saopaulo            | Semaranga           | yes           |
| <i>L. fainei</i>          | Hurstbridge         | Hurstbridge         | opportunistic |
| <i>L. inadai</i>          | Lyme                | Lyme                | opportunistic |
| <i>L. broomii</i>         | n/a                 | n/a                 | opportunistic |
| <i>L. licerasiae</i>      | Varillal            | Iquitos             | opportunistic |
| <i>L. biflexa</i>         | Patoc               | Semaranga           | no            |
| <i>L. wolbachii</i>       | Codice              | n/a                 | no            |
| <i>L. genomospecies 3</i> | Holland             | Holland             | no            |

Adapted from (Bharti et al., 2003, Levett et al., 2006, Matthias et al., 2008)

n/a = not available

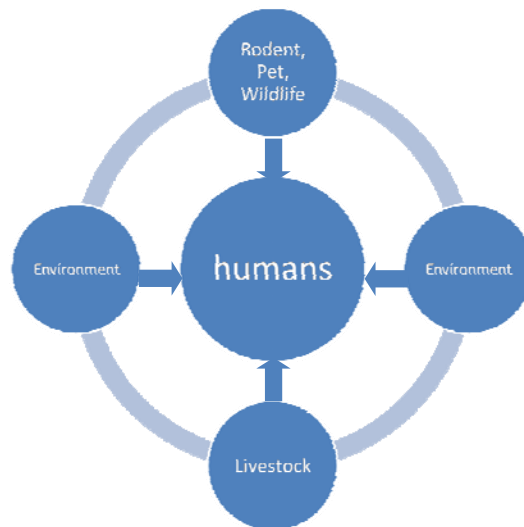


Figure 1.3 Transmission of leptospirosis

Occupation is a significant risk factor for human leptospirosis. Direct contact with infected animals accounts for most infections in farmers, meat workers, meat inspectors, veterinarians, rodent control workers and other occupations that require contact with animals (Demers et al., 1985, Terry et al., 2000, Thornley et al., 2002). Indirect contact is important for sewer workers, miners, soldiers, fish farmers, rice planters and banana farmers (Johnston et al., 1983, Padre et al., 1988, Robertson et al., 1981, Smythe et al., 2000). Tradition and religion may determine the degree of contact between humans and animals such as pigs, dogs and cattle. Perception of illness, tolerance of pain and suffering, expectation and understanding of medical care, availability and type of medical care all contribute to whether or not a person will be recognized or reported as having leptospirosis (Faine et al., 1999).

Leptospire are able to survive in alkaline soils, mud, swamps, streams, rivers, organs and tissues of live or dead animals and diluted milk. Survival of pathogenic leptospire in the environment is dependent on several factors including pH, temperature, and the presence of inhibitory compounds. In general, they are sensitive to dryness, heat, acids and basic disinfectants (Alston & Broom, 1958, Faine et al., 1999). Under laboratory conditions, leptospire in water at room temperature remain viable for several months at pH 7.2 to 8.0 (Crawford et al., 1971). When soil was contaminated with urine from infected rats or voles, leptospire survived for approximately 14 days (Karaseva et al., 1973). Survival of serovar Pomona has been documented for 42 days in soil under simulated winter conditions, 183 days in wet soil and 94 days in river water (Miller et al., 1991).

The presence of a maintenance host is crucial to the endemic existence of leptospirosis. A maintenance host is defined as a species in which infection is persistent and shedding of viable organisms in the urine lasts for months, and may even last for a lifetime (Marshall & Manktelow, 2002). Infection is usually acquired at an early age, and the prevalence of chronic excretion in the urine increases with the age of animal. Animals may be a maintenance host for some serovars but incidental host for others that may cause severe or

fatal disease (Hathaway et al., 1983). Specific leptospiral serovars have been associated with different mammalian hosts (Farr, 1995).

The epidemiology of leptospirosis has been described in three patterns. The first occurs in temperate climates where few serovars (mainly Hardjo, Pomona, Tarasovi and Grippotyphosa) are involved and human infection mostly occurs by direct contact with infected animals through farming. Under these conditions, it is likely that leptospirosis could be controlled by a vaccination programme. The second pattern occurs in tropical wet areas, where there are many more serovars infecting humans and animals and also with a larger number of reservoir species, including rodents, farm animals and pets. The incidence is much higher than in a temperate climate due to longer survival of leptospires in warm and humid conditions (Faine et al., 1999). The disease is seasonal with the likelihood of infection increasing during periods of high rainfall. Poor drainage, high water-holding capacity of soil, flooding and high density of carrier and susceptible animals are risk factors that correlate with a high incidence of leptospirosis (Heath & Johnson, 1994). Control of rodent populations, drainage of wet areas and occupational hygiene are all necessary for the prevention of human leptospirosis. The third pattern comprises rodent-borne infection in urban environments, most commonly associated with poverty and poor housing and sanitary conditions. These are also the areas where large outbreaks of leptospirosis are most likely to occur following floods, hurricanes or other disasters (Faine et al., 1999).

## **1.7 Pathogenesis**

Pathogenesis of leptospirosis is claimed to be similar for every animal species including humans. Leptospire enter the host via small abrasions, breaches of the surface integument, conjunctiva, mucous membrane and genital tract (Faine et al., 1999). They do not localize at the site of entry but spread rapidly through the blood stream and lymphatics to target tissues. Motility of leptospires may play an important role at the beginning of infection and spreading of organisms to various organs (Bharti et al., 2003). Leptospire do not cause an acute inflammatory response to their presence in tissue. Non-pathogenic strains are cleared rapidly by reticulo-endothelial phagocytosis whereas pathogenic strains can evade this process and multiply exponentially both in bloodstream and tissues.

The incubation period depends on infective dose, growth rate of organisms, their toxicity, and host immunity. In a carrier state, leptospires may persist in certain tissues that are immunologically privileged sites such as proximal renal tubules, the brain, anterior chamber of the eye and the genital tract. A study of renal colonization in a rat model have shown that leptospires initially disseminate throughout various organs, followed by clearance except from the kidneys, suggesting that kidneys are the most immune-privileged site (Athanazio et al., 2008). In the host renal tubule, leptospires migrate through the interstitial space to settle on the brush border and later excrete in host urine which is the common source of infection to other animals and humans (Faine et al., 1999).



The mechanisms by which leptospires cause disease are not well understood. Attachment to cell surface and toxicity seem to be important properties of virulent leptospires. A number of putative virulence factors have been suggested, but with few exceptions, their role in pathogenesis remains unclear (Levett, 2001). However, the advent of whole-genome sequencing of leptospires has greatly impacted on development of bioinformatics. This helps to reveal proteins independent of their abundance without the need of culturing organisms. Functional genomics studies, including transcription profile, gene cloning and protein expression complement *in silico* analysis have helped the understanding of pathogenesis of leptospires (Atzingen et al., 2008).

### 1.7.1 Attachment

Several attachment *in vitro* assays have shown that virulent leptospires adhere to fibroblastic cells (Vinh et al., 1984), renal epithelial cells (Ballard et al., 1986) and human endothelial cells (Thomas & Higbie, 1990). Leptospires have been shown to be phagocytosed by macrophages only in the presence of specific immune serum and complement (Vinh et al., 1982, Cinco et al., 1981, Pereira et al., 1998, McGrath et al., 1984). This suggests that the outer envelope of pathogenic leptospires possesses an antiphagocytic activity (Levett, 2001). In one study, it has been shown that pathogenic leptospires attached to neutrophils but were not killed by them (Wang et al., 1984). Leptospiral lipopolysaccharide (LPS) is claimed to stimulate adherence of neutrophils which is mediated by platelet-activating-factor (PAF) (Isogai et al., 1997). This suggests a role of LPS in the development of thrombocytopenia (Levett, 2001).

In the past decade, there has been a belief that interaction by leptospires with extracellular matrix (ECM) of host cells plays an important role in the colonization (Barbosa et al., 2006). The first step in adhesion of bacteria to the target cell membrane involves molecular recognition between surface proteins of both cell types (Merien et al., 2000). Normally, pathogens may gain access to ECM component after tissue trauma by cuts and abrasions, chemical injury or bacterial toxin and lytic enzymes (Ljungh & Wadstrom, 1996). In mammals, ECM is composed of two main macromolecules i.e. glycosaminoglycans and fibrous protein such as collagen, elastin, laminin and fibronectin (Barbosa et al., 2006). Fibronectin, a type of glycoprotein that is distributed widely in host cells was proposed to be the primary target for leptospires to adhere. Several fibronectin-binding proteins such as 36-kDa (Merien et al., 2000), LigA, LigB (Choy et al., 2007), Lsa21 (Atzingen et al., 2008) and Lsa 24 (Barbosa et al., 2006) have been studied and claimed as potential virulence factors of leptospires. Recently, *Leptospira* lipoprotein Loa22 was also proposed as an essential for *in vivo* infection of pathogenic leptospires (Nally et al., 2007, Ristow et al., 2007).

### 1.7.2 Toxins

Endotoxin activity has been reported in several serovars of leptospires. Leptospiral LPS preparations exhibit activity in biological assays for endotoxin similar to other Gram-negative bacteria. Some studies have shown that leptospiral LPS has less lethal potency than *E. coli* (De-Souza & Koury, 1992, Isogai et al., 1990). This may be due to the fact that leptospiral LPS stimulates innate immunity via a TLR2-dependent mechanism whereas other Gram-negative LPS stimulates innate immunity via TLR4-dependent mechanism (Werts et al., 2001).

Haemolysins have been suggested to be phospholipases, that act on erythrocytes (Thompson & Manktelow, 1986) and other cell membranes containing the substrate phospholipids, leading to cytolysis (Lee et al., 2002). Haemolysins from several serovars have been characterized. For example, serovar Pomona is known for production of haemolytic disease in cattle while serovar Ballum creates similar symptoms in hamsters (Levett, 2001). The haemolysins of serovars Hardjo and Pomona are claimed to be have sphingomyelinase C activity (Bernheimer & Bey, 1986, del Real et al., 1989). Also, phospholipase C activity has been reported in serovar Canicola (Yanagihara et al., 1982). However, a haemolysin gene (SphH) from serovar Lai is not associated with sphingomyelinase or phospholipase C and is clearly demonstrated to form pores in mammalian cells (Lee et al., 2000, Lee et al., 2002). Recently, haemolysins were further divided into 2 groups, sphingomyelinase and non-sphingomyelinase hemolysins (Zhang et al., 2005). The sphingomyelinase haemolysin gene Sph2 was also claimed to induce apoptosis in human liver cells (Zhang et al., 2008).

Cytotoxic activity of leptospires was first observed in supernatant extract from serovars Pomona and Copenhageni (Cinco et al., 1980). It has also been detected in the plasma of infected animals (Knight et al., 1973). This toxin elicited a typical cytopathic effect, with infiltration of macrophages and polymorphonuclear cells (Yam et al., 1970, Diamant et al., 2002). Later, a glycolipoprotein (GLP) fraction of leptospires was claimed to be the cause and shown to be toxic to cultured fibroblasts displaying endotoxic properties (Vinh et al., 1986). A similar fraction from serovar Canicola inhibits  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity either in purified preparation or in isolated nephron segments from rabbit kidney (Younes-Ibrahim et al., 1995). The hypothesis is that GLP will be released after lysis of leptospires by the host immune response. Then, it will cause an impairment of  $\text{Na}^+$  and  $\text{K}^+$ -ATPase which increase nonesterified unsaturated fatty acids (NEUFAs) and bilirubin serum levels and decrease albumin concentrations leading to hepatic icterus and renal dysfunction (renal failure) of the host (Burth et al., 2005).

## 1.8 Clinical signs

### 1.8.1 Humans

Human infection with leptospirosis may present variable clinical manifestations ranging from subclinical disease with undifferentiated febrile illness, to jaundice, renal failure and potentially lethal pulmonary disease (Bharti et al., 2003). Severe leptospirosis is frequently reported with infection by serovars Icterohaemorrhagiae, Copenhageni and Lai (Cachay & Vinetz, 2005). However, disease severity depends largely on geographic location, ecology of local maintenance host (Levett, 2001), human host genetics and dose of infection (Cachay & Vinetz, 2005).

Most people infected by *Leptospira* will develop mild fever with flu-like symptoms. People usually do not seek medical attention or may be misdiagnosed by unaware practitioners resulting in under-reporting of the true leptospirosis incidence (Heuer et al., 2008). In more severe cases, the disease will begin with moderate fever with a combination of symptoms such as general malaise, chills, headache, muscle pain and weakness, pneumonia, anaemia, conjunctival suffusion and photophobia. Aseptic meningitis may also occur in young patients. The disease usually lasts two to three weeks and a long recovery period is common (Levett, 2001, Torten & Marshall, 1994). Weil's syndrome represents the most severe form of leptospirosis characterized by jaundice and acute renal failure that occurs in the second phase of the illness. The case fatality rate may reach 15% despite supportive care (Ko et al., 1999). However, the incidence of Weil's syndrome is low. Pulmonary and cardiac involvement have also been reported in human leptospirosis (Chakurkar et al., 2008, Dolhnikoff et al., 2007).

### 1.8.2 Animals

In animals, clinical features of leptospirosis are similar across species (Faine et al., 1999). Various signs of disease can occur because of interactions between host adaptation, virulence of the serovars and current immune status of the host (Heath & Johnson, 1994). Clinical signs are usually mild or subclinical when infected by host-adapted serovars. However, when infected by non-host-adapted serovars, the clinical signs can range from mild to severe icterohaemorrhagic disease depending on age and host immunity. Normally, severe cases are observed more commonly in young than in older animals (Ellis, 1994). Clinical signs of acute leptospirosis usually start with elevated temperatures accompanied by anorexia, dull appearance, agalactia, haemolytic anaemia, haematuria, haemoglobinuria, jaundice, renal failure, all of which may progress to death. Animals that recover may have poor growth rates and significant renal lesions at slaughter (Faine et al., 1999). Chronic leptospirosis usually involves infection by host-adapted serovars which can take the form of abortion, stillbirth, weak offspring, chronic interstitial nephritis, poor reproductive performance and poor growth (Ellis, 1994, Ayanegui-Alcérreca, 2006). However, it should be noted that abortion caused by host-adapted serovars tends to occur sporadically compared with abortion storms that occur

with non-host-adapted serovars (Grooms, 2006). Due to the diverse symptomatology of leptospirosis, it is difficult to diagnose animal leptospirosis based on clinical signs alone (Torten & Marshall, 1994).

## **1.9 Pathology**

The main pathologic lesions of leptospirosis in animals and humans are similar, commonly marked in the kidney, liver, lung and heart except in peracute cases which only show signs of acute septicaemia. The differences are determined by serovar, the host immune responses and chronic-carrier state where leptospires survive, grow and pass on infection (Faine et al., 1999). The primary lesion of leptospirosis in both animals and humans is damage of the endothelial cell membrane of the capillaries which is believed to be caused by leptospiral toxin. It is characterized by development of vasculitis and inflammatory infiltrates composed of monocytic cells, plasma cells, histiocytes and neutrophils. With gross lesions, petechial haemorrhages are common and organs are discoloured if an icteric status is present (Levett, 2001). The vascular changes predispose ischemic damage, leading to necrosis of the target organs i.e. kidney, liver, lung, brain, placenta and muscle (Faine et al., 1999). These result in the common findings of leptospirosis which are nephritis, hepatitis and jaundice, pulmonary haemorrhage and oedema, encephalopathy and meningitis, placentitis and abortion or stillbirth, interstitial myocarditis and acute muscle tenderness (Chakurkar et al., 2008, Dolhnikoff et al., 2007, Faine et al., 1999). However, the severity of the disease depends on each individual.

The main histopathological lesions considered to be typical of leptospirosis are in the kidney. Cortical cellular necrosis, petechiae and ecchymotic haemorrhages particularly in the glomeruli and the proximal convoluted tubule are observed along with infiltration of mononuclear cells, interstitial nephritis and fibrosis. Leptospires may be seen within renal tubular epithelium on microscopic examination using Warthin-Starry stain (Langston & Heuter, 2003, Faine et al., 1999). Hepatic lesions included centrilobular necrosis, hepatocellular vacuolation and sometimes retention of bile (Fairley et al., 1986). Pulmonary congestion and haemorrhage are common histological findings in human pulmonary involvement in leptospirosis (Dolhnikoff et al., 2007).

## **1.10 Immune response**

Immunity to leptospires includes type-1 or cell mediated immunity (CMI) and type-2 or humoral mediated immunity (HMI). Formerly, it was thought that protective immunity to leptospiral infection was entirely humoral because it is highly serovar-specific (Adler & Faine, 1977). It has been proposed that immunity is linked to antibodies directed against leptospiral polysaccharide (LPS) which is a complex antigenic structure (Adler & Faine, 1977, Faine et al., 1999, Sonrier et al., 2001). Also, LPS has been shown to stimulate the innate immune system which comprises the cells and mechanisms that defend the host in a non-specific manner via the TLR2-dependent mechanism, unlike other Gram-negative

organisms that stimulate antibody production via the TLR4-dependent mechanism (Werts et al., 2001). The antibody response is classic with peak Immunoglobulin M (IgM) antibodies appearing first, quickly followed by Immunoglobulin G (IgG) antibodies. Anamnestic responses develop after re-exposure to organisms of the same serovar (Heath & Johnson, 1994). The level of antibody at any time point is influenced by its initial level, the rates of decay and its continued production. The duration of persistence of antibodies after either natural infection or immunization varied considerably between studies in both humans and animals. It is not possible to exclude subclinical reinfection as a counter stimulus to the decay in antibody titers (Faine et al., 1999).

Recently, the role of CMI in leptospiral infection has been realized. Insight into the protective response by CMI has been gained from study of a monovalent killed *L. borgpetersenii* vaccine in cattle. Those studies have demonstrated high level of interferon- $\gamma$  (IFN- $\gamma$ ) produced by CD4+ T cells and gamma delta ( $\gamma\delta$ ) T cells which correspond to CMI (Naiman et al., 2001, Naiman et al., 2002, Brown et al., 2003). Another study has demonstrated that leptospiral glycolipoprotein (GLP) extracted from pathogenic leptospires induced cellular activation of peripheral blood mononuclear cells (PBMC) through the secretion of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-10 (IL-10) whereas leptospiral GLP extracted from non-pathogenic leptospires did not (Diament et al., 2002). Naive human PMBCs from healthy individuals have also been shown to produce a large quantity of IFN- $\gamma$ -producing  $\gamma\delta$  T cells after *in vitro* stimulation by *L. interrogans*. Furthermore, in patients with acute leptospirosis, there was a significant increase of  $\gamma\delta$  T cells in peripheral blood (Klimpel et al., 2003). Thus, much remains to be established to fully understand the mechanisms of host immunity to leptospiral infection and to develop novel efficient leptospiral vaccines (Bharti et al., 2003, Koizumi & Watanabe, 2005).

### **1.11 Diagnosis**

The diverse clinical signs of leptospirosis make laboratory confirmation essential. However, interpretation of results must be cautious since they only represent the evidence of leptospiral infection, not necessarily disease causation. Consistent criteria are required in combination with clinical or pathology findings to confirm the diagnosis of leptospirosis. In animal populations, especially livestock, it is appropriate to diagnose leptospiral infection on a herd basis to provide morbidity or prevalence statistics that determine the magnitude of problem and determine control programs.

There are two main imperatives of leptospirosis diagnostic tests, namely detection and identification with the approach depending on the intention of diagnosis. On an individual animal basis, methods for detection *per se* are sufficient to confirm the infection and initiate a treatment. However, to deal with leptospirosis in a broader perspective, methods for serovar identification will provide much more information especially for understanding its epidemiology and determining control programmes. Isolation of *Leptospira* organisms from

clinical samples is the definitive method since it can both detect and identify the organism, using serological (CAAT) or molecular typing. However, isolation is uncommonly used because it is technically demanding, tediousness, complex, time consuming (e.g. 16 weeks before declaring a sample negative) and expensive. Thus, a variety of alternative methods have been developed in an attempt to provide more rapid and less expensive diagnosis of leptospirosis. These include methods that demonstrate organisms or antigens, that detect host antibody, and that detect genomic materials or DNA. Each method has its own purposes, advantages and disadvantages as summarized in Table 1.2.

#### **1.11.1 Direct examination and antigen detection**

Leptospire may be visualized in urine by dark-field microscopy because of their characteristic shape and movement but this method suffers from poor sensitivity and specificity (Vijayachari et al., 2001b). It requires a high degree of operator skill since the organisms can be mistaken for other cell debris. Approximately  $10^4$  to  $10^5$  leptospire/ml are necessary for one cell per field to be visible under dark-field microscopy of urine (Turner, 1970, Langston & Heuter, 2003). A variety of staining methods for histological sections such as silver staining (Skilbeck & Chappel, 1987), Warthin-Starry staining (Elliott, 1988), immunofluorescent staining (Skilbeck, 1986) and immunohistochemical staining (Yener & Keles, 2001) have been applied to increase the sensitivity of microscopic examination of leptospire on histological sections. Recently, a monoclonal antibody-based method has been evaluated to detect leptospiral antigens in cattle urine, claimed to detect as few as  $10^3$  leptospire/ml of urine (Suwimonteerabutr et al., 2005).

#### **1.11.2 Antibody detection**

Since *Leptospira* require several weeks to culture, laboratory diagnosis often depends on the demonstration of antibodies against *Leptospira* in sera. Serological tests are either serogroup-specific or genus-specific (Levett, 2004). Formerly, agglutination tests were developed such as the microscopic agglutination test (MAT) which is read under dark-field microscope, and macroscopic slide agglutination test (MSAT) which is read by the naked eye (Galton et al., 1958). The MAT is the most widely used standard reference test because of its high sensitivity and specificity and the ability to identify to serogroup or serovar level (O'Keefe, 2002, Vijayachari et al., 2001a). By comparison, the MSAT is a low performance test with poor reproducibility (Marin-Leon et al., 1997). A recent study reported MAT sensitivity ranging from 91.1% to 100.0% and specificity ranging from 94.3% to 100% (McBride et al., 2007b). However, the MAT has some limitations including the requirement for maintenance of a large number of live *Leptospira* cultures for antigens and complexity of methodology and interpretation which limits its use in the standard laboratory (Myers, 1976, Palmer, 1988).

Interpretation of MAT results is complicated by a number of factors including cross-reactivity of antibodies, antibodies induced by vaccination, and lack of consensus about what

antibody titers are indicative of infection. An agglutinating titer of  $\geq 1:200$  is considered significant in humans, in combination with present clinical illness, whereas  $\geq 1:100$  is considered significant in animals as evidence of previous exposure (Levett, 2001). However, the diagnostic cut point titer at  $\geq 1:100$  may be exceeded in vaccinated animals or may not be reached in host-adapted serovar infection (Grooms & Bolin, 2005). Different studies using different cut points make it difficult for comparison between studies. Paired sera are required to confirm the stage of infection. If the titer remains low, it may be assumed that it was due to previous infection but if the titer increases, it may be assumed due to recent infection. A fourfold or greater rise in titer between paired sera taken 7 to 10 days apart is considered diagnostic in both humans and animals (Faine et al., 1999).

Due to the complexity of the MAT, many rapid screening immunological tests have been developed and evaluated for determination of specific leptospiral IgM or IgG antibodies such as the complement fixation test (CF) (Terzin, 1956), Microcapsule Agglutination test (MCAT) (Arimitsu et al., 1982, Sehgal et al., 1997), haemolysin test (HL) (Cox, 1957), indirect immunofluorescent antibody test (IFA) (Appassakij et al., 1995), indirect haemagglutination test (IHA) (Imamura et al., 1974, Sulzer et al., 1975), dipstick assay (Smits et al., 1999, Smits et al., 2000) and enzyme-linked immunosorbent assay (ELISA) (Adler et al., 1980, Terpstra et al., 1985). Several specific monoclonal antibodies have been developed and combined with ELISA format in an attempt to further identify antibodies to serovar level (Surujballi et al., 1997, Yan et al., 1999).

Whole-cell *Leptospira*-based serologic assays are the most widely used methods for laboratory diagnosis of leptospirosis. Several commercial kits have been developed based on ELISA and immunochromatographic methods such as IgM ELISA, Dip-S-Tick (PanBio Inc.) and LeptoTek Dri-Dot (Biomérieux). These tests were extensively validated for their sensitivity and specificity against the MAT (Effler et al., 2002, McBride et al., 2007b, Ooteman et al., 2006). In general, the sensitivity of these tests were low in the acute phase (33% to 67%) but improved in the convalescent phase (84% to 100%) of the disease with high specificity (88% to 100%) (McBride et al., 2007a, McBride et al., 2007b, Ooteman et al., 2006). However, some studies reported that whole-cell *Leptospira* assays had low specificity (Blacksell et al., 2006, Vijayachari et al., 2002).

Recently, recombinant protein-based serologic tests have been developed because of their specificity and reproducibility. A recombinant flagella antigen immunocapture assay was described for serodiagnosis of bovine leptospirosis (Bughio et al., 1999). *Leptospira* immunoglobulin-like (Lig) proteins were evaluated as a serodiagnostic marker for human leptospirosis using an immunoblot assay. It has resulted in an improvement in detecting acute leptospiral infection, with sensitivity of 85% to 96% and specificity of 90% to 100% (Croda et al., 2007). There has also been an attempt to use the conserved regions of the Lig proteins as the serodiagnostic marker in differentiating between vaccinated animals and naturally infected animals (Palaniappan et al., 2004).

### 1.11.3 Molecular detection

Diagnosis of leptospirosis requires an assay able to detect a small number of leptospires in blood or urine and that can be performed rapidly, so infection can be detected and treated at an early stage. For epidemiological studies such an assay should be sensitive and allow many samples to be processed simultaneously. DNA-based techniques have been introduced into the field of leptospirosis research. Formerly, leptospiral DNA in clinical samples has been detected by DNA-hybridization with labelled radioactive ( $^{32}\text{P}$ ) or non-radioactive (biotin) probes. The principle is that if the nucleotide sequence of the samples match with those of the probes, DNA-hybridization will occur and this can be detected by autoradiographic or colourimetric methods depending on the type of probes. However, these techniques are probably no more sensitive than immunological or immunohistochemical methods (Terpstra et al., 1987, Terpstra et al., 1986). Polymerase Chain Reaction (PCR) assay has been demonstrated to be a sensitive and rapid technique. It involves *in vitro* enzymatic amplification of a target DNA sequence through a series of polymerizations carried out by a thermostable DNA polymerase. The specificity of the assay can be adjusted by the choice of short DNA fragment called “Primer”. First designed primers were developed from the serovar Hardjo DNA library in order to detect DNA of leptospires in cattle urine. The PCR assay is also claimed to be more sensitive than culture from urine (Van Eys et al., 1989). This technique was later improved by modification of the DNA extraction method and was claimed to detect as few as 5 to 10 leptospires/ml (Gerritsen et al., 1991). Another study has designed primers from 16S rRNA gene of serovar Canicola and claimed to detect as few as 10 cells of leptospires in urine, CSF and blood when combined with the DNA hybridization technique (Merien et al., 1992). However, primers designed from previous two studies were unable to differentiate between pathogenic and non-pathogenic *Leptospira*.

Thus, another study has developed combined primer sets of G1/G2 and B64-I/B64-II that can detect only pathogenic *Leptospira* by assessing them with a large number of reference strains belonging to all pathogenic and non-pathogenic *Leptospira* species (Gravekamp et al., 1993). These primers were widely used and validated in later studies for detection of leptospiral DNA in clinical samples from both animals and humans (Bal et al., 1994, Fonseca Cde et al., 2006, Brown et al., 1995, Ooteman et al., 2006, Parma et al., 1997, Wagenaar et al., 2000). The limit of detection of these PCR techniques varied from 1 cell/ml to 1,000 cells/ml depending on type of sample tested and DNA preparation methods. One study reported a sensitivity of 91% and a specificity of 100% when using these primers on cattle urine compared with culture (Wagenaar et al., 2000). In addition, several studies have more recently reported newly designed primers (Branger et al., 2005a, Woo et al., 1997b, Fearnley et al., 2008, Slack et al., 2006, Shukla, 2003). Others employ a multiplex PCR technique (Tansuphasiri et al., 2006, Kositanont et al., 2007) which uses more than one primer pair in a single reaction in an attempt to distinguish between pathogenic and non-pathogenic *Leptospira*.



PCR was claimed to be a promising method for the quick and early detection of leptospires in clinical samples in the period before antibodies become detectable (Brown et al., 1995, Fonseca Cde et al., 2006, Kee et al., 1994). The nested PCR technique which requires two rounds of PCR reaction with outer primer and inner primer pairs was developed to increase sensitivity and specificity of PCR in order to detect pathogenic leptospires (Bomfim et al., 2008, Nassi et al., 2003).

Recently, an extension of PCR that has significant application to the diagnosis of leptospirosis is the real-time PCR. These assays are rapid and sensitive and can quantify the amount of DNA in clinical samples without the use of conventional agarose gel electrophoresis. Most of the real-time PCR techniques that have been reported for detection of leptospiral DNA involve the use of double-stranded DNA intercalating dye, such as SyBr Green I (Levett et al., 2005b, Merien et al., 2005, Slack et al., 2006) or the use of fluorescent labelled probes such as Taqman (Cox et al., 2005, Woo et al., 1998, Smythe et al., 2002, Slack et al., 2007) or fluorescent resonance energy transfer (FRET) (Fearnley et al., 2008) to emit a signal at a specific wavelength for detection of amplified PCR product. All reported real-time PCR assays were able to differentiate between pathogenic and non-pathogenic *Leptospira*. Moreover, DNA sequencing of PCR products combined with a nucleotide Basic Local Alignment Search Tool (BLASTn) on the National Centre for Biotechnology Information (NCBI) database were able to identify the genomospecies of *Leptospira* DNA sample (Fearnley et al., 2008, Slack et al., 2006). Primer pairs Lepto F/ Lepto R designed from 16s rRNA gene were able to detect leptospiral DNA in serum and seeded urine samples (Smythe et al., 2002). This same technique was modified and reported with a sensitivity of 96.4% and a specificity of 99.5% on patient serum samples compared with bacterial culture as the gold standard. The detection limit is reported at 10 cells/reaction (Slack et al., 2007). This technique has also been used as a tool to predict a carrier state of the flying fox from kidney and urine sample (Cox et al., 2005). Another study has used a real-time PCR assay to measure the concentration of leptospiral DNA in patient's sera and reported the range of 80 to 39,000 cells/ml which can be associated with the prognosis of leptospirosis (Merien et al., 2005).

#### **1.11.4 Molecular typing**

Due to the cumbersomeness of serology techniques for identification of leptospires, there has been interest in developing molecular techniques for this purpose. DNA-DNA hybridization is considered to be the gold standard technique for species-level identification of leptospires (Brendle et al., 1974, Brenner et al., 1999, Levett et al., 2006, Ramadass et al., 1992, Yasuda et al., 1987). However, it is seldom used because of its complexity. Assays involving digestion of chromosomal DNA by several restriction enzymes such as restriction endonuclease analysis (REA) (Marshall et al., 1981, Thiermann et al., 1986, Brown & Levett, 1997, Savio et al., 1994, Djordjevic et al., 1993, Skilbeck & Davies, 1989), restriction fragment length polymorphism (RFLP) (Corney & Colley, 1996, Zuerner et al., 1993), pulsed-field gel electrophoresis (PFGE) (Herrmann et al., 1992), ribotyping (Perolat et al.,

1993, Perolat et al., 1994) and multilocus enzyme electrophoresis (MEE) (Letocart et al., 1999) have also been used for identification of leptospires. All these techniques however, suffer from disadvantages such as requirement for special expensive equipment, laborious and time-consuming procedure, poor reproducibility, ambiguous interpretation of data and most importantly, requirement of a large quantity of purified DNA. As a result, several methods based on analysis of amplified PCR product from leptospiral DNA have been developed.

PCR-based methods for identification of leptospires are rapid and require only small amounts of DNA. RFLP has been improved by performing the technique on amplified PCR product known as PCR-RFLP (Woo et al., 1997a). This technique has been reported in the detection and differentiation of *Leptospira* in clinical samples such as bovine semen (Heinemann et al., 2000) and human blood (Kawabata et al., 2001). DNA fingerprinting using arbitrary primers with low stringency known as arbitrary primed PCR (AP-PCR) or random amplified polymorphic DNA (RAPD) has been studied extensively (Corney et al., 1993, Gerritsen et al., 1995, Letocart et al., 1997, Perolat et al., 1994, Ralph et al., 1993, Ramadass et al., 1997, de Caballero et al., 1994). This technique is simple, rapid and can be performed without previous information of leptospiral DNA base sequences. However, the use of non-specific primers in this technique results in low specificity and reproducibility.

Thus, the low-stringency single specific primer PCR (LSSP-PCR) technique was subsequently developed to solve these issues (Bomfim & Koury, 2006). Another DNA fingerprinting technique using repetitive extragenic palindrome (REP) elements from the leptospiral genome has been developed. This REP-PCR technique has been reported in identification of *Leptospira interrogans* (Zuerner et al., 1995). Fluorescent amplified fragment length polymorphism (FAFLP) has been developed for a molecular epidemiology study of leptospirosis in India. This technique combines the power of RFLP with the flexibility of PCR. The main principle is that the leptospiral genomic DNA will be cut into small fragments by restriction enzymes and bound to fluorescent labelled adapters that are designed to be complementary to PCR primers. Then, the PCR amplified fragments will be detected by fluorescent reading equipment such as DNA sequencers (Vijayachari et al., 2004).

Recently, investigation of multi-locus variable number of tandem repeat methodology (MLVA) has been described for identification of *Leptospira interrogans* (*sensu stricto*) at the serovar level (Majed et al., 2005, Slack et al., 2005). This technique detects the differences in copy number of tandem repeats from selected VNTR loci which are polymorphic and allows for genotyping. It has proven to be a simple and reproducible method with highly discriminatory power. Moreover, it can also be performed under standard laboratory conditions without sophisticated equipment (Slack et al., 2005). However, this technique may suffer from an inaccuracy of agarose gel electrophoresis and problems associated with transfer of data between different laboratories. These issues can be resolved by DNA sequence-based identification methods.

Table 1.2 Summary of laboratory diagnostic tests for leptospirosis, including sample required and advantages and disadvantages

| Test                         | Sample                                | Advantage                                                 | Disadvantage                                    |
|------------------------------|---------------------------------------|-----------------------------------------------------------|-------------------------------------------------|
| Isolation                    |                                       |                                                           |                                                 |
| Bacterial culture            | Blood, urine, kidney, CSF             | Gold standard, ready for typing                           | Easy to contaminate, cumbersome, time consuming |
| Direct and antigen detection |                                       |                                                           |                                                 |
| Dark field microscopy        | Urine                                 | Simple                                                    | Low sensitivity                                 |
| Histological staining        | Kidney                                | Simple                                                    | Low sensitivity                                 |
| Immunological staining       | Kidney                                | Increased sensitivity                                     | Requires special reagent or equipment           |
| Monoclonal antibody          | Urine                                 | Increased sensitivity                                     | Complicated, expensive                          |
| Antibody detection           |                                       |                                                           |                                                 |
| MAT                          | Serum                                 | Gold standard, serogroup specific                         | Biohazard, laborious                            |
| MSAT                         |                                       | Simple                                                    | Low sensitivity                                 |
| CF                           |                                       | Non biohazard                                             | Genus specific                                  |
| MCAT                         |                                       | Early detection                                           | Genus specific                                  |
| HL                           |                                       | Non biohazard                                             | Genus specific                                  |
| IFA                          |                                       | Non biohazard                                             | Requires fluorescent microscope                 |
| IH                           |                                       | Non biohazard                                             | Genus specific                                  |
| Dipstick                     |                                       | Simple and rapid                                          | Genus specific                                  |
| ELISA                        |                                       | Early detection (IgM), can combine with modern technology | Genus specific                                  |
| Molecular detection          |                                       |                                                           |                                                 |
| DNA-hybridization            | DNA from isolates or clinical samples | High specificity                                          | Limitation to probe                             |
| PCR                          |                                       | High sensitivity/specificity                              | Easy to contaminate                             |
| Real-time PCR                |                                       | Not rely on gel picture                                   | Expensive equipment                             |
| Serological typing           |                                       |                                                           |                                                 |
| CAAT                         | Viable isolates                       | Gold standard                                             | Cumbersome, biohazard                           |
| Molecular Typing             |                                       |                                                           |                                                 |
| DNA hybridization            | DNA from isolates                     | Gold standard                                             | Cumbersome                                      |
| REA                          |                                       | High discrimination                                       | Complicated                                     |
| RFLP                         |                                       | Rapid identification                                      | Ambiguous data                                  |
| PFGE                         |                                       | High discrimination                                       | Expensive equipment, Complicated                |
| MEE                          |                                       | High discrimination                                       | Complicated                                     |
| PCR-RFLP                     |                                       | Ability to perform directly on clinical sample            | Ambiguous data                                  |
| AP-PCR or RAPD               |                                       | Rapid identification                                      | Poor reproducibility, ambiguous data            |
| LSSP-PCR                     |                                       | Increased reproducibility                                 | Ambiguous data                                  |
| REP-PCR                      |                                       | High reproducibility                                      | Ambiguous data                                  |
| FAFLP                        |                                       | High discrimination                                       | Requires special equipment                      |
| MLVA                         |                                       | High discrimination                                       | Rely on gel picture                             |
| Single gene sequencing       |                                       | High reproducibility, unambiguous data                    | One gene may not be polymorphic enough          |
| MLST                         |                                       | High discrimination, unambiguous data                     | Require special equipment                       |

Analysis of the nucleotide arrangement of the nucleic acid strand forms a powerful tool to compare multiple leptospiral isolates. The sequencing of a gene at the same locus helps to characterize different species of *Leptospira*. The usual target for DNA-sequence based identification of *Leptospira* species is the 16S rRNA gene as previously described (Fearnley et al., 2008, Hookey et al., 1993, Postic et al., 2000, Levett et al., 2005a). However, the 16S rRNA gene is claimed to be not polymorphic enough due to its conserved nature. Several genes such as *rpoB* gene (La Scola et al., 2006) and *gyrB* gene (Slack et al., 2006) were proposed as alternatives for identification of *Leptospira* species. Multilocus sequence typing (MLST) is the most recent method that has been developed for genotypic classification of *Leptospira* (Ahmed et al., 2006, Thaipadungpanit et al., 2007). It generates the DNA sequence data of *Leptospira*, which is unambiguous and suitable for creating electronically accessible databases (Ahmed et al., 2006). In general, MLST requires the amplification of several housekeeping genes which evolve slowly by PCR and then compare the amplified product by DNA sequencing method. MLST has a high discriminatory power, reproducibility and robustness. The only limitation of MLST is that it requires highly skilled personnel and expensive equipment which will limit its use to sophisticated laboratories.

### 1.12 Treatment

Several studies have evaluated *in vitro* susceptibility of leptospires to antimicrobial agents (Ellinghausen, 1983, Hospenthal & Murray, 2003, Oie et al., 1983, Shalit et al., 1989). They show a high degree of efficacy of a broad range of antibiotics such as  $\beta$ -lactams, fluoroquinolones, macrolides, ketolides, tetracyclines, cephalosporins and streptomycin but not for chloramphenicol and sulphonamides. However, the relevance of the *in vitro* findings to clinical outcome is yet to be reported. Several studies have shown that ampicillin, ofloxacin and oxytetracycline fail to eliminate leptospires from the kidneys despite being effective *in vitro* (Ketterer & Dunster, 1985, Truccolo et al., 2002). Laboratory animals have been used to assess the *in vivo* efficacy of antibiotics to experimentally-induced leptospirosis (Alt & Bolin, 1996, Alexander & Rule, 1986, Moon et al., 2006, Moon et al., 2007). One study has employed real-time PCR to measure the density of leptospires in organs of experimentally infected hamsters after treatment with antibiotics (Truccolo et al., 2002).

Currently, penicillin and doxycycline are the drugs of choice for treatment of human leptospirosis because they have been studied in randomized controlled clinical trials (Edwards et al., 1988, McClain et al., 1984, Watt et al., 1988, Prescott, 1991). The treatment is most effective when started early during acute illness. For patients who develop jaundice, pulmonary symptoms and acute renal failure, supportive treatments such as fluid therapy and dialysis are required (Levett, 2001). Data from a randomized double-blind controlled field trial in the United States Army have shown the potential of low dose doxycycline (200mg/week) administered orally as a short-term chemoprophylaxis for leptospirosis (Takafuji et al., 1984).

In animals, especially pets, the treatment for acute leptospirosis is generally similar to humans (Langston & Heuter, 2003). However, animals are usually treated with dihydrostreptomycin or oxytetracycline (Thiermann, 1984). In case of an outbreak, prophylactic antibiotic treatment of all animals in the mob or herd will markedly decrease the incidence of disease. Combination of prophylactic treatment with whole-herd vaccination and animal isolation is effective (Dean et al., 2005).

Another perspective in the treatment of leptospirosis in animals is to eliminate the carrier state in chronic leptospirosis. Several studies have assessed the ability of antibiotics to clear leptospiral organisms from cattle (Alt et al., 2001, Ellis et al., 1985, Gerritsen et al., 1994, Gerritsen et al., 1993, Smith et al., 1997). Single dihydrostreptomycin injection at a dose rate of 25mg/kg was claimed to eliminate the shedding status of both experimentally-infected and naturally-infected cows with serovar Hardjobovis (Gerritsen et al., 1994, Gerritsen et al., 1993). However, this is in contrast to another study which reported the failure of dihydrostreptomycin at the same dose rate to remove *Leptospira* serovar Hardjoprajitno from the genital tract and kidney (Ellis et al., 1985). The variation between these studies may result from the serovar susceptibility to antibiotics and detection methods of infection. There is an attempt to find an alternative drug to dihydrostreptomycin because of concerns over drug residues and safety, since it can be ototoxic in some animals. Amoxycillin is proposed to be efficacious in eliminating the carrier status of cattle infected with serovar Hardjobovis and also is removed rapidly from the body (Smith et al., 1997). Recently, newer antibiotics such as tulathromycin and ceftiofur crystalline free acids suspension have been shown to clear serovar Hardjobovis organisms from the urine and kidney tissue of experimentally infected cattle (Cortese et al., 2007).

### **1.13 Control**

Control of leptospirosis requires sophisticated and holistic approaches that incorporate a range of strategies. The arguments go beyond the obvious need to prevent clinical illness and economic losses in domestic animals. There are also needs to minimize the risk of human infection by controlling exposure from animals (Ellis, 1994). Control of sporadic leptospirosis (animals infected with non-host-adapted serovars) requires identification and reduction of the maintenance hosts and/or immunization of the incidental host whereas control of endemic leptospirosis (animal infected with host-adapted serovars) requires different strategies. The key factor is to limit the direct and indirect transmission of the organisms between susceptible hosts, carriers and contaminated environment (Heath & Johnson, 1994). This can be achieved through elimination of carriers in the maintenance host populations by antibiotic treatment, reducing transmission risk factors by management and through prevention by vaccination.

Leptospiral vaccination has been shown to reduce clinical and subclinical disease and bacterial shedding in dairy cattle and pigs, resulting in a significant reduction of animal and human cases of leptospirosis in New Zealand (Thornley et al., 2002). Currently, all licensed leptospiral vaccines are killed whole cell vaccines containing local serovars since the immunity is serovar-specific (Koizumi & Watanabe, 2005). Leptospire are grown in protein medium and inactivated by mechanical (heat, pressure) or chemical (formalin, phenol, methiolate) means. Vaccines are designed to induce the production of antibodies against the LPS found on the surface of leptospire (Brown et al., 2003). Vaccines should be given to young animals when maternal immunity wanes and a booster should be given four to six weeks later and thereafter at one to two year intervals (Torten & Marshall, 1994). In cattle and deer, commercially available vaccines containing serovar Hardjobovis alone or in combination with serovar Pomona have been shown to decrease the incidence of infection and the duration and intensity of urinary shedding (Bolin & Alt, 2001, Mackintosh et al., 1980a, Marshall et al., 1979, Allen et al., 1982, Ayanegui-Alcérreca, 2006). Vaccination has also shown the ability to induce CMI by production of IFN- $\gamma$ -producing cells (Brown et al., 2003, Naiman et al., 2001, Naiman et al., 2002). Clearing of *Leptospira* from an endemically infected herd by combining the use of an aggressive vaccination program e.g. whole-herd vaccination (Little et al., 1992) with antibiotic treatment, may be possible (Alt et al., 2001) provided external sources of infection are eliminated.

There were attempts to develop leptospiral vaccine candidates from LPS and protein antigens (Koizumi & Watanabe, 2005, Wang et al., 2007). It has been found from one study that LPS vaccine prepared from non-pathogenic *L. biflexa* serovar Patoc can effectively protect hamsters against *L. interrogans* serovar Manilae (Matsuo et al., 2000). Another study demonstrated cross-protective immunity induced by protein extracts administered to gerbils (Sonrier et al., 2001). Results from those studies suggest the possibility of developing new vaccines that protect against various serovars. Until now, several leptospiral protein antigens such as OmpL1 and LipL41 (Haake et al., 1999), LipL32 (also known as Hap-1) (Branger et al., 2001) and Lig proteins (Koizumi & Watanabe, 2004) have been shown to elicit protective immunity in animal models. Moreover, whole genome analysis of available *Leptospira* DNA sequences has been used to identify candidate antigens for development of leptospiral vaccine (Gamberini et al., 2005). Recently, a DNA vaccine has been developed and shown to induce protection against a lethal challenge of *L. interrogans* serovar Canicola in gerbils (Branger et al., 2005b). DNA vaccine technology is claimed to be a promising new approach for vaccination against leptospirosis due to several advantages such as easy construction, low cost mass production, simple administration, temperature stability and ability to induce both HMI and CMI responses (Branger et al., 2005b, Wang et al., 2007).

### 1.14 Effects of leptospirosis on animal production

Farm animals infected with non-host-adapted leptospiral serovars may develop a significant illness leading to loss of production (reproduction or growth) or death, e.g. infection of cattle with serovar Pomona. While measuring clinical disease is easy, to measure the animal production costs associated with subclinical leptospirosis is a more complex task because it is determined by interactions between management factors and factors intrinsic to animals in any given environment which influence prevalence, and effects are often difficult to detect without systematic recording of management outcomes such as pregnancy, calving and weaning rates, and growth rate. Reproductive efficiency is a major contributing factor to the economic viability of any livestock industry. *Leptospira* organisms are proven to infect the reproductive organs and affect reproductive performance of livestock such as cattle (Ellis et al., 1986c, Ellis & Thiermann, 1986b, Thiermann, 1982), pigs (Ellis et al., 1986a, Ellis et al., 1986b, Ellis & Thiermann, 1986a), sheep and goats (Lilenbaum et al., 2008) and deer (Ayanegui-Alcérreca et al., 2007). Several studies have attempted to find and explain the association between subclinical leptospirosis and reproductive performance especially in cattle and pig industries (Dhaliwal et al., 1996c, Boqvist et al., 2002, Kazami et al., 2002, Kasimanickam et al., 2007, Grooms, 2006). One study analysed fertility data and demonstrated the reduction in conception rates of cows infected with *Leptospira* serovar Hardjo (Dhaliwal et al., 1996b). Another study from the same author also demonstrated that vaccination against *Leptospira* serovar Hardjo improved fertility in cows (Dhaliwal et al., 1996a). This is consistent with reports in other species such as pigs in Brazil where seroreactivity to *Leptospira* is proven to be associated with reproductive losses (Ramos et al., 2006). In New Zealand farmed deer, one study has shown an improvement in weaning rate to 97% in vaccinates, compared with 88% in controls in the presence of evidence of leptospiral infection (Ayanegui-Alcérreca et al., 2007).

The effects of leptospiral infection on growth of livestock animals have not been studied widely. One study reported that seropositivity for *Leptospira* did not affect daily weight gain of beef cattle (Fava et al., 2004). This is contrary to the data from a study in farmed deer in New Zealand which reported that yearling deer with evidence of serological infection during the previous nine months were 3.7 kg lighter at 12 months of age than those without seroconverting (Ayanegui-Alcérreca et al., 2007). More research is needed to understand the role of leptospiral infection in growth response of livestock.

### 1.15 Leptospirosis in New Zealand

Leptospirosis outbreaks in New Zealand livestock were first reported in the 1950s (Bruere, 1952, Hartley, 1952, Kirschner et al., 1952). It has now been recognized in humans, domestic animals and wildlife (Blackmore et al., 1976, Hathaway, 1981). Until now, eight serovars in two genomospecies were isolated in New Zealand as summarized in Table 1.3. Cattle and farmed deer are recognized as a maintenance host for serovar Hardjo (Note: serovar Hardjo in

New Zealand is now known as Hardjobovis), pigs for serovar Pomona and Tarassovi, Norway rats (*Rattus norvegicus*) for serovar Copenhageni, black rats (*Rattus rattus*) for serovar Ballum, brush tail possums (*Trichosurus vulpecula*) for serovar Balcanica. Serovar Australis and Canicola have been isolated from human patients and are not considered to be endemic (Midwinter & Fairley, 1999). All other species reported with leptospirosis were considered to be accidental hosts (Hathaway, 1981, Marshall & Manktelow, 2002).

### 1.15.1 Humans

Leptospirosis is the most common notifiable occupationally-acquired zoonosis in New Zealand. The major source of infection in humans is from domestic animals, with clearly definable high risk occupational groups, consisting of farmers, meat workers, veterinarians and forestry related personnel (Schollum & Blackmore, 1982, Thornley et al., 2002). After the first report of leptospirosis in a human in the 1950s (Kirschner & Gray, 1951), leptospirosis was made notifiable under the Health Act of 1956 and all human cases must be recorded (Thornley et al., 2002) and risk factor data from the notifying doctor are entered into a surveillance database (EpiSurv). The disease is also under laboratory surveillance and testing is done by ESR (Environmental Science and Research). These laboratory and notification data are brought together nationally by ESR for the New Zealand Ministry of Health (Keenan, 2007).

Table 1.3 Classification of *Leptospira* species in New Zealand and their maintenance host

| Species               |                          | Serovar     | Serogroup           | Reservoir host                 |
|-----------------------|--------------------------|-------------|---------------------|--------------------------------|
| Sensu lato            | Sensu stricto            |             |                     |                                |
| <i>L. interrogans</i> | <i>L. borgpetersenii</i> | Hardjobovis | Sejroe              | Cattle, Deer                   |
| <i>L. interrogans</i> | <i>L. interrogans</i>    | Pomona      | Pomona              | Pig                            |
| <i>L. interrogans</i> | <i>L. interrogans</i>    | Copenhageni | Icterohaemorrhagiae | Norway rat                     |
| <i>L. interrogans</i> | <i>L. borgpetersenii</i> | Ballum      | Ballum              | Black rat<br>Mouse<br>Hedgehog |
| <i>L. interrogans</i> | <i>L. borgpetersenii</i> | Balcanica   | Sejroe              | Possum                         |
| <i>L. interrogans</i> | <i>L. borgpetersenii</i> | Tarassovi   | Tarassovi           | Pigs                           |
| <i>L. interrogans</i> | <i>L. interrogans</i>    | Australis   | Australis           | n/a                            |
| <i>L. interrogans</i> | <i>L. interrogans</i>    | Canicola    | Canicola            | n/a                            |

Adapted from (Ayanegui-Alcérrec, 2006, Marshall & Manktelow, 2002)

n/a = not available

In the early 1980s, the reported annual incidence of leptospirosis in humans in New Zealand was one of the highest in the world and the highest rates of infection were from dairy farming areas (Mackintosh et al., 1980b). After cattle and pig vaccination became widespread, the annual incidence of human cases declined from 5.7 to 2.9 cases per 100,000 (Thornley et al., 2002). The average annual incidence of human leptospirosis from 2001 to 2003 was 4.0 cases per 100,000 based on laboratory-identified cases. However, this is likely



to be an underestimate because many individuals with leptospirosis may not seek medical attention because the symptoms may have been mild and short-lived, and possible misdiagnosis by general practitioners (Baker & Lopez, 2004, Heuer et al., 2008). A recent cross-sectional serological survey of meat workers in a lamb-only abattoir in the Hawkes Bay area revealed a 9.5% seroprevalence of either serovar hardjobovis or Pomona (Benschop et al., 2008). Infection with serovar Hardjobovis in humans in New Zealand has been associated with contact with cattle, sheep and deer whilst infection with serovar Pomona has been associated with pigs, cattle, sheep and deer (Bell, 2005, Brown, 2005, Thornley et al., 2002, Baker & Lopez, 2004).

### 1.15.2 Deer

Previous reports have shown that leptospirosis is a well recognized clinical disease in New Zealand farmed deer (Ayanegui-Alcérreca et al., 2003, Wilson et al., 1998, Ayanegui-Alcérreca et al., 2007). The first substantial case report of leptospirosis in farmed deer was in the 1980s (Fairley et al., 1986) but recently a substantial epidemiological study of this disease in farmed deer has been reported (Ayanegui-Alcérreca, 2006). *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona are the most commonly detected serovars. *Leptospira borgpetersenii* serovar Tarassovi and *Leptospira interrogans* serovar Copenhageni were also reported (Flint et al., 1988, Wilson et al., 1998) but it is likely that positive titers to those serovars resulted from cross-reactivity (Ayanegui-Alcérreca, 2006). Young animals are generally more susceptible to disease than adults and may suffer severe outbreaks and high mortality rates (Ayanegui-Alcérreca et al., 2007). It is suggested that the transmission of the infection and disease usually occurs before 1 year of age (Wilson & McGhie, 1993, Ayanegui-Alcérreca, 2006).

Data from a regional seroprevalence survey of 110 farms (Ayanegui-Alcérreca et al., 2010) showed evidence that 81% of farmed deer herds in New Zealand were infected with *Leptospira*. Serovar Hardjobovis was found in 61% of herds, Pomona in 4% and a combination of both serovars in 16%. At the individual level, serovar Hardjobovis was found in 54% of deer, Pomona in 2% and a combination of both serovars in 7%. No differences were found between regions. It is proposed that farmed deer in New Zealand are maintenance hosts for serovar Hardjobovis and an accidental host for serovar Pomona and that deer play an important role in the infection cycle of leptospirosis on New Zealand multi-species livestock farms (Ayanegui-Alcérreca, 2006).

Clinical cases of leptospirosis in farmed deer were associated with serovar Pomona with exception of two associated with serovar Hardjobovis (Wilson & McGhie, 1993). A recent case report of Pomona outbreak on a Southland deer farm described clinical signs such as weakness, lethargy, red urine and sudden death (Dean et al., 2005). The necropsy findings of deer leptospirosis include enlarged kidneys with small to large numbers of white spots and fibrotic scarring, jaundice with a swollen liver and red urine in the bladder (Fairley et al., 1986, Wilson et al., 1998). Histopathological lesions in kidneys reveal infiltration of

mononuclear cells, interstitial nephritis and fibrosis. Hepatic lesions included hepatitis, centrilobular necrosis, hepatocellular vacuolation and haemosiderin pigmentation (Fairley et al., 1986).

Research has shown immunological response to commercially available leptospiral vaccine in farmed deer with no interference with colostrum-derived antibody (Ayanegui-Alcérreca, 2006). Little was known about the effect of leptospiral vaccine on deer growth and reproduction, but this has been the subject of recent research (Subharat et al., 2008).

### **1.15.3 Cattle**

In the late 1970s and early 1980s, several studies were conducted to investigate the seroprevalence of leptospiral infection in cattle, particularly dairy cattle, in New Zealand. The data from those studies confirmed that Hardjo infection was endemic and that cattle were a maintenance host for this serovar but not for serovar Pomona (Hathaway, 1981, Bahaman et al., 1984). A serological survey of dairy cattle in the Taranaki district showed that 62% of the animals had a titer to serovar Hardjo and 4% had a titer to serovar Pomona. It was suggested that leptospiral infection in cattle occurs during the first two years of age (Bahaman et al., 1984). Data from a serological survey in beef herds in the Hawkes Bay area showed that 100% of 50 herds had a positive titers to serovar Hardjo with 64% of individuals having a titer of  $\geq 1:96$ , and 44% a titer of  $\geq 1:384$  (Matthews et al., 1999).

### **1.15.4 Sheep**

An early epidemiological study of leptospirosis in sheep from 45 lines at a slaughterhouse revealed evidence of serovar Hardjo infection in 20% of animals and serovar Pomona infection in 3.8% (Blackmore et al., 1982). That study also suggested that sheep were unlikely to be a maintenance host and not a significant source of infection of other species with serovar Hardjo (Blackmore et al., 1982). More recent data from an abattoir study during 2004-5 (Dorjee et al., 2005) showed that 85.7% and 23.4% of lines of hoggets and lambs, respectively, were seropositive for Hardjobovis and 28.6% and 10.6% of lines of hoggets and lambs, respectively, were seropositive for Pomona. Seroprevalences for Hardjobovis and Pomona at the individual animal level were 6.1% and 1.6%, respectively. The within-line prevalence of Hardjobovis ranged from 3.3% to 15.4% for lambs and from 3.3% to 60% for hoggets while the within-line prevalence of serovar Pomona ranged from 3.3% to 6.7% for lambs and 3.3% to 40.0% for hoggets. A low but persistent seroprevalence of Hardjobovis in sheep throughout the two years of study indicates low endemicity to this serovars, whereas Pomona infections appear to be sporadic (Dorjee et al., 2008). The data from this study also suggests that approximately 13 of every 1,000 sheep slaughtered are potentially shedding live leptospire and pose a zoonotic risk to meat workers in the sheep abattoir (Dorjee et al., 2008).

## 1.16 Conclusion

This review has summarized current knowledge on important aspects of leptospirosis with particular relevance to the research presented in this thesis. The history of leptospirosis along with its aetiology, current taxonomy and classification, as well as its general epidemiology, clinical signs and pathology has been described. Current information on pathogenesis mechanisms, immune response and advances in diagnostic methods has been clarified and the treatment and control of leptospirosis has been discussed with the focus on vaccination and vaccines.

The ability to rapidly determine whole genome sequences of *Leptospira* has been a breakthrough in leptospirosis research during the past few years. The strategy of reverse genetics in which the leptospiral genome is analysed by computer programs has been applied for identification of leptospiral virulence factors. This has helped understanding of the pathogenesis and immunity-induced mechanisms of leptospires that will also lead to the development of novel diagnostic tests and identification of leptospiral candidate antigens for vaccines.

To our knowledge, this review provides the most current information on animal production related to leptospirosis. It is clear that information available in this area is limited. More study is needed to establish an understanding of the role of leptospiral infection in terms of production response in livestock, since this may be an important stimulus for farmers to control leptospirosis with dual animal production and health and human health implications.

Lastly, the epidemiology of leptospirosis in both humans and New Zealand livestock has been reviewed. Because livestock farming in New Zealand is becoming increasingly multi-species including deer, cattle and sheep, stimulated by potential for financial stability and biological benefits, there is an increasing risk of diseases such as leptospirosis that can be transferred between species. Thus, understanding the role of each potential host on mixed-species farms is essential to the overall understanding of the epidemiology of leptospirosis, and has particular relevance to implementation of control measures on-farm aimed at reducing the clinical and subclinical incidence of disease, and similarly, reduction of risk to humans in New Zealand.

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## **Chapter 2**

### **Growth response and bacterial shedding in urine following vaccination for leptospirosis in young farmed deer**

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(Raw data of this chapter are in Appendix 2a, 2b and 2c)



## Abstract

**AIM:** To investigate growth response and shedding of leptospires in urine following serovar Hardjobovis and Pomona leptospiral vaccination in rising-one-year-old farmed red deer.

**METHODS:** Five previously *Leptospira* seropositive commercial deer farms were recruited. Between 2 - 20 March 2007, 435 mixed-sex, 3-month-old deer received a single dose of streptomycin (25 mg/kg) to minimise leptospiral infection. They were randomly allocated to streptomycin treated vaccinate (SV) and streptomycin treated control (SC) groups. Half (n=217) received a 2ml subcutaneous injection of a bivalent whole-cell killed leptospiral vaccine (Leptavoid-2) followed by a booster four weeks later. They were grazed with unvaccinated controls (SC) (n=218). These animals were isolated from other 3-month-old deer on each property which constituted a no-streptomycin control (NSC) group. In May, after 51 to 55 days, SV and SC deer were amalgamated with NSC deer on each property to be exposed to natural leptospiral challenge. Natural challenge of *Leptospira* was monitored in SC deer by serology against *L. borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona using a standard microscopic agglutination test (MAT), in May, August and November. Urine was collected in March, May, August and November from females in all groups for detection of leptospiral shedding by using bacterial culture and real-time PCR. Male SV and SC deer were weighed in March, May, August and November.

**RESULTS:** Vaccination induced antibody to both Hardjobovis and Pomona in most animals in May, with a range of titres between 1:24 to 1:96 and 1:24 to 1:1536, respectively. Serologically, three of the five farms had evidence of Hardjobovis infection in male SC deer (seroprevalence range 6.7 – 87.5%) and four had evidence in female SC deer (seroprevalence range 4.3 – 77.8%) with titres up to 1:768. All were seronegative to Pomona. Vaccinated male deer (SV group) on the three farms with evidence of Hardjobovis infection had a higher growth rate (12.4 – 26.5g/d) than controls (SC group). Between August and November, the period corresponding to the time of seroconversion, growth was on average 49g/day ( $p=0.0004$ ) higher in the SV group. On two farms with evidence of urinary shedding by real-time PCR, no shedding was found in vaccinated deer (SV) but the proportion shedding in SC and NSC deer was 23.5 and 52.6%, respectively. Shedding was reduced by vaccination from 56% (5/9) to 0% (0/11) on Farm 1 and from 12% (3/25) to 0% (0/25) on Farm 4, the overall difference of 24.5% being highly significant ( $p=0.0012$ ).

**CONCLUSION:** The bivalent leptospiral vaccine used significantly improved growth and prevented urinary shedding of young deer exposed to Hardjobovis providing evidence of vaccine efficacy and that Hardjobovis can cause adverse effects on productivity of young deer.

**KEY WORDS:** *Leptospirosis, farmed deer, vaccine, growth, urine shedding, Hardjobovis, Pomona, New Zealand*

**List of abbreviations**

ADG = Average daily gain

CMI = Cell-mediated immunity

EMJH = Ellinghausen-McCullough-Johnson-Harris medium

MAT = Microscopic agglutination test

NSC = No-streptomycin control

PBS = Phosphate-buffered saline

RD = Risk difference

SC = Streptomycin treated control

SV = Streptomycin treated vaccine

## 2.1 Introduction

Leptospirosis is a widespread zoonotic disease that affects most mammals and is an important cause of production loss in livestock throughout the world. Infection occurs via skin abrasions or mucous membranes followed by primary localisation in the kidney from where leptospire are voided in urine to expose other animals or humans (Faine *et al.* 1999).

In New Zealand (NZ) farmed deer, *Leptospira borgpetersenii* serovar Hardjobovis infection is endemic whilst *Leptospira interrogans* serovar Pomona is sporadic (Ayanegui-Alcérreca *et al.* 2007). A regional leptospirosis sero-prevalence survey of 110 deer farms in NZ showed evidence that 81% were seropositive, with Hardjobovis alone in 61% of herds, Pomona alone in 4% and a combination of both in 16% of herds (Ayanegui-Alcérreca *et al.* 2010). Infection and disease in farmed deer usually occurs before 1 year of age (Ayanegui-Alcérreca 2006) and evidence suggests that most if not all clinical cases were associated with serovar Pomona (Wilson and McGhie 1993). Subclinical effects of these serovars on farmed deer have not been investigated.

Control of leptospirosis on deer farms should be aimed not only at preventing clinical illness and economic loss in animals, but also at minimising the risk of exposure to humans. Human leptospirosis incidence in New Zealand is among the highest in developed countries and is largely associated with livestock including farmed deer (Baker and Lopez 2004; Bell 2005). Farmed deer shed leptospire in urine for seven weeks on average (Ayanegui-Alcérreca 2006) but shedding may extend up to a year as reported in cattle (Ellis and Michna 1977; Hellstrom and Marshall 1978; Leonard *et al.* 1992). Vaccination is likely to be the most effective tool for reducing shedding rates in deer. Several commercial vaccines have been reported to decrease the incidence of infection, renal colonisation and duration and intensity of urinary shedding after experimental or natural challenge of serovar Hardjobovis and Pomona in cattle (Bolin and Alt 2001; Mackintosh *et al.* 1980; Marshall *et al.* 1979; Marshall *et al.* 1982) and pigs (Hodges 1977; Hodges *et al.* 1985).

Preliminary research into a leptospiral vaccine in farmed deer has shown reduced urine shedding, shedding duration, and positive kidney culture incidence in currently infected herds (Ayanegui-Alcérreca 2006). The vaccine has not been evaluated in deer which were leptospira-free prior to vaccination. Additionally, that author showed that in one herd, yearling deer, with evidence of infection during the growth period, were on average 3.7 kg lighter at 12 months of age than those without evidence of infection. By contrast, a study in beef cattle showed that seropositivity for *Leptospira* did not affect daily weight gain (Fava *et al.* 2004). Thus, the growth response shown by Ayanegui-Alcérreca (2006) in deer requires replication, and if replicated, more data are needed on the relationship between prevalence and production responses at the herd level to assist in determination of likely cost-benefits of vaccination.

The purpose of this study was to replicate and further evaluate the effect of a bivalent leptospiral vaccine on growth and leptospiral shedding in young farmed deer under pastoral conditions typical of New Zealand, using different methodology to previous studies.

## 2.2 Materials and methods

### 2.2.1 Ethical approval

All procedures involving the use of animals in this project were approved by the Massey University Animal Ethics Committee under protocols 06/68 and 06/149.

### 2.2.2 Farms and animals

During August to November 2006, 20 commercial red deer (*Cervus elaphus*) farms, but possibly containing some wapiti (*C.e. canadensis*) genes, in the Manawatu and Hawkes Bay regions were recruited for a cross-sectional survey of leptospirosis to identify *Leptospira* seroprevalence. In early 2007, based on farmer willingness to participate and suitable numbers of animals, 435 mixed-sex, 3-month-old deer from five candidate farms with positive *Leptospira* serological status were enrolled. The location of farm, number of animals, sex and date of sampling are summarised in Table 2.1.

**Table 2.1 Location, number of deer in each group, sex (F = Female, M = Male) and vaccination and sampling dates for each farm.**

| Farm | Location   | No. of animals |    |    |    |     | Vaccination        |                | Blood and urine sampling |     |     |
|------|------------|----------------|----|----|----|-----|--------------------|----------------|--------------------------|-----|-----|
|      |            | SC             |    | SV |    | NSC | Sensitiser*<br>Mar | Booster<br>Apr | May                      | Aug | Nov |
|      |            | F              | M  | F  | M  | F+M |                    |                |                          |     |     |
| 1    | Hawkes Bay | 15             | 15 | 15 | 15 | 60  | 20                 | 19             | 10                       | 3   | 21  |
| 2    | Manawatu   | 27             | 22 | 27 | 21 | 80  | 15                 | 17             | 8                        | 8   | 6   |
| 3    | Hawkes Bay | 21             | 20 | 20 | 21 | 110 | 2                  | 5              | 26                       | 1   | 16  |
| 4    | Hawkes Bay | 28             | 20 | 28 | 20 | 170 | 13                 | 13             | 3                        | 6   | 8   |
| 5    | Hawkes Bay | 24             | 26 | 25 | 25 | 120 | 8                  | 5              | 1                        | 10  | 13  |

SC = Streptomycin treated control; SV = Streptomycin treated and vaccinated; NSC = No streptomycin control

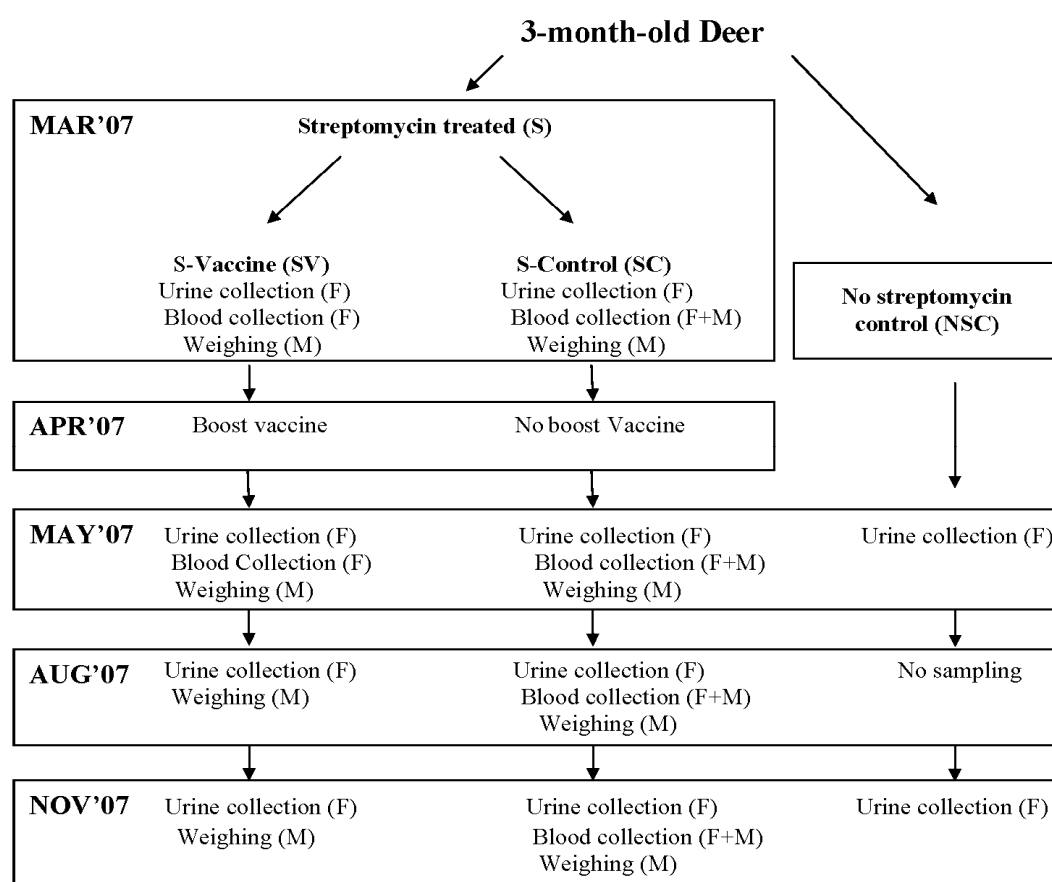
\* Also blood and urine sampled

### 2.2.3 Trial design

A summary of the study design is shown in Figure 2.1. The study commenced with newly weaned 3-4 month-old deer in March 2007. Males (n=205) were used to evaluate growth response (average daily gain in grams (ADG)) to vaccination. Females (n=230) were used to evaluate shedding of leptospires in urine using real-time PCR and bacterial culture. These deer were randomly selected from the total available (Day 1), separated from their cohorts, ear tagged and treated with streptomycin (Vibrostrep, Stockguard Animal Health Limited, NZ) at 25 mg/kg intramuscularly to attempt to eliminate any residual leptospiral infection

(Ellis *et al.* 1985; Mackintosh 1993). Half of the streptomycin treated animals were vaccinated with a bivalent leptospiral vaccine (group SV) whereas the other half were left as unvaccinated controls (group SC). In the SV group, males were weighed whereas females were blood and urine sampled. In the SC group, males were weighed and blood sampled whereas females were urine and blood sampled. These two groups were kept isolated from other animals in a “low risk” area of the farm to reduce risk of re-exposure to leptospires while immunity developed to the vaccine. In April (Day 28), the SV group received a booster vaccination.

In May (Day 56), these two groups (SV and SC) were re-united with their original cohort group that did not receive antibiotic treatment (no streptomycin control (NSC)) to become exposed to natural infection. The weighing and blood and urine sampling regimes for these deer were repeated in August and November. For the NSC group, 20 females were randomly urine sampled at joining (May) and again in November.



**Figure 2.1 Vaccination study design (Groups within the same boxes grazed together) (M = male; F = female).**

## 2.2.4 Vaccination

A commercial bivalent leptospiral vaccine (Leptavoid-2, Intervet/Schering-Plough Animal Health Limited, NZ) was administered subcutaneously by inserting the needle into a hand-held skin fold.

### 2.2.5 Blood sampling

Deer were physically restrained for blood collection from the jugular vein using a new 20 gauge needle and a 10 ml evacuated blood tube with no anticoagulant. Blood samples were left to clot at room temperature before centrifugation at 3,000 rpm (1512 x *g*) for 15 minutes and storage of serum at -20°C.

### 2.2.6 Serology

The microscopic agglutination test (MAT) was used to test serum reactivity to laboratory standardised *Leptospira* serovars Hardjibovis and Pomona. The method has been developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the Control of Leptospirosis” (Faine 1982). An initial serum dilution of 1:12 and two-fold serial dilution of serum covering the range of 1:24 to 1:3072 were tested. Titre was recorded as the reciprocal of the highest dilution at which  $\geq 50\%$  of leptospires were agglutinated. The cut-off point at  $\geq 1:48$  was used for both serovars to determine positivity (Blackmore *et al.* 1982; Dorjee *et al.* 2008).

### 2.2.7 Growth

**2.2.7.1 Sample size:** The number of samples required to evaluate leptospiral vaccine efficacy in terms of growth response to leptospirosis infection was calculated assuming that at least 4 kg difference in total weight gain from March to September between vaccinated and control deer was to be shown significant. This critical difference was based on previous results (Ayanegui-Alcérreca 2006). Thirty-six deer per group (vaccinated and control) were required to achieve 95% confidence and 80% power.

**2.2.7.2 Weight:** Male deer were weighed at intervals individually by digital scale. The animal identification number was recorded and matched with weight. Average daily gain (ADG: g/d) was calculated for periods March to May, May to August, August to November and overall between March and November.

**2.2.7.3 Statistical analysis:** Data were recorded and maintained using Microsoft Excel (Microsoft Corp, Redmond WA, USA) and checked for data entry errors against written records and by descriptive analysis (min/max values, data plots) before performing analysis. Statistical analyses were performed using SAS v9.1 (SAS Institute, Cary NC, USA). Linear regression analysis was used to determine the effect of vaccination on weight gain only on farms with serological evidence of leptospiral infection. The response ADG was regressed on study periods (1, 2, and 3), vaccination status and farm in a three-way interaction. The analysis adjusted for correlation of period-ADG within animal using a marginal model (GEE) with a 1<sup>st</sup> order autoregressive correlation pattern. A value of  $p \leq 0.05$  denoted statistical significance.

## 2.2.8 Urine shedding of leptospires

**2.2.8.1 Sample size:** The number of samples required to evaluate leptospiral vaccine efficacy in terms of urine shedding of leptospires was calculated by assuming a shedding rate of 2% in vaccinated deer and 10% in controls (Ayanegui-Alcérreca 2006). One hundred and eight deer per group (vaccinated and control) were needed to achieve 95% confidence and 80% power.

**2.2.8.2 Urine collection and processing:** Urination was induced by administration of furosemide (“Salix”, Intervet, NZ) at 1-1.5 mg/kg intramuscularly (Fairley *et al.* 1984; Warren and Whelan 1981). A new 70 ml plastic collector was held beneath the vulva after urination began (Figure 2.2). As much middle-stream urine as possible was collected and immediately held at 4°C. After transport to the laboratory, urine was centrifuged at 3,000 rpm ( $1512 \times g$ ) for 10 minutes to provide sediments which were re-suspended with 400 µl of phosphate-buffered saline (PBS) to neutralise the pH (Levett 2001).



**Figure 2.2 Urine collection from female R1yo deer.**

**2.2.8.3 Culture and serotyping:** The culture method used was developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the Control of Leptospirosis” (Faine 1982). Half of the re-suspended urine sediments (200 µl) were inoculated into 5 ml of Ellinghausen-McCullough-Johnson-Harris (EMJH) medium with an addition of antibiotic (5'-fluoracil) for contamination inhibition. From this inoculated medium, a 100 µl aliquot was transferred to another 5 ml of medium, followed by further transfer of 100 µl to another 5ml of medium. All three inoculated media were incubated aerobically at 28-30°C and examined every two weeks under dark-field microscopy. Cultures that showed no growth within four months were discarded and declared as negative. Isolates of leptospires were serotyped against standardised antisera of *Leptospira* serovars Hardjobovis and Pomona.

**2.2.8.4 DNA extraction and Real-time PCR:** Half of the re-suspended urine sediment (200 µl) was extracted for leptospiral DNA using a High Pure Template Kit (Roche, Germany) as per manufacturer's instructions. The real-time PCR technique was a modification of the method described by Slack *et al.* (2006). SYTO9 (Invitrogen, Oregon, USA) was used as fluorescent double-stranded DNA specific intercalating dye (Monis *et al.* 2005) for real-time PCR assays. The assay was performed in a Rotor-Gene 6000 machine (Corbett Research, Mortlake, Australia) using primers 2For 5'-tgagccaagaagaacaagctaca-3' and 504Rev 5'-matggttcrcrtttccgaaga-3' (Slack *et al.* 2006). Each 25µl reaction contained 2µl of DNA extracted from samples, 1.5µM SYTO9, 1X PCR buffer, 1.5mM MgCl<sub>2</sub>, 200µM dNTPs, 12.5pmol of 2For primer, 12.5pmol of 504Rev primer, 0.1% bovine serum albumin (BSA), 1 unit of Taq DNA polymerase and double distilled water. Thermal cycling consisted of initial denaturation at 95°C for 10min followed by 40 cycles of denaturation at 95°C for 10 sec, annealing at 60°C for 20 sec and extension at 72°C for 20 sec. Melting temperature (T<sub>m</sub>) of PCR product was determined by melting curve analysis. It was performed by heating the PCR product from 70°C to 90°C and monitoring fluorescence change every 0.1°C. Confirmation of positive samples was determined by melting temperature (T<sub>m</sub>) of the PCR product compared with the positive control. The T<sub>m</sub> of positive samples was found to be between 83°C and 84°C. The positive control used for the real-time PCR assay was field isolates of *Leptospira* serovar Hardjobovis and the negative control was double distilled water.

**2.2.8.5 Statistical analysis:** The risk differences (RD) between SV and SC deer shedding, with 95% confidence intervals, were stratified by farm and computed using the Mantel-Haenszel procedure. A value of  $P \leq 0.05$  was selected to confirm that the RD was not equal to zero.

## 2.3 Results

Twenty deer (2 male and 5 female SV, 8 male and 5 female SC) were lost to follow-up during the study because of accidental death and disappearance. There were 100 SV and 95 SC male deer included in growth response analyses and 110 SV and 110 SC female deer included in shedding response analyses.

### 2.3.1 Serology

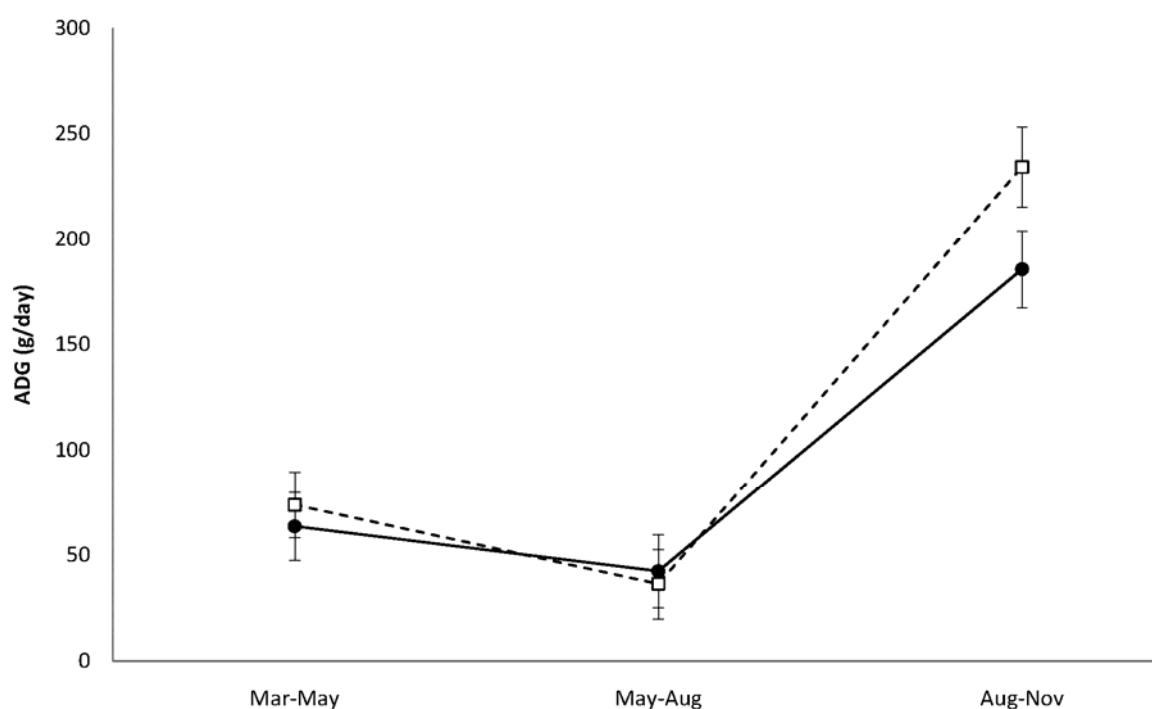
Serology results for females are shown in Table 2.2. Prior to vaccination (March), some deer on Farms 1 and 2 were seropositive to Hardjobovis but non-vaccinates were seronegative in May, suggesting that the initial titres were to maternal antibody. In May, 21 - 26 days after booster vaccination, 62/110 (59.6%) female SV deer were seropositive to Hardjobovis with titres ranging from 1:24 to 1:96, and 101/110 (97.1%) were seropositive to Pomona with titres ranging from 1:24 to 1:1536. In the female SC group, seroprevalence to Hardjobovis in November varied from 0 to 77.8%. Serology results for male deer are presented in Table 2.3. Male deer in the SC group on Farms 1-3 were seropositive for Hardjobovis by November,



with seroprevalence ranging from 5 to 87.5%. Farm 4 was seropositive for Hardjobovis only in female deer (n=2, 7.8%), with titres 1:192 and 1:768. No samples were seropositive for Pomona.

### 2.3.2 Growth

Weight data for each farm are shown in Table 2.4, and means ( $\pm$  95% CI) for vaccinates and controls are presented in Figure 2.3. The ADG of SV deer from three farms with evidence of Hardjobovis infection was 48.7 g/day (95% CI = 22.4 – 73.6) higher than the ADG of SC between August and November (Figure 2.3) ( $p=0.0004$ ), the period when most seroconversions occurred. On Farms 1, 2 and 3, seropositive to Hardjobovis, the overall mean differences in ADG between vaccinates and controls were 26.5 g/day ( $p<0.05$ ), 12.4 g/day ( $p>0.05$ ) and 16.3 g/day ( $p>0.05$ ), respectively. This resulted in a mean live weight difference in November of 6.5 kg, 3.1 kg, and 4.0 kg on Farms 1 – 3 respectively, that were seropositive, and of 0 kg and -1.2 kg on Farms 4 and 5, respectively, that were seronegative.



**Figure 2.3 Mean ADG ( $\pm$ 95% CI) at each weighing period in vaccinates (SV (--□--)) and controls (SC (—●—)) on farms with evidence of Hardjobovis infection (Note: seroconversion occurred principally between August and November).**

**Table 2.2 Seroprevalence (%) and (reciprocal titre range) for Hardjibovis (H) and Pomona (P) in female deer on each farm from March (pre-vaccination) to November (Deer were grazed in contact with untreated cohorts after the May sampling).**

| Farm | No. of animals |    | March (pre-vaccination) |       |              |       | May   |       |                 |                   | August      |       | November         |       |
|------|----------------|----|-------------------------|-------|--------------|-------|-------|-------|-----------------|-------------------|-------------|-------|------------------|-------|
|      | SC             | SV | SC                      |       | SV           |       | SC    |       | SV              |                   | SC          |       | SC               |       |
|      |                |    | H                       | P     | H            | P     | H     | P     | H               | P                 | H           | P     | H                | P     |
| 1    | 14             | 14 | 7.1<br>(48)             | 0 (-) | 14.3<br>(48) | 0 (-) | 0 (-) | 0 (-) | 64.3<br>(24-96) | 100<br>(24-768)   | 0 (-)       | 0 (-) | 77.8<br>(48-192) | 0 (-) |
| 2    | 25             | 26 | 4.0<br>(48)             | 0 (-) | 11.5<br>(48) | 0 (-) | 0 (-) | 0 (-) | 50.0<br>(24-96) | 92.3<br>(24-1536) | 8.3<br>(96) | 0 (-) | 4.3<br>(48)      | 0 (-) |
| 3    | 21             | 18 | 0 (-)                   | 0 (-) | 0 (-)        | 0 (-) | 0 (-) | 0 (-) | 38.9<br>(24-96) | 77.8<br>(24-384)  | 0 (-)       | 0 (-) | 12.5<br>(48-96)  | 0 (-) |
| 4    | 26             | 27 | 0 (-)                   | 0 (-) | 0 (-)        | 0 (-) | 0 (-) | 0 (-) | 76.9<br>(24-96) | 88.5<br>(24-384)  | 0 (-)       | 0 (-) | 7.8<br>(192-768) | 0 (-) |
| 5    | 24             | 25 | 0 (-)                   | 0 (-) | 0 (-)        | 0 (-) | 0 (-) | 0 (-) | 40.9<br>(24-96) | 100<br>(24-384)   | 0 (-)       | 0 (-) | 0 (-)            | 0 (-) |

**Table 2.3 Seroprevalence (%) and (reciprocal titre range) for Hardjibovis (H) and Pomona (P) in male deer on each farm from March (pre-vaccination) to November (Deer were grazed in contact with untreated cohorts after the May sampling).**

| Farm | No. of animals | March (pre-vaccination) |       | May      |          | August        |       | November      |       |
|------|----------------|-------------------------|-------|----------|----------|---------------|-------|---------------|-------|
|      |                | SC                      |       | SC       |          | SC            |       | SC            |       |
|      |                | H                       | P     | H        | P        | H             | P     | H             | P     |
| 1    | 15             | 13.3 (48)               | 0 (-) | 6.7 (48) | 6.7 (48) | 0 (-)         | 0 (-) | 87.5 (48-192) | 0 (-) |
| 2    | 20             | 0 (-)                   | 0 (-) | 0 (-)    | 0 (-)    | 11.1 (96-192) | 0 (-) | 5.0 (48)      | 0 (-) |
| 3    | 18             | 0 (-)                   | 0 (-) | 0 (-)    | 0 (-)    | 0 (-)         | 0 (-) | 6.7 (48)      | 0 (-) |
| 4    | 18             | 0 (-)                   | 0 (-) | 0 (-)    | 0 (-)    | 0 (-)         | 0 (-) | 0 (-)         | 0 (-) |
| 5    | 24             | 0 (-)                   | 0 (-) | 0 (-)    | 0 (-)    | 0 (-)         | 0 (-) | 0 (-)         | 0 (-) |

**Table 2.4 Mean ADG (and 95% CI) of vaccinated (SV) and control (SC) male deer on each farm for each period (Deer were grazed in contact with untreated cohorts after the May weighing).**

| Farm | Period                  |                          |                       |                       |                           |                           |                           |                           | ADG difference<br>(Mar – Nov) |
|------|-------------------------|--------------------------|-----------------------|-----------------------|---------------------------|---------------------------|---------------------------|---------------------------|-------------------------------|
|      | Mar - May               |                          | May - Aug             |                       | Aug - Nov                 |                           | Overall (Mar - Nov)       |                           |                               |
|      | SC                      | SV                       | SC                    | SV                    | SC                        | SV                        | SC                        | SV                        | SV - SC                       |
| 1    | 119.0<br>(90.5 – 147.4) | 169.9<br>(126.9 – 213.0) | 46.5<br>(14.9 – 78.2) | 21.7<br>(6.2 – 37.3)  | 204.6*<br>(181.4 – 227.8) | 254.3*<br>(230.8 – 277.7) | 126.8*<br>(112.9 – 140.6) | 153.3*<br>(144.0 – 162.6) | 26.5*                         |
| 2    | 45.3<br>(23.0 – 67.7)   | 65.3<br>(50.3 – 80.3)    | 67.1<br>(49.5 – 84.7) | 71.6<br>(59.9 – 83.4) | 166.2<br>(142.7 – 189.8)  | 183.1<br>(169.8 – 196.5)  | 101.4<br>(89.3 – 113.4)   | 113.8<br>(106.4 – 121.2)  | 12.4                          |
| 3    | 31.4<br>(18.1 – 43.9)   | 4.7<br>(-14.9 – 24.2)    | 2.4<br>(-14.6 – 19.4) | 3.5<br>(-6.8 – 13.8)  | 173.7<br>(121.1 – 226.3)  | 273.2<br>(225.3 – 321.1)  | 95.1<br>(76.2 – 113.9)    | 111.4<br>(100.7 – 122.1)  | 16.3                          |
| 4    | 125.1<br>(88.6 – 161.7) | 105.9<br>(63.3 – 148.5)  | 34.6<br>(13.0 – 56.3) | 43.6<br>(22.8 – 64.4) | 190.9<br>(152.4 – 229.4)  | 185.6<br>(152.8 – 213.1)  | 113.0<br>(95.7 – 130.2)   | 113.0<br>(99.5 – 125.9)   | 0                             |
| 5    | 61.3<br>(41.8 – 80.7)   | 79.5<br>(59.1 – 100.0)   | 32.0<br>(19.6 – 44.3) | 26.1<br>(14.1 – 38.2) | 235.2<br>(208.8 – 261.6)  | 231.6<br>(205.9 – 257.4)  | 114.3<br>(101.5 – 127.1)  | 109.5<br>(95.1 – 123.8)   | -4.8                          |

\*Statistically significant at P<0.05

### 2.3.3 Urine culture/PCR for *Leptospira*

Urine culture and PCR data from samples collect in November are summarised in Table 2.5. There was no evidence of urinary shedding of *Leptospira* in vaccinated deer. Urinary shedding was identified by culture and/or real-time PCR on Farms 1 and 4 and only in control (both SC and NSC) groups. The prevalence of urinary shedding determined by real-time PCR in SC and NSC groups in Farm 1 was 55.6% and 83.3%, respectively, whereas on Farm 4 prevalence was 12.0% and 25.0%, respectively. All animals shedding on Farm 1 had titres to Hardjobovis in the range 1:48 to 1:192 whereas only one of three PCR positive animals on Farm 4 had a titre to Hardjobovis (1:192). All *Leptospira* isolates derived from urine culture from Farm 1 were identified as serovar Hardjobovis.

Shedding was reduced by vaccination from 56% (5/9) to 0% (0/11) on Farm 1 and from 12% (3/25) to 0% (0/25) on Farm 4. Stratified analysis of shedding results from farms with evidence of urinary shedding (Farms 1 and 4) revealed an average risk difference (RD<sub>mh</sub>) of -24.5% (95%CI -38.9% – -10.1%) between the SV and SC deer (p=0.0012), indicating a difference in shedding between vaccinates and controls.

**Table 2.5 Number of deer (and %) urine culture and real-time PCR *Leptospira* positive in SC, SV and NSC groups in November.**

| Farm | Group      |            |          |          |            |             |
|------|------------|------------|----------|----------|------------|-------------|
|      | SC         |            | SV       |          | NSC        |             |
|      | Culture    | PCR        | Culture  | PCR      | Culture    | PCR         |
| 1    | 1/9 (11.1) | 5/9 (56%)  | 0/11 (0) | 0/11 (0) | 6/18 (33%) | 15/18 (83%) |
| 2    | 0/24 (0)   | 0/24 (0)   | 0/24 (0) | 0/24 (0) | 0/20 (0)   | 0/20 (0)    |
| 3    | 0/19 (0)   | 0/19 (0)   | 0/16 (0) | 0/16 (0) | 0/19 (0)   | 0/19 (0)    |
| 4    | 0/25 (0)   | 3/25 (12%) | 0/25 (0) | 0/25 (0) | 0/20 (0)   | 5/20 (25%)  |
| 5    | 0/21 (0)   | 0/21 (0)   | 0/22 (0) | 0/22 (0) | 0/20 (0)   | 0/20 (0)    |

## 2.4 Discussion

This study has confirmed the data of Ayanegui-Alcérreca (2006) showing both a reduction of growth attributable to exposure to leptospires, and that vaccination was effective in reducing the urinary shedding rate of leptospires. This paper also confirmed that serovar Hardjobovis has caused sub-clinical production loss on deer farms, and that this can be controlled by vaccination.

The present study was designed to mimic in a research context, the situation in which a whole infection-free herd was vaccinated and then challenged. It was intended to better evaluate the effect of a vaccine on growth and shedding outcomes than possible by vaccination in the face of endemic infection. Rising-one-year-old deer were selected for this study since a previous study has demonstrated that infection was more common in this than other age groups (Ayanegui-Alcérreca 2006). Female deer were selected for the urine shedding study for ease of urine sampling and male deer were selected for the growth study since they are more likely to be slaughtered at one year of age. Urine shedding observed by both culture and real-time PCR in November, in the NSC group along with the evidence of serology in SC group, confirmed that natural challenge by Hardjobovis was present in at least two herds.

The weak MAT antibody response to vaccination against Hardjobovis in this study was similar to that described in cattle (Allen *et al.* 1982; Mackintosh *et al.* 1980; Marshall *et al.* 1979) and deer (Ayanegui-Alcérreca 2006). However, it has been proposed that the efficacy of killed leptospiral vaccine in terms of protection is derived from cell-mediated immunity (CMI) which is not detected by MAT. A similar monovalent vaccine (Sv. Hardjobovis) used in cattle studies was claimed to induce CMI by production of IFN- $\gamma$ -producing cells (Brown *et al.* 2003; Naiman *et al.* 2001; Naiman *et al.* 2002) and prevented renal colonisation and urinary shedding after experimental challenge of serovar Hardjobovis (Bolin and Alt 2001).

Evidence of MAT response from control and vaccinated deer at the start of the trial from Farms 1 and 2 could be due to maternally derived antibodies as reported by Ayanegui-Alcérreca (2006), since titre prevalence declined at the May and August samplings. Subsequent increasing MAT titres to Hardjobovis after joining with NSC deer and evidence of *Leptospira* shedding from SC and NSC deer have demonstrated the existence of natural challenge by Hardjobovis on four of five farms. This supports that our study model was effective in terms of natural challenge of leptospires and mimicking the effect of the vaccine in previously un-infected animals. However, since seroprevalence was low, it would appear that exposure to leptospires was limited on all but Farm 1. A severe drought occurring in the study area in 2007 may have played an important role in limiting the transmission and survival of leptospires during the year of study (Hellstrom and Marshall 1978).

In New Zealand, farmed deer are believed to be a maintenance host for *Hardjobovis* with high susceptibility to infection but low pathogenicity (Ayanegui-Alcérreca *et al.* 2007). However, while the subclinical effect of this serovar on growth response has been preliminarily investigated demonstrating a negative effect, that observation required replication since such response challenges conventional beliefs about subclinical effects of leptospiral infection in host-adapted species. Farm 1 which experienced a high seroprevalence of *Hardjobovis* and confirmed infection using urine PCR, showed a statistically significant greater weight gain in vaccinated animals. The difference of ADG was approximately 27 g/day, which resulted in a mean of 6.5 kg in November, after a growing period of 241 days following vaccination.

On Farms 2 and 3 which also showed evidence of *Hardjobovis* infection, the differences in mean live weight in November of 3.1 kg and 4.0 kg respectively, were not statistically significant. On Farm 4 and 5 with no evidence of *Hardjobovis*, no evidence of mean live weight difference was observed. This finding is consistent with the previous report of Ayanegui-Alcérreca (2006) that deer with evidence of leptospiral infection during the growth period were 3.7 kg lighter at 12 months of age than those without evidence of infection. That data, combined with the observation of a significant difference in this study were only on the farm with the highest seroprevalence and urine shedding rate, supports that herd mean responses, and therefore economic responses to vaccination are seroprevalence-related.

When the ADG of deer on the three farms with *Hardjobovis* infection was combined and analysed by period, a significantly higher weight gain (49 g/day) was found in vaccinated deer during August to November (94 days) when almost all of the seroconversion occurred. This corresponded to a 4.6 kg mean difference between vaccinates and controls in November.

An important argument for control of leptospirosis is its potential impact upon humans. Zoonotic infections of human with *Leptospira* represent a significant public health problem in New Zealand. This study indicates that a suitable vaccine is capable of reducing the public health hazard of *Leptospira* shedding in deer urine as in dairy cattle (Thornley *et al.* 2002). The detection of leptospiuria was based on real-time PCR rather than culture since a recent study has shown that culture of field-sampled deer urine suffered high contamination rates and real-time PCR was claimed to be more sensitive and 100% specific, and detects only pathogenic *Leptospira* spp. (Slack *et al.* 2006).

The prevalence of urine shedding in NSC deer was high (83.3%) on Farm 1 where seroprevalence to *Hardjobovis* was 87.5% in non-vaccinates, suggesting strong natural challenge. The prevalence of urinary shedding in NSC deer was lower (25.0%) on Farm 4, and only one of three control deer with evidence of urine shedding had a titre to *Hardjobovis* of 1:192. This suggests a scenario of recent infection with low spreading rate during the sampling period of this farm. There was no evidence of urine shedding from vaccinated deer on both two farms suggesting vaccine protection. The calculated Mantel-Haenszel RD has shown that vaccination could reduce the risk of deer shedding leptospires by 24.5%

( $p=0.0012$ ). This finding is consistent with previous reports in cattle (Bolin and Alt 2001) and deer, albeit in already infected animals (Ayanegui-Alcérreca 2006).

Prevention of urinary shedding of leptospires in farmed deer could reduce the risk of leptospirosis for other animals and humans. As the majority of notified human leptospirosis cases in farmers and meat workers is associated with Hardjobovis (Baker and Lopez 2004; Thornley *et al.* 2002), it should be possible to formulate a vaccination programme for farmed deer which will reduce the occupational exposure to humans while also providing positive economic returns if challenge is sufficiently high.

This study demonstrated that vaccination of young deer with a bivalent leptospiral vaccine has the potential to significantly improve mean weight gain in infected herds and prevent urinary shedding after natural challenge with Hardjobovis. It also provides the first evidence of adverse subclinical effects on deer production by serovar Hardjobovis alone.

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## **Chapter 3**

### **Reproduction response following vaccination for leptospirosis in New Zealand farmed deer**

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(Raw data of this chapter are in Appendix 3a and 3b)

## Abstract

**AIM:** To investigate reproduction response in terms of weaning rate, following leptospiral vaccination against serovars Hardjobovis and Pomona in rising-two-year-old (R2yo) farmed red deer hinds.

**METHODS:** Six previously *Leptospira* positive commercial deer farms were recruited. In mid-February 2007, 382 R2yo hinds on six farms received a single dose of streptomycin (25 mg/kg) to minimise leptospiral infection. They were randomly allocated to streptomycin treated vaccinate (SV) and streptomycin treated control (SC) groups. SV hinds (n=191) received a 2ml S/C injection of a bivalent whole-cell killed leptospiral vaccine (Leptavoid-2) followed by a booster four weeks later and were grazed with SC hinds (n=191). These animals were isolated from other no-streptomycin control (NSC) hinds on each property. After the rut (June), all SV and SC hinds were amalgamated with all NSC hinds on the property for maximum exposure to natural leptospiral challenge. Natural challenge of *Leptospira* was evidenced in SC hinds by serology against serovars (*L. borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona) using the microscopic agglutination test (MAT) and in NSC hinds by detection of organism shedding in urine through bacterial culture and real-time PCR. Pregnancy scanning was done in May/June 2007 by rectal ultrasound to determine conception rate. Only pregnant vaccinated and control hinds were included for analyses. In late October, prior to calving, the pregnant SV and SC hinds were checked for carriage of foetus to term by abdominal ballotment and udder palpation to determine calving rate. In March 2008, at weaning, SV and SC hinds were examined for lactation status by udder observation and palpation and the calves were counted for determination of weaning rate.

**RESULTS:** One hundred and thirty hinds (n=130) from two properties were excluded for failure to conceive or because they were sold for management reasons. Exclusion rates were similar for SV and SC hinds. Of 252 remaining hinds, 203 (80.6%) were scanned pregnant and remained in the study. Serology revealed evidence of Hardjobovis infection on all four remaining farms, and a single hind was serologically positive for Pomona between October and March of the following year. Real-time PCR from urine samples confirmed urine shedding in the NSC group on two farms. The calving rate of the SV and SC groups averaged 97.9% (range 94.7 – 100%) and 97.2% (range 94.1 – 100%), respectively ( $p>0.05$ ). The weaning rates of SV and SC groups were 88.7% (range 77.8 – 94.7%) and 83.0% (range 76.5 – 87.5%), respectively ( $p=0.015$ ).

**CONCLUSIONS:** Vaccination for leptospirosis resulted in no difference in calving rate, but a significantly higher weaning rate than unvaccinated controls suggesting that vaccination reduced pre-weaning mortality. This reduced reproductive performance was attributable to serovar Hardjobovis.

**KEY WORDS:** *Leptospirosis, farmed deer, vaccine, reproduction, calving rate, weaning percentage, Hardjobovis, Pomona, New Zealand*

**List of abbreviations**

EMJH = Ellinghausen-McCullough-Johnson-Harris medium

MAT = Microscopic agglutination test

NSC = No-streptomycin control

PBS = Polyphosphate-buffered saline

PCR = Polymerase chain reaction

Rpm = Revolutions per minute

R2yo = Rising-two-year-old

SC = Streptomycin treated control

SV = Streptomycin treated vaccinate

### 3.1 Introduction

Leptospirosis is a worldwide disease of livestock with potential major economic impact on reproductive efficiency (Grooms 2006). *Leptospira* infection occurs via skin abrasions or mucous membranes. In livestock, following leptospiremia, leptospires localise and persist primarily in the kidney and genital tract. Leptospires are shed in urine which serves as a source of infection to other animals (Faine *et al.* 1999). Reproductive efficiency is a major factor contributing to the economic viability of livestock farming. Leptospirosis can reduce reproductive outcomes and therefore have a substantial economic impact (Grooms 2006).

Considerable data have been published about the effect of leptospirosis on reproduction in cattle (Dhaliwal *et al.* 1996a; Dhaliwal *et al.* 1996c, 1996d; Ellis 1994; Ellis and Thiermann 1986) and pigs (Kazami *et al.* 2002; Ramos *et al.* 2006). Chronic leptospirosis causes impaired fertility, neonatal death, abortions and decreased milk production (Lilenbaum *et al.* 2008). However, little is known of the effect of leptospirosis on reproduction of pastoral farmed deer since this is a relatively new industry and largely limited to New Zealand.

In New Zealand (NZ) farmed deer, *Leptospira borgpetersenii* serovar Hardjobovis infection is endemic whereas *Leptospira interrogans* serovar Pomona is sporadic (Ayanegui-Alcérreca *et al.* 2007). A NZ regional leptospirosis prevalence survey of 110 farms showed evidence that 81% of farmed deer herds in New Zealand were seropositive with serovar Hardjobovis in 61% of herds, Pomona in 4% and a combination of both in 16% of herds (Ayanegui-Alcérreca *et al.* 2010). Infection and disease in farmed deer usually occurs before 1 year of age (Ayanegui-Alcérreca 2006) and most clinical cases have been associated with serovar Pomona (Wilson and McGhie 1993).

Vaccination is one of the most effective tools for controlling leptospirosis. Several commercial monovalent and bivalent vaccines have been reported to prevent renal colonisation and urinary shedding after experimental or natural challenge in cattle (Bolin and Alt 2001; Mackintosh *et al.* 1980; Marshall *et al.* 1979) and pigs (Hodges *et al.* 1985). One study in vaccinated and non-vaccinated cows showed a correlation between renal and foetal infection in cows that became infected with serovar Hardjobovis after experimental challenge (Bolin *et al.* 1989). Vaccination improved the fertility of cattle (Dhaliwal *et al.* 1996b). Preliminary research into a leptospiral vaccine in farmed deer in New Zealand has shown a nine percentage point (88 vs. 97%) improvement in weaning rate in vaccinated deer on a farm with evidence of serovar Hardjobovis and Pomona infection (Ayanegui-Alcérreca *et al.* 2007). However, that observation requires replication to fully investigate and validate reproductive effects of leptospiral infection.

The purpose of this study was to determine the effect of a bivalent leptospiral vaccine on reproductive performance, measured as weaning rate of confirmed pregnant hinds, in leptospiral infected farmed deer herds under pastoral conditions in New Zealand.

## 3.2 Materials and methods

### 3.2.1 Ethical approval

All procedures involving manipulations on animals in this project have been approved by the Massey University Animal Ethics Committee under protocols 06/68 and 06/149.

### 3.2.2 Farms, animals and sample size

From August to November 2006, 20 commercial red deer (*Cervus elaphus*) farms but possibly containing some wapiti (*C.e. canadensis*) genes in the Manawatu and Hawkes Bay region were recruited for a cross-sectional survey of leptospirosis to identify *Leptospira* seroprevalence. Based on *Leptospira* seropositivity, farmer's willingness to participate, and presence of suitable number of animals, six candidate farms were enrolled in early 2007. R2yo hinds were chosen. The number required to evaluate leptospiral vaccine efficacy in terms of reproduction outcomes was calculated by assuming a nine percentage point difference of weaning rate between vaccinates and controls based on previously collected data (Ayanegui-Alcérreca *et al.* 2007). An estimated 105 hinds per group were required to achieve 95% confidence and 80% power for demonstrating significance of a difference of nine percentage points or more. Thus, it was proposed to use a minimum of 30 hinds per group on each of six farms assuming 70% of R2yo hinds would conceive and allowing for some loss to follow up. The farm location, final number of animals and vaccination and sampling dates for each farm are presented in Table 3.1.

**Table 3.1 Location, number of deer in each group, vaccination and sampling date for each farm recruited for the study.**

| Farm | Location   | No. animals |    |     | Vaccination |         | Pregnancy determination |              |          |
|------|------------|-------------|----|-----|-------------|---------|-------------------------|--------------|----------|
|      |            | SC          | SV | NSC | Sensitiser* | Booster | Post-rut                | Pre-calving* | Weaning* |
| 1    | Manawatu   | 20          | 20 | 25  | 19 Feb      | 19 Mar  | 21 May                  | 1 Nov        | 5 Mar    |
| 2    | Manawatu   | 42          | 42 | 60  | 12 Feb      | 15 Mar  | 12 Jun                  | 6 Nov        | 3 Mar    |
| 3    | Hawkes Bay | 32          | 32 | 50  | 16 Feb      | 22 Mar  | 6 Jun                   | 26 Oct       | 20 Mar   |
| 4    | Hawkes Bay | 31          | 33 | 40  | 23 Feb      | 3 Apr   | 31 May                  | 25 Oct       | 13 Mar   |
| 5    | Hawkes Bay | 34          | 36 | 43  | 21 Feb      | 22 Mar  | 15 Apr                  | n/a          | n/a      |
| 6    | Hawkes Bay | 30          | 30 | 40  | 14 Feb      | 22 Mar  | 29 May                  | n/a          | n/a      |

SC = Streptomycin treated control; SV = Streptomycin treated and vaccinated; NSC = No streptomycin control; n/a = not available as these farms were lost to follow up

\* Also blood sampled

### 3.2.3 Trial design

The study design for each farm is shown in Figure 3.1. Hinds (n=382) were randomly selected from the total number available in February 2007 (Day 1), and separated from their cohorts which remained on-farm. They were treated with streptomycin (Vibrostep, Stockguard Animal Health Limited, NZ) at 25mg/kg intramuscularly to attempt to eliminate any residual leptospiral infection (Mackintosh 1993). Half were simultaneously vaccinated with a bivalent vaccine (“Leptavoid-2” Intervet-Shering Plough) (SV group) whereas the other half were blooded sampled and acted as unvaccinated control (SC group). These two groups were grazed together but isolated from other deer (constituting a no streptomycin or vaccine control group (NSC)) on the property to reduce risk of re-exposure to leptospires while vaccine-induced immunity developed. In March 2007 (Day 28), the vaccinated animals received a booster vaccination. Natural mating was undertaken.

Late May to early June 2007 the SV and SC groups were joined with naturally mated cohort hinds (NSC group), to become exposed to natural infection. All trial hinds (SV, SC and NSC) on each property were scanned for pregnancy by rectal ultrasound 28 to 60 days post-mating and the SC group was blood sampled. The pregnancy status shortly prior to calving was examined in late October to early November 2007 either by udder palpation, abdominal palpation or scanning to determine carriage of foetus to term. The SC hinds were blood sampled again on that occasion. At weaning in March 2008, all hinds were examined for lactation status by udder examination to determine whether they reared a calf to weaning. Calves were counted to establish weaning rate (calves weaned/hinds scanned pregnant). The SC hinds were blood sampled again on that date. For the NSC group, 20 were randomly urine sampled when joined with the SV and SC groups (June) and at weaning (March) to determine leptospiral shedding status.

### 3.2.4 Vaccination

A commercial bivalent leptospiral vaccine (Leptavoid-2, Intervet/Schering-Plough Animal Health Limited, NZ) was administered subcutaneously by inserting the needle into a hand-held skin fold.

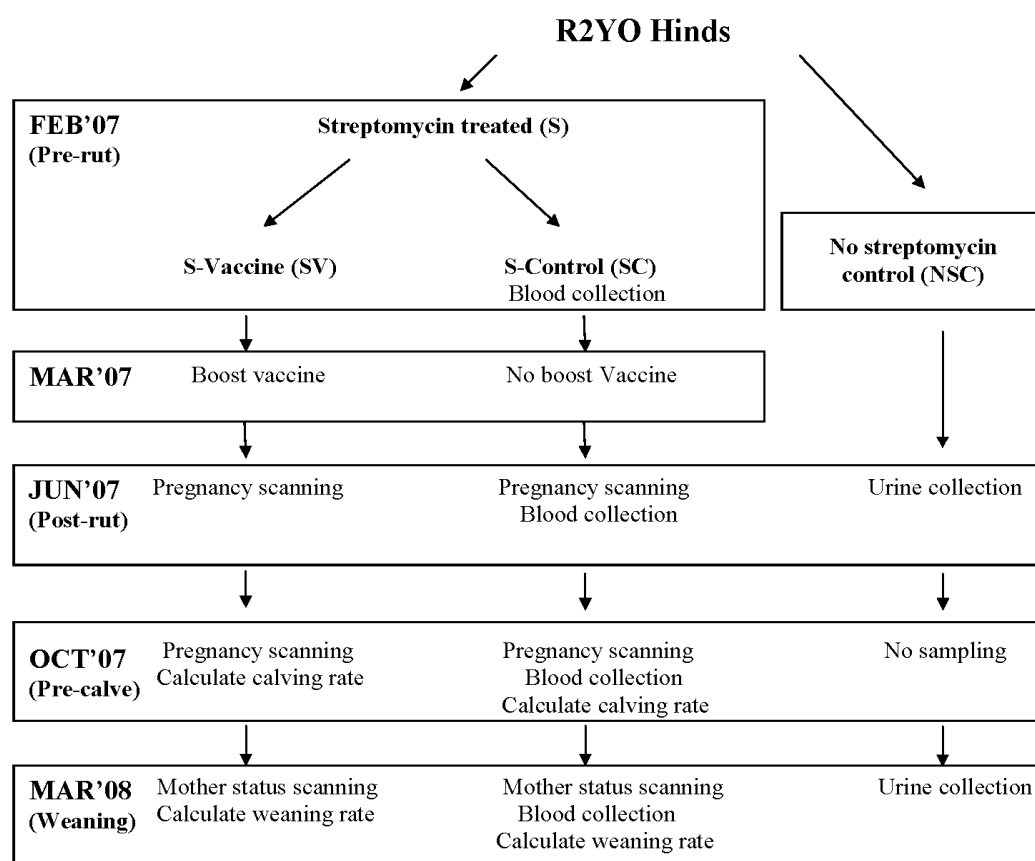
### 3.2.5 Blood sampling

Deer were physically restrained for blood collection from the jugular vein using a sterile 20 gauge needle and a 10 ml evacuated blood tube with no anticoagulant. Blood samples were left to clot at room temperature before centrifugation at 3,000 rpm (1512 x g) for 15 minutes and sera stored at -20°C.

### 3.2.6 Serology

The microscopic agglutination test (MAT) was used to test serum reactivity to laboratory standardised *Leptospira* serovars Hardjobovis and Pomona. The method has been developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the

Control of Leptospirosis” (Faine 1982). An initial serum dilution of 1:12 and two-fold serial dilution of serum covering the range of 1:24 to 1:3072 was tested. Titre was recorded as the reciprocal of the highest dilution at which  $\geq 50\%$  of leptospire had agglutinated. A cut-off point of  $\geq 1:48$  was used for both serovars to determine serological positivity (Blackmore *et al.* 1982; Dorjee *et al.* 2008).



**Figure 3.1 Vaccination study design (Groups within the same box were grazing together).**

### 3.2.7 Urine shedding

**3.2.7.1 Urine collection and processing:** Urination was induced by administration of furosemide (“Salix”, Intervet, NZ) at 1-1.5 mg/kg intramuscularly (Fairley *et al.* 1984; Warren and Whelan 1981). A sterile 70 ml plastic collector was held beneath the vulva after urination began. As much middle-stream urine as possible was collected and immediately held at 4°C. After transport to the laboratory, urine was centrifuged at 3,000 rpm ( $1512 \times g$ ) for 10 minutes to provide sediment which was re-suspended with 400  $\mu$ l of polyphosphate-buffered saline (PBS) to neutralise the pH (Levett 2001).

**3.2.7.2 Culture and serotyping:** The culture method used was developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the Control of Leptospirosis” (Faine 1982). Half of the re-suspended urine sediments (200  $\mu$ l) were inoculated into 5 ml of Ellinghausen-McCullough-Johnson-Harris (EMJH) medium



with an addition of antibiotic (5'-Fluouracil) for contamination inhibition. From this inoculated medium, a 100 µl aliquot was transferred to another 5 ml of medium, followed by further transfer of 100 µl to another 5ml of medium. All three inoculated media were incubated aerobically at 28-30°C and examined every two weeks under dark-field microscopy. Cultures that showed no growth within four months were discarded and declared as negative. Isolates of leptospire were serotyped against standardised antisera of *Leptospira* serovars Hardjobovis and Pomona.

**3.2.7.3 DNA extraction and Real-time PCR:** Half of re-suspended urine sediments (200 µl) were extracted for leptospiral DNA using a High Pure Template Kit (Roche, Germany) as per manufacturer's instructions. The real-time PCR technique was a modification of the method described by Slack *et al.* (2006). SYTO9 (Invitrogen, Oregon, USA) was used as fluorescent double-stranded DNA specific intercalating dye (Monis *et al.* 2005) for real-time PCR assays. The assay was performed in a Rotor-Gene 6000 machine (Corbett Research, Mortlake, Australia) using primers 2For 5'-tgagccaagaagaacaagctaca-3' and 504Rev 5'-matgggtccrctttccgaaga-3' (Slack *et al.* 2006). Each 25µl reaction contained 2µl of DNA extracted from samples, 1.5µM SYTO9, 1X PCR buffer, 1.5mM MgCl<sub>2</sub>, 200µM dNTPs, 12.5pmol of 2For primer, 12.5pmol of 504Rev primer, 0.1% bovine serum albumin (BSA), 1 unit of Taq DNA polymerase and double distilled water. Thermal cycling consisted of initial denaturation at 95°C for 10min followed by 40 cycles of denaturation at 95°C for 10sec, annealing at 60°C for 20sec and extension at 72°C for 20 sec. Melting temperature (T<sub>m</sub>) of the PCR product was determined by melting curve analysis. It was performed by heating the PCR product from 70°C to 90°C and monitoring fluorescence change every 0.1°C. Confirmation of positive samples was determined by melting temperature (T<sub>m</sub>) of the PCR product compared with the positive control. The T<sub>m</sub> of positive samples was found to be between 83°C and 84°C. The positive control used for the real-time PCR assay was field isolates of *Leptospira* serovar Hardjobovis and the negative control was double distilled water.

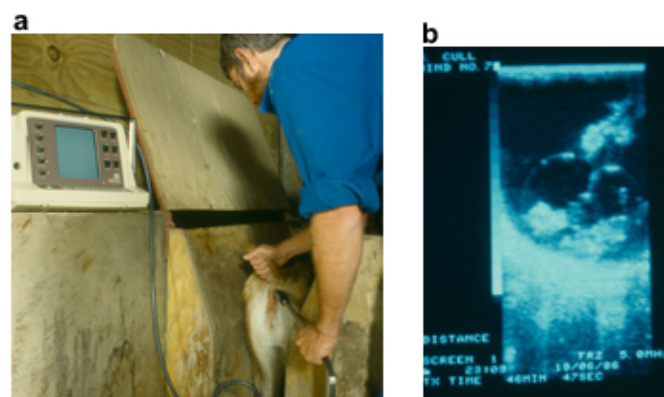
### **3.2.8 Pregnancy determination and calf rearing**

Rectal ultrasound scanning for pregnancy was done in SV and SC hinds in late May to early June (Figure 3.2). During late gestation, late October to early November, pregnancy was confirmed using one or more of udder and/or abdominal palpation, visual assessment and lower abdominal flank ultrasound scanning to determine calf loss during pregnancy. At weaning in March, udder palpation and calf count were used to determine calf loss from birth to weaning (Beatson *et al.* 2000).

### **3.2.9 Statistical analysis**

Data were recorded and maintained using Microsoft Excel (Microsoft Corp, Redmond WA, USA) and checked for data entry errors against written records before performing analysis. All statistical analyses were performed using SAS v9.1 (SAS institute, Cary NC, USA).

Logistic regression using generalised estimating equations (GEE) was used to evaluate the effect of vaccination on the proportion of scanned pregnant hinds carrying their foetus to term (calving rate) and of hinds rearing a calf (weaning rate). This was a matched design approach because hinds were randomly allocated to vaccine and control within farm using farm as the subject effect in recognition of the matched design. The consequence of a matched analysis is that only within-farm difference in weaning rates contributes to the hypothesis tested, and hence, the variance of the contrast is likely reduced. A value of  $p \leq 0.05$  was selected for statistical significance. The risk difference (RD) was calculated as a measure of vaccine efficacy.



**Figure 3.2 (a) Rectal ultrasound scanning of a hind (b) Ultrasound image of a deer foetus**

### 3.3 Results

#### 3.3.1 Pregnant hinds included in study analysis

Of 382 R2yo hinds, 130 from Farms 5 and 6 were removed from the study because there were no hinds on Farm 5 scanned pregnant due to undiagnosed reasons, whereas on Farm 6, the owner sold all the hinds in the study because of drought conditions. Of 252 hinds on the remaining four farms (Farms 1-4), 203 (80.6%) conceived and remained in the study. They comprised 97 vaccinated and 106 control hinds. Data are presented from the four remaining farms.

#### 3.3.2 Serology

Serology results are presented in Table 3.2. At the start of the trial (February), SC hinds from Farms 2, 3 and 4 were seropositive to serovar Hardjobovis. All hinds on Farm 1 were seronegative despite a previously established infection status of deer on that farm. The seroprevalence for Hardjobovis was lower in June on Farms 2-4 and increased through to March. Farm 1 developed a high seroprevalence between June and October and maintained it until March. For Pomona, there was only a single seropositive hind on Farm 1.

### 3.3.3 Urine culture/PCR

No *Leptospira* organisms were isolated by culture from any urine samples of NSC hinds in June, when joined with SC and SV deer. There was PCR evidence of *Leptospira* shedding: in 4/20 and 2/20 of NSC hinds on Farm 1 when groups were joined and at weaning, respectively. On Farm 4, 6/20 NSC hinds were urine PCR positive at weaning only. No shedding was observed in deer on Farms 2 and 3.

**Table 3.2 Seroprevalence (%) and (reciprocal titre range) for Hardjobovis (H) and Pomona (P) in streptomycin treated control hinds (group SC) on each farm from February (pre-vaccination of SV group) to March the following year. (Deer were grazed in contact with untreated cohorts from the June sampling).**

| Farm | No. of animals | Feb (pre-vaccination) |          | Jun              |          | Oct                |          | Mar                |             |
|------|----------------|-----------------------|----------|------------------|----------|--------------------|----------|--------------------|-------------|
|      |                | H                     | P        | H                | P        | H                  | P        | H                  | P           |
| 1    | 17             | 0<br>(-)              | 0<br>(-) | 0<br>(-)         | 0<br>(-) | 43.8<br>(96 – 384) | 0<br>(-) | 46.7<br>(48 – 384) | 6.7<br>(48) |
| 2    | 24             | 87.5<br>(48 – 1536)   | 0<br>(-) | 8.3<br>(48)      | 0<br>(-) | 14.3<br>(48 – 96)  | 0<br>(-) | 28.6<br>(48 – 96)  | 0<br>(-)    |
| 3    | 32             | 55.0<br>(48 – 192)    | 0<br>(-) | 9.1<br>(48 – 96) | 0<br>(-) | 40.0<br>(48 – 96)  | 0<br>(-) | 41.4<br>(48)       | 0<br>(-)    |
| 4    | 33             | 32.0<br>(48 – 192)    | 0<br>(-) | 9.6<br>(48 – 96) | 0<br>(-) | 12.9<br>(48 – 192) | 0<br>(-) | 21.2<br>(48 – 96)  | 0<br>(-)    |

### 3.3.4 Calving rate

Calving rates were similar in the two groups SV and SC (Table 3.3). The average calving rate (i.e. carriage of foetus to term) of the scanned pregnant vaccinated hinds was 97.9% (range 94.7 – 100%) and in the control hinds was 97.2% (range 94.1 – 100%;  $p>0.05$ ). There was no interaction between vaccination and farm, thus vaccination had no measurable impact in calving rates on all farms at a statistically significant level.

**Table 3.3 Calving rate (number of hinds carrying foetus to term/number scanned pregnant (%)) of hinds in control (SC) and vaccinated (SV) groups, measured in November, and the difference.**

| Farm      | SC             | SV            | Difference (SV – SC) |
|-----------|----------------|---------------|----------------------|
| 1         | 16/17 (94.1)   | 18/18 (100.0) | 5.9                  |
| 2         | 24/24 (100.0)  | 18/19 (94.7)  | -5.3                 |
| 3         | 31/32 (96.9)   | 31/31 (100.0) | 3.1                  |
| 4         | 32/33 (97.0)   | 28/29 (96.6)  | -0.4                 |
| All farms | 103/106 (97.2) | 95/97 (97.9)  | 0.7                  |

### 3.3.5 Weaning rate

The weaning data are summarised in Table 3.4. The mean weaning rate of the scanned pregnant vaccinated hinds (SV) was 88.7% whereas the mean weaning rate of the scanned pregnant control hinds (SC) was 83%. Logistic regression analysis showed that the 5.7% (95%CI = 1.2% – 8.9%) higher weaning rate of the vaccinated group was significant (p=0.015).

**Table 3.4 Weaning rate (number weaned/number scanned pregnant (%)) of hinds in control (SC) and vaccinated (SV) groups in March 2008, and the difference.**

| Farm             | SC                   | SV                  | Difference<br>(SV – SC) |
|------------------|----------------------|---------------------|-------------------------|
| 1                | 13/17 (76.5)         | 14/18 (77.8)        | 1.3                     |
| 2                | 21/24 (87.5)         | 18/19 (94.7)        | 7.2                     |
| 3                | 27/32 (84.3)         | 29/31 (93.5)        | 9.2                     |
| 4                | 27/33 (81.8)         | 25/29 (86.2)        | 4.4                     |
| <b>All farms</b> | <b>88/106 (83.0)</b> | <b>86/97 (88.7)</b> | <b>5.7</b>              |

### 3.4 Discussion

This study has complemented and is consistent with a previous finding by Ayanegui-Alcérreca (2006) that vaccination of deer against leptospirosis can improve the weaning rate on farms that have evidence of leptospiral infection. This study found a significant 5.7 percentage point increase in weaning rates whereas the study of (Ayanegui-Alcérreca 2006) reported a nine percentage points increase in weaning rates due to vaccination. That study was on multiparous hinds. Our study was designed to mimic in a research context and as far as practical, a situation in which a whole infection-free herd was vaccinated and then challenged by leptospire. This was intended to better evaluate the efficacy of a vaccine on reproduction outcomes than possible with vaccination in the face of endemic infection. R2yo hinds were selected for this study since this is the reproductively mature age group most likely to be seronegative when exposed to leptospire, and hence most likely to demonstrate a response to vaccination should leptospira infection occur and affect reproductive performance.

Increasing titres and prevalence to serovar Hardjobovis on all four remaining study farms after joining with NSC hinds confirmed that there was natural challenge in those animals, and observation of urine shedding by PCR confirmed active exposure to the SV and SC groups. Seropositivity to Pomona during this study was sparse, suggesting that the reproduction response observed was due primarily, if not in full, to serovar Hardjobovis.

Despite no *Leptospira* organisms being isolated from urine samples of NSC hinds, real-time PCR revealed evidence of *Leptospira* shedding on Farms 1 and 4. The sensitivity of PCR for identification of leptospires in urine is higher than that of isolation, but is likely to be less than 100%, particularly when deer shedding low numbers of organisms (see Chapter 5). Real-time PCR evidence combined with seroconversion of SC deer on all four farms suggest that the study model was effective in terms of providing natural challenge by leptospires.

In New Zealand, farmed deer are believed to be a maintenance host for serovar Hardjobovis with high susceptibility to infection but low pathogenicity (Ayanegui-Alcérreca *et al.* 2007). Ayanegui-Alcérreca (2006) was the first to investigate potential subclinical effects of this serovar on reproduction of farmed deer demonstrating a weaning rate of 97% in vaccinated hinds and 88% in non-vaccinated in-contact controls. However, that study did not differentiate whether losses were pre-, peri- or post-natal, whereas the present study included observations on calf survival peri- or post-partum. In dairy cattle, persistent infection by Hardjobovis was reported to cause reduced fertility, reduced conception rates (Dhaliwal *et al.* 1996d), increased number of services per conception and prolonged calving interval. It is also a cause of early embryonic death, abortion, stillbirth and weak calf syndrome (Smyth *et al.* 1999). Reports about serovar Pomona suggest that it can cause more severe clinical effects such as abortion storm (Gilmour 2007; Knott and Dadswell 1970), but the sporadic occurrence renders Pomona less economically important than Hardjobovis (Givens 2006). This may equally apply to farmed deer.

There is paucity of information about effects of leptospiral vaccination on animal reproduction. One study in Queensland, Australia reported a reduction of abortion rates in beef cows treated with antibiotics and vaccinated against Hardjobovis (Holroyd 1980). However, another study in the USA failed to observe a difference in calving rate between vaccinated and unvaccinated beef cows despite the finding that beef cows positive to Hardjobovis as determined by increasing titre and urine shedding achieved lower calving rates (Kasimanickam *et al.* 2007). The authors postulated that inefficacy of vaccine and low level of challenge may be the contributing factors in failure to observe the difference between vaccinated and control cows.

In the present study, there was no difference in calving rate, i.e. carriage of foetus to term, between vaccinated and control hinds suggesting no evidence of prenatal losses. However, the weaning rate increased by 5.7 percentage points (6.9% improvement, range 1.5 – 10.7% between farms) due to vaccination. Therefore, the adverse effect of leptospiral infection was limited to the interval from calving to weaning, e.g. stillbirth, mis-mothering and/or birth of weak non-viable calves. The apparent absence of foetal loss may be explained by the initial streptomycin treatment which happened pre-mating and appeared to reduce re-infection rates until after June.

The reason for a lower weaning rate in our study compared with that of (Ayanegui-Alcérreca 2006) may be a relatively low challenge of the four trial farms and possibly the absence of

Pomona as deer in that report were infected by both serovars at higher prevalence and higher titres to both Hardjobovis (seroprevalence 85%; titres up to 1:384) and Pomona (seroprevalence 100%; titres up to 1:1536). In addition, Pomona is believed to be more pathogenic and to cause more severe effects than Hardjobovis in farmed deer (Ayanegui-Alcérreca *et al.* 2007), although there is limited robust data. Furthermore, infection with Hardjobovis may interfere with milk production of hinds. Several studies have shown that Hardjobovis infection can cause mastitis and milk drop syndrome in cattle (Ellis *et al.* 1976; Hoare and Claxton 1972) and agalactia in sheep (McKeown and Ellis 1986). This effect on milk production, if happen, could potentially decrease the success of calf rearing in farmed deer.

Findings from Farms 2, 3 and 4, but not Farm 1, suggest that the reproduction response to vaccination correlated with seroprevalence. Since the weaning rates of both SC and SV hinds were lower on Farm 1 (76.5 and 77.8%) than on the other farms (81.8 – 94.7%) whereas the calving rates were not, we speculate that specific events had occurred on Farm 1 between birth and weaning that impaired calf rearing. Such events could be related to general management or level of nutrition, which in total could have negated any effect of *Leptospira* vaccination on calf rearing.

We conclude that this study concurred with a previous report of improvement of reproductive performance associated with leptospirosis vaccination. It also confirmed that *Leptospira* serovar Hardjobovis can reduce reproductive performance on deer farms and that vaccination with a bivalent *Leptospira* vaccine has the potential to significantly improve weaning rates after natural challenge with this serovar. Further research is needed to determine the mechanism of this response and the relative importance of serovars Hardjobovis and Pomona in causing reproductive loss on deer farms.

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## **Chapter 4**

### **Longitudinal serological survey and herd-level risk factors for *Leptospira* serovars Hardjobovis and Pomona on deer farms with sheep and/or beef cattle**

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(Farm questionnaire and raw data of this chapter are in Appendix 4a and 4b)

## Abstract

**AIM:** To investigate serology of *Leptospira* serovars Hardjobovis and Pomona on deer farms with sheep and/or beef cattle in the lower North Island of New Zealand and to identify associations between putative risk factors and serological status of each livestock species.

**METHODS:** Serological screening was conducted on commercial deer farms with sheep and/or beef cattle between August and October each year in 2006 to 2008 to determine seroprevalence of two leptospiral serovars. Serum samples were obtained from species farmed on deer only (n=3), deer and beef cattle (n=3), deer and sheep (n=2) and deer, beef cattle and sheep (n=12) farms in the Manawatu, Hawkes Bay and Wairarapa regions. On each farm every year, 20 or more rising 2-year-old replacement animals were randomly sampled from each species (1,173 deer, 817 beef cattle, 1,300 sheep). The microscopic agglutination test (MAT) was used to detect leptospiral antibodies against *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona. A cut point titre of  $\geq 1:48$  determined a positive sample. A questionnaire was completed by the farm owner or manager. Bivariate analysis was used to test for simple associations between potential risk factors and seropositive status of *Leptospira*, at one or more of the sampling dates, in deer herds for each serovar, using Fisher's exact test. Prevalence Ratio and 95% confidence intervals were computed to determine the strength of association between risk factors and the risk for seropositivity to *Leptospira* in deer.

**RESULTS:** One farm having deer and sheep was lost to follow-up after the first year. The proportion of deer, cattle and sheep herds/flocks seropositive for Hardjobovis over three samplings averaged 64.9%, 69.2% and 64.1%, respectively. Equivalent results for serovar Pomona were 29.8%, 23.0% and 10.3%, respectively. At the individual animal level, 19.9% of deer, 39.1% of cattle and 30.1% of sheep were Hardjobovis positive, 8.8% of deer, 6.2% of cattle and 1.9% of sheep were Pomona positive and 4.3% of deer, 4.8% of cattle and 1.5% of sheep were positive for both serovars. Deer herds were more likely to be Hardjobovis positive when large (n>560) (PR=1.46, p=0.036), when deer were on farms with hilly topography (PR=4.67, p<0.001) and when deer were co-grazing with Hardjobovis positive cattle herds (PR=1.93, p=0.022) or sheep flocks (PR=1.70, p=0.007). Deer herds were more likely to be Pomona positive when deer were co-grazing with Pomona positive cattle herd (RR=7.50, p=0.050). Deer herd were less likely to be Pomona positive when the farm had an open herd replacement policy (PR=0.28, p=0.007).

**CONCLUSIONS:** Exposure to *Leptospira* was widely distributed in deer, sheep and beef cattle in the lower North Island of New Zealand and concurrent infection of more than one species was common. Co-grazing of deer with other leptospiral positive species on farm tended to increase the risk of seropositivity to both Hardjobovis and Pomona in deer herds.

**KEY WORDS:** *Leptospirosis, deer, cattle, sheep, serology, Hardjobovis, Pomona, epidemiology, risk factors, mixed-species grazing, New Zealand*

**List of abbreviations**

MAT = Microscopic agglutination test

## 4.1 Introduction

Livestock farming in New Zealand has become increasingly multi-species including deer, beef cattle and sheep on the same property (Hilson 2007; Wilson 2007). Previous reports have shown that leptospirosis is a well recognised clinical disease in New Zealand livestock including farmed deer (Ayanegui-Alcérreca *et al.* 2007; Dean *et al.* 2005), cattle (Mackintosh 1981) and sheep (Thornton 1994; Vermunt *et al.* 1994). Leptospirosis has been shown to cause mortality, reproductive failure and production losses in deer (Ayanegui-Alcérreca 2006), cattle (Gilmour 2007; Smyth *et al.* 1999) and sheep (Lilenbaum *et al.* 2008).

Several studies have been conducted to establish the epidemiology of leptospirosis separately in deer (Ayanegui-Alcérreca 2006; Wilson *et al.* 1998), cattle (Bahaman *et al.* 1984; Matthews *et al.* 1999) and sheep (Dorjee *et al.* 2005). Data from a regional seroprevalence survey of 110 deer farms (Ayanegui-Alcérreca *et al.* 2010) showed 81% of farmed deer herds in New Zealand were seropositive, with *Leptospira* serovar Hardjobovis alone in 61% of herds, Pomona in 4% and a combination of both serovars in 16%. At the individual animal level, serovar Hardjobovis was found in 54% of deer, Pomona in 2% and a combination of both serovars in 7%. No differences were found between regions.

In the late 1970s and early 1980s, several studies were conducted to investigate the seroprevalence of leptospirosis in cattle, particularly dairy cattle, in New Zealand. A serological survey in Taranaki showed that 62% of dairy cattle were positive for serovar Hardjo and 4% for serovar Pomona and that infection usually occurred during the first two years of life (Bahaman *et al.* 1984). Data from a serological survey of leptospirosis in beef herds in the Hawke's Bay area showed that all of 50 herds had positive titres for serovar Hardjo whereas 64% of the individuals had a titre of  $\geq 1:96$ , and 44% had a titre of  $\geq 1:384$  (Matthews *et al.* 1999).

An early epidemiological study of leptospirosis in sheep from 45 lines at a slaughterhouse revealed Hardjo (now Hardjobovis) infection in 20% of animals and serovar Pomona in 3.8% (Blackmore *et al.* 1982). More recent abattoir data from 68 lines comprising 65 different properties showed that 85.7% and 23.4% of lines of hoggets and lambs respectively, were seropositive for Hardjobovis and 28.6% and 10.6% respectively, were seropositive for Pomona. Seroprevalence for Hardjobovis and Pomona at the individual animal level was 6.1% and 1.6% respectively. The within-line prevalences of Hardjobovis ranged from 3.3% to 15.4% for lambs and from 3.3% to 60% for hoggets while that for serovar Pomona ranged from 3.3% to 6.7% for lambs and 3.3% to 40.0% for hoggets (Dorjee *et al.* 2005).

Despite these individual species surveys and epidemiological studies of leptospirosis in pastoral farming in New Zealand, no mixed-species surveys or epidemiological studies have been carried out. Understanding the role of each potential host on mixed-species farms is important to understanding the disease, and has particular relevance to choice and

implementation of control measures on-farm aimed at reducing the clinical and subclinical incidence of disease, and reduction of risk to humans.

This paper describes a preliminary study conducted in the lower North Island of New Zealand designed to provide baseline data for future serological and molecular epidemiological study of leptospirosis and to evaluate potential risk factors for seropositive status of deer herds on mixed-species farms.

## **4.2 Materials and methods**

### **4.2.1 Ethical approval**

All procedures involving the use of animals in this project have been approved by the Massey University Animal Ethics Committee under protocol No. 06/68.

### **4.2.2 Farms and animals**

Twenty commercial farms, three with deer only and 17 with deer and sheep and/or cattle in the Manawatu, Hawkes Bay and Wairarapa regions in the lower North Island of New Zealand were recruited. Candidate farms were selected based on the farmer's willingness to participate, history of no previous leptospirosis vaccination, and the presence of suitable animals and handling facilities. The farms recruited were deer only (n=3), deer and beef cattle (n=3), deer and sheep (n=2) and deer, beef cattle and sheep (n=12). A herd or flock was defined as animals of the same species (*i.e.* deer herd, cattle herd and sheep flock) grazed on the same farming property. Two farms had a closed herd policy, breeding their own replacements for all classes of stock.

### **4.2.3 Study design**

A longitudinal seroprevalence survey design was chosen to determine the *Leptospira* status of both individual animals and herds/flocks from 2006 to 2008. Between August and October each year, jugular (sheep and deer) or coccygeal vein (cattle) (Figure 4.1) blood samples were taken using a 20 gauge needle and 10mL evacuated glass tubes without anticoagulant from a randomly selected sub-sample of replacement mobs that were more than one year old of each species on farm. The required numbers of samples per farm were 20 deer, 20 cattle and 60 sheep, as calculated by FreeCalc software. Estimates were based on a MAT sensitivity of 85% and specificity of 95%, a minimum within-farm seroprevalence of 30% to be detected in deer and cattle herds based on previous observations, and an average herd size of 350, to provide 95% confidence and 80% power. To categorise deer and cattle herds as positive, at least 3 of 20 serum samples had to be positive at the titre of 1:48. To categorise sheep flocks as positive, initially at least 5 of 60 serum samples had to be positive based on a MAT sensitivity of 85% and specificity of 95%, a minimum within-farm seroprevalence of 12% to be detected in sheep based on previous observations, and an average herd size of 1000, to provide 95% confidence and 80% power. However, based on higher than expected within

flock seroprevalence of 42% in the first year (2006), the number of samples required from sheep was reduced to 20 per farm in 2007 and 2008 with the same criteria to consider flock positive as for deer and cattle. In total, 3,290 animals were sampled including 1,173 deer, 817 beef cattle and 1,300 sheep. Farm owners or managers were asked to complete a questionnaire at both the 2007 and 2008 samplings to obtain information on farm demographics, geography, animal replacement policy, neighbours, grazing management, vaccination programme and history of clinical leptospirosis since the previous sampling.



**Figure 4.1 Blood collection from cattle (a), sheep (b) and deer (c).**

#### **4.2.4 Serology**

Blood samples were left to clot at ambient temperature. Subsequently, samples were centrifuged at 3,000 rpm (1512 x *g*) for 15 minutes to extract serum for the microscopic agglutination test (MAT). MAT was used to test serum reactivity to laboratory standardised *Leptospira* serovar Hardjobovis and Pomona to determine the *Leptospira* status of the individual animals and herds. The method has been developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the Control of Leptospirosis” (Faine 1982). An initial serum dilution of 1:12 and two-fold serial dilution of serum covering the range of 1:24 to 1:3072 were tested. Titre was recorded as the reciprocal of the highest dilution at which  $\geq 50\%$  of leptospires were agglutinated. To consider a sample positive, the cut-off point at 1:48 was used for both serovars (Blackmore *et al.* 1982; Dorjee *et al.* 2008).

#### 4.2.5 Statistical analysis

Data collected were recorded and maintained using Microsoft Excel (Microsoft Corp, Redmond WA, USA). Data entries were checked against the written records for errors before performing analysis. The proportion of leptospiral antibody-positive deer, cattle herds and sheep flocks were calculated for both *Leptospira* serovars Hardjobovis and Pomona for descriptive analysis. Seroprevalence for each species was calculated as: (number of antibody positive animals/number of sampled animals) x 100 for each herd. Initially, it was evaluated whether the serological status of a herd correlated in subsequent years using the intra-class-correlation criterion. Where correlation was present, statistical hypothesis tests were adjusted by a variance inflation factor (McDermott and Schukken 1994). Due to the small number of herds, only simple bivariate analysis was used to test for associations between herd level variables shown in Table 4.2 and serological status of herds in each year for each serovar. Statistical inferences were based on Fisher's Exact Test. For variables with more than two levels that implied an increase of exposure, associations were tested for the presence of an increasing or decreasing trend (Rothman and Greenland 1998a). Associations were calculated as prevalence ratios (PR) and 95% confidence interval (Rothman and Greenland 1998b) to express the strength of association between risk factors and the risk for seropositivity to *Leptospira* in deer herds. A value of  $p \leq 0.05$  denoted statistical significance.



**Table 4.1 Summary of farm characteristics based on 2007 and 2008 questionnaire data.**

| Farm | Location       | Mean herd/flock size |        |       | Farm geography* | Replacement policy* | Neighbour waterway* | Co-graze with cattle | Co-graze with sheep | Past clinical leptos |
|------|----------------|----------------------|--------|-------|-----------------|---------------------|---------------------|----------------------|---------------------|----------------------|
|      |                | Deer*                | Cattle | Sheep |                 |                     |                     |                      |                     |                      |
| 1    | Palmerston Nth | 71                   | -      | -     | Flat            | Open                | Yes                 | No                   | No                  | No                   |
| 2    | Masterton      | 191                  | -      | -     | Flat            | Open                | Yes                 | No                   | No                  | No                   |
| 3    | Waipawa        | 425                  | -      | -     | Flat            | Open                | Yes                 | No                   | No                  | No                   |
| 4    | Palmerston Nth | 99                   | 9      | -     | Flat            | Open                | Yes                 | Yes                  | No                  | No                   |
| 5    | Dannevirke     | 652                  | 826    | -     | Hilly           | Open                | Yes                 | Yes                  | No                  | No                   |
| 6    | Waipukurau     | 820                  | 162    | -     | Hilly           | Open                | Yes                 | Yes                  | No                  | Yes                  |
| 7    | Pohangina      | 751                  | -      | 360   | Hilly           | Open                | Yes                 | No                   | Yes                 | Yes                  |
| 9    | Pohangina      | 304                  | 155    | 1783  | Hilly           | Open                | Yes                 | Yes                  | Yes                 | Yes                  |
| 10   | Apiti          | 813                  | 76     | 1260  | Hilly           | Open                | No                  | Yes                  | Yes                 | No                   |
| 11   | Pohangina      | 900                  | 91     | 630   | Hilly           | Open                | Yes                 | Yes                  | Yes                 | No                   |
| 12   | Rangiwahia     | 559                  | 60     | 10120 | Hilly           | Open                | Yes                 | Yes                  | Yes                 | No                   |
| 13   | Dannevirke     | 210                  | 12     | 560   | Hilly           | Open                | Yes                 | No                   | Yes                 | No                   |
| 14   | Takapau        | 1201                 | 270    | 2660  | Hilly           | Closed              | Yes                 | Yes                  | Yes                 | Yes                  |
| 15   | Takapau        | 652                  | 150    | 1170  | Hilly           | Open                | Yes                 | Yes                  | No                  | No                   |
| 16   | Waipawa        | 824                  | 1020   | 3900  | Hilly           | Open                | Yes                 | Yes                  | No                  | No                   |
| 17   | Pohangina      | 824                  | 36     | 232   | Hilly           | Open                | Yes                 | Yes                  | No                  | No                   |
| 18   | Pohangina      | 234                  | 250    | 2400  | Hilly           | Closed              | No                  | Yes                  | Yes                 | No                   |
| 19   | Takapau        | 667                  | 90     | 950   | Hilly           | Open                | Yes                 | Yes                  | Yes                 | No                   |
| 20   | Colyton        | 305                  | 161    | 900   | Hilly           | Open                | Yes                 | Yes                  | No                  | No                   |

\* Variables for statistical analysis described in Table 4.2

**Table 4.2 Description of variables used for statistical analysis.**

| <b>Variable</b>                        | <b>Description</b>                                                                                                                                                                                                                                                          |
|----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Deer herd size                         | Total number of deer on farm including all classes<br>Large = deer herd size > median (n=560)<br>Small = deer herd size < median                                                                                                                                            |
| Farm geography                         | Hilly = >50% of area of farms are hilly<br>Flat = >50% of area of farms are flat                                                                                                                                                                                            |
| Replacement policy                     | Open = introduce animals on a regular basis from other farms<br>Closed = do not introduce animals from other farms                                                                                                                                                          |
| Neighbour waterway                     | Yes = waterway from neighbour passes through farm<br>No = no waterway from neighbour passes through farm                                                                                                                                                                    |
| Graze with Hardjobovis positive cattle | 4 = co-grazed with Hardjobovis positive cattle<br>3 = not co-grazed but Hardjobovis positive cattle present on farm<br>2 = co-grazed with Hardjobovis negative cattle<br>1 = not co-grazed, no Hardjobovis positive cattle present on farm<br>0 = no cattle on the property |
| Graze with Hardjobovis positive sheep  | 4 = co-grazed with Hardjobovis positive sheep<br>3 = not co-grazed but Hardjobovis positive sheep present on farm<br>2 = co-grazed with Hardjobovis negative sheep<br>1 = not co-grazed, no Hardjobovis positive sheep present on farm<br>0 = no sheep on the property      |
| Graze with Pomona positive cattle      | 4 = co-grazed with Pomona positive cattle<br>3 = not co-grazed but Pomona positive cattle present on farm<br>2 = co-grazed with Pomona negative cattle<br>1 = not co-grazed, no Pomona positive cattle present on farm<br>0 = no cattle on the property                     |
| Graze with Pomona positive sheep       | 4 = co-grazed with Pomona positive sheep<br>3 = not co-grazed but Pomona positive sheep present on farm<br>2 = co-grazed with Pomona negative sheep<br>1 = not co-grazed, no Pomona positive sheep present on farm<br>0 = no sheep on the property                          |

## 4.3 Results

### 4.3.1 Farm characteristics

Farm characteristics are summarised in Table 4.1. During the study one farm (Farm 8: deer and sheep) was lost to follow-up in 2007 and 2008 since the farmer withdrew, thus data from 19 farms are presented and analysed. The median deer herd size was 560 animals (range 71 – 1,201) and used to differentiate the size of farm as large or small in study analyses. The median herd/flock size of cattle and sheep were 150 (range 9 – 1,020) and 1,170 (range 232 – 10,120), respectively. Four farms were described as flat whereas the remainder were hilly. Effective area of farms ranged between 10 and 1,376 hectares. Deer fenced areas ranged between 10 and 350 hectares. Sixteen farms (84%) had at least one waterway from neighbouring, stock carrying properties that passed through farm. All the mixed and multi-species farms co- and/or cross-grazed other stock with deer herds during both years studied. No leptospiral vaccination was recorded from these farms despite four having clinical leptospirosis confirmed by a veterinarian in the five years prior to this study. Farms 6, 7 and 9 had records of clinical leptospirosis in hinds, weaner deer and sheep, respectively and Farm 13 in both weaner deer and cows within the same year.

### 4.3.2 Herd level infection

Summary of within-herd seroprevalence, reciprocal titre range and herd/flock serological status for Hardjobovis and Pomona are presented in Tables 4.3 and 4.4, respectively. The proportion of seropositive deer, cattle and sheep herds/flocks (both serovars) over three samplings averaged 72%, 74% and 64%, respectively. The proportion of Hardjobovis seropositive deer, cattle and sheep herds/flocks over three samplings was 42.1%, 52.8% and 53.9%, respectively. The proportion of Pomona seropositive deer, cattle and sheep herds/flocks over three samplings was 7.0%, 4.8% and 0%, respectively. Dual infections with serovars Hardjobovis and Pomona were found in 22.5% of deer herds, 16.3% of cattle herds and 10.3% of sheep flocks (Table 4.5). Persistence and non-persistence of *Leptospira* status for Hardjobovis and Pomona in deer, cattle and sheep herds/ flocks is shown in Table 4.6. For Hardjobovis positive deer, cattle and sheep herds/flocks, 42.1%, 42.9% and 46.2% remained positive for at least two consecutive years, respectively. For Pomona positive deer, cattle and sheep herds/flocks, 26% of deer herds remained positive for at least two consecutive years.

### 4.3.3. Individual animal level infection

Individual animal level seroprevalence data are shown in Table 4.7. Overall, 19.9% of deer (n = 1,154) were positive for Hardjobovis, 8.8% were positive for Pomona and 4.3% were positive for both. For cattle (n = 817), 39.1% were positive for Hardjobovis, 6.2% were positive for Pomona and 4.8% were positive for both. For sheep (n = 1,244) 30.1% were positive for Hardjobovis, 1.9% were positive for Pomona and 1.5% were positive for both serovars.

**Table 4.3 Within-herd/flock seroprevalence (No +/No sampled) (herd/flock status in parenthesis) and positive reciprocal titre range to Hardjobovis in each species on farms each year.**

| Farm                                   | Deer                    |        |                         |        |                         |        | Cattle                   |         |                          |        |                          |         | Sheep                  |         |                         |        |                         |        |
|----------------------------------------|-------------------------|--------|-------------------------|--------|-------------------------|--------|--------------------------|---------|--------------------------|--------|--------------------------|---------|------------------------|---------|-------------------------|--------|-------------------------|--------|
|                                        | 06                      |        | 07                      |        | 08                      |        | 06                       |         | 07                       |        | 08                       |         | 06                     |         | 07                      |        | 08                      |        |
|                                        | Prev                    | Titre  | Prev                    | Titre  | Prev                    | Titre  | Prev                     | Titre   | Prev                     | Titre  | Prev                     | Titre   | Prev                   | Titre   | Prev                    | Titre  | Prev                    | Titre  |
| 1                                      | 1/19<br>(-)             | 48     | 1/19<br>(-)             | 96     | 1/20<br>(-)             | 96     | NP                       | NP      | NP                       | NP     | NP                       | NP      | NP                     | NP      | NP                      | NP     | NP                      | NP     |
| 2                                      | 0/20<br>(-)             | 0      | 5/19<br>(+)             | 48     | 2/19<br>(-)             | 48     | NP                       | NP      | NP                       | NP     | NP                       | NP      | NP                     | NP      | NP                      | NP     | NP                      | NP     |
| 3                                      | 1/20<br>(-)             | 48     | 0/20<br>(-)             | 0      | 1/20<br>(-)             | 48     | NP                       | NP      | NP                       | NP     | NP                       | NP      | NP                     | NP      | NP                      | NP     | NP                      | NP     |
| 4                                      | 1/21<br>(-)             | 96     | 4/16<br>(+)             | 48     | 0/20<br>(-)             | 0      | 3/5<br>(+)               | 96-192  | 0/8<br>(-)               | 0      | 0/7<br>(-)               | 0       | NP                     | NP      | NP                      | NP     | NP                      | NP     |
| 5                                      | 3/21<br>(+)             | 48-96  | 1/20<br>(-)             | 384    | 0/20<br>(-)             | 0      | 13/20<br>(+)             | 48-384  | 0/10<br>(-)              | 0      | 2/20<br>(-)              | 48-192  | NP                     | NP      | NP                      | NP     | NP                      | NP     |
| 6                                      | 8/20<br>(+)             | 48-96  | 12/20<br>(+)            | 48-192 | 13/20<br>(+)            | 48-768 | 16/20<br>(+)             | 48-384  | 17/20<br>(+)             | 48-192 | 1/20<br>(-)              | 192     | NP                     | NP      | NP                      | NP     | NP                      | NP     |
| 7                                      | 6/20<br>(+)             | 48-96  | 6/20<br>(+)             | 48-96  | 6/15<br>(+)             | 48-384 | NP                       | NP      | NP                       | NP     | NP                       | NP      | 0/30<br>(-)            | 0       | 0/19<br>(-)             | 0      | 0/19<br>(-)             | 0      |
| 9                                      | 6/20<br>(+)             | 48-96  | 0/19<br>(-)             | 0      | 4/18<br>(+)             | 48-192 | 10/17<br>(+)             | 48-96   | 7/19<br>(+)              | 48-192 | 5/20<br>(+)              | 48-96   | 18/60<br>(+)           | 48-384  | 10/17<br>(+)            | 48-96  | 12/20<br>(+)            | 48-192 |
| 10                                     | 0/20<br>(-)             | 0      | 6/19<br>(+)             | 48-96  | 10/20<br>(+)            | 48-96  | 16/20<br>(+)             | 48-192  | 5/18<br>(+)              | 48-192 | 11/20<br>(+)             | 48-768  | 3/60<br>(-)            | 48-192  | 7/20<br>(+)             | 48-192 | 12/20<br>(+)            | 96-768 |
| 11                                     | 3/19<br>(+)             | 48-96  | 17/20<br>(+)            | 48-96  | 9/20<br>(+)             | 48-96  | 0/20<br>(-)              | 0       | 5/20<br>(+)              | 48-192 | 1/20<br>(-)              | 48      | 20/60<br>(+)           | 48-384  | 9/20<br>(+)             | 96-768 | 7/20<br>(+)             | 48-192 |
| 12                                     | 3/20<br>(+)             | 48     | 4/20<br>(+)             | 48-96  | 11/15<br>(+)            | 48-96  | 3/20<br>(+)              | 48-384  | 7/20<br>(+)              | 48-192 | 13/20<br>(+)             | 48-1536 | 1/60<br>(-)            | 96      | 13/20<br>(+)            | 48-96  | 17/19<br>(+)            | 96-768 |
| 13                                     | 9/14<br>(+)             | 48-96  | 9/20<br>(+)             | 48-96  | 0/20<br>(-)             | 0      | 17/19<br>(+)             | 48-384  | NP                       | NP     | NP                       | NP      | 0/60<br>(-)            | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0      |
| 14                                     | 9/18<br>(+)             | 48-384 | 4/20<br>(+)             | 48-96  | 1/20<br>(-)             | 48     | 20/20<br>(+)             | 48-768  | 0/20<br>(-)              | 0      | 0/19<br>(-)              | 0       | 41/60<br>(+)           | 48-768  | 6/20<br>(+)             | 48-192 | 0/18<br>(+)             | 0      |
| 15                                     | 5/19<br>(+)             | 48-96  | 2/19<br>(-)             | 48-384 | 7/18<br>(+)             | 48-96  | 13/19<br>(+)             | 48-192  | 11/18<br>(+)             | 48-384 | 0/19<br>(-)              | 0       | 55/59<br>(+)           | 48-384  | 13/19<br>(+)            | 48-96  | 10/20<br>(+)            | 48-96  |
| 16                                     | 8/20<br>(+)             | 48-192 | 3/20<br>(+)             | 48     | 5/20<br>(+)             | 48-192 | 13/20<br>(+)             | 48-384  | 0/20<br>(-)              | 0      | 0/19<br>(-)              | 0       | 5/60<br>(+)            | 192-384 | 0/20<br>(-)             | 0      | 9/20<br>(+)             | 48-384 |
| 17                                     | 0/20<br>(-)             | 0      | 13/19<br>(+)            | 48-192 | 1/20<br>(-)             | 96     | 6/15<br>(+)              | 48-384  | 7/7<br>(+)               | 48-768 | 18/20<br>(+)             | 48-384  | 38/60<br>(+)           | 48-768  | 4/20<br>(+)             | 48-96  | 7/20<br>(+)             | 48-192 |
| 18                                     | 4/20<br>(+)             | 48-96  | 8/20<br>(+)             | 48-192 | 0/20<br>(-)             | 0      | 7/20<br>(+)              | 192-768 | 17/20<br>(+)             | 48-192 | 11/20<br>(+)             | 38-96   | 8/60<br>(+)            | 48-1536 | 1/19<br>(-)             | 384    | 0/20<br>(-)             | 0      |
| 19                                     | 6/20<br>(+)             | 48-384 | 9/40<br>(+)             | 48-192 | 5/39<br>(+)             | 48     | 20/20<br>(+)             | 96-768  | 12/40<br>(+)             | 48-96  | 34/39<br>(+)             | 48-384  | 37/60<br>(+)           | 48-768  | 9/20<br>(+)             | 48-768 | 9/20<br>(+)             | 48-192 |
| 20                                     | 7/21<br>(+)             | 48-192 | 10/20<br>(+)            | 48-96  | 10/20<br>(+)            | 48-384 | 5/18<br>(+)              | 48-768  | 4/20<br>(+)              | 48-96  | 0/10<br>(-)              | 0       | 4/46<br>(+)            | 48-384  | 0/19<br>(-)             | 0      | 2/20<br>(-)             | 96-192 |
| <b>Mean<br/>prev (%)<br/>(min-max)</b> | <b>31.5 (15.0-64.0)</b> |        | <b>39.0 (15.0-85.0)</b> |        | <b>45.0 (25.0-68.8)</b> |        | <b>63.1 (15.0-100.0)</b> |         | <b>53.3 (20.- 100.0)</b> |        | <b>58.6 (25.0-100.0)</b> |         | <b>41.9 (8.3-95.0)</b> |         | <b>45.9 (20.0-68.4)</b> |        | <b>56.2 (35.0-89.5)</b> |        |

Prev = Prevalence, + = Herd/flock positive, - = Herd/flock negative, NP = Not present

**Table 4.4 Within-Herd/flock seroprevalence (No +/No sampled) (herd/flock status in parenthesis) and positive reciprocal titre range to Pomona in each species on farms each year.**

| Farm                                   | Deer                    |         |                         |        |                         |         | Cattle                  |        |                         |        |                         |         | Sheep        |        |                       |        |                         |        |
|----------------------------------------|-------------------------|---------|-------------------------|--------|-------------------------|---------|-------------------------|--------|-------------------------|--------|-------------------------|---------|--------------|--------|-----------------------|--------|-------------------------|--------|
|                                        | 06                      |         | 07                      |        | 08                      |         | 06                      |        | 07                      |        | 08                      |         | 06           |        | 07                    |        | 08                      |        |
|                                        | Prev                    | Titre   | Prev                    | Titre  | Prev                    | Titre   | Prev                    | Titre  | Prev                    | Titre  | Prev                    | Titre   | Prev         | Titre  | Prev                  | Titre  | Prev                    | Titre  |
| 1                                      | 0/19<br>(-)             | 0       | 0/19<br>(-)             | 0      | 0/20<br>(-)             | 0       | NP                      | NP     | NP                      | NP     | NP                      | NP      | NP           | NP     | NP                    | NP     | NP                      | NP     |
| 2                                      | 0/20<br>(-)             | 0       | 0/19<br>(-)             | 0      | 0/19<br>(-)             | 0       | NP                      | NP     | NP                      | NP     | NP                      | NP      | NP           | NP     | NP                    | NP     | NP                      | NP     |
| 3                                      | 0/20<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | NP                      | NP     | NP                      | NP     | NP                      | NP      | NP           | NP     | NP                    | NP     | NP                      | NP     |
| 4                                      | 7/21<br>(+)             | 96-384  | 4/16<br>(+)             | 48-192 | 9/20<br>(+)             | 48-1536 | 2/5<br>(+)              | 48-96  | 0/8<br>(-)              | 0      | 0/7<br>(-)              | 0       | NP           | NP     | NP                    | NP     | NP                      | NP     |
| 5                                      | 10/21<br>(+)            | 48-384  | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/19<br>(-)             | 0      | 19/20<br>(+)            | 96-1536 | NP           | NP     | NP                    | NP     | NP                      | NP     |
| 6                                      | 1/20<br>(-)             | 192     | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | 1/20<br>(-)             | 48     | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | NP           | NP     | NP                    | NP     | NP                      | NP     |
| 7                                      | 1/20<br>(-)             | 192     | 1/20<br>(-)             | 96     | 1/15<br>(-)             | 48      | NP                      | NP     | NP                      | NP     | NP                      | NP      | 0/30<br>(-)  | 0      | 0/19<br>(-)           | 0      | 0/19<br>(-)             | 0      |
| 9                                      | 2/20<br>(-)             | 48-192  | 3/19<br>(+)             | 48-192 | 11/18<br>(+)            | 48-192  | 2/17<br>(-)             | 48-192 | 8/19<br>(+)             | 48-96  | 7/20<br>(+)             | 48-1536 | 19/60<br>(-) | 96-384 | 1/17<br>(-)           | 48     | 6/20<br>(+)             | 48-384 |
| 10                                     | 1/20<br>(-)             | 192     | 12/19<br>(+)            | 48-384 | 4/20<br>(+)             | 48-96   | 2/20<br>(-)             | 48-192 | 2/18<br>(-)             | 48-96  | 2/20<br>(-)             | 96      | 1/60<br>(-)  | 192    | 3/20<br>(+)           | 48-96  | 0/20<br>(-)             | 0      |
| 11                                     | 0/19<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0      | 2/20<br>(-)             | 192-384 | 0/60<br>(-)  | 0      | 0/20<br>(-)           | 0      | 0/20<br>(-)             | 0      |
| 12                                     | 0/20<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/15<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | 0/60<br>(-)  | 0      | 1/20<br>(-)           | 96     | 0/19<br>(-)             | 0      |
| 13                                     | 0/14<br>(-)             | 0       | 0/20<br>(-)             | 0      | 1/20<br>(-)             | 48      | 0/19<br>(-)             | 0      | NP                      | NP     | NP                      | NP      | 0/60<br>(-)  | 0      | 0/20<br>(-)           | 0      | 0/20<br>(-)             | 0      |
| 14                                     | 6/18<br>(+)             | 48-384  | 10/20<br>(+)            | 48-384 | 2/20<br>(-)             | 48      | 5/20<br>(+)             | 96-384 | 1/20<br>(-)             | 96     | 0/19<br>(-)             | 0       | 2/60<br>(-)  | 96-768 | 1/20<br>(-)           | 48     | 0/18<br>(-)             | 0      |
| 15                                     | 0/19<br>(-)             | 0       | 0/19<br>(-)             | 0      | 0/18<br>(-)             | 0       | 1/19<br>(-)             | 96     | 0/18<br>(-)             | 0      | 0/19<br>(-)             | 0       | 0/59<br>(-)  | 0      | 0/19<br>(-)           | 0      | 0/20<br>(-)             | 0      |
| 16                                     | 0/20<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/20<br>(-)             | 0      | 4/19<br>(+)             | 48-192  | 0/60<br>(-)  | 0      | 0/20<br>(-)           | 0      | 0/20<br>(-)             | 0      |
| 17                                     | 0/20<br>(-)             | 0       | 1/19<br>(-)             | 48     | 1/20<br>(-)             | 192     | 0/15<br>(-)             | 0      | 0/7<br>(-)              | 0      | 0/20<br>(-)             | 0       | 1/60<br>(-)  | 48     | 0/20<br>(-)           | 0      | 0/20<br>(-)             | 0      |
| 18                                     | 7/20<br>(+)             | 48-1536 | 12/20<br>(+)            | 48-384 | 12/20<br>(+)            | 48-192  | 2/20<br>(-)             | 96-192 | 0/20<br>(-)             | 0      | 6/20<br>(+)             | 48-192  | 0/60<br>(-)  | 0      | 0/19<br>(-)           | 0      | 0/20<br>(-)             | 0      |
| 19                                     | 0/20<br>(-)             | 0       | 4/40<br>(+)             | 48-96  | 0/39<br>(-)             | 0       | 0/20<br>(-)             | 0      | 0/40<br>(-)             | 0      | 10/39<br>(+)            | 48-384  | 0/60<br>(-)  | 0      | 15/20<br>(+)          | 48-384 | 5/20<br>(+)             | 48-192 |
| 20                                     | 8/21<br>(+)             | 48-192  | 11/20<br>(+)            | 48-192 | 9/20<br>(+)             | 48-384  | 0/18<br>(-)             | 0      | 3/20<br>(+)             | 96-192 | 0/10<br>(-)             | 0       | 0/46<br>(-)  | 0      | 1/19<br>(-)           | 96     | 2/20<br>(-)             | 48-384 |
| <b>Mean<br/>prev (%)<br/>(min-max)</b> | <b>37.9 (33.3-50.0)</b> |         | <b>41.3 (15.8-63.2)</b> |        | <b>46.2 (20.0-60.1)</b> |         | <b>32.5 (25.0-40.0)</b> |        | <b>32.5 (15.0-50.0)</b> |        | <b>46.9 (21.1-95.0)</b> |         | <b>0 (0)</b> |        | <b>45 (15.0-75.0)</b> |        | <b>23.3 (15.0-30.0)</b> |        |

Prev = Prevalence, + = Herd/flock positive, - = herd/flock negative, NP = Not present

**Table 4.5 Number (and %) of herds/flocks serologically positive for Hardjobovis and Pomona each year.**

| Species | No. of herds/flocks sampled |    |    | No. of positive herds/flocks and (%) |             |             |            |            |             |             |             |             |              |              |              |
|---------|-----------------------------|----|----|--------------------------------------|-------------|-------------|------------|------------|-------------|-------------|-------------|-------------|--------------|--------------|--------------|
|         |                             |    |    | Hardjobovis                          |             |             | Pomona     |            |             | Both        |             |             | Overall      |              |              |
|         | 06                          | 07 | 08 | 06                                   | 07          | 08          | 06         | 07         | 08          | 06          | 07          | 08          | 06           | 07           | 08           |
| Deer    | 19                          | 19 | 19 | 9<br>(47.4)                          | 8<br>(42.1) | 7<br>(36.8) | 1<br>(5.3) | 1<br>(5.3) | 2<br>(10.5) | 4<br>(20.0) | 6<br>(31.6) | 3<br>(15.8) | 14<br>(73.6) | 15<br>(78.9) | 12<br>(63.2) |
| Cattle  | 15                          | 14 | 14 | 12<br>(80.0)                         | 8<br>(57.1) | 3<br>(21.4) | 0<br>(0)   | 0<br>(0)   | 2<br>(14.3) | 2<br>(13.3) | 2<br>(14.2) | 3<br>(21.4) | 14<br>(93.3) | 10<br>(71.4) | 8<br>(57.1)  |
| Sheep   | 13                          | 13 | 13 | 9<br>(69.2)                          | 6<br>(46.2) | 6<br>(46.2) | 0<br>(0)   | 0<br>(0)   | 0<br>(0)    | 0<br>(0)    | 2<br>(15.4) | 2<br>(15.4) | 9<br>(69.2)  | 8<br>(61.5)  | 8<br>(61.5)  |

**Table 4.6 Number (and %) of herds/flocks persistently positive or negative for Hardjobovis and Pomona or change status between years.**

| Serovar     | Species | No. of herds/ flocks | Herd/ flock <i>Leptospira</i> status |                       |                      |
|-------------|---------|----------------------|--------------------------------------|-----------------------|----------------------|
|             |         |                      | Consistently positive                | Consistently negative | Change between years |
| Hardjobovis | Deer    | 19                   | 8 (42.1)                             | 2 (10.5)              | 10 (52.6)            |
|             | Cattle  | 14                   | 6 (42.9)                             | 0 (0)                 | 8 (57.1)             |
|             | Sheep   | 13                   | 6 (46.2)                             | 2 (15.4)              | 5 (38.5)             |
| Pomona      | Deer    | 19                   | 5 (26.3)                             | 11 (57.9)             | 3 (15.8)             |
|             | Cattle  | 14                   | 0 (0)                                | 6 (42.9)              | 8 (57.1)             |
|             | Sheep   | 13                   | 0 (0)                                | 10 (76.9)             | 3 (23.1)             |

**Table 4.7 Number (%) of animals seropositive to Hardjobovis and Pomona alone or combined each year.**

| Species            | No. tested |     |     | No. of positive animals and (%) |               |               |             |             |              |             |             |             |               |               |               |
|--------------------|------------|-----|-----|---------------------------------|---------------|---------------|-------------|-------------|--------------|-------------|-------------|-------------|---------------|---------------|---------------|
|                    |            |     |     | Hardjobovis                     |               |               | Pomona      |             |              | Both        |             |             | Overall       |               |               |
|                    | 06         | 07  | 08  | 06                              | 07            | 08            | 06          | 07          | 08           | 06          | 07          | 08          | 06            | 07            | 08            |
| Deer               | 372        | 398 | 384 | 67<br>(18.0)                    | 90<br>(22.6)  | 73<br>(19.0)  | 30<br>(8.1) | 35<br>(8.8) | 37<br>(9.6)  | 13<br>(3.3) | 23<br>(5.8) | 13<br>(3.4) | 110<br>(29.6) | 148<br>(37.2) | 123<br>(32.0) |
| Cattle             | 273        | 271 | 273 | 151<br>(55.3)                   | 88<br>(32.5)  | 80<br>(29.3)  | 4<br>(1.5)  | 12<br>(4.4) | 35<br>(12.8) | 11<br>(4.0) | 3<br>(1.1)  | 15<br>(5.5) | 166<br>(60.8) | 103<br>(38.0) | 130<br>(47.6) |
| Sheep              | 735        | 253 | 256 | 225<br>(30.6)                   | 63<br>(24.9)  | 86<br>(33.6)  | 1<br>(0.1)  | 14<br>(5.2) | 8<br>(3.1)   | 5<br>(0.6)  | 8<br>(3.2)  | 5<br>(1.9)  | 231<br>(31.4) | 85<br>(33.6)  | 99<br>(38.7)  |
| <b>All species</b> | 1,380      | 922 | 913 | 443<br>(32.1)                   | 241<br>(26.1) | 239<br>(26.2) | 35<br>(2.5) | 61<br>(6.6) | 80<br>(8.8)  | 29<br>(2.0) | 34<br>(3.7) | 33<br>(3.6) | 507<br>(36.7) | 336<br>(36.4) | 352<br>(38.6) |

#### **4.3.4 Association between leptospiral positive deer herds and risk factors**

There were no correlations between serological status of a herd sampling in subsequent years using the intra-class-correlation criterion. Repeated tests of animals from the same herd were therefore regarded as independent ( $n = 3 \text{ years} * 19 \text{ herds} = 57 \text{ herd-years}$ ). The prevalence ratio for factors associated with a deer herd being leptospiral seropositive for Hardjobovis and Pomona are summarised in Tables 4.8 and 4.9, respectively.

For Hardjobovis, there was a higher PR of a deer herd being seropositive when the deer herd size was large, the farm was hilly and when deer were co-grazing with Hardjobovis positive cattle or sheep. There were trends for increases in the proportion of Hardjobovis positive deer herds when exposed to Hardjobovis positive cattle herds or sheep flocks at different level of co-grazing. No association was found between replacement policy and joint water sources with neighbouring farms.

For Pomona, there was a significantly higher PR of a deer herd being seropositive when co-grazing with Pomona positive cattle herds. There were trends for increases in seropositivity to Pomona in deer herds when exposed to Pomona positive cattle herds but not sheep flocks at different level of co-grazing. There was a lower PR of a deer herd being seropositive if the farm brought in stock from other farms. No association was found between deer herd size, farm geography and a waterway from a neighbour and seropositivity of deer herds for Pomona.



**Table 4.8 Prevalence Ratio (PR), 95% confidence interval (CI) and p-value for risk factors associated with a deer herd being seropositive for Hardjobovis (Hardjo).**

| <b>Risk factors</b>                                           | <b>Herds<br/>Hardjo+</b> | <b>Herds<br/>Hardjo-</b> | <b>PR</b> | <b>95% CI</b> | <b>p-value</b> |
|---------------------------------------------------------------|--------------------------|--------------------------|-----------|---------------|----------------|
| Deer herd size                                                |                          |                          |           |               |                |
| - Large (> 560 animals)                                       | 21                       | 6                        | 1.46      | 1.00 – 2.16   | 0.036          |
| - Small (< 560 animals)                                       | 16                       | 14                       | Ref       |               |                |
| Farm geography                                                |                          |                          |           |               |                |
| - Hill                                                        | 35                       | 10                       | 4.67      | 1.30 – 16.67  | <0.001         |
| - Flat                                                        | 2                        | 10                       | Ref       |               |                |
| Replacement policy                                            |                          |                          |           |               |                |
| - Open herd (Buy in stock)                                    | 33                       | 18                       | 0.97      | 0.53 – 1.77   | 0.346          |
| - Closed herd                                                 | 4                        | 2                        | Ref       |               |                |
| Neighbour waterway                                            |                          |                          |           |               |                |
| - Yes                                                         | 32                       | 16                       | 1.20      | 0.65 – 2.23   | 0.235          |
| - No                                                          | 5                        | 4                        | Ref       |               |                |
| Co-grazing with Hardjobovis + cattle*                         |                          |                          |           |               |                |
| Co-grazed with Hardjobovis positive cattle                    | 17                       | 5                        | 1.93      | 1.00 – 3.74   | 0.022          |
| Not co-grazed but Hardjobovis positive cattle present on farm | 5                        | 2                        | 1.79      | 0.82 – 3.88   | 0.149          |
| Co-grazed with Hardjobovis negative cattle                    | 6                        | 2                        | 1.88      | 0.90 – 3.92   | 0.104          |
| Not co-grazed, no Hardjobovis positive cattle present on farm | 3                        | 2                        | 1.50      | 0.58 – 3.82   | 0.298          |
| No cattle on the property                                     | 6                        | 9                        | Ref       |               |                |
| Co- grazing with Hardjobovis + sheep*                         |                          |                          |           |               |                |
| Co-grazed with Hardjobovis positive sheep                     | 12                       | 1                        | 2.77      | 1.41 – 5.42   | <0.001         |
| Not co-grazed but Hardjobovis positive sheep present on farm  | 9                        | 3                        | 2.25      | 1.08 – 4.67   | 0.026          |
| Co-grazed with Hardjobovis negative sheep                     | 1                        | 1                        | 1.50      | 0.32 – 6.94   | 0.479          |
| Not co-grazed, no Hardjobovis positive sheep present on farm  | 9                        | 3                        | 2.25      | 1.08 – 4.67   | 0.026          |
| No sheep on the property                                      | 6                        | 12                       | Ref       |               |                |

Ref = Reference category, + = positive

\*significant for increased trend at  $p \leq 0.05$

**Table 4.9 Prevalence Ratio (PR), 95% confidence interval (CI) and p-value for risk factors associated with a deer herd being seropositive for Pomona (Pom).**

| Risk factors                                             | Herds Pom+ | Herds Pom- | PR    | 95% CI       | p-value |
|----------------------------------------------------------|------------|------------|-------|--------------|---------|
| Deer herd size                                           |            |            |       |              |         |
| - Large (> 560 animals)                                  | 6          | 21         | 0.61  | 0.26 – 1.42  | 0.116   |
| - Small (< 560 animals)                                  | 11         | 19         | Ref   |              |         |
| Farm geography                                           |            |            |       |              |         |
| - Hilly                                                  | 14         | 31         | 1.24  | 0.43 – 3.64  | 0.263   |
| - Flat                                                   | 3          | 9          | Ref   |              |         |
| Replacement policy                                       |            |            |       |              |         |
| - Open herd (Buy in stock)                               | 12         | 39         | 0.28  | 0.15 – 0.52  | 0.007   |
| - Closed herd                                            | 5          | 1          | Ref   |              |         |
| Neighbour waterway                                       |            |            |       |              |         |
| - Yes                                                    | 12         | 36         | 0.45  | 0.21 – 1.01  | 0.063   |
| - No                                                     | 5          | 4          | Ref   |              |         |
| Co-grazing with Pomona + cattle*                         |            |            |       |              |         |
| Co-grazed with Pomona positive cattle                    | 3          | 3          | 7.50  | 1.00 – 58.59 | 0.050   |
| Not co-grazed but Pomona positive cattle present on farm | 3          | 0          | 15.00 | 2.26 – 99.64 | 0.005   |
| Co-grazed with Pomona negative cattle                    | 6          | 18         | 3.75  | 0.50 – 28.17 | 0.131   |
| Not co-grazed, no Pomona positive cattle present on farm | 5          | 4          | 8.30  | 1.15 – 60.46 | 0.014   |
| No cattle on the property                                | 1          | 14         | Ref   |              |         |
| Co-grazing with Pomona + sheep                           |            |            |       |              |         |
| Co-grazed with Pomona positive sheep                     | 3          | 1          | 3.38  | 1.20 – 9.48  | 0.072   |
| Not co-grazed but Pomona positive sheep present on farm  | -          | -          | -     | -            | -       |
| Co-grazed with Pomona negative sheep                     | 2          | 9          | 0.82  | 0.18 – 3.75  | 0.354   |
| Not co-grazed, no Pomona positive sheep present on farm  | 8          | 19         | 1.33  | 0.47 – 3.78  | 0.236   |
| No sheep on the property                                 | 4          | 14         | Ref   |              |         |

Ref = Reference category, + = positive

\*significant for increased trend at  $p \leq 0.05$

## 4.4 Discussion

This study provided preliminary, regionally-based data on leptospirosis on mixed-species farms. Data will be used to inform more substantial studies of leptospirosis on mixed-species farms in New Zealand in the future. It demonstrated that infection by *Leptospira* serovars Hardjobovis and Pomona was common in mixed species commercial farms in the lower North Island of New Zealand, and has described some risk factors for deer seropositivity. Deer in larger herds, grazing hill country and grazing with Hardjobovis seropositive cattle

and sheep were at higher risk of being seropositive to this serovar than deer grazed alone or on flat land. Similarly, deer on properties with cattle seropositive for Pomona were more likely to be seropositive to that serovar. Deer on farms that had an open replacement policy were less likely to be seropositive for Pomona.

The predominant serovar observed in this study was Hardjobovis which is consistent with previous findings in deer (Ayanegui-Alcérreca *et al.* 2003; Wilson *et al.* 1998), cattle (Bahaman *et al.* 1984; Matthews *et al.* 1999) and sheep (Blackmore *et al.* 1982; Dorjee *et al.* 2005) in New Zealand. Sampling was conducted between August and October in each year which is the wet season in the study region. Wet conditions are suitable for survival and transmission of leptospires (Hellstrom and Marshall 1978) optimising the chance of observing active infections on farms in this study. The target sampling group was young replacements of each species in order to observe transmission to the next generation and persistence of the herd/flock leptospiral status, as demonstrated to occur in deer herds (Ayanegui-Alcérreca 2006).

The 63 – 79% seroprevalence of leptospirosis in deer at the herd level presented here is lower than the 81% national seroprevalence demonstrated in a New Zealand-wide prevalence survey but was similar to data for the Manawatu/Hawkes Bay regions (Ayanegui-Alcérreca *et al.* 2010). Moreover, this was similar to that described in an earlier survey from the Manawatu area (Wilson *et al.* 1998). These data support the assertion that farmed deer in New Zealand are a maintenance host for Hardjobovis and accidental host for Pomona. However, the seroprevalence of Hardjobovis alone presented here, at the herd and individual animals levels, (42% and 20%, respectively) were lower than reported by Ayanegui-Alcérreca *et al.* (2010) (61% and 54%, respectively). The likely reason is the difference of titre cut point used for each study since Ayanegui-Alcérreca *et al.* (2010) used a titre cut point at 1:24 for serovar Hardjobovis, while the present study used a titre cut point at 1:48. The latter cut point has been shown to result in less non-specific agglutination and cross reaction (Blackmore *et al.* 1982). Climatic and other factors may alter seroprevalence between years.

Data for beef cattle from this study identifying that serovar Hardjobovis infections were endemic and serovar Pomona infections were sporadic agrees with previously data from dairy cattle (Bahaman *et al.* 1984) which showed the seroprevalence of Hardjobovis in cattle herds was 70% whereas Pomona infections were 21%. The 43% seroprevalence of Hardjobovis in individual cattle in this study was lower than 68% reported in a survey in beef herds in Hawke's Bay when using the same titre cut points (Matthews *et al.* 1999). The likely reason is the difference of age of animals sampled in each study since Matthews *et al.* (2009) sampled mixed age cattle while the present study sampled replacement animals. In a survey in dairy cattle herds in Taranaki, individual animal seroprevalence for Pomona was 58.5% (Bahaman *et al.* 1984).

During the first year of this study, 60 sheep per farm were sampled to determine the flock leptospiral status, based on estimated seroprevalence using published data. However, since

the within-flock prevalence of leptospirosis in sheep at the first sampling was higher than expected, the number of animals/farm required subsequently reduced to 20. The 64% seroprevalence of Hardjobovis in sheep at the flock level in this study was lower than 86% reported previously in hoggets ((Dorjee *et al.* 2005). The likely reason is the different criteria to determine the status of positive sheep flock since Dorjee *et al.* (2005) determined the flock status as 1 seropositive of 30 sheep while the present study determined the flock status by 5 seropositive of 60 in 2006 and 3 seropositive of 20 samples in 2007 and 2008. However, the 30% seroprevalence of Hardjobovis in adult sheep at the individual animal level in this study was similar to that in ewes (31%) but higher than in hoggets (23%) reported by Blackmore *et al.* (1982). Dorjee *et al.* (2005) reported a seroprevalence of 16.0% in hoggets at the same titre cut point as used in the present study (1:48). The reason that seroprevalence of Hardjobovis in sheep was higher than previous studies may be that this study was based on deer farms and deer is likely to be a maintenance host for this serovar (Ayanegui-Alcérreca *et al.* 2007), although climatic effects may have contributed.

The 10% seroprevalence of Pomona in sheep at the flock level in this study was lower than in a previous study that reported a seroprevalence of 28.6% in hogget lines (Dorjee *et al.* 2005). Nevertheless, the 3.4% seroprevalence of Pomona in sheep at the individual animal level in this study was similar to 3.8% reported by Blackmore *et al.* (1982) and 4% by Dorjee *et al.* (2005).

Blackmore *et al.* (1982) proposed that sheep were not a maintenance host for Hardjobovis but the seroprevalence of this serovar from this and other studies which are in the same range as in cattle and deer suggest that sheep may act as a maintenance host. This proposition is supported by other studies (Cousins *et al.* 1989; Gerritsen *et al.* 1994). The proposition by Blackmore *et al.* (1982) was almost 30 years prior to this study, thus it could be that during the past years, livestock farming in New Zealand has become increasingly multi-species including deer, beef cattle and sheep on the same property (Hilson 2007; Wilson 2007) affecting the disease dynamic, potentially altering the host status of sheep in the epidemiology of infection. However, proof that sheep are maintenance host for Hardjobovis requires evidence of long term leptospirosis by urine culture or PCR and strain typing, and epidemiological studies on sheep-only farms, which was beyond the scope of this study.

There was a higher proportion of farms (13/15) that had at least one livestock species persistently positive to Hardjobovis in two consecutive years than to Pomona (3/5). This further supports that all three livestock species may be maintenance hosts for Hardjobovis as proposed for deer (Ayanegui-Alcérreca *et al.* 2007), cattle (Hathaway 1981) and possibly sheep (Cousins *et al.* 1989; Gerritsen *et al.* 1994). Observations here were consistent with other observations of endemicity of this serovar in that more than 67% of deer herds and 50% of sheep flocks seronegative to Hardjobovis in 2006 had become seropositive in 2007 suggesting that *Leptospira* negative herds/flocks at a single sampling are likely to become positive on a later date. This finding is critical for risk evaluation in terms of farmer decision making to control or vaccinate their animals.

The persistence of seropositivity to Pomona was found particularly in deer but not in cattle and sheep on some farms, which demonstrates the potential for Pomona to be transferred to replacement stock on infected farms if the risk factors persist. This finding was consistent with previous longitudinal study in deer which reported persistence of this serovar over at least a two-year period (Ayanegui-Alcérreca 2006). It has been reported that Pomona can survive for at least 42 days in New Zealand soil under simulated winter conditions (Hellstrom and Marshall 1978) and is maintained in pigs (Hathaway 1981). Nevertheless, evidence that Pomona could persist for a long period in farmed deer justifies further investigation into the role of this species in maintaining this serovar at either individual or population levels. It may be possible that for Pomona, deer fall between a sporadic and maintenance host at the individual animal level and a maintenance host at the population level.

The Hardjobovis or Pomona status of repeated herd tests in subsequent years was not measurably correlated. Thus, statistical hypothesis tests did not have to be adjusted for correlation in the data. Given that initial analysis at the animal level revealed strong within-herd correlation, the animal level analyses that were appropriately adjusted for the correlation did not provide reliable estimates and all significant factors were at the herd level, therefore, all analysis was done on the herd basis and each herd-year could be assumed to be an independent observation.

Some environmental factors were tested for association with leptospiral status of deer herds, but only grazing hill pasture was significantly positively associated with deer herd status, and only for serovar Hardjobovis. This may be because most farms studied were hilly and there was high prevalence of Hardjobovis observed in deer herds. However, it is possible that hilly terrain can facilitate the organism spreading because the source of water for stock on hill pastures is more likely to be natural waterways than reticulated water. This risk factor will be investigated further in future studies.

Herd-level risk factors for seropositivity to *Leptospira* have been studied in other countries, mainly in dairy cattle, for serovar Hardjo. Larger herd size, co-grazing with infected livestock, access to contaminated water sources and introduction of other animals on farm have been reported to be associated with seroprevalence of leptospirosis (Lilenbaum and Santos 1996). By contrast, in the present study, an open herd replacement policy was associated with a 72% reduction in the risk of seropositivity of deer herds for serovar Pomona, although not for Hardjobovis. This apparent effect could, however, be spurious since the majority of studied farms were open herds and there was a low prevalence of Pomona in deer herds. Larger deer herd size (>560 animals) was positively associated with deer herd status with serovar Hardjobovis but not Pomona. Some reports suggest that seroprevalence is related to herd size in cattle (Ellis 1994; Lilenbaum and Santos 1996) although other studies in cattle reported that herd size was not a risk factor for leptospiral infection (Alonso-Andicoberry *et al.* 2001; Espi *et al.* 2000).

An increasing level of co-grazing exposure of deer with cattle or sheep (as described in Table 4.2) was associated with significant increasing trends of seropositivity to Hardjobovis and Pomona in deer herds. This finding was consistent with observations of infection in a cattle herd that co-grazing with infected cattle and sheep (Lilenbaum and Santos 1996). It suggests that inter-species transmission of leptospires may occur on farms in New Zealand. However, further studies of a greater number of mixed-species farms including bacterial isolation and importantly, strain typing, are needed to confirm that between-species transmission is occurring, since it is possible that host adaptation within serovars or different strains may occur.

There was variation in seroprevalence between species and between years within and between species observed in this study. Climatic changes between years of study could be one of the contributing factors. There was a correlation between humidity and seroprevalence of serovars Hardjobovis and Pomona in cattle reported in Queensland, Australia (Elder *et al.* 1986). A correlation between seroconversion for Hardjobovis in cattle and the wettest period of the year in New Zealand has also been reported (Hellstrom and Marshall 1978). However, it was not appropriate to analyse climatic variables in this study because there could be many confounding factors between farms which we did not observe. This will be subject to further study in a larger epidemiological study of leptospirosis on mixed-species farms.

In summary, this study demonstrates that leptospiral infection is prevalent across species on mixed-species farms in the lower North Island of New Zealand. The seroprevalence of leptospiral infection in deer, beef cattle and sheep was moderate to high at the herd/flock level, but lower at the individual animal level. Hardjobovis was the predominant serovar with Pomona at a lower seroprevalence. Co-grazing of farmed deer with leptospiral infected cattle and/or sheep is a significant risk for deer herds to be seropositive to *Leptospira*. This study provides baseline data for more intensive serological and molecular epidemiological study of leptospirosis on mixed-species farms in the future.

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## **Chapter 5**

### **Evaluation of a SYTO9 real-time PCR to detect and identify pathogenic *Leptospira* species in kidney tissue and urine of New Zealand farmed deer**

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## Abstract

A SYTO9 real-time PCR assay for detection of pathogenic *Leptospira* species based on amplification of DNA gyrase subunit B (*gyrB*) gene has been optimized and evaluated for sensitivity and specificity on kidney and urine samples from New Zealand farmed deer. The detection limit was approximately  $10^3$  cells/ml or equivalent to 2 to 10 copies/reaction. Comparison of this PCR assay on deer kidneys (n = 268) using culture as the gold standard revealed a sensitivity and a specificity of 85.0% and 99.2%, respectively. For deer urine (n=113), the assay was compared with known inoculated samples as the gold standard and revealed a sensitivity and specificity of 96.7% and 100%, respectively. The detectable concentration of leptospires shed naturally in deer urine collected on-farm (n = 28) was  $3.7 \times 10^3$  to  $1.7 \times 10^6$  cells/ml. To assess the assay's capability for identifying pathogenic *Leptospira* species, 14 field isolates of serovars Hardjobovis and Pomona were amplified for PCR product, purified and sequenced. When compared with the NCBI database using a BLASTn search, sequence data matched with *L. borgpetersenii* serovar Hardjobovis in 13 samples and *L. interrogans* serovar Pomona in one sample which was consistent with the microscopic agglutination test (MAT). Sequence analysis of purified PCR product amplified directly from kidney and urine samples also yielded serovar-comparable MAT results. Results from this study suggest that this real-time PCR assay is a useful tool for rapid, economic, sensitive and specific detection of pathogenic leptospires in clinical samples from farmed deer for diagnostic purposes and epidemiological, vaccine and other research. It can also be used for estimating the concentration of leptospires in urine and identifying *Leptospira* species in combination with DNA sequencing.

## 5.1 Introduction

Leptospirosis is an emerging zoonotic disease with worldwide distribution. It is caused by infection with pathogenic *Leptospira*, helical shaped motile spirochetes that belong to the family *Leptospiraceae* genus *Leptospira*. Humans and animals usually get infected by direct contact with blood, urine or kidney of infected animals or indirectly by contact with surface water, mud and soil that is contaminated with pathogenic leptospires from excretor animals (Levett, 2001). There have been over 200 serovars of pathogenic *Leptospira* within 13 species isolated and described (Bharti et al., 2003). Six serovars within two species of *L. borgpetersenii* and *L. interrogans* were isolated in animals in New Zealand (Hathaway, 1981, Midwinter et al., 1999).

In New Zealand, livestock, including farmed deer, play an important role in the spread of leptospirosis to both animals and humans because they excrete organisms into the environment via urine, and at slaughter, put meat workers at risk via urine and kidney tissue. Leptospirosis is a well recognised clinical disease and sub-clinical infection in New Zealand farmed deer (Ayanegui-Alcérreca et al., 2010, Wilson et al., 1998). *Leptospira borgpetersenii* serovar Hardjobovis and *L. interrogans* serovar Pomona are the most commonly detected serovars (Ayanegui-Alcérreca et al., 2007). A regional leptospirosis sero-

prevalence survey of 110 deer farms in NZ showed evidence that 81% were seropositive, with Hardjobovis alone in 61% of herds, Pomona alone in 4% and a combination of both in 16% of herds (Ayanegui-Alcérreca et al., 2010). It is proposed that farmed deer in New Zealand are maintenance hosts for Hardjobovis and an accidental host for Pomona and that deer play an important role in the infection cycle of leptospirosis on New Zealand livestock farms (Ayanegui-Alcérreca, 2006).

Culture of *Leptospira* organisms from clinical samples is the definitive method that allows identification of infecting serovars. However, culture is rarely used because it is technically demanding, tedious, complex, time consuming, prone to contamination and expensive. The Microscopic Agglutination Test (MAT) is most widely used as the standard serology test because of its high sensitivity and specificity and the ability to identify leptospiral infection to serogroup or serovar level (O'Keefe, 2002, Vijayachari et al., 2001). However, the MAT does not reflect the carrier status of the host. It also has drawbacks including the maintenance of live *Leptospira* cultures for antigens and complexity of methods and interpretation that limits its use in the laboratory (Myers, 1976, Palmer, 1988). Conventional methods such as dark-field microscopy and Warthin-Starry staining to detect leptospire in clinical samples are unreliable because of their low sensitivity (O'Keefe, 2002).

With the introduction of Polymerase Chain Reaction (PCR), rapid detection of small numbers of leptospire in clinical samples has become practical due to specific amplification of leptospiral DNA. PCR can also determine the shedding and carrier status of leptospire, detecting leptospiral DNA in cattle urine (Bal et al., 1994, Bomfim et al., 2006), pig kidney (Fearnley et al., 2008), and cattle semen and vaginal fluids (Lilenbaum et al., 2008). There have also been applications of PCR for rapid diagnosis by detection of leptospire in blood (Fonseca Cde et al., 2006, Slack et al., 2007).

Real-time PCR has further improved the diagnosis of leptospirosis. A new double-stranded DNA intercalating dye from the SYTO family has been introduced as an alternative to conventional SyBr Green I. SYTO9 has been shown to produce robust and consistent DNA melting curves that are not affected by DNA concentration and can be used with a broad range of dye concentrations without causing PCR inhibition. It is easier to use than SyBr Green I, particularly for adapting conventional assays to a real-time format and for DNA melting curve analysis (Monis et al., 2005).

Recently, the DNA gyrase subunit B gene (*gyrB*) has been proposed as an alternative target to 16S rRNA gene for species identification. The *gyrB* gene is a single-copy gene, present in all bacteria, which encodes the ATPase domain of the enzyme DNA gyrase essential for DNA replication. The amino acid sequences of *gyrB* gene allow the comparison of bacterial taxonomy (Dauga, 2002). Recently, *gyrB* gene has been used as a target for identification of *Leptospira* species and is claimed as an alternate identification gene (Kawabata et al., 2006, Slack et al., 2006).

This paper reports the evaluation and validation of a quantitative real-time PCR using SYTO9 detection technology in combination with amplification and sequencing of a partial fragment of *gyrB* gene for detection and identification of pathogenic *Leptospira* species in urine and kidney samples from New Zealand farmed deer. We evaluated the detection limit, sensitivity and specificity in clinical contexts, examining kidney tissue and urine and comparing results with the gold standard, culture. We also report the range of concentration of leptospires shed naturally in deer urine, identification of pathogenic *Leptospira* species based on DNA sequence of *gyrB* gene PCR product from field isolates of deer kidney and urine, and phylogenetic analysis.

## **5.2 Materials and methods**

### **5.2.1 Sample sources**

Deer kidneys (n=268, range 8 – 25 per farm) paired with blood samples as available (n=209, range 0 – 25 per farm) were collected from 19 randomly selected slaughter lines from 18 farms at Deer Slaughter Premises (DSPs) in Feilding, Manawatu and Makarewa, Southland of New Zealand during the period of November 2006 to November 2008. Deer were rising-one-year-old or older of both sexes. There were no data on previous exposure to *Leptospira*. Additionally, urine (n=111, range 15 – 28 per farm) collected from female rising-one-year-old deer on five commercial deer farms in the Manawatu and Hawkes Bay regions of New Zealand in November 2007 as part of vaccine efficacy study (Chapters 2 and 3) were used in this study. Sampled animals were phenotypically red deer (*Cervus elaphus*) but possibly containing some wapiti (*Cervus elaphus canadensis*) genes.

### **5.2.2 Sample collection and preparation**

#### **5.2.2.1 Blood**

Blood samples collected at DSPs were by free flow into a new 10 ml plain blood tube after animal sticking, immediately after stunning, at the beginning of the slaughter line. On farms, blood samples were collected from the jugular vein of physically restrained deer using a new 20-gauge needle and a 10 ml evacuated blood tube with no anticoagulant. Blood was held at 4°C before transport to the laboratory for centrifugation at 3,000 rpm (1,512 x g) for 15 minutes after which serum was aliquoted into new, labeled 1.5ml microcentrifuge tubes and stored at -20°C.

#### **5.2.2.2 Kidney**

Whole kidneys were collected at the evisceration and inspection area of the slaughter board after capsule removal, put into labeled sterile plastic bags aseptically and held at 4°C before transport to the laboratory. Within 6-24 hours of collection, the kidney surface was swabbed with 70% alcohol and randomly aspirated from several sites over the entire kidney into a 5ml, sterile syringe using a sterile 16-gauge needle. Half of the extracted kidney tissue (approximately 50mg) was used for bacterial culture and the other half used for DNA

extraction for real-time PCR. Processed kidney sample was later split into two portions. One half was stored in freezer at -20°C whereas the other half was stored in 10% formalin.

#### **5.2.2.3 Urine**

Urination was induced by administration of furosemide (“Salix”, Intervet, NZ) at 1-1.5 mg/kg intramuscularly (Fairley et al., 1984, Warren et al., 1981). A new 70 ml plastic collector was held beneath the vulva after urination began. As much middle-stream urine as possible was collected and immediately held at 4°C. After transport to the laboratory, urine was centrifuged at 3,000 rpm ( $1512 \times g$ ) for 10 minutes to provide sediment which was re-suspended with 400 µl of phosphate-buffered saline (PBS) to neutralize the pH (Levett, 2001). Half (200µl) was used for bacterial culture and half was used for DNA extraction for real-time PCR. *Leptospire*-free deer urine was determined by no presence of organism or its DNA in bacterial culture and real-time PCR along with no detectable antibodies against *Leptospira* serovars Hardjobovis and Pomona in blood from the same animals.

#### **5.2.3 Serology**

The MAT was used to test serum reactivity to laboratory standardized *Leptospira* serovar Hardjobovis and Pomona. The method has been developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the Control of Leptospirosis” (Faine, 1982). A titer of  $\geq 1:48$  was considered positive for both serovars (Blackmore et al., 1982, Dorjee et al., 2008).

#### **5.2.4 Bacterial culture**

Ellinghausen-McCullough-Johnson-Harris (EMJH) was used as a selective medium with an addition of antibiotic (5'-fluoracil) for contamination inhibition. The processed samples (kidney and urine) were inoculated and sub-cultured into two consecutive tubes, incubated at 28-30°C and examined every two weeks under dark-field microscopy for four months. Isolates of leptospire were serotyped against the standardized antisera of *Leptospira* serovar Hardjobovis and Pomona. These methods were adapted from the standard protocols from the Massey University Leptospirosis Research Unit, based on “Guidelines for the Control of Leptospirosis” (Faine, 1982).

#### **5.2.5 DNA extraction**

DNA was extracted from 50mg of kidney tissue and 200µl of urine using the High Pure Template Kit (Roche, Germany) as per manufacturer’s instructions. DNA was eluted in a final volume of 200µl.

#### **5.2.6 PCR amplification**

The real-time PCR assay was modified from an assay described by (Slack et al., 2006). Magnesium and primer titration was performed to determine the optimal concentration to give the lowest cycle threshold (CT) value. SYTO9 (Invitrogen, USA) was used as a fluorescent, double-stranded DNA-specific, intercalating dye (Monis et al., 2005) for all real-

time PCR assays. The assay was performed in a Rotor-Gene 6000 machine (Corbett Research, Australia) using primers 2For 5'-tgagccaagaagaacaagctaca-3' and 504Rev 5'-matggttcrcrtttccgaaga-3' (Slack et al., 2006). Each 25µl reaction contained 2µl of DNA extracted from samples, 1.5µM SYTO9, 1X PCR buffer, 1.5mM MgCl<sub>2</sub>, 200µM dNTPs, 12.5pmol of 2For primer, 12.5pmol of 504Rev primer, 0.1% Fraction V bovine serum albumin (BSA) (Sigma, USA), 1 unit of Taq DNA polymerase and double distilled water. Thermal cycling consisted of initial denaturation at 95°C for 10min followed by 40 cycles of denaturation at 95°C for 10sec, annealing at 60°C for 20sec and extension at 72°C for 20sec. Melting temperature (T<sub>m</sub>) of PCR product was determined by melting curve analysis. It was performed by heating the PCR product from 70°C to 90°C and monitoring fluorescence change every 0.1°C. Confirmation of positive samples was determined by T<sub>m</sub> of PCR product or performing electrophoresis of PCR product in a 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. The positive control used was *Leptospira* serovar Hardjobovis and the negative control was double distilled water.

#### **5.2.7 PCR product purification**

The amplified PCR product was purified to remove excess primers and dNTPs using the High Pure PCR Product Purification Kit (Roche, Germany) as per manufacturer's instructions. Purified PCR product was eluted in a final volume of 50µl.

#### **5.2.8 DNA sequencing and phylogenetic analysis**

The purified PCR products were forwarded to the Allan Wilson Centre Genome Service, Massey University, New Zealand for ABI sequencing using the ABI3730 DNA capillary instrument. Purified PCR products were sequenced in both orientations by the dideoxy-chain termination method using 2For and 504Rev specific primers. The sequence data were assembled and trimmed to a minimum of two contiguous sequences using the MT Navigator software (Perkin Elmer, USA). Unknown sequence data were submitted to a nucleotide Basic Local Alignment Search Tool (BLASTn) available at the National Center for Biotechnology Information (NCBI) website. Analysis of DNA sequences was performed using the Geneious Basic version 4.6.1 software. Multiple alignments of the DNA sequence were performed for phylogenetic comparison using the neighbor joining method.

#### **5.2.9 Detection limit**

The limit of leptospiral DNA detection by real-time PCR was evaluated using artificially inoculated, leptospire-free, deer urine that was negative by culture and was from MAT negative animals. Urine samples were prepared by mixing 180µl of leptospire-free deer urine with 20µl of *Leptospira* serovar Hardjobovis (field isolate suspensions at a concentration of  $2 \times 10^8$  cells/ml measured in a Petroff-Hausser counting chamber) (Faine, 1982). The next step was seven serial, ten-fold dilutions from  $10^7$ - $10^1$  cells/ml of leptospire-free urine. The diluted samples were subsequently subjected to DNA extraction (see section 5.2.5). Two microliters of extracted DNA samples obtained from all seven dilutions were used as templates in the

PCR. The procedure was repeated with four different field isolates of *Leptospira* serovar Hardjovovis for confirmation of detection limit.

#### **5.2.10 Diagnostic sensitivity and specificity**

Two experiments were carried out to determine the sensitivity and specificity of real-time PCR on kidney and on urine samples.

For kidneys, fresh tissue samples from 268 deer were subjected directly to real-time PCR and compared with culture as the gold standard using the techniques described above. Blood samples from 209 of those animals were subjected to MAT for serovar identification. The suitability of long term storage of kidney tissue as either frozen or formalin-fixed samples for real-time PCR was compared with fresh samples from 11 kidneys. The agreement between results of the real-time PCR and kidney culture was tested by Kappa statistic.

For urine, the sensitivity and specificity of real-time PCR was determined by creating artificially inoculated urine samples at the lowest detection limit (as described in 5.2.9 above) to mimic a natural gold standard. Thirty aliquots of three different field isolates of *Leptospira* serovar Hardjovovis containing approximately  $2 \times 10^8$  cells/ml were diluted in phosphate buffered saline (PBS) pH 7.4 to  $10^4$  cells/ml. Thereafter, 20 $\mu$ l of the diluted aliquot were inoculated with 180 $\mu$ l of 30 different samples of leptospire-free deer urine to achieve a concentration of  $10^3$  cells/ml. All samples were chilled at 4°C overnight to simulate the usual transport time for field-collected samples. All 30 inoculated urine samples and 83 leptospire-free deer urine samples were subjected to DNA extraction and real-time PCR as below to determine sensitivity and specificity.

#### **5.2.11 Quantification of urinary shedding**

Twenty-eight real-time PCR positive deer urine samples from an on-farm vaccine efficacy study (see Chapter 2) were used for estimating concentrations of leptospires shed naturally in deer urine. A standard curve was constructed for each PCR run from serial dilutions of leptospires inoculated in leptospire-free deer urine as described in 5.2.9 above. Each sample was tested in duplicate in each PCR run to estimate repeatability. The concentration of leptospires was calculated using Rotor-Gene 6000 series software (Corbett Research, Australia). The concentration of leptospires was compared with culture from the same animals to determine the lowest concentration of leptospires in field-collected deer urine that could be cultured.

#### **5.2.12 Identification of pathogenic *Leptospira* species**

To assess ability to identify pathogenic *Leptospira* species, 14 isolates of unknown serovar from kidney and urine within this study (as in 5.2.10 above), three real-time PCR positive deer kidney tissue samples and seven real-time PCR positive deer urine samples were amplified for the product of *gyrB* gene. The amplified PCR products were then purified and forwarded to the Allan Wilson Genome Service Centre, Massey University, Palmerston North, New Zealand, for DNA sequencing. The sequence data were BLASTn searched to



assess homologies with sequences in the NCBI database. Phylogenetic analysis was performed to compare the DNA sequence between isolates.

## 5.3 Results

### 5.3.1 Detection limit

The lowest detection limit was approximately  $10^3$  cells/ml or equivalent to 2-10 copies/reaction. This was shown by both visualization of specific 500bp DNA fragments in the gel (Fig. 5.1, Lane 5) and melting curve analysis by real-time PCR revealing that concentrations below  $10^3$  cells/ml could not be distinguished from the negative control (Fig. 5.2, Line 5). This pattern was identical for all 5 different field isolates that were subjected to serial dilutions.

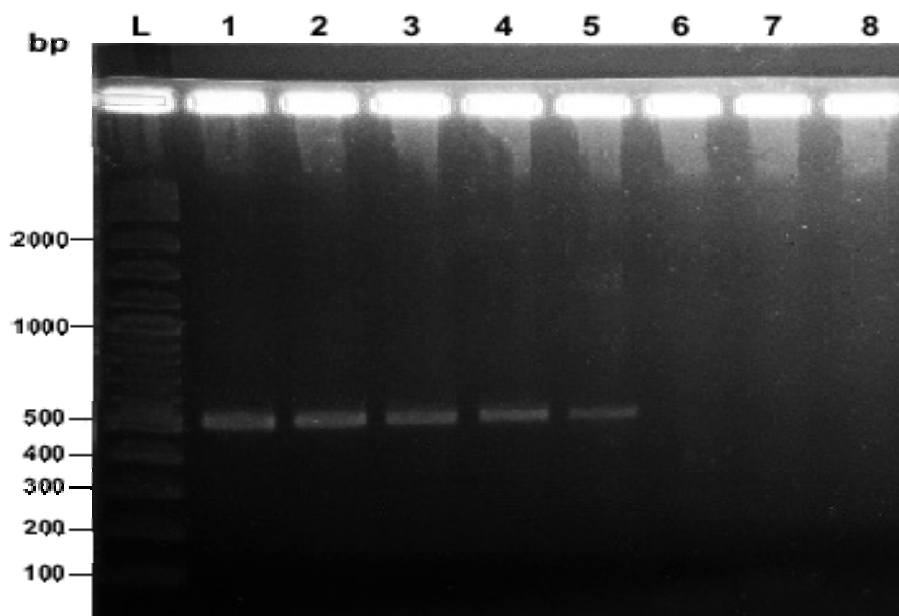


Fig. 5.1 Representative gel electrophoresis showing the limit of detection (Lane 5) of real-time PCR in deer urine dilution of field isolates of *Leptospira* serovar Hardjobovis. Lane L: 2-Log DNA ladder ranging from 100bp-10,000bp; Lanes 1-7:  $10^7$  -  $10^1$  cells/ml, respectively; Lane 8: negative control.

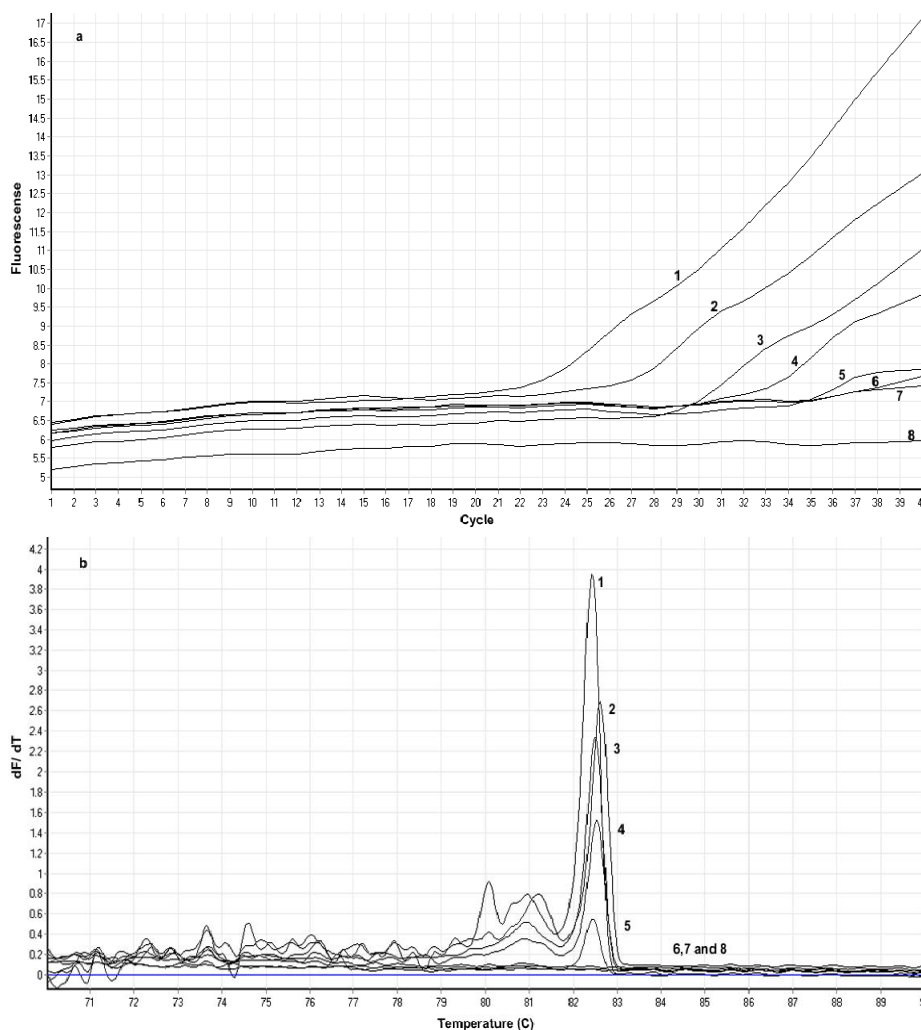


Fig. 5.2 Representative a) cycling and b) melt curve analysis showing the limit of detection of real-time PCR in deer urine dilutions of field isolates of *Leptospira* serovar Hardjobovis . Line 1: 10<sup>7</sup> cells/ml; Line 2: 10<sup>6</sup> cells/ml; Line 3: 10<sup>5</sup> cells/ml; Line 4: 10<sup>4</sup> cells/ml; Line 5: 10<sup>3</sup> cells/ml; Line 6: 10<sup>2</sup> cells/ml; Line 7: 10<sup>1</sup> cells/ml; Line 8: negative control.

### 5.3.2 Diagnostic sensitivity and specificity

Results of culture and real-time PCR for kidney samples corresponding to MAT are summarized in Table 5.1. Of 268 kidney samples, 17 were both culture and PCR positive. Two samples were culture negative but PCR positive and three were culture positive but PCR negative. MAT results were available for four of the five of those samples, all of which were positive.

Data of real-time PCR on kidney against culture and its performance are presented in Table 5.2. The sensitivity and specificity were 85.0% and 99.2%, respectively. The positive predictive value, negative predictive value and Kappa (agreement test) were 89.5%, 98.8% and 86.2%, respectively. Of 11 real-time PCR-positive, fresh kidney samples, seven (64%) were positive after being frozen at -20°C and none were positive after being fixed in 10% formalin.

Table 5.1 Kidney culture, real-time PCR and MAT results from 19 slaughter lines.

| Line | No. tested | No. positive |        | No. MAT positive |        | Titer range |            |
|------|------------|--------------|--------|------------------|--------|-------------|------------|
|      |            | Culture      | PCR    | Hardjovovis      | Pomona | Hardjovovis | Pomona     |
| 1    | 10         | 3            | 3      | 9                | 7      | 1:48-1:192  | 1:96-1:768 |
| 2    | 20         | 4            | 4      | 15               | 0      | 1:48-1:96   | -          |
| 3    | 20         | 0            | 0      | 0                | 4      | -           | 1:48       |
| 4    | 10         | 0            | 0      | 0                | 0      | -           | -          |
| 5    | 8          | 0            | 0      | 1                | 0      | 1:48        | -          |
| 6    | 13         | 4            | 2      | 9                | 4      | 1:48-1:192  | 1:96-1:192 |
| 7    | 14         | 0            | 0      | 0                | 2      | n/a         | 1:48-1:768 |
| 8    | 14         | 0            | 0      | 2                | 0      | 1:48        | -          |
| 9    | 15         | 2            | 2      | 7                | 2      | 1:48-1:96   | 1:48-1:96  |
| 10   | 15         | 0            | 0      | 0                | 0      | -           | -          |
| 11   | 12         | 0            | 0      | 3                | 1      | 1:48-1:192  | 1:48       |
| 12   | 14         | 1            | 1      | 4                | 3      | 1:48-1:192  | 1:48-1:384 |
| 13   | 10         | 0            | 0      | 5                | 1      | 1:48-1:192  | 1:48       |
| 14   | 13         | 0            | 0      | 4                | 0      | 1:48        | -          |
| 15   | 15         | 0            | 0      | n/a              | n/a    | n/a         | n/a        |
| 16   | 13         | 0            | 0      | n/a              | n/a    | n/a         | n/a        |
| 17   | 14         | 6            | 5      | n/a              | n/a    | n/a         | n/a        |
| 18   | 13         | 0            | 0      | n/a              | n/a    | n/a         | n/a        |
| 19   | 25         | 0            | 2      | 22               | 0      | 1:48-1:3072 | n/a        |
| All  | 268        | 20/268       | 19/268 | 81/209           | 24/209 | 1:48-1:3072 | 1:48-768   |

n/a = not available

Data of real-time PCR on urine against inoculated samples at the detection limit of  $10^3$  cells/ml and its performance are presented in Table 5.3. Of 30 inoculated urine samples, 29 were positive by real-time PCR. None of 83 leptospire-free urine samples were positive by real-time PCR. The analytical sensitivity and specificity of the SYTO9 PCR assay in comparison to inoculated samples as the gold standard were 96.7% and 100.0%, respectively.

Table 5.2 Result of real-time PCR on kidney against culture and its performance (%) with 95% CI in brackets.

|                           | Kidney           |                  |
|---------------------------|------------------|------------------|
|                           | Culture positive | Culture negative |
| PCR positive              | 17               | 2                |
| PCR negative              | 3                | 246              |
| Sensitivity               | 85.0 (62.1-96.8) |                  |
| Specificity               | 99.2 (97.1-99.9) |                  |
| Positive predictive value | 89.5 (66.8-98.7) |                  |
| Negative predictive value | 98.8 (96.5-99.8) |                  |
| Kappa                     | 86.2 (74.2-98.2) |                  |

Table 5.3 Result of real-time PCR on urine against inoculated samples at the detection limit of  $10^3$  cells/ml and its performance (%) with 95% CI in brackets.

|              | Urine            |                 |
|--------------|------------------|-----------------|
|              | Inoculated       | Leptospire-free |
| PCR positive | 29               | 0               |
| PCR negative | 1                | 83              |
| Sensitivity  | 96.7 (82.8-99.9) |                 |
| Specificity  | 100 (96.5-100)   |                 |

### 5.3.3 Quantification of urinary shedding

The concentration of leptospires from 28 real-time PCR positive deer urine samples was between 3,690 and 1,714,450 cells/ml (Table 5.4). The lowest concentration of leptospires that was cultured from deer urine was 10,120 cells/ml (No culture result was available for sample 23, with a lower count, due to contamination). All seven isolates derived from urine culture were serotyped as serovar Hardjobovis using standard antisera typing method.

### 5.3.4 Identification of pathogenic *Leptospira* species

The *gyrB* gene sequences of 14 unknown isolates were compared to those deposited on the NCBI database with BLASTn search. Thirteen isolates were 100% homologous with the DNA sequence of *L. borgpetersenii* serovar Hardjobovis (NCBI accession no. CP000350.1 and CP000348.1) and one isolate was 100% homologous with three different *gyrB* gene sequences of *L. interrogans* serovar Pomona (NCBI accession no. AY896738.1), *L. interrogans* serovar Medanensis (AY896746.1) and *L. interrogans* serovar Canicola (AY896745.1). There was 100% agreement between the *gyrB* gene sequencing and conventional antisera typing of 13 isolates being serovar Hardjobovis and one isolate being serovar Pomona. Phylogenetic analysis has confirmed 100% similarity between 13 isolates of serovar Hardjobovis that all differed from the single Pomona isolate. In addition, DNA sequences of three amplicons derived directly from deer kidney and seven amplicons derived directly from deer urine were 100% homologous with the DNA sequence of *L. borgpetersenii* serovar Hardjobovis (NCBI accession no. CP000350.1 and CP000348.1) when a BLASTn search was performed.

Table 5.4 Concentration of leptospires in real-time PCR positive urine from deer on commercial farms, and corresponding culture results.

| Sample | Farm region | Concentration (cells/ml) | Culture |
|--------|-------------|--------------------------|---------|
| 1      | Manawatu    | 11,213                   | –       |
| 2      | Manawatu    | 1,205,170                | –       |
| 3      | Manawatu    | 270,179                  | +       |
| 4      | Manawatu    | 59,575                   | C       |
| 5      | Manawatu    | 48,237                   | –       |
| 6      | Manawatu    | 1,071,519                | +       |
| 7      | Manawatu    | 859,663                  | +       |
| 8      | Manawatu    | 10,120                   | +       |
| 9      | Manawatu    | 167,652                  | –       |
| 10     | Manawatu    | 1,011,509                | –       |
| 11     | Manawatu    | 78,673                   | –       |
| 12     | Manawatu    | 38,646                   | C       |
| 13     | Manawatu    | 165,583                  | C       |
| 14     | Manawatu    | 703,169                  | +       |
| 15     | Manawatu    | 325,092                  | C       |
| 16     | Manawatu    | 12,207                   | C       |
| 17     | Manawatu    | 32,026                   | C       |
| 18     | Manawatu    | 305,271                  | C       |
| 19     | Manawatu    | 1,618,695                | +       |
| 20     | Manawatu    | 1,714,450                | +       |
| 21     | Hawkes Bay  | 22,190                   | –       |
| 22     | Hawkes Bay  | 5,092                    | –       |
| 23     | Hawkes Bay  | 3,690                    | C       |
| 24     | Hawkes Bay  | 136,343                  | –       |
| 25     | Hawkes Bay  | 598,999                  | –       |
| 26     | Hawkes Bay  | 565,415                  | –       |
| 27     | Hawkes Bay  | 363,936                  | –       |
| 28     | Hawkes Bay  | 5,849                    | –       |

+ = positive, – = negative, C = contaminated

## 5.4 Discussion

This study evaluated and validated the performance of a diagnostic PCR assay for use on deer kidney tissue and urine as a research and diagnostic tool for determining infection, carrier and shedding status of New Zealand farmed deer. A SYTO9 real-time PCR was developed using primers previously designed for a conserved region of *gyrB* sequences of pathogenic *Leptospira* species (Slack et al., 2006) for use on the Corbett Rotor-Gene system. It was the first study using real-time PCR directly on kidney and urine samples from farmed deer with evaluation against culture as the gold standard. To our knowledge, it was also the first study to quantify pathogenic leptospires shed naturally in animal urine. The study also identified specific *Leptospira* species by DNA sequencing of PCR amplicons.

Based on the method of Slack et al (2006) using a Lightcycler system, we optimized PCR conditions to suit the Rotor-Gene instrument. SYTO9 ds-DNA intercalating dye was adopted as it has shown to have advantage over SyBr Green I (Monis et al., 2005) being easily adapted using standard conventional PCR reagents. That this assay does not need a specific probe like Taqman or FRET makes it cheaper and able to run on a larger scale than other assays.

The lowest detection limit of the assay was determined by DNA extracted from known concentrations of leptospires measured in a Petroff-Hausser counting chamber and dark field microscopy as described by (Faine, 1982). The detection limit of the PCR using the DNA extracted from dilution of leptospires mixed with leptospire-free deer urine was approximately  $10^3$  cells/ml or equivalent to 2 to 10 copies/reaction. This was consistent with another study which reported the detection limit of  $10^3$  cells/ml in inoculated human serum (Ooteman et al., 2006) and 3 to 10 copies/reaction in inoculated human serum and urine (Levett et al., 2005). However, this was in contrast to (Gerritsen et al., 1991) who reported the detection limit of 10 cells/ml in bovine urine.

The assay showed 85% sensitivity (95% CI 62.1%-96.8%), 99% specificity (95% CI 97.1%-99.9%) and “excellent” agreement (Kappa = 86.2%, 95%CI 74.2%-98.1%) with kidney culture as the gold standard. Failure of the PCR to detect leptospires in some culture positive kidney samples may be due to leptospire concentrations below detection limit. Grooms et al., (2005) claimed that DNA extracted from tissue can contain inhibitors interfering with PCR reactions. A possible reason for two PCR positive kidney samples being culture negative may be lack of viable organisms at the time of processing since those samples were from a DSP in Southland, a long distance from the laboratory, requiring more than 24 hours before processing. Samples from the other DSP were placed in culture within approximately six hours of collection. It is notable that the source animals for those two samples were seroreactive to serovar Hardjobovis with high titers consistent with recent exposure and/or current infection with leptospires. Hence, we assert that there is a high probability that the real-time PCR result does indicate infection.

Long term storage of kidney samples for use in DNA extraction and real-time PCR was investigated on a limited number of specimens in this study. The lowered sensitivity from frozen compared with fresh samples and the inability to detect DNA in formalin fixed tissues suggest that kidney samples need to be processed fresh to achieve the highest sensitivity.

Approximately 30% of urine cultures from deer on farms were contamination whereas real-time PCR returned several positive results from such samples. Determination of the analytical sensitivity of real-time PCR was therefore undertaken using urine artificially inoculated with leptospires showing the lowest detection limit to be  $10^3$  cells/ml. The analytical sensitivity at this concentration and above was 96.7% (95% CI 82.8%-99.9%) and the specificity was 100% (95% CI 90.5%-100%). These results were comparable with the findings of Slack et al (2007) who reported a diagnostic sensitivity of 96.4% and a specificity

of 99.5% on patient sera using primer pairs Lepto F/ Lepto R. Those primers which were designed from the 16s rRNA gene detected leptospiral DNA in both serum and seeded urine samples (Smythe et al., 2002).

In addition to detection of the organism *per se*, the ability of real-time PCR to quantify the concentration of leptospires in clinical samples provides diagnostic benefits, for example in evaluating antibiotic efficiency by post-treatment clearance of leptospires in urine. The technique may also be used for epidemiological studies involving *Leptospira* shedding and possibly even surface contamination when studying potential risk factors for disease transmission. One study has developed a real-time PCR assay to measure the concentration of leptopiral DNA in patient's sera and reported a range of 80 to 39,000 cells/ml that informed the prognosis for patients suffering from leptospirosis (Merien et al., 2005). To our knowledge, this study is the first reporting detectable concentrations of *Leptospira* serovar Hardjobovis shed in field samples of deer urine, finding a range from  $3.7 \times 10^3$  to  $1.7 \times 10^6$  cells/ml. This defined the detection limit of this PCR assay at  $10^3$  cells/ml although it is possible that deer may shed lower concentrations of leptospires in urine. The lowest concentration of leptospires (sv. Hardjobovis) that could be cultured from deer urine was  $10^4$  cells/ml which was higher than that from a previous report of culture of  $10^2$  cells/ml in water and  $10^3$  cell/ml in bovine semen (sv. Hardjoprajitno) (Heinemann et al., 2000). Considering that urine samples collected on-farm are prone to contamination, as shown in this study (approximately 30% of samples were contaminated), this detection limit of urine culture is plausible and likely explains the higher sensitivity of real-time PCR over urine culture. However, urine from other sources (e.g. humans) collected under aseptic conditions may yield a higher proportion of positive cultures.

A limitation of PCR-based diagnosis of leptospiral infection is the inability to identify the infecting leptospires at the species, serogroup or serovar level (Lilenbaum et al., 2008, Merien et al., 2005). The infecting serovar may be predicted by combining real-time PCR results with the MAT since data from our study have shown that approximately 85% of kidneys positive to real-time PCR were from animals positive to the MAT. However, caveats to this conjecture are in those cases of early infection when the host immunity has not been activated, or in the event of dual or multiple serovar infections.

Species identification can be addressed by amplicon sequencing of PCR product of *gyrB* gene (Slack et al., 2006). All 14 unknown isolates and 10 real-time PCR positive samples subjected to PCR amplification and sequencing of *gyrB* gene resulted in 100% homology with sequences deposited on the NCBI database when a BLASTn search was performed. In addition, the BLASTn search results matched fully with standard antisera typing showing that 13 isolates were identified as serovar Hardjobovis and one as serovar Pomona. The BLASTn search results and phylogenetic analysis agreed with the findings of Slack et al. (2006) that sequencing of *gyrB* gene could differentiate pathogenic leptospires to species level but not to serovar level. However, since serovars Hardjobovis and Pomona are the most prevalent serovars in New Zealand livestock and that they belong to different species (*L. borgpetersenii*

and *L. interrogans*), these techniques represent a rapid tool for identifying pathogenic *Leptospira* species and the likely serovar from clinical samples. Similar deduction would be appropriate in regions where only a few species and serovars of pathogenic leptospires are encountered. For more accurate genotypic classification, Multilocus Sequence Typing (MLST) is the most up-to-date method that has been developed and claimed to have high discriminatory power, reproducibility and robustness (Ahmed et al., 2006, Thaipadungpanit et al., 2007).

In conclusion, we have validated a real-time PCR assay based on SYTO9 technology for the detection of pathogenic leptospires in kidney and urine samples of farmed deer in New Zealand. The assay was clinically evaluated against the gold standard (culture) and found to have comparable diagnostic sensitivity, specificity and predictive values for kidney samples, and better sensitivity for urine samples. This assay identifies pathogenic *Leptospira* species when combined with DNA sequencing method. This method is simple, rapid and easily adapted with conventional PCR reagents which make it cheaper than commercial real-time PCR reagents. It has potential for application as a research tool for the determination of carrier or shedding status of animal species for epidemiology studies and evaluation of vaccine efficacy, and as a clinical diagnostic tool on tissue and urine and potentially blood samples for rapid diagnosis in animals and humans.

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## **Chapter 6**

### **Investigation of possible novel leptospiral serovars in farmed deer in New Zealand**

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(Raw data of this chapter are in Appendix 6a, 6b and 6c)

## Abstract

**AIM:** To investigate possible novel leptospiral infection in farmed deer in New Zealand.

**METHODS:** In September 2006, five serum samples from a serum bank from each of 70 farms sampled for a previous national seroprevalence survey were forwarded to the World Health Organisation/Food and Agriculture Organisation/World Organisation for Animal Health (WHO/FAO/OIE) reference laboratory for leptospirosis in Brisbane, Australia, to test for 23 reference panel serovars, most believed to be exotic to New Zealand, using the microscopic agglutination test (MAT). In addition to apparent cross-reactivity to some serovars, there was evidence of Arborea, a serovar novel to New Zealand, on eleven farms. Subsequently 126 additional banked sera samples from nine out of those eleven farms (8 - 20/farm) were sent to the reference laboratory for similar serology in July, 2007. Two farms in the Southland region were considered seropositive for Arborea. Culture of deer kidneys (n=43) from these two farms collected at a Deer Slaughter Premise (DSP) in Makarewa was attempted in November 2007 and November 2008. Sera from those deer were sent to the Brisbane laboratory for serology confirmation by MAT.

**RESULTS:** From the initial 350 deer sera, 96 (27.4%) and 19 (5.4%) samples were seroreactive to Hardjobovis and Pomona, respectively. There was evidence of cross-reactivity between Hardjobovis with Medanensis and Szwajizak. Serology for Tarassovi, Grippotyphosa, Celledoni, Australis, Zanoni, Robinsoni, Canicola, Kremastos, Bulgarica, Cynopteri, Ballum, Bataviae, Djasiman, Javanica, Panama, Shermani and Topaz was negative or sporadic, generally with titres of 1:50 and therefore likely non-specific. Fourteen samples (4.0%) from eleven farms were seroreactive to serovar Arborea, justifying further investigation. The remaining 126 banked sera samples from nine out of those eleven farms were tested for serovar Arborea in July 2007 revealing a seroprevalence of 15.4% and 30.0% on two farms, respectively. None of 43 deer kidney and sera samples collected subsequently from those two farms were culture or serology positive to serovar Arborea.

**CONCLUSIONS:** While there was serological evidence for Arborea in deer, attempts to isolate the organism were unsuccessful. Since sample size for the follow-up investigation was insufficient to validate presence or absence of infection, further study should be undertaken to verify the status of this serovar of *Leptospira* in New Zealand in both deer and other livestock species.

**KEY WORDS:** *Leptospirosis, farmed deer, exotic, serology, culture, Arborea, New Zealand*

**List of abbreviations**

DSP = Deer slaughter premise

EMJH = Ellinghausen-McCullough-Johnson-Harris medium

MAT = Microscopic agglutination test

## 6.1 Introduction

Leptospirosis is considered to be an emerging zoonotic disease with worldwide distribution. It is caused by infection with pathogenic leptospires, helical shaped motile spirochaetes which belong to the family *Leptospiraceae*, genus *Leptospira*. A serological taxonomy system (*sensu lato*) classifies *Leptospira* into serovars on the basis of surface antigen patterns has determined over 200 serovars (Bharti *et al.* 2003). In New Zealand, leptospirosis has been recognised in humans, domestic animals and wildlife (Blackmore *et al.* 1976; Hathaway 1981). To date, six serovars have been isolated from animals in New Zealand. Cattle and farmed deer are recognised as maintenance hosts for *L. borgpetersenii* serovar Hardjobovis, pigs for *L. interrogans* serovar Pomona and *L. borgpetersenii* serovar Tarassovi, Norway rats (*Rattus norvegicus*) for *L. interrogans* serovar Copenhageni, black rats (*Rattus rattus*) for *L. borgpetersenii* serovar Ballum, brush tail possums (*Trichosurus vulpecula*) for *L. borgpetersenii* serovar Balcanica (Midwinter and Fairley 1999).

Leptospirosis has been reported as a clinical disease in farmed deer since the 1980s (Fairley *et al.* 1986). A recent review confirmed that serovar Hardjobovis and Pomona are the most commonly detected serovars in this species (Ayanegui-Alcérreca *et al.* 2007) although serovar Copenhageni has also been isolated from deer. Serological evidence for Tarassovi, Ballum and Balcanica has been reported in deer (Flint *et al.* 1988; Wilson *et al.* 1998) but it is likely that positive titres to those serovars resulted from cross-reactivity (Ayanegui-Alcérreca 2006). A national prevalence survey of serovar Hardjobovis, Pomona and Copenhageni from 110 farms in nine regions of New Zealand was undertaken recently (Ayanegui-Alcérreca *et al.* 2010) demonstrating widespread distribution and 81% seroprevalence. However, no screening for other serovars has been reported from deer or other animals in New Zealand, so it is possible that further serovars may be present in this country. Knowledge of the serovars present in livestock populations is important for both animal disease control and public health.

This paper reports a collaborative study between the Massey University Deer Research Group and the WHO/FAO/OIE reference laboratory for leptospirosis in Brisbane to investigate potentially novel leptospiral serovars in New Zealand farmed deer based on a serum bank from an earlier national seroprevalence survey (Ayanegui-Alcérreca *et al.* 2010). Based on serology results, attempts were initiated to isolate serovar Arborea which had previously not been found in New Zealand.

## 6.2 Materials and methods

### 6.2.1 Study design

Initially, a cross-sectional study design was chosen to determine the individual and herd seroprevalence to leptospiral serovars based on stored samples from a recent seroprevalence study (Ayanegui-Alcérreca *et al.* 2010). In August 2006, 350 stored sera from 70 farms were

randomly selected (5/farm). The samples were couriered frozen to the WHO/FAO/OIE reference laboratory for leptospirosis in Brisbane for serological test (MAT) on a panel of 23 serovars. This sample number was based on MAT sensitivity of 90 %, specificity of 99% (McBride *et al.* 2007), within-herd prevalence of 50% and herd size of 350 to provide 95% confidence and 80% power. Based on serological results, further samples from nine of the farms were submitted for serological screening. Subsequently, further blood and kidney tissue samples were collected from two farms that presented with evidence of serovar Arborea.

## 6.2.2 Serum samples

**6.2.2.1 Initial serological screening:** Sera were selected from a serum bank from a recent national seroprevalence study of leptospirosis in farmed deer. The serum bank contained approximately 20 serum samples stored at -20°C, from 9 - 30 month-old red or red x wapiti from each of 110 farms as described by (Ayanegui-Alcérreca *et al.* 2010). Five sera were randomly selected from 70 farms. The number of farms selected by region is presented in Table 6.1.

**6.2.2.2 Follow-up serology:** Based on initial screening that revealed seroreactivity to Arborea on nine farms, 126 additional serum samples from those farms (8 - 20/farm) were submitted for screening for Arborea in July 2007 to estimate within-herd prevalence. Serology was done concurrently with serovar Ballum which may cross-react with Arborea in the MAT to determine specificity.

**6.2.2.3 Additional blood and tissue sampling:** Follow-up serology confirmed seropositivity to Arborea, on two farms in Southland. Between November 2007 and November 2008, 43 deer (Farm 4 = 8, Farm 5 = 35) were blood and kidney sampled on two visits at a Deer Slaughter Premise (DSP) in Makarewa, Southland as shown in Table 6.4.

**Table 6.1 Number of farms tested by region**

| Region            | Number of farms |
|-------------------|-----------------|
| Otago             | 13              |
| West Coast        | 5               |
| Northland         | 7               |
| Canterbury        | 9               |
| Bay of Plenty     | 10              |
| Manawatu-Wanganui | 7               |
| Waikato           | 2               |
| Southland         | 14              |
| Hawkes Bay        | 3               |
| <b>Total</b>      | <b>70</b>       |



### 6.2.3 Additional sample collection and processing

**6.2.3.1 Blood:** Blood samples were collected by free flow into a new 10 ml plain blood tube after animal sticking, immediately after stunning. Blood samples were left to clot at room temperature. Samples were then centrifuged at 1512 *g* for 10 minutes after which serum was aliquoted into new, labelled 1.5ml microcentrifuge tubes and stored at -20°C. The sera were couriered frozen to WHO/FAO/OIE reference laboratory for leptospirosis in Brisbane for serology.

**6.2.3.2 Kidney:** Kidney samples were taken at the evisceration and inspection area of the slaughter line. Whole kidneys, without renal capsule, were put into labelled sterile plastic bags aseptically and held at 4°C before transport back to the laboratory. Later, the kidney surface was swabbed with 70% alcohol and randomly aspirated from several sites over the entire kidney into a 5ml sterile syringe using a sterile 16-gauge needle. Approximately 50 mg of kidney tissue was used for bacterial culture.

### 6.2.4 Laboratory procedures

**6.2.4.1 Serology:** Microscopic Agglutination Test (MAT) was used to test serum reactivity against a standardised reference leptospiral panel of *L. interrogans* serovars Pomona, Copenhageni, Canicola, Australis, Szwajizak, Medanensis, Zanoni, Robinsoni Bataviae, Djasiman and Kremastos, *L. borgpetersenii* serovars Hardjobovis, Tarassovi, Arborea, Ballum and Javanica, *L. kirschneri* serovars Grippotyphosa, Bulgarica, Cynopteri and Panama, *L. weilii* serovars Celledoni and Topaz and *L. santarosai* serovar Shermani to determine the leptospiral titre status of individual animals and herds. An initial serum dilution of 1:25 and two-fold serial dilution of serum covering the range of 1:50 to 1:6400 were tested. Titre was recorded as the reciprocal of the highest dilution at which  $\geq 50\%$  of leptospires were agglutinated (Stallman 1984). To consider a sample positive, the cut-off point at 1:50 was used for all serovars which was regarded as evidence of past or present exposure. To consider a herd positive, at least one serum sample had to be positive at the minimum dilution of 1:50.

**6.2.4.2 Bacterial culture:** The culture method has been developed by the Leptospirosis Research Unit, IVABS, Massey University based on “Guidelines for the Control of Leptospirosis” (Faine 1982). Ellinghausen-McCullough-Johnson-Harris (EMJH) was used as a selective medium with an addition of 5'-fluoracil for contamination inhibition. The medium was inoculated with processed kidney, incubated at 28-30°C and examined every two weeks for four months under dark-field microscopy.

## 6.3 Results

### 6.3.1 Initial serological screening

Serology data at herd and individual level of each serovar testing positive on at least one farm, with reciprocal titre range and evidence of cross-reaction are presented in Table 6.2. Forty herds (57.1%) and 96 individual samples (27.4%) were seroreactive to Hardjobovis with evidence of cross reactivity to Kremastos, Szwajizak, Medanensis, Panama and Djasiman. Nine herds (12.9%) and 19 individual samples (5.4%) were seroreactive to Pomona with evidence of cross reactivity to Grippytyphosa, Topaz, Cynopteri and Djasiman. Eleven herds (15.7%) and 14 individual samples (4.0%) were seroreactive to Arborea with evidence of cross reactivity to Ballum, Javanica, Panama and Topaz. One individual sample (0.3%) in one herd (1.5%) was seroreactive to Bataviae with no evidence of cross reactivity. There was no evidence of seroreactivity to Copenhageni, Tarassovi, Celledoni, Australis, Zanoni, Robinsoni, Canicola, Bulgarica and Shermani.

**Table 6.2 Herd and individual seroprevalence for seroreactive serovars with range of number positive/herd, reciprocal titre range and potential evidence of cross-reaction, from initial screening of 70 herds (5 samples/herd).**

| Serovar       | No. of herds positive (%) | Number of samples positive/herd | No. of individuals positive (%) | Reciprocal titre range | Potentially cross-reacting serovar (%)                                             |
|---------------|---------------------------|---------------------------------|---------------------------------|------------------------|------------------------------------------------------------------------------------|
| Hardjobovis   | 40 (57.1)                 | 1 – 5                           | 96 (27.4)                       | 50 – 400               | Kremastos (6.3), Szwajizak (22.9), Medanensis (38.5), Panama (1.0), Djasiman (2.1) |
| Pomona        | 9 (12.9)                  | 1 – 5                           | 19 (5.4)                        | 50 – 1600              | Grippytyphosa (5.3), Topaz (15.8), Cynopteri (5.3), Djasiman (5.3)                 |
| Grippytyphosa | 1 (1.5)                   | 1                               | 1 (0.3)                         | 50                     | Pomona (100.0)                                                                     |
| Kremastos     | 5 (7.1)                   | 1 – 2                           | 6 (1.7)                         | 50                     | Hardjobovis (100.0)                                                                |
| Szwajizak     | 17 (24.3)                 | 1 – 3                           | 22 (6.3)                        | 50 – 200               | Hardjobovis (100.0)                                                                |
| Medanensis    | 23 (32.9)                 | 1 – 3                           | 37 (10.6)                       | 50 – 200               | Hardjobovis (100.0)                                                                |
| Cynopteri     | 1 (1.5)                   | 1                               | 1 (0.3)                         | 100                    | Pomona (100.0)                                                                     |
| Arborea       | 11 (15.7)                 | 1 – 3                           | 14 (4.0)                        | 50 – 800               | Ballum (7.1)                                                                       |
| Ballum        | 1 (1.5)                   | 1                               | 1 (0.3)                         | 50                     | Arborea (100.0)                                                                    |
| Bataviae      | 1 (1.5)                   | 1                               | 1 (0.3)                         | 50                     | None                                                                               |
| Djasiman      | 4 (5.7)                   | 1                               | 4 (1.1)                         | 50 – 100               | Hardjobovis (50.0), Pomona (25.0)                                                  |
| Javanica      | 1 (1.5)                   | 1                               | 1 (0.3)                         | 100                    | Arborea (100)                                                                      |
| Panama        | 6 (8.6)                   | 1 – 2                           | 7 (2.0)                         | 50 – 200               | Arborea (57.1), Hardjobovis (14.3)                                                 |
| Topaz         | 2 (2.9)                   | 1 – 3                           | 4 (1.1)                         | 50 – 200               | Pomona (75.0), Arborea (25.0)                                                      |

### 6.3.2 Follow-up serology

The within-herd prevalence and reciprocal titre range for Arborea and Ballum from initial and follow-up serum band samples combined from nine farms initially positive for Arborea are presented in Table 6.3. Three farms were classified with evidence of Arborea with within-herd prevalences of 12.5%, 20.0% and 5.6% on Farms 4, 5 and 6, respectively. Evidence of cross reactivity with Ballum was found only on Farm 6.

### 6.3.3 Culture for Arborea

Bacterial culture and serology results of serum and kidney samples collected at a DSP from Farms 4 and 5, considered positive to Arborea after follow-up sampling, are summarised in Table 6.4. None of 43 kidney samples were culture positive whereas one sample from Farm 5 was seroreactive to both Arborea and Ballum.

**Table 6.3 Seroprevalence and reciprocal titre range for Arborea and Ballum on each farm positive for Arborea at initial screening (Table 6.2) and follow-up, combined, and farm location.**

| Farm | Region        | No. of samples | Arborea            |                        | Ballum             |                        |
|------|---------------|----------------|--------------------|------------------------|--------------------|------------------------|
|      |               |                | Seroprevalence (%) | Reciprocal titre range | Seroprevalence (%) | Reciprocal titre range |
| 1    | Canterbury    | 20             | 5.0                | 800                    | 0                  | –                      |
| 2    | Bay of Plenty | 14             | 7.1                | 50                     | 0                  | –                      |
| 3    | Southland     | 20             | 5.0                | 50                     | 0                  | –                      |
| 4    | Southland     | 13             | 15.4               | 50                     | 0                  | –                      |
| 5    | Southland     | 20             | 30.0               | 50 – 100               | 0                  | –                      |
| 6    | Manawatu      | 23             | 13.0               | 50 – 100               | 4.3                | 50                     |
| 7    | Southland     | 16             | 6.3                | 50                     | 0                  | –                      |
| 8    | Canterbury    | 25             | 4.0                | 50                     | 0                  | –                      |
| 9    | Southland     | 20             | 5.0                | 50                     | 0                  | –                      |

**Table 6.4. Kidney culture and seroprevalence with reciprocal titre range, for Arborea and Ballum on two farms most likely to be infected, based on follow-up serological screening.**

| Farm | No. of samples | Culture (%) | Arborea            |                        | Ballum             |                        |
|------|----------------|-------------|--------------------|------------------------|--------------------|------------------------|
|      |                |             | Seroprevalence (%) | Reciprocal titre range | Seroprevalence (%) | Reciprocal titre range |
| 4    | 8              | 0           | 0                  | –                      | 0                  | –                      |
| 5    | 35             | 0           | 2.9                | 50                     | 2.9                | 50                     |

## 6.4 Discussion

This study provides data on potentially exotic leptospiral serovars in farmed deer in New Zealand. Initial screening suggested serovar Arborea in animals from eleven herds. Follow-up screening suggested infection was possible in two herds but subsequent tests failed to confirm infection with this serovar. There was no herd-level evidence for other serovars not already known to be present in New Zealand.

These results confirmed endemicity of Hardjobovis, sporadicity of Pomona as observed from a previous national seroprevalence study (Ayanegui-Alcérreca *et al.* 2010). However, the seroprevalence of Hardjobovis at the herd and individual level from this study (57.1% and 27.4%, respectively) was lower than that of Ayanegui-Alcérreca *et al.* (2010) (77.7% and 60.8%, respectively). The difference in individual animal seroprevalence is likely to be due to the difference of titre cut point used for each study since Ayanegui-Alcérreca *et al.* (2010) used a titre cut point at 1:24 for Hardjobovis, while the present study used a titre cut point at 1:50. The difference in herd prevalence is likely to be due to the smaller number of animals sampled for initial screening (n=5) compared with approximately 20 for the study by Ayanegui-Alcérreca *et al.* (2010). For Pomona, the seroprevalence at the herd and individual level from this study (12.9% and 5.5%, respectively) is consistent with those of Ayanegui-Alcérreca *et al.* (2010) (20.0% and 8.4%, respectively), while the titre cut point used was similar (1:50 vs. 1:48). Additionally, differences in sensitivity of tests between laboratories could contribute to differences in individual animal seroprevalence observed between studies. However, a Kappa (agreement test) between results from the Massey University laboratory (Ayanegui-Alcérreca *et al.* 2010) compared with those from the reference laboratory used in this study was 0.81 for Hardjobovis and 1.0 for Pomona at the herd level demonstrating excellent agreement.

A high degree of cross reaction between serovar Hardjobovis, Szwajizak and Medanensis was observed. This is consistent with previous reports in cattle (Black *et al.* 2001) and possums (Eymann *et al.* 2007). It is generally accepted that the serovar with the highest antibody titre likely represents the infective serovar (O'Keefe *et al.* 2002). For other serovars such as Tarassovi, Grippotyphosa, Celledoni, Australis, Zaroni, Robinsoni, Canicola, Kremastos, Bulgarica, Cynopteri, Ballum, Bataviae, Djasiman, Javanica, Panama, Shermani and Topaz, their serology was either negative, or sporadic, with titres of 1:50 and therefore likely to be due to non-specific reactivity.

Serovar Arborea has never been reported in New Zealand. Serovar Arborea was first isolated in Europe in the 1940s and belongs to the serogroup Ballum. It appears to be newly established in Queensland, Australia and is an emerging source of human leptospirosis (Slack *et al.* 2006) with cases in people involved with horticulture and livestock based occupations. Commonly, this serovar is maintained in rodents such as the house mouse (*Mus domesticus*) and black rats (*Rattus rattus*) (Slack *et al.* 2006; Vanasco *et al.* 2000). In addition, one study in Australia suggested that the brush tail possum (*Trichosurus vulpeca*), which is widespread

in New Zealand, can be an accidental host for Arborea (Eymann *et al.* 2007). Importation of goods from other countries and human travel are possible routes means of introduction of Arborea to New Zealand.

In this study Arborea seroprevalence at both herd and individual animal levels was as high as that recorded commonly for serovar Pomona. Initial screening found titres to Arborea in five herds in Southland, three in Manawatu, two in Canterbury, one in Bay of Plenty. The two herds (Farm 4 and 5) with the highest prevalence after follow-up screening, were chosen for kidney and further blood sampling (n=43) as they were farms most likely to be culture positive. However, we failed to isolate any leptospiral organism from those 43 kidney samples and MAT detected only one deer positive to Arborea which cross-reacted with Ballum at a low titre. We therefore considered this as being non-specific for both serovars. Based on MAT sensitivity of 90% and specificity of 99%, and the previous with-in herd prevalence of 15.4%, a result of 8 negative samples on Farm 4 could be expected by chance. It was therefore insufficient to conclude that the Arborea-prevalence was zero in this herd and we tried unsuccessfully to obtain more samples from this farm. By contrast, 34 negative samples from 35 tested on Farm 5 suggest that the probability of Arborea infection in this herd was likely to be absent. It appears that evidence for Arborea infection was either non-specific in the first instance or had cleared from this herd in the interval since it was initially detected.

Failure to isolate Arborea organisms from kidney samples is consistent with previous studies on the relationship between seropositivity and culture in sheep (Dorjee *et al.* 2008). Similarly, it is uncommon for seronegative deer to be kidney culture positive, with culture from only 5% of seropositive deer (Ayanegui-Alcérreca 2006). Theoretically, the probability for failure of growing an isolate from kidneys from 43 seropositive deer is 11% when the true prevalence is 10% and culture sensitivity is 50%. Thus, a sample of 43 was not large enough to provide substantial confidence for the detection of at least one isolate. Since Arborea belongs to the same serogroup as Ballum which is well-established in black rats (*Rattus rattus*) in New Zealand (Midwinter and Fairley 1999), positive serology for Arborea could potentially be confounded by cross reaction. However, evidence of serology from this study suggests Arborea titres were not due to cross-reaction since positive samples showed no or little evidence of cross reaction with Ballum.

In summary, this study provides serological data on a possible exotic leptospiral organism in farmed deer in New Zealand. The serological findings suggest that Arborea infection may have occurred on the two farms studied. It is possible that an accidental exposure to Arborea may have occurred via indirect contact with free-living rodents or wildlife that carried the organism. However, further serological screening and culture is required to be totally confident that this serovar is indeed present or not in livestock, rodents and wildlife in New Zealand.

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## **Chapter 7**

### **Detection of *Leptospira* spp. in an early foetus but not uterine tissue of non-pregnant farmed deer**

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(Raw data of this chapter are in Appendix 7)



## Abstract

**AIM:** To find evidence for uterine localisation and foetal infection of *Leptospira* in New Zealand farmed deer during and shortly after the breeding season using culture and real-time PCR.

**METHODS:** Between February and July 2008, 120 kidney, 116 blood, 120 uterine samples and 27 foetuses were collected from 120 mixed-age hinds from lines from nine farms at a Deer Slaughter Premise at Feilding. Kidney, uterine and foetal renal/peri-renal samples were subjected to bacterial culture using Ellinghausen-McCullough-Johnson-Harris (EMJH) medium and real-time polymerase chain reaction (PCR) using DNA gyrase subunit B gene primers. Blood samples were tested for antibodies against *Leptospira borgpetersenii* serovar Hardjobovis and *Leptospira interrogans* serovar Pomona using the microscopic agglutination test (MAT).

**RESULTS:** Seven of 120 kidney samples were positive by culture and five of these, but no others, were positive by real-time PCR. Of 120 uterine samples, none were culture or PCR positive. None of 27 foetus samples were culture positive but one, collected on July 11, was positive by real-time PCR. Thirty four of 116 serum samples (29.3%) were positive to serovar Hardjobovis and 13 (11.2%) to serovar Pomona. The PCR positive foetus was from a dam that was kidney culture negative, but which had a titre of 1:192 for Hardjobovis.

**CONCLUSIONS:** Attempts to isolate *Leptospira* from the genital tracts and early foetuses of farmed deer were unsuccessful. However, molecular evidence suggested foetal infection in one case. This finding justifies further study of the role of leptospires in the genital tract and foetus as a cause of reproductive loss in farmed deer.

**KEY WORDS:** *Leptospirosis, farmed deer, uterus, foetus, culture, real-time PCR, New Zealand*

### **List of abbreviations**

BSA = Bovine serum albumin

DNA = Deoxyribonucleic acid

EMJH = Ellinghausen-McCullough-Johnson-Harris medium

MAT = Microscopic agglutination test

PCR = Polymerase chain reaction

RPM = Revolutions per minute

T<sub>m</sub> = Melting temperature

## 7.1 Introduction

*Leptospira* serovars Hardjobovis and Pomona are well-known pathogens causing reproductive losses in livestock worldwide (Grooms 2006). Pathogenic leptospires are helical-shaped bacteria which penetrate through skin abrasions or mucous membranes, localising and persisting primarily in the proximal renal tubules of kidney but have also been found in the genital tracts of sexually mature cattle (Ellis and Thiermann 1986; Ellis *et al.* 1986a) and pigs (Ellis *et al.* 1985; Ellis *et al.* 1986b). Leptospires localising in renal tubules are intermittently excreted in urine which serves as a source of infection to other animals (Faine *et al.* 1999; Levett 2001). Seven serovars have been isolated from various animal hosts in New Zealand (Hathaway 1981; Midwinter and Fairley 1999), including Hardjobovis, Pomona and Copenhageni from deer (Ayanegui-Alcérreca *et al.* 2007).

Leptospiral infection has been shown to affect fertility in several domestic animal species including cattle (Dhaliwal *et al.* 1996a), pigs (Ramos *et al.* 2006), goats and sheep (Lilenbaum *et al.* 2008). It increases the number of services required for conception and the calving interval in dairy cattle (Dhaliwal *et al.* 1996a). It also causes early embryonic death, abortion, stillbirth and weak newborn in cattle (Smyth *et al.* 1999) and pigs (Kazami *et al.* 2002). There have been attempts to determine the presence of leptospires in the genital tracts of these species to establish an association with reproductive loss. In cattle, leptospires have been isolated from placenta of experimentally infected heifers (Ellis and Michna 1977), oviduct and uterus of non-pregnant cows (Ellis and Thiermann 1986; Ellis *et al.* 1986a) and viable and aborted fetuses (Ellis *et al.* 1982a; Ellis *et al.* 1982b; Langoni *et al.* 1999). In pigs, leptospires have been isolated from the oviduct, uterus and foetus of aborted sows (Ellis *et al.* 1985; Ellis *et al.* 1986b). These data suggest that leptospires have the ability to cross the placenta and invade the foetus of those species.

Several techniques such as immunofluorescence and enzyme-linked immunosorbent assay (ELISA) have been applied to determine the presence of leptospiral antigen or antibody in genital discharges of cattle (Dhaliwal *et al.* 1996b; Dhaliwal *et al.* 1996c). Recently, polymerase chain reaction (PCR) has been used to detect leptospires in vaginal fluids of sheep and goats (Lilenbaum *et al.* 2008) and aborted cattle fetuses (Richtzenhain *et al.* 2002). Real-time PCR assay has been developed based on conventional PCR and is rapid and sensitive, requiring no post-PCR manipulations (Slack *et al.* 2007). It has been used for detection of leptospires in pig kidney tissue, aborted foetus (Fearnley *et al.* 2008) and flying fox urine (Cox *et al.* 2005).

Leptospirosis is a well established infection and disease in New Zealand farmed deer (Ayanegui-Alcérreca *et al.* 2007; Wilson *et al.* 1998). A regional prevalence survey of 110 commercial deer herds in New Zealand showed that 81% had evidence of exposure to leptospires: 61% of the herds to *Leptospira borgpetersenii* serovar Hardjobovis and 4% to *Leptospira interrogans* serovar Pomona, and 16% to both serovars (Ayanegui-Alcérreca *et al.* 2010). Thus serovar Hardjobovis is endemic in most herds whereas serovar Pomona occurs

sporadically (Ayanegui-Alcérreca *et al.* 2007). Transmission of the organism and disease commonly occur in deer before one year of age (Ayanegui-Alcérreca 2006; Wilson and McGhie 1993). Infection and clinical disease has been well described in New Zealand farmed deer (Ayanegui-Alcérreca *et al.* 2007) but abortion has not. Abortion has been demonstrated following artificial infection with serovar Pomona in white tailed deer in North America (Trainer *et al.* 1961). While Ayanegui-Alcérreca (2006) described a significant (10%) improvement in weaning rate in naturally infected multiparous hinds following vaccination in a herd with dual serovar Hardjovovis and Pomona infection, and Subharat *et al.* (2008) showed an average of 5% greater weaning rate in this age-group in five other herds with Hardjovovis infection, nothing is known of the localisation of this organism in the genital tract or foetus of farmed deer.

This paper reports a study to explore evidence for vertical transmission by determining the presence of pathogenic *Leptospira* in the uterus and foetus of non-pregnant and early pregnant farmed deer in the North Island of New Zealand using bacterial culture and real-time PCR.

## **7.2 Materials and methods**

### **7.2.1 Animals**

From February to July 2008, 120 mixed age hinds, phenotypically red deer (*Cervus elaphus*), but possibly containing some wapiti (*C.e. canadensis*) genes, from randomly selected lines from nine commercial deer farms with no previous information on leptospiral infection were sampled at the Deer Slaughter Premise (DSP) in Feilding, Manawatu, New Zealand. In total, 116 blood, 120 kidney and 120 uterine samples were collected and 27 fetuses were available from samples collected in July. Based on sample size calculation using FreeCalc software, the number of reproductive tissue samples required for finding at least one carcass with evidence of leptospira in reproductive tissue was estimated to be 115 (95% confidence; 80% power) based on bacterial culture sensitivity of 50% and specificity of 100% and an assumed true infection prevalence of 5%.

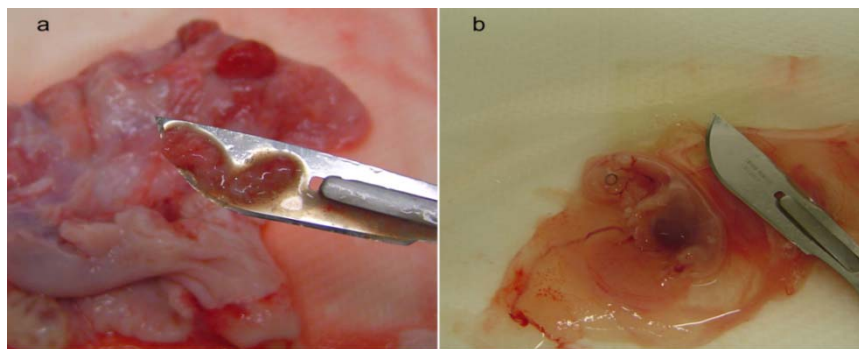
### **7.2.2 Sample collection and processing**

**7.2.2.1 Blood:** Blood samples were collected by free flow into a new 10 ml plain blood tube after animal sticking, immediately after stunning. Blood was held at 4°C before transport back to the laboratory where samples were centrifuged at 3,000 rpm (1,512 x g) for 15 minutes after which serum was aliquoted into new labelled 1.5ml microcentrifuge tubes and stored at -20°C for serology.

**7.2.2.2 Kidney:** Hind kidney samples were taken at the evisceration and inspection area of the slaughter line, put into labelled sterile plastic bags aseptically and held at 4°C before transport back to the laboratory. Later, the kidney surface was swabbed with 70%

alcohol and randomly aspirated from several sites over the entire kidney into a 5ml sterile syringe using a sterile 16-gauge needle. Half of the extracted kidney tissue (approximately 50mg) was used for bacterial culture and the other half used for DNA extraction for real-time PCR.

**7.2.2.3 Uterus and foetus:** Whole uteri with or without a foetus were collected at the same time as the kidney. They were placed into labelled sterile plastic bags aseptically and held at 4°C before transport to the laboratory where they were processed aseptically within a biohazard cabinet. The uterus was cut open and the uterine body and horn mucosal epithelium randomly excised using a scalpel. Foetuses, if available, were incised and tissue removed from the area of kidney and visceral organs (Figure 7.1). Approximately 50mg was used for each of bacterial culture and DNA extraction for real-time PCR.



**Figure 7.1 Processing of uterus and foetus samples. (a) Scraping of uterine epithelium (b) Deer foetus from pregnant uterus.**

### 7.2.3 Laboratory procedures

**7.2.3.1 Serology:** The microscopic agglutination test (MAT) was used to test serum reactivity to laboratory standardised *Leptospira* serovars Hardjobovis and Pomona. The method has been developed by the Leptospirosis Research Unit, IVABS, Massey University based on the guidelines for the control of leptospirosis (Faine 1982). For both serovars a titre of  $\geq 1:48$  was considered positive (Blackmore *et al.* 1982; Dorjee *et al.* 2008).

**7.2.3.2 Bacterial culture and serotyping:** Ellinghausen-McCullough-Johnson-Harris (EMJH) was used as a selective medium with an addition of antibiotic (5-Fluorouracil) for contamination inhibition. The medium was inoculated with processed kidney, uterus and foetus samples, incubated at 28-30°C and examined every two weeks for four months under dark-field microscopy. Isolates of leptospires were serotyped against standardised antisera of *Leptospira* serovars Hardjobovis and Pomona. These methods were adapted from the Leptospirosis Laboratory SOP, IVABS, Massey University based on the guidelines for the control of leptospirosis (Faine 1982).

**7.2.3.3 DNA extraction and real-time PCR:** Leptospiral DNA was extracted from 50mg of processed samples (kidney, uterus and foetus) using the High Pure Template Kit (Roche, Germany) as per manufacturer's instructions. DNA was eluted in a final volume of 200µl. The real-time PCR technique was a modification of the method described by Slack *et al.* (2006). SYTO9 (Invitrogen, Oregon, USA) was used as fluorescent double-stranded DNA specific intercalating dye (Monis *et al.* 2005) for all real-time PCR assays. The assay was performed in a Rotor-Gene 6000 machine (Corbett Research, Mortlake, Australia) using primers 2For 5'-tgagccaagaagaacaagctaca-3' and 504Rev 5'-matggttcrcrtttccgaaga-3' (Slack *et al.* 2006). Each 25µl reaction contained 2µl of DNA extracted from samples, 1.5µM SYTO9, 1X PCR buffer, 1.5mM MgCl<sub>2</sub>, 200µM dNTPs, 12.5pmol of 2For primer, 12.5pmol of 504Rev primer, 0.1%bovine serum albumin (BSA), 1 unit of Taq DNA polymerase and double distilled water. Thermal cycling consisted of initial denaturation at 95°C for 10min followed by 40 cycles of denaturation at 95°C for 10sec, annealing at 60°C for 20sec and extension at 72°C for 20sec. Melting temperature (T<sub>m</sub>) of PCR product was determined by melting curve analysis. It was performed by heating the PCR product from 70°C to 90°C and monitoring fluorescence change every 0.1°C. Confirmation of positive samples was determined by melting temperature (T<sub>m</sub>) of the PCR product compared with the positive control. The T<sub>m</sub> of positive samples was found to be between 83°C and 84°C. The positive control used for the real-time PCR assay was field isolates of *Leptospira* serovar Hardjobovis and the negative control was double distilled water.

## 7.3 Results

### 7.3.1 Serology

There was evidence of seroreactivity to *Leptospira* serovar Hardjobovis in 34 of 116 serum samples (29.3%) and for serovar Pomona in 13 serum samples (11.2%). One of nine lines had no animals seropositive to either serovar. Results are summarised in Table 7.1.

**Table 7.1 Details of blood samples, the number and percentage seropositive, and the positive reciprocal titre range for each line.**

| Line       | Sampling date | No. of samples | No. seropositive (%) |                  | Positive reciprocal titre range |                 |
|------------|---------------|----------------|----------------------|------------------|---------------------------------|-----------------|
|            |               |                | Hardjobovis          | Pomona           | Hardjobovis                     | Pomona          |
| 1          | 11 Feb        | 13             | 9 (69.2)             | 4 (30.8)         | 48 - 192                        | 96 - 192        |
| 2          | 11 Feb        | 14             | 0 (0)                | 2 (14.3)         | -                               | 48 - 768        |
| 3          | 18 Feb        | 14             | 2 (14.3)             | 0 (0)            | 48                              | -               |
| 4          | 18 Feb        | 15             | 7 (46.7)             | 2 (13.3)         | 48 - 96                         | 48 - 96         |
| 5          | 21 Feb        | 15             | 0 (0)                | 0 (0)            | -                               | -               |
| 6          | 11 Jul        | 8              | 3 (37.5)             | 1 (12.5)         | 48 - 192                        | 48              |
| 7          | 11 Jul        | 14             | 4 (28.6)             | 3 (21.4)         | 48 - 192                        | 48 - 384        |
| 8          | 17 Jul        | 10             | 5 (50.0)             | 1 (10.0)         | 48 - 1:192                      | 48              |
| 9          | 17 Jul        | 13             | 4 (30.8)             | 0 (0)            | 48                              | -               |
| <b>All</b> |               | <b>116</b>     | <b>34 (29.3)</b>     | <b>13 (11.2)</b> | <b>48 - 192</b>                 | <b>48 - 768</b> |

### 7.3.2 Bacterial culture

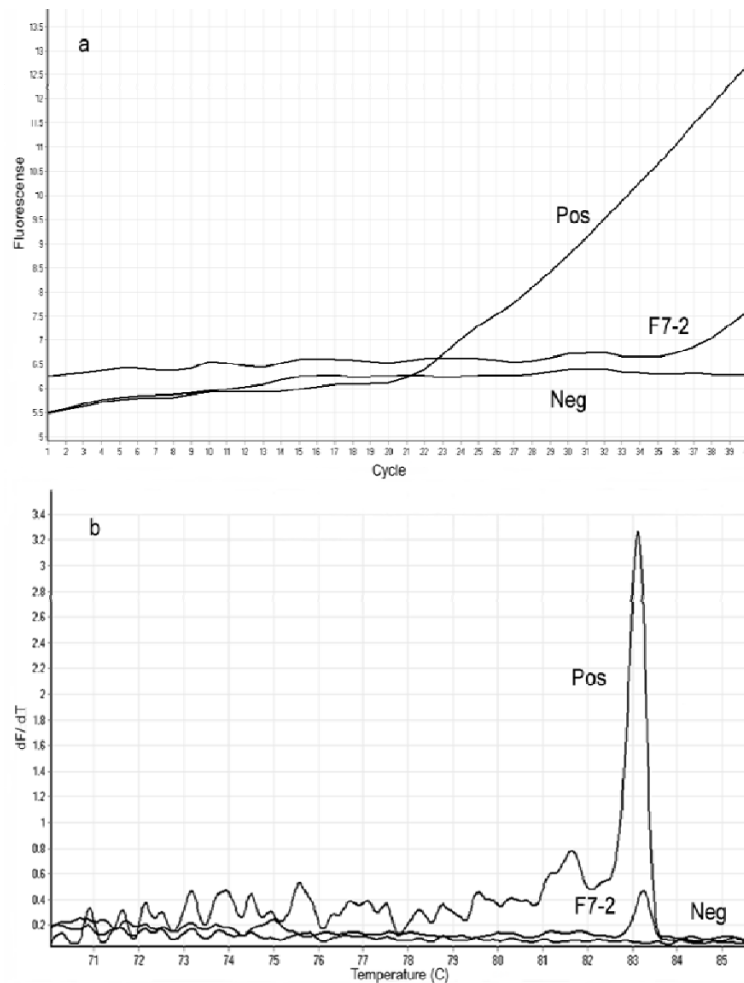
Seven of 120 kidney samples (5.8 %) from three farms were culture positive. All seven isolates were confirmed as serovar Hardjobovis. Five culture positive hinds were seropositive for Hardjobovis. None of 120 uteri or 27 fetuses was culture positive. Bacterial culture and PCR results are summarised in Table 7.2.

### 7.3.3 Real-time PCR

Five of 120 kidney samples (4.2%) were positive by real-time PCR, all of which were culture positive (Table 7.2). None of 120 uteri were positive by real-time PCR. One of 27 foetal samples was positive by real-time PCR (Figure 7.2). While this foetus was from a kidney culture and PCR positive line the dam was culture and PCR negative but seropositive to Hardjobovis (titre 1:192).

**Table 7.2 Results of bacterial culture and real-time PCR of kidney, uterus and foetus samples**

| Line       | Sampling date | Kidney       |              | Uterus       |              | Foetus      |             |
|------------|---------------|--------------|--------------|--------------|--------------|-------------|-------------|
|            |               | Culture      | PCR          | Culture      | PCR          | Culture     | PCR         |
| 1          | 11 Feb        | 4/13         | 2/13         | 0/13         | 0/13         | n/a         | n/a         |
| 2          | 11 Feb        | 0/14         | 0/14         | 0/14         | 0/14         | n/a         | n/a         |
| 3          | 18 Feb        | 0/14         | 0/14         | 0/14         | 0/14         | n/a         | n/a         |
| 4          | 18 Feb        | 2/15         | 2/15         | 0/15         | 0/15         | n/a         | n/a         |
| 5          | 21 Feb        | 0/15         | 0/15         | 0/15         | 0/15         | n/a         | n/a         |
| 6          | 11 Jul        | 0/12         | 0/12         | 0/12         | 0/12         | 0/8         | 0/8         |
| 7          | 11 Jul        | 1/14         | 1/14         | 0/14         | 0/14         | 0/11        | 1/11        |
| 8          | 17 Jul        | 0/10         | 0/10         | 0/10         | 0/10         | 0/1         | 0/1         |
| 9          | 17 Jul        | 0/13         | 0/13         | 0/13         | 0/13         | 0/7         | 0/7         |
| <b>All</b> |               | <b>7/120</b> | <b>5/120</b> | <b>0/120</b> | <b>0/120</b> | <b>0/27</b> | <b>1/27</b> |



**Figure 7.2 Real-time PCR results for foetus: (a) cycling of positive control (Pos), positive foetus (no.2 from line 7: F7-2) and negative control (Neg); (b) Melt curve of positive control (Pos), the positive foetus (F7-2) and negative control (Neg).**

## 7.4 Discussion

Leptospirosis is well-recognised and prevalent in farmed deer in New Zealand with a recent survey (Ayanegui-Alcérreca *et al.* 2010) estimating that 81% of herds were infected. Two studies have shown reduction of up to 10% in weaning percentage from hinds, associated with leptospirosis (Ayanegui-Alcérreca 2006; Subharat *et al.* 2008). Localisation of leptospire in the uterus may lead to foetal infection, with subsequent reproductive losses. The presence of leptospire in the genital tract has not been investigated. This is the first reported attempt to identify leptospire in uteri or foetuses of farmed deer. The study was designed to determine whether leptospiral infection could be detected in the uterus of non-pregnant deer in the late summer and in uterus and foetus (as available) from deer in the late autumn and early winter period corresponding to approximately the first three months of gestation.

Real-time PCR provided evidence of infection of one foetus, which was approximately three months of age, being sampled July 11. This tentatively indicates that leptospires may pass from dam to foetus in deer early in pregnancy. Uterine infection has been demonstrated in cattle from day 121 of gestation to term (Ellis *et al.* 1982b). Foetal infection with leptospires may contribute to reproductive wastage in deer as in other livestock species. While this observation is based on PCR evidence but not culture, validation of the PCR for leptospires in deer tissue used here has been shown to be 99.2% specific (see Chapter 5). Moreover, this foetal PCR evidence is consistent with positive MAT results of its dam with a titre of 1:192. Therefore, foetal infection is likely to be a valid inference.

The lines of hinds sampled for this study were randomly selected with no previous information of leptospiral infection. The chance of detecting leptospires could have been enhanced by selecting the lines from farms with a history of leptospiral infection or abortion as described in previous studies in cattle (Ellis *et al.* 1982a; Langoni *et al.* 1999) and pigs (Ellis *et al.* 1985; Ellis *et al.* 1986b) or the lines with kidney lesions. However, this is difficult logistically, and costly. Further, if only yearlings were sampled, the possibility of detecting infection may be higher because it is the age group that is recently infected (Ayanegui-Alcérreca 2006) whereas the older deer are more likely to be immune and less likely to be shedding or harbouring the organism. An association between reproductive wastage and leptospirosis was observed in both adults and the younger age group (Ayanegui-Alcérreca 2006; Subharat *et al.* 2008). No studies of reproductive performance related to leptospirosis have been conducted in older deer.

The animal level seroprevalence of 29.3% to *Leptospira* serovar Hardjobovis and 11.2% to serovar Pomona in this study was consistent with a recent report (Subharat *et al.* 2007) on deer in the same catchment area, but was lower than 45.0% and 12.2% reported previously from this area at the same DSP despite using a higher titre cut-point at 1:96 (Wilson *et al.* 1998). As transmission of leptospires is favoured by warm and humid conditions, the low prevalence observed might be associated with climatic effects since the summer and autumn preceding sample collection experienced particularly low rainfall. Ayanegui-Alcérreca (2006) reported differences in seroprevalence associated with high and low rainfall summer and autumn seasons. This study therefore likely suffered from a lower than optimum number of infected deer. The predominance of serovar Hardjobovis was expected for this population based on earlier reports (Ayanegui-Alcérreca 2006; Wilson *et al.* 1998) confirming that the serological status for this study was representative of leptospiral infection in deer herds in the region.

Bacterial culture and real-time PCR from kidney samples was done concurrently with uterine and foetal samples to provide complementary evidence of the infection status of the dam, which is not available from serology alone. The kidney is the most immune privileged site for this organism (Athanasio *et al.* 2008). In this study, culture and/or real-time PCR failed to detect the organism from any uterus, or all but one foetus, despite some of those animals being kidney culture and/or real-time PCR positive. The animal from which the PCR positive



foetus was derived was seropositive for serovar Hardjobovis (titre 1:192) but was negative for kidney culture and real-time PCR. The reason the foetal tissue was PCR but not culture positive may be that culture has specific limitations, including fastidious growth of the organism and contamination (Faine *et al.* 1999) and that PCR does not depend on the microbiological viability of the organism.

Failure of culture and real-time PCR to detect leptospires in uterine tissue may be because the concentration of leptospires scraped from uterine epithelium was below the detection limit of both techniques, or alternatively, the uterus of deer is rarely, if ever, infected by leptospires. Although, there have been some studies demonstrating isolation of leptospires from the uterus in non-pregnant cattle (Ellis *et al.* 1986a; Ellis *et al.* 1986b) this may not be the case in farmed deer. Different leptospiral strains are reported to behave differently. Within serovar Hardjobovis, some strains have been shown to persist primarily in kidney whereas other strains have a predilection for the genital tract and yet others persist in both organs (Ellis 1994). The seropositive adult deer in this study may have been exposed some time previously, developed immunity and subsequently cleared the infection. It is also possible that infection of the uterus only occurs in the acute or early phase of infection during the period of haematogenous spread, but none of the studied animals were in this phase of the infection process at the time of sampling.

The PCR evidence of leptospires from the deer foetus in this study suggests foetal infection. Foetal infection is claimed to cause stillbirth, abortion and weak offspring in cattle. With serovar Pomona infections, abortion usually occurs in the last three months of gestation whereas for serovar Hardjobovis infections, abortion has been observed from early gestation through to term (Ellis 1994). Ellis *et al.* (1982a) has demonstrated lower prevalence of leptospires isolated from normal foetuses when compared with aborted foetuses from a previous study (Ellis *et al.* 1982a). The difference in prevalence of leptospires isolated from normal and aborted foetuses suggest that leptospires, particularly serovar Hardjo, play an important role in bovine abortion (Ellis *et al.* 1982b). A more recent study has supported that theory by isolating Hardjo from aborted cattle foetuses (Langoni *et al.* 1999).

Real-time PCR assay may be a useful tool for detection of pathogenic leptospires in deer foetuses. The DNA gyrase subunit B gene primers used in this study are claimed to be genus-specific and detects only pathogenic *Leptospira* (Slack *et al.* 2006). It offers several advantages over the standard bacterial culture including being less time-consuming, inexpensive and detects leptospiral DNA from lysed or inactive organism. However, real-time PCR does not differentiate the serovar of causative leptospires which limits its use as a sole test for epidemiological study of infection (Lilenbaum *et al.* 2008) or diagnosis of serovar. However, this study has shown a 100% correlation between MAT and cultured serovar from the same animals (5 of 7) suggesting that PCR should also correspond to MAT serovar. Previous observations support this relationship (Ayanegui-Alcérreca 2006). This interpretation, however, needs to take account of the stage of infection when the immunity of the host has not been activated and in the event of dual or multiple serovar infections.

Techniques such as DNA sequencing (Slack *et al.* 2006) and restriction fragment length polymorphism (Heinemann *et al.* 2000) of the PCR product are under investigation to overcome this problem.

In conclusion, this study failed to isolate or show the molecular evidence of pathogenic leptospires from the uterus of deer in the early autumn and the period of early gestation. Nevertheless, we have demonstrated evidence of pathogenic leptospires in a deer foetus using a real-time PCR assay. Thus early infection of the foetus may play a role in adverse effects of leptospirosis on deer reproductive performance demonstrated earlier (Ayanegui-Alcérreca 2006; Subharat *et al.* 2008). This observation, while based on sound experimental design, is tentative, due to the small number of animals involved. However, it indicates that further research in both early and late pregnancy is warranted to fully investigate and confirm the presence of this organism and its impact on reduced reproductive performance in farmed deer.

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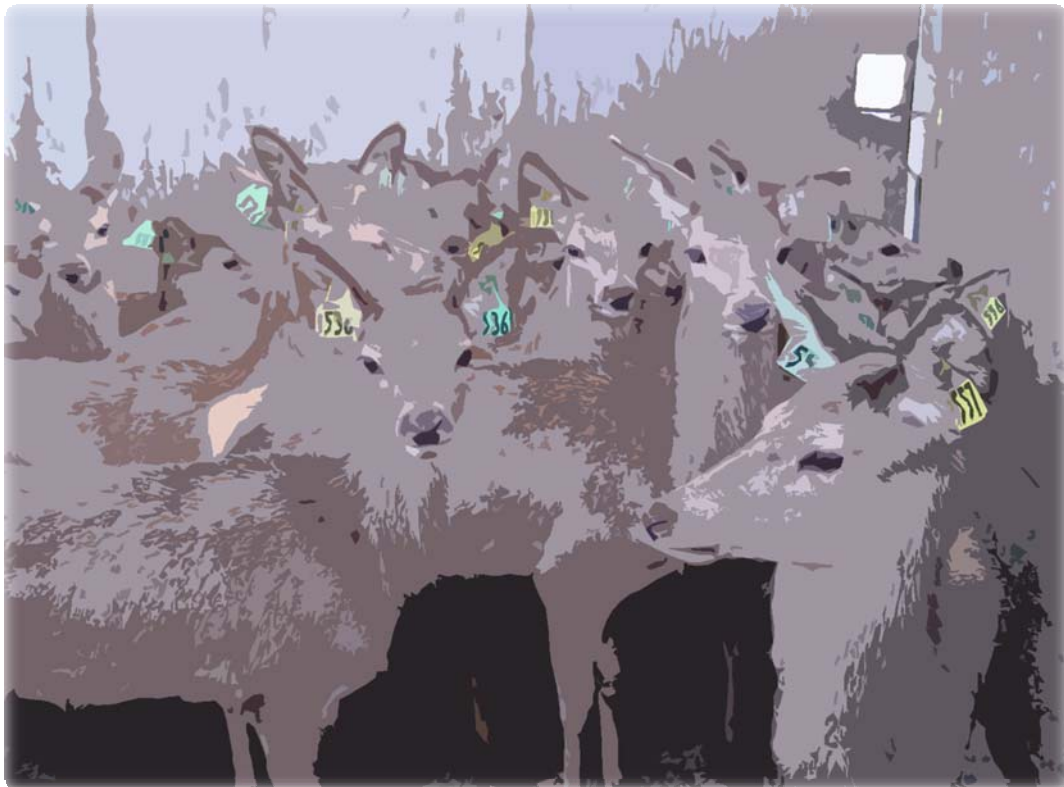
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# Chapter 8

## General Discussion



## 8.1 Introduction

The studies presented in this thesis were designed to further investigate the epidemiology of leptospirosis in New Zealand farmed deer based on current knowledge (Ayanegui-Alcérreca 2006), to develop and validate a novel molecular technique for diagnostic purposes and to enhance understanding of control measures for leptospirosis by means of vaccination. The study employed serology, bacteriology and a newly developed real-time PCR to determine infection and vaccine efficacy under natural challenge conditions. It concentrated largely on *Leptospira* serovars Hardjobovis and Pomona since they are the two most common serovars reported in farmed deer in New Zealand (Ayanegui-Alcérreca *et al.* 2007).

This chapter discusses the overall research findings in this thesis summarised broadly as epidemiology, diagnosis and vaccination control. This chapter also critiques experimental design and methodology, and addresses limitations and suggested areas for future research. It also proposes action to control leptospirosis for the deer industry.

## 8.2 Epidemiology of leptospirosis

### 8.2.1 Leptospirosis on mixed-species farms

Recent data from a regional seroprevalence survey (Ayanegui-Alcérreca *et al.* 2010) showed evidence that 81% of farmed deer herds in New Zealand were infected with *Leptospira* and no differences were found between regions. It was proposed that farmed deer in New Zealand are maintenance hosts for serovar Hardjobovis and accidental hosts for serovar Pomona and that deer may play an important role in the infection cycle of leptospirosis on multi-species livestock farms (Ayanegui-Alcérreca 2006). Livestock farming in New Zealand is becoming increasingly multi-species with approximately 85% of farms with deer also farming beef cattle and/or sheep on the same property (Hilson 2007; Wilson 2007). Despite several studies having been conducted to gain information on the epidemiology of leptospirosis separately in deer (Ayanegui-Alcérreca 2006; Wilson *et al.* 1998), cattle (Bahaman *et al.* 1984; Matthews *et al.* 1999) and sheep (Dorjee *et al.* 2005), epidemiological links between these species on farms are unclear. It is important to understand the role played by each potential host on mixed-species farms in the epidemiology of the disease to determine choice and implementation of appropriate control measures aimed at reducing the incidence of clinical and sub-clinical disease, and reduction of risk to humans.

Chapter 4 describes a pilot longitudinal seroprevalence study of leptospirosis on 19 deer farms with sheep and/or beef cattle in the lower North Island of New Zealand. This study had a dual purpose, with herd screening being for both identification of farms for vaccine studies of growth and reproduction (Chapters 2 and 3), and this

multi-species study *per se*. It is the first mixed-species epidemiological study carried out on-farm in New Zealand attempting to identify potential risk factors influencing *Leptospira* serological status of the deer herd.

Results from this study suggested that leptospirosis was prevalent on mixed-species farms in the lower North Island. The predominant serovar was Hardjobovis whereas Pomona was less common. Seroprevalence was consistent with that reported in single-species deer (Ayanegui-Alcérreca *et al.* 2003; Wilson *et al.* 1998), cattle (Bahaman *et al.* 1984; Matthews *et al.* 1999) and sheep studies (Blackmore *et al.* 1982; Dorjee *et al.* 2005).

Co-grazing with infected sheep and/or cattle was significantly positively associated with deer herd status to both serovar Hardjobovis and Pomona. This finding suggests inter-species transmission. Nevertheless, appropriate molecular typing of leptospiral organisms from each species on a farm is required to determine whether there are livestock-host adapted strains or not, and, to test the theory of cross-species infection. Multilocus Sequence Typing (MLST) is the candidate technique and is currently being validated within the Leptospirosis Research Unit, IVABS, Massey University (Subharat *et al.* 2009).

Another important finding from this study was the 64.1% flock prevalence of *Leptospira* serovar Hardjobovis in sheep. Blackmore *et al.* (1982) randomly sampled carcasses of lambs and proposed that sheep were not a maintenance host for Hardjobovis but the seroprevalence of Hardjobovis from this and other studies (Cousins *et al.* 1989; Gerritsen *et al.* 1994a) suggest that sheep may indeed act as a maintenance host. Lamb carcasses may not be a suitable source population as it tends to underestimate population prevalence. Conventionally, only cattle and deer in New Zealand are regarded as maintenance host for this serovar. Thus, if leptospiral infection can be controlled efficiently in those species, for example by vaccination, and sheep do not maintain the organism, theoretically, leptospiral infection could be indirectly eliminated from sheep. This would be attractive for sheep farmers since vaccination of this species would be more costly against returns at the individual animal level than for cattle and deer. This proposition needs further investigation since sheep comprise the largest population of livestock in New Zealand and it could prove significant for effective control strategies for leptospirosis on mixed-species farms.

Proof that sheep is a maintenance host for Hardjobovis requires evidence of long-term leptospiuria by urine culture or PCR and molecular typing to see whether they carry species-specific *Leptospira* organisms that are different from cattle and farmed deer with no evidence of continuous challenge from other species. One possible way to test whether sheep is the maintenance host for Hardjobovis could be to compare the flock prevalence of this serovar between sheep-only farms and sheep on mixed-species



farms. If the flock prevalence of *Hardjobovis* from sheep-only farms was lower than that of the sheep on multi-species farm, this may suggest that sheep are spill over host and may not maintain *Hardjobovis*. However, control for confounding factors for such study must be appropriate. Another possible way to test this could be to vaccinate deer and cattle on farms that have sheep on the property and monitor the seroprevalence of *Leptospira* in sheep for a long period. If the seroprevalence in sheep declined over time, it would suggest that sheep were not a maintenance host and deer and or cattle were necessary for maintenance in sheep.

The study in Chapter 4 provides a basis for future studies on mixed-species farms. Due to the limited number of farms and incomplete farm categories (no cattle-only, sheep-only and cattle and sheep farms), data from this study were not suitable for multivariable analysis. To permit such analyses, a study should be larger, involve all regions of New Zealand and include all categories of farms.

Sera stored in a serum bank from this study could contribute to a future analysis for possible novel serovars of *Leptospira* in cattle and sheep although it will only represent the herds/flocks from the lower North Island of New Zealand.

### **8.2.2 Possible novel serovar of *Leptospira* in NZ**

The availability of the serum bank from a previous regional seroprevalence study (Ayanegui-Alcérreca *et al.* 2010) provided a unique opportunity to further investigate possible novel serovars of *Leptospira* in farmed deer (Chapter 6). A collaborative study between Massey University Deer Research Group (MUDRG) and the World Health Organisation/Food and Agriculture Organisation/World Organisation for Animal Health (WHO/FAO/OIE) reference laboratory for leptospirosis in Brisbane was initiated in 2006 to investigate exotic serovars, and allow inter-laboratory comparisons for serology for *Hardjobovis* and *Pomona*. To our knowledge, this study was the first in 30 years to investigate for possible novel leptospiral serovars against full reference panels in any animal species in New Zealand. Serology against 23 reference serovars confirmed the endemicity of *Hardjobovis* and sporadicity of *Pomona*. It also revealed seropositivity to *Arborea* which has never been found in New Zealand in 11/70 (15.7%) deer herds tested. This finding was reported to MAF-Biosecurity as a possible new organism according to requirements, although no follow-up was undertaken.

The pattern of serological evidence suggested that *Arborea* titres were not due to cross-reaction since positive samples showed no or little evidence of being positive for *Ballum*, which is from the same serogroup and is found in New Zealand. However, attempts to isolate the organism from kidney samples of farmed deer from two seropositive farms in 2007 and 2008 were unsuccessful. Since follow-up was opportunistic, attempts to investigate for *Arborea* were limited by time and financial

resources. Thus, results cannot exclude the presence of this serovar from New Zealand. Further serological screening and culture are required to give greater confidence that this serovar is indeed present or not in deer, other livestock, rodents, wildlife or humans in New Zealand. This could be focused initially in the Southland region where the present serological evidence of Arborea was clustered. The sample size for deer could be estimated based on prevalence data from this study. However, such study will require significant resources.

### 8.3 Diagnosis

Highly sensitive and specific diagnostic tests are desirable for epidemiological study of infections such as leptospirosis. Chapter 5 describes development and validation of a molecular diagnostic tool for detection of leptospiral organisms in deer clinical samples. The rationale for developing this technique was to address shortcomings of conventional methods in detection of leptospires in clinical samples, such as dark-field microscopy and Warthin-Starry staining, neither of which are sufficiently sensitive (O'Keefe 2002). Furthermore, culture, which is usually used as the gold standard, is tedious, complex, time-consuming and prone to contamination. Real-time Polymerase Chain Reaction (PCR) assay was chosen to overcome many of those shortcomings.

This study evaluated and validated the performance of a real-time PCR assay for use on deer kidney tissue and urine as a research and diagnostic tool for determining infection, carrier and shedding status of deer. It was the first study to use real-time PCR directly on deer kidney and urine samples with evaluation against culture as the gold standard. Furthermore, it was also the first study to quantify pathogenic leptospires shed naturally in deer urine. This technique is also able to identify specific *Leptospira* species by DNA sequencing of PCR amplicons. This research confirmed that the real-time PCR assay was a useful tool for rapid, cost-effective, sensitive and specific detection of pathogenic leptospires in clinical samples, particularly urine.

Urine samples for validation of this real-time PCR assay were from the vaccination validation study (Chapters 2 and 3) whereas kidney samples were from randomly selected lines at deer slaughter premises. Results have shown that the real-time PCR was highly sensitive and specific compared with culture. A limitation of this study was that urine samples were highly contaminated, thus it was not possible to robustly evaluate clinical sensitivity of real-time PCR on urine samples against culture. However, if deer urine samples could be collected more aseptically, for example, from urinary bladder puncture at slaughter, it would likely reduce the contamination rate in culture and make it possible to fully evaluate clinical sensitivity of urine real-time PCR.

The technique can be applied for future epidemiological studies involving *Leptospira* shedding in other species and possibly even environmental contamination when studying potential risk factors for disease transmission. It may also be applied for early diagnosis of human leptospirosis by detection of leptospiral DNA in blood during the leptospiremia phase as reported in other studies (Fonseca Cde *et al.* 2006; Levett *et al.* 2005; Merien *et al.* 2005).

The ability of real-time PCR to quantify the concentration of leptospires in clinical samples provides diagnostic benefits, for example in evaluating the concentration of the organisms shed in urine during different stages of infection, or even antibiotic efficiency evaluated by post-treatment clearance of leptospires in urine. Merien *et al.* (2005) used this assay to measure the concentration of leptospiral DNA in patient's sera, informing the prognosis for those suffering from leptospirosis.

This real-time PCR, in combination with DNA sequencing, provides a rapid tool for identifying pathogenic *Leptospira* species and the likely serovar from clinical samples. Since the two most common serovars found in New Zealand (Hardjobovis and Pomona) belong to different species, it is possible to conjecture the infection serovar by this technique. Nevertheless, this would be appropriate only in regions where few species and serovars of pathogenic leptospires were encountered. For more accurate genotypic classification where multiple serovars may be present, Multilocus Sequence Typing (MLST) is the most up-to-date method that has been developed for typing and is claimed to have high discriminatory power to strain level, reproducibility and robustness (Ahmed *et al.* 2006; Thaipadungpanit *et al.* 2007). This technique is currently being developed and validated at the Leptospirosis Research Unit, IVABS, Massey University (Platero 2009; Subharat *et al.* 2009). *Leptospira* isolates from this study including all DNA extracted samples from both urine and kidney have been stored as reference material for future development and validation of new techniques.

Research in Chapter 6 provided an opportunity to compare the results of Microscopic Agglutination Tests (MAT) for Hardjobovis and Pomona between the Leptospirosis Research Unit, IVABS, Massey University and the WHO/FAO/OIE reference laboratory for leptospirosis in Brisbane. The Kappa (K) agreement test has shown excellent agreement of MAT between the two laboratories for both serovars Hardjobovis (K=0.81) and Pomona (K=1.0) at the herd level and moderate agreement for Hardjobovis (K=0.41) and substantial agreement for Pomona (K=0.68) at individual animal level. Variation between laboratories at the individual animal level was expected due to the subjectivity of test interpretation, as is widely accepted and reported elsewhere (Faine 1982; Levett 2001). Because of the subjectivity of interpretation of a MAT titre, only one person should read positive MAT results for all samples of a given study to ensure consistency and validity of results. If this is not possible, appropriate training is required in combination with blind testing against

known results to ensure the greatest consistency between personnel. Serum samples from this study were stored in the deer serum bank and are currently available for such training.

#### **8.4 Vaccination control of leptospirosis**

Control for leptospirosis requires a range of strategies. The key is to limit direct and indirect transmission of the organisms between susceptible host, carrier and contaminated environment (Heath and Johnson 1994). Vaccination is likely to be effective and practical in reduction of transmission of *Leptospira* to both animal and human cases as has been shown earlier in cattle and pigs (Marshall and Manktelow 2002). It is recognised as a tool aimed at reducing the carrier state as a means of limiting the infection cycle. Little *et al.* (1992) claimed that vaccination is the best option in controlling for leptospirosis under pastoral systems.

The purpose of research in Chapters 2 and 3 was to determine the effect of a commercial bivalent leptospiral vaccine (Leptavoid-2, Intervet/Schering-Plough Animal Health Limited, NZ) on leptospiral shedding, growth and reproduction of farmed deer under New Zealand pastoral conditions involving natural challenge. Generally, the conventional method for evaluation of vaccine efficacy is to vaccinate animals and expose them to artificial challenge in a controlled environment (Bolin *et al.* 1989). However, this may not reflect the natural challenge of *Leptospira* as the infective dose and route of administration may be different from artificial challenge. Further, challenge models for testing vaccine efficacy for infectious disease agents that can cause potentially serious clinical disease in experimental subjects present animal welfare concerns. Alternatives to challenge models for vaccine efficacy studies, such as serological equivalence and field studies are becoming accepted.

This study attempted to investigate the efficacy of vaccine under natural challenge to address those issues. However, in a natural challenge situation, it is likely that some animals may be infected prior to vaccination, confounding the apparent effect of the vaccine. The study was therefore designed to simulate an infection-free herd scenario, as much as possible, created by the use of streptomycin to attempt to eliminate the subclinical kidney infection and shedding state, prior to vaccination. This was followed by exposure to natural challenge. Streptomycin treated deer initially showed no evidence of increasing titres or *Leptospira* shedding. This changed once they were joined with no-streptomycin treated control animal; their subsequent increase in titres and shedding demonstrating that the study model was effective in terms of natural challenge with *Leptospira* and mimicked the effect of vaccine in previously uninfected animals. It is proposed that this design better evaluated the effect of a vaccine on growth and shedding outcomes than vaccination in the face of endemic infection. The latter model would likely under-estimate the true effect of the vaccine in a situation of low or zero challenge, which is what would be expected over time in

a herd with a prolonged vaccination programme. The detection of leptospirosis in this trial was based on real-time PCR since high contamination rates in urine culture from earlier studies thwarted vaccine efficacy evaluation in terms of urine shedding (Ayanegui-Alcérreca *et al.* 2010).

The major limitation of this study was the severe drought season which resulted in the deer on studied farms being underfed. Moreover, dry conditions likely reduced organism survival and transmission, contributing to a low natural challenge that resulted in the low observed seroprevalence in most herds. Further, one farm had to drop out from the study because the farmer sold most of their stock due to insufficient feed. Severe drought also delayed the time taken for deer to reach slaughter weight. Thus, the relevance of kidney samples to observe carrier status and kidney lesions at slaughter was reduced. The collection of kidney samples from those deer in the following year was not an option since it would have been beyond the study period and the possible expected efficacy of the vaccine as shown in other species (Hancock *et al.* 1984). In addition, animals in this study only experienced *Hardjobovis* infection unlike a previous study by (Ayanegui-Alcérreca 2006) that reported some dual infections of both *Hardjobovis* and *Pomona*. Evidence suggests that serovar *Hardjobovis* is rarely if ever associated with clinical disease (Wilson and McGhee, 1993), thus it is assumed that *Pomona* is more pathogenic in deer as it appears to be in sheep and cattle. Thus, if this study had *Pomona* infection, the effect observed for both growth and reproduction response could have been greater. Nevertheless, importantly, the presence of only one serovar allowed this study to confirm for the first time that serovar *Hardjobovis* alone appeared to be capable of causing sub-clinical production loss, both growth and reproduction, on deer farms.

#### **8.4.1 Growth response**

Not much was known of the effect of leptospiral infection on growth of farmed deer prior to this study. Preliminary studies on individual animal data in one herd, showed that yearling deer with evidence of infection during the previous 8-month growth period were 3.7 kg lighter than those without the evidence of infection (Ayanegui-Alcérreca 2006). However, that observation needed replication before robust claims of the effect of leptospirosis on growth could be made. The present study was designed to evaluate growth response due to vaccination at the herd level. A significant growth response was confirmed. This was despite the drought conditions likely predisposing to lower prevalence and poor feed quality and quantity, thus it may be that the magnitude of the difference may have been greater if optimum feeding was available, and had the climate been less extreme.

Data from this study suggested that the magnitude of the vaccination response at the herd level, and hence the economics of vaccination, will depend on the prevalence of infection, and serovar. No response would be expected in uninfected herds but

significant responses are likely in high prevalence situations. Data from Farm 1 in Chapter 2 which experienced a 87% seroprevalence of Hardjibovis and confirmed infection using urine PCR, showed a difference of average daily gain (ADG) at 27 g/day, which resulted in a mean liveweight difference of 6.5 kg from March to November, after a growth period of 241 days. This was a result of sufficient magnitude to be statistically significant on that farm alone. Based on this data, the marginal return on investment would be \$29 per deer (assuming \$8.0/kg carcass weight, 56% dressing of liveweight), for an investment of \$3.20 (\$2.60 for vaccine and \$0.60 for labour and sundry costs), a 9.1 times return on vaccination cost (Wilson *et al.* 2009). Chapter 2 presents data on seroprevalence cut-points for a cost-effective response to vaccination.

Analysing growth response due to vaccination at the herd level, rather than at individual animal level as reported by Ayanegui-Alcérreca (2006), may have underestimated the true effect at animal level because some animals in the control group had no serological evidence of being infected. However, data from this study did not withstand such analysis since the prevalence on each of infected farms (Farm 1, 2 and 3 in Chapter 2) were either too high or low. Thus, the number of animals in the control group representing infected vs. non-infected was too small to draw any conclusion. Furthermore, to combine those data from each infected farm together was not appropriate since it would be confounded by factors such as management, feeding and genetics. The strong causal animal-level argument is that these deer were allocated to vaccine and control groups at random and kept under identical conditions on each farm.

The greatest effect of vaccine on growth in Chapter 2 was on Farm 1 with the highest liveweight observed in November (Table 8.1). It suggests that other than herd seroprevalence and management and climatic factors, the magnitude of vaccination response may also be influenced by the genetic growth potential on individual farms because deer with higher growth potential may lose more growth than deer with a low growth potential. However, this proposition needs to be tested to fully understand the magnitude of the effect of leptospirosis on growth in a range of circumstances.

The mechanism for leptospirosis causing reduction in growth is unknown. It is possible that localisation of leptospire in the kidney caused alteration of kidney function, pain that influenced food intake, or continued immune challenge on the cost of energy in feed. It is common to observe kidney lesions in deer associated with leptospiral infection, with the worst-affected lines generally having the highest seroprevalence and titres (Wilson *et al.* 1998). Thus, metabolic function could have been affected particularly during the establishment phase of infection, resulting in reduction of growth rate and food conversion efficiency. It was notable that the greatest reduction in ADG occurred in the period corresponding with the period of greatest seroconversion providing further evidence of a causal relationship. Activation

of the immune system during infection and stress may change the priority of partitioning nutrients from growth to host defence (Colditz 2002).

**Table 8.1 Mean liveweight (kg) (and range) of control and vaccinated male deer on each farm in November and the difference (from Chapter 2).**

| Farm | Control                | Vaccinate              | Difference<br>(Vaccinate – control) |
|------|------------------------|------------------------|-------------------------------------|
| 1*   | 89.7<br>(76.0 – 101.0) | 99.1<br>(89.0 – 107.0) | 9.4                                 |
| 2*   | 70.8<br>(48.0 – 86.5)  | 72.6<br>(58.0 – 86.5)  | 1.8                                 |
| 3*   | 71.0<br>(48.4 – 87.6)  | 77.0<br>(65.6 – 91.2)  | 6.0                                 |
| 4    | 82.1<br>(57.5 – 96.5)  | 80.8<br>(61.5 – 91.0)  | -1.3                                |
| 5    | 81.7<br>(66.5 – 104.0) | 78.7<br>(58.5 – 97.5)  | -3.0                                |

\* Farms with evidence of leptospiral infection in male deer

#### 8.4.2 Shedding response

In order to control leptospirosis, consideration goes beyond the obvious need to prevent clinical illness and economic loss in deer. There is also a need to minimise the risk of human infection by controlling exposure from them. It is clear from reported statistics and farmer and veterinary anecdotes that there is a risk of transmission from deer to humans with an apparently relatively higher risk to those at Deer Slaughter Premises (Bell 2005; Brown 2005). A recent cross-sectional survey of 1,895 farmers in 2008-09 has shown that human leptospirosis incidence was 8-fold higher in farmers reporting that their deer were affected by leptospirosis than farmers where leptospirosis was not observed in deer (Verdugo and Heuer 2009). This confirms the specific public health implication of leptospirosis in deer. Furthermore, there is also risk of transmission to other in contact animals of the same or different species. Transmission of *Leptospira* is usually via urine containing organisms. The study in Chapter 2 has investigated whether leptospiral vaccination prevented urinary shedding if the animal received the vaccine, therefore developing immunity before exposure to the organisms.

That study has shown 100% prevention of shedding, based on urine PCR. This was in deer given streptomycin to eliminate the carrier state and vaccinated, followed by a period of reduced risk of exposure to infection while immune responses developed, followed by natural challenge. That there were no vaccinated deer shedding leptospires in urine provides strong evidence that vaccine is behaving in a similar manner to that reported in cattle and pigs (Hodges *et al.* 1985; Marshall *et al.* 1982). This complements a previous report using the same type of vaccine (Leptavoid-3, Intervet/Schering-Plough Animal Health Limited, NZ) which was shown to reduce

the incidence of urine shedding, based on dark field microscopy, by 44% in young deer herds already infected and continuously exposed to infection (Ayanegui-Alcérreca 2006). Hancock *et al.* (1984) suggested it is unlikely that vaccination will prevent shedding in all animals already infected at the time of vaccination. The present study demonstrated that vaccine did prevent leptospiral shedding when immuno-naïve deer received vaccine prior to infection. On a whole farm basis, elimination of shedding is likely to be possible over a prolonged period of whole herd vaccination as persistently infected animals are replaced by vaccinated un-infected animals (Little *et al.* 1992). To date, there are no data for the efficacy of streptomycin in eliminating *Leptospira* shedding in farmed deer. However, there is evidence that naturally infected cows stop shedding leptospires after a single treatment with dihydrostreptomycin at the same dose used in this study (Gerritsen *et al.* 1994b). Given the similarity of infection in farmed deer it is likely that streptomycin has the same effect in this species.

Since human leptospirosis incidence in New Zealand is among the highest in developed countries and is largely associated with livestock including farmed deer (Baker and Lopez 2004; Bell 2005), results from this study suggest that leptospiral vaccination in farmed deer should reduce this risk to humans. This could potentially reduce the cost for potential medical expenses or the need to employ replacement labour during illness and recuperation for the farmer and meat worker due to leptospirosis. It could also reduce the hidden national cost of this disease since almost 50% of leptospirosis patients require hospitalisation (Wilson *et al.* 2009).

#### **8.4.3 Reproduction response**

It is well known that *Leptospira* serovars Hardjobovis and Pomona are pathogens causing reproductive losses in livestock worldwide (Grooms 2006). Reports about Pomona suggest that it can cause more severe clinical effects such as abortion storms (Gilmour 2007; Knott and Dadswell 1970), but the sporadic occurrence renders Pomona less economically important than Hardjobovis (Givens 2006). This may also apply to farmed deer. Chronic leptospirosis causes impaired fertility, neonatal death, abortions and decreased milk production (Lilenbaum *et al.* 2008). Previous study in adult hinds reported a nine percentage point higher weaning rate (10.2% improvement) in vaccinated compared with control hinds in a herd with dual Hardjobovis and Pomona infection (Ayanegui-Alcérreca 2006). The present study has complemented and is consistent with that study, replicating that vaccination of deer against leptospirosis can improve the weaning rate on farms with evidence of leptospiral infection. This study found an overall significant 5.7 percentage point increase in weaning rates (6.9% improvement) due to vaccination. However, it was not appropriate to estimate the odds ratio of seropositive vs. seronegative hinds rearing calf in the control group due to the low level of challenge. Therefore, better evidence was provided by comparing weaning rates of vaccinated and control deer



allocated at random and kept under identical conditions. Nevertheless, this should be subjected to further study with a larger sample size, and target the farms with a high level of challenge, although the latter is difficult to achieve reliably because it is difficult to predict seroprevalence because of large seasonal differences.

Based on weaner deer value in March at \$225/animal, the average gain of 6.9% reported in Chapter 3 would result in a return on vaccination of \$15.5 per hind, and the highest response of 10.7% would result in a return of \$24.1 per hind. The break-even point for economic return on vaccination in terms of reproduction is an improvement in weaning rate of 1.4%. Wilson *et al.* (2009) suggested that more than 50% of farmers might expect an economic return for vaccination of yearling hinds based on surveys of seroprevalence in deer herds, and this financial value of progeny.

The pilot study in Chapter 7 which investigated the presence of pathogenic *Leptospira* in the uterus and foetus of non-pregnant and early pregnant farmed deer could establish an association with reproductive loss. Leptospire have been isolated from several reproductive organs in cattle such as placenta (Ellis and Michna 1977), oviduct and uterus (Ellis and Thiermann 1986; Ellis *et al.* 1986) and viable and aborted foetuses (Ellis *et al.* 1982a; Ellis *et al.* 1982b; Langoni *et al.* 1999). However, nothing is known of the localisation of this organism in the genital tract or foetus of farmed deer. This study has demonstrated evidence of pathogenic leptospire in a deer foetus, using a real-time PCR assay, but it failed to isolate or show molecular evidence of pathogenic leptospire from the uterus of deer. Early infection of the foetus may play a role in the adverse effects of leptospirosis on deer reproductive performance demonstrated in this study and elsewhere (Ayanegui-Alcérreca 2006; Subharat *et al.* 2008).

Data from Chapter 3 suggested that reproductive losses due to leptospiral infection were peri- or post-natal, but pre-natal losses should not be ruled out since abortion has been demonstrated following artificial infection with serovar Pomona in white tailed deer (Trainer *et al.* 1961). This could be serovar-related since only Hardjobovis infection was found in the present study. In dairy cattle, reproductive losses have been shown to be pre-, peri and post-natal. Persistent infection by Hardjobovis was reported to cause reduced conception rates and fertility (Dhaliwal *et al.* 1996), increased number of services per conception and prolonged calving interval. It is also a cause of early embryonic death, abortion, stillbirth and weak calf syndrome (Smyth *et al.* 1999).

Since this study was done only in the early autumn, when most hinds are in early gestation, and found evidence in only one deer, further research in both early and later pregnancy and in non-pregnant hinds during the corresponding period is needed to fully investigate and confirm the presence of this organism and its role in reducing reproductive performance. Primiparous hinds should be the target group since they are

more likely to be recently infected. The lower weaning rate in this class of animal rather than adults is consistent with the hypothesis of an infectious pathogen being a cause of the reproductive loss. Thus, adoption of vaccination may have an important positive impact on the reproductive efficiency of farmed deer by improving the retention rate of young hinds, hence increasing the average breeding life, and improving production efficiency by requiring fewer replacement hinds to be retained.

#### **8.4.4 Implementation of vaccination and control**

The production (growth and reproduction) increase through vaccination in deer shown from this study suggested a likely economical effect on most farms over a period of time. It is estimated that about 67% of deer farms could achieve economic response (Wilson *et al.* 2009) in any one year, and since the status of farms is not static, it is likely that almost all farms will experience infection and therefore risk negative effects of leptospirosis on a long-term basis. In the absence of public subsidies for the control of human leptospirosis, vaccination against leptospirosis in livestock for the purpose of protection of people needs to be economically attractive for farmers to motivate the investment.

There are three commercial vaccines labelled for use in deer in New Zealand, namely Leptavoid-2, Leptavoid-3 (Intervet/Schering-Plough Animal Health Limited, NZ) and Leptosshield (Pfizer Animal Health Limited, NZ). However, only Leptavoid-2 and Leptavoid-3 have been tested for vaccine efficacy on farmed deer. While there is no evidence that other vaccines are not efficacious it should not be assumed that all vaccines will have the same effect until supported by scientifically sound evidence (Wilson *et al.* 2009).

It is likely that continued whole herd vaccination would reduce the incidence of disease and infection, as shown in dairy cattle. Evidence from a longitudinal study of cattle in Luing Island, Scotland demonstrated that when whole herd immunity was sustained by vaccination for at least five years, Hardjobovis infection could be eliminated (Little *et al.* 1992). However, elimination could be challenged by potential sources of re-infection such as introduction of infected and shedding stock, and contaminated waterways from neighbouring properties and wildlife vectors. The principles of risk evaluation in relation to animal health decision-making, including vaccination should be considered (Wilson *et al.* 2009).

Prior to consideration of vaccination, serological testing can determine the leptospiral infection status of a herd. Animals to target for sampling are those 1-2 years of age since the seroprevalence in that group has been shown generally to be higher than in other age classes (Ayanegui-Alcérreca 2006). A minimum of 20 samples should be collected to enable the presence or absence of infection to be evaluated with reasonable confidence as determined in Chapter 4. However, if a herd is believed on

the basis of this testing to be uninfected it therefore lacks herd-level immunity. It may be that if the organism is introduced, the morbidity and mortality rate may be higher than in an endemically infected herd where natural immunity exists. For a vaccination regime, Ayanegui-Alcérreca (2006) recommended that young deer should be sensitised at 3-months old followed by a booster a month later. All classes of hinds should be boosted one month prior to calving to enhance maternal derived antibody. Stags should be boosted annually at a convenient time that fits normal management practice. While this proposed programme is based on a range of epidemiological and vaccine studies, it is recommended that it should be tested in longitudinal studies on a number of deer farms for its effectiveness.

Maintaining farmed deer in a closed herd would reduce the risk of introducing infection. A quarantine program is an option when introducing new stock to the herd in order to minimise potential transmission. This applies particularly to amalgamation of young weaner deer from different sources when they are most susceptible. Wilson and McGhee (1993) noted that transport and amalgamation of deer from several sources, particularly of young deer in the autumn would appear to be the most significant risk factor for disease outbreaks in that age group. If replacement deer are introduced to the herd, their serological status could be tested. If seropositive, infection could be reduced by treatment with Streptomycin at 25 mg/kg (Mackintosh 1993) immediately prior to shipping and quarantined when arriving at farms. Deer introduced to the herd should be vaccinated and boosted prior to introduction. Optimum nutrition and animal health (e.g. trace elements and internal parasites) could improve resilience to clinical disease, but would unlikely affect infection *per se* (Wilson *et al.* 2009). Transmission risks such as waterways on farm could be fenced out from grazing. Drainage, reducing the risk of surface water contaminated with urine would reduce risk of transmission.

Recent data from 237 deer herds surveyed in 2008-09 revealed that 11 herds (4.6%) had clinical leptospirosis confirmed or suspected in the past three years (Verdugo and Heuer 2009). This figure could well be an under-estimate due to unawareness of the disease by farming and veterinary sectors. The risk of clinical disease depends on management, environment, climate, immuno-competence, and exposure to various sources of infection, thus making it impossible to predict even with reasonable confidence the magnitude of herd, mob or individual animal level losses. A clinical outbreak can be controlled by the use of an antibiotic and vaccination, the former providing short-term effect and the latter providing longer term protection. However, this would rely on early detection since some outbreaks have been explosive, with large losses before detection and diagnosis (Wilson *et al.* 2009).

Cessation of a vaccination programme after a period of whole herd vaccination will result in an increased in risk of clinical disease unless a total prevention of future exposure can be assured. This is because the unvaccinated generation will be

immuno-naïve and therefore at higher risk of disease, should they be exposed to the organism, than a naturally infected herd that has developed herd-level immunity by continuous natural challenge, although disease can still occur in this circumstance. There is no information on the efficacy or duration that vaccine protects leptospiral infection and/or disease in farmed deer. However, studies in cattle and dogs (Hancock *et al.* 1984; Klaasen *et al.* 2003) suggested that the immunity could persist for one year then an annual booster is needed. Thus, a decision to vaccinate has long-term implications (Wilson *et al.* 2009).

Since most deer farming properties are mixed-species, vaccination control programmes need to consider all species on-farm. However, the need to vaccinate all species will depend on establishment of whether sheep are maintenance hosts for *Hardjibovis* or not. For example, if sheep are not maintenance hosts, vaccination of cattle and deer may indirectly reduce or eliminate the risk of infection in sheep. Research into this question needs to be commenced as soon as possible in order to provide evidence for appropriate control measures for leptospirosis on mixed-species farms.

## **8.5 Proposed industry action**

Research undertaken within this thesis is clearly relevant to the 2007 Deer Industry New Zealand (DINZ) productivity strategy which is “*to improve deer farmer profitability and industry sustainability through improved growth and reproductive productivity over the next 5 years to 2012*” (Pearse and Fung 2007). Epidemiological studies provide valuable information on disease distribution and risk factors associated with leptospirosis, whereas the newly developed real-time PCR will be a useful tool for diagnosis and the case definition for future research. Vaccination has proven to be an efficacious means of control and likely to return a cost-effective production response on as many as 67% of deer farms in a single production cycle. The demonstrated effect on growth of up to 6.5 kg contributed approximately 60% of the targeted improvement proposed by the productivity strategy (increase of 5.4 kg carcass weight equivalent to 10 kg live weight at the point of sale) (Pearse and Fung 2008). Furthermore, increasing the weaning rate by 5.7 percentage points contributed to 60% of the strategic productivity target (improvement in weaning percentage of first calving hinds from 72% to 80%) (Pearse and Fung 2008). This suggests that the targeted increase in productivity set by the strategy could be achieved by controlling subclinical leptospirosis. Thus the achievable gains in productivity are likely larger than those defined in the industry’s productivity strategy.

Deer farmers need to be well informed about the risk of leptospirosis, production losses and their relationships to human infection, its epidemiology and control options that are provided and discussed in this thesis, which builds upon recent research within the Massey University Deer Research Group and Leptospirosis Research Unit.

The deer industry organisation, DINZ, should highlight these findings to producers to ensure that deer farmers make well-informed and therefore best-practice decisions tailored to individual circumstances. The New Zealand Veterinary Association (NZVA) should take part in providing up-to-date information for farmers about disease risks in both human and animal health perspectives, production losses associated with leptospirosis and available options for disease control. The best option for control is vaccination and leptospiral vaccines in New Zealand are under prescription by a veterinarian (IVS 2010). Thus the veterinarian has a dual incentive to promote awareness of leptospirosis to their deer farmer clients: improvement of productivity on client's farms, and business revenue. This will, in the long run, help increase the productivity of deer farming in terms of growth and reproduction.

Last but not least, due to the nature of farming in New Zealand which involves multi-species including deer, cattle and sheep, on the same property, DINZ should coordinate with the sheep and beef industries to control leptospirosis. Data of disease dynamics between each species on farms are still lacking and need further investigation. In addition, there is no research into subclinical production losses in sheep and beef cattle in New Zealand similar to that studied in this thesis. Research into these aspects should be undertaken to determine how these effects can be built into an overall extension strategy by the industry. It will be ideal that all industries take part in sharing information and provide funding together for further research since it is unlikely that control for leptospirosis in one species of interest on farms will be efficient.

## **8.6 Areas for further research**

This study has provided significant data on epidemiology of leptospirosis on mixed-species deer farms, a recent molecular diagnostic technique for *Leptospira* and control by means of vaccination. However, it has also raised more research questions and identified several areas that should be addressed in further research. These are summarised below:

- A nationwide epidemiological study of leptospirosis on mixed-species farm that include all farm categories should be conducted to fully understand disease dynamics, production effects and identify potential risk factors for leptospiral infection. Data available from the present study could be used for study design and sample size calculation.
- A study to test whether sheep act as a maintenance host for *Leptospira* should be conducted. This could be done by longitudinal observations on seroprevalence of sheep on farms with or without presence of other species or by vaccinating other species on farms and seeing whether seroprevalence of sheep declined over time. However, appropriate control for other sources of infection is critical.

- Inter-species transmission of *Leptospira* between species on farm should be investigated using MLST and/or other genetic analyses on isolates arising from this study and the mixed-species study proposed above. It should also compare within and between regions of New Zealand.
- Investigation of the possible presence of *Arborea* should be continued and expanded to other livestock species including wildlife vectors and humans to confirm or refute its identity. Southland should be the first region to be targeted for sampling based on data from the study.
- Sheep and beef cattle should be investigated for exotic serovars in the same manner as performed in farmed deer from this study. Serum samples of beef cattle and sheep stored in the serum bank from this study could be randomly selected and used as a preliminary resource that represents Manawatu region.
- The recently developed real-time PCR should be validated, for example by comparison with other reported PCRs, culture and/or MAT using Bayesian methods. Stored DNA samples from this study are available for this purpose.
- A study involving a whole-herd vaccination programme should be conducted. Serology and urinary shedding should be monitored longitudinally to observe the duration of immunity. The study should include all species present on farm. In addition, testing whether sheep are maintenance host as mentioned above could be adjunct with this study.
- Growth and reproduction response in deer observed from this study should be replicated by using the same study design or long term vaccination over at least two successive seasons without prior antibiotic treatment. However, it should try to enrol large number of animals and farms that are high risk to experience natural challenge of *Leptospira*.
- A study to confirm the presence of leptospiral organisms in the female reproductive tract and foetus should be conducted. It should aim for both early and late pregnancy periods. While primiparous hinds should be the primary target group since they are more likely to be recently infected, adult hinds should also be evaluated.

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# Appendix



## Appendix 2a. Raw data of average daily gain (ADG) and serology in male deer.

(Period: 1 = March-May, 2 = May-August, 3 = August-November)

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 1        | 1        | control | 1      | 39.2        | 0           | 0      |
| 2        | 1        | control | 1      | 176.5       | 0           | 0      |
| 3        | 1        | control | 1      | 137.3       | 0           | 0      |
| 4        | 1        | control | 1      | 137.3       | 0           | 0      |
| 5        | 1        | control | 1      | 98.0        | 0           | 0      |
| 6        | 1        | control | 1      | 176.5       | 0           | 0      |
| 7        | 1        | control | 1      | 156.9       | 0           | 0      |
| 8        | 1        | control | 1      | 117.6       | 24          | 0      |
| 9        | 1        | control | 1      | 176.5       | 0           | 0      |
| 10       | 1        | control | 1      | 137.3       | 0           | 0      |
| 11       | 1        | control | 1      | 0           | 0           | 0      |
| 12       | 1        | control | 1      | 176.5       | 48          | 48     |
| 13       | 1        | control | 1      | 137.3       | 0           | 0      |
| 14       | 1        | control | 1      | 58.8        | 0           | 0      |
| 15       | 1        | control | 1      | 58.8        | 0           | 0      |
| 16       | 1        | vaccine | 1      | 176.5       | -           | -      |
| 17       | 1        | vaccine | 1      | 137.3       | -           | -      |
| 18       | 1        | vaccine | 1      | 196.1       | -           | -      |
| 19       | 1        | vaccine | 1      | 196.1       | -           | -      |
| 20       | 1        | vaccine | 1      | 215.7       | -           | -      |
| 21       | 1        | vaccine | 1      | 117.6       | -           | -      |
| 22       | 1        | vaccine | 1      | 176.5       | -           | -      |
| 23       | 1        | vaccine | 1      | 372.5       | -           | -      |
| 24       | 1        | vaccine | 1      | 19.6        | -           | -      |
| 25       | 1        | vaccine | 1      | 137.3       | -           | -      |
| 26       | 1        | vaccine | 1      | 19.6        | -           | -      |
| 27       | 1        | vaccine | 1      | 176.5       | -           | -      |
| 28       | 1        | vaccine | 1      | 235.3       | -           | -      |
| 29       | 1        | vaccine | 1      | 156.9       | -           | -      |
| 30       | 1        | vaccine | 1      | 215.7       | -           | -      |
| 1        | 1        | control | 2      | 162.8       | 0           | 0      |
| 2        | 1        | control | 2      | 46.5        | 0           | 0      |
| 3        | 1        | control | 2      | 81.4        | 0           | 0      |
| 4        | 1        | control | 2      | 11.6        | 0           | 0      |
| 5        | 1        | control | 2      | 34.9        | 0           | 0      |
| 6        | 1        | control | 2      | 11.6        | 0           | 0      |
| 7        | 1        | control | 2      | -23.3       | 0           | 0      |
| 8        | 1        | control | 2      | -           | -           | -      |
| 9        | 1        | control | 2      | 93.0        | 0           | 0      |
| 10       | 1        | control | 2      | 11.6        | 0           | 0      |
| 11       | 1        | control | 2      | 34.9        | 0           | 0      |
| 12       | 1        | control | 2      | -           | 0           | 24     |
| 13       | 1        | control | 2      | -           | 0           | 0      |
| 14       | 1        | control | 2      | 0           | 0           | 0      |
| 15       | 1        | control | 2      | 93.0        | -           | -      |
| 16       | 1        | vaccine | 2      | 104.7       | -           | -      |
| 17       | 1        | vaccine | 2      | 0           | -           | -      |
| 18       | 1        | vaccine | 2      | 0           | -           | -      |
| 19       | 1        | vaccine | 2      | 23.3        | -           | -      |
| 20       | 1        | vaccine | 2      | 11.6        | -           | -      |
| 21       | 1        | vaccine | 2      | 11.6        | -           | -      |
| 22       | 1        | vaccine | 2      | 11.6        | -           | -      |
| 23       | 1        | vaccine | 2      | -11.6       | -           | -      |
| 24       | 1        | vaccine | 2      | 23.3        | -           | -      |
| 25       | 1        | vaccine | 2      | -23.3       | -           | -      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 26       | 1        | vaccine | 2      | 58.1        | -           | -      |
| 27       | 1        | vaccine | 2      | 23.3        | -           | -      |
| 28       | 1        | vaccine | 2      | 23.3        | -           | -      |
| 29       | 1        | vaccine | 2      | 23.3        | -           | -      |
| 30       | 1        | vaccine | 2      | 46.5        | -           | -      |
| 1        | 1        | control | 3      | -           | -           | -      |
| 2        | 1        | control | 3      | 238.5       | -           | -      |
| 3        | 1        | control | 3      | 211.0       | 192         | 0      |
| 4        | 1        | control | 3      | 174.3       | 96          | 0      |
| 5        | 1        | control | 3      | 192.7       | 96          | 0      |
| 6        | 1        | control | 3      | 201.8       | 96          | 0      |
| 7        | 1        | control | 3      | 119.3       | 192         | 0      |
| 8        | 1        | control | 3      | -           | -           | -      |
| 9        | 1        | control | 3      | 211.0       | 192         | 0      |
| 10       | 1        | control | 3      | 238.5       | 48          | 0      |
| 11       | 1        | control | 3      | 247.7       | -           | -      |
| 12       | 1        | control | 3      | -           | -           | -      |
| 13       | 1        | control | 3      | -           | -           | -      |
| 14       | 1        | control | 3      | 211.0       | 24          | 0      |
| 15       | 1        | control | 3      | -           | -           | -      |
| 16       | 1        | vaccine | 3      | -           | -           | -      |
| 17       | 1        | vaccine | 3      | 247.7       | -           | -      |
| 18       | 1        | vaccine | 3      | 247.7       | -           | -      |
| 19       | 1        | vaccine | 3      | -           | -           | -      |
| 20       | 1        | vaccine | 3      | 275.2       | -           | -      |
| 21       | 1        | vaccine | 3      | -           | -           | -      |
| 22       | 1        | vaccine | 3      | 284.4       | -           | -      |
| 23       | 1        | vaccine | 3      | -           | -           | -      |
| 24       | 1        | vaccine | 3      | -           | -           | -      |
| 25       | 1        | vaccine | 3      | -           | -           | -      |
| 26       | 1        | vaccine | 3      | 293.6       | -           | -      |
| 27       | 1        | vaccine | 3      | 211.0       | -           | -      |
| 28       | 1        | vaccine | 3      | 220.2       | -           | -      |
| 29       | 1        | vaccine | 3      | -           | -           | -      |
| 30       | 1        | vaccine | 3      | -           | -           | -      |
| 31       | 2        | control | 1      | 74.1        | 0           | 0      |
| 32       | 2        | control | 1      | -37.0       | 0           | 0      |
| 33       | 2        | control | 1      | 120.4       | 0           | 0      |
| 34       | 2        | control | 1      | -46.3       | 0           | 0      |
| 35       | 2        | control | 1      | -9.3        | 0           | 0      |
| 36       | 2        | control | 1      | 74.1        | 0           | 0      |
| 37       | 2        | control | 1      | 46.3        | 0           | 0      |
| 38       | 2        | control | 1      | 83.3        | 0           | 0      |
| 39       | 2        | control | 1      | 37.0        | 0           | 0      |
| 40       | 2        | control | 1      | 37.0        | 0           | 0      |
| 41       | 2        | control | 1      | 46.3        | 0           | 0      |
| 42       | 2        | control | 1      | 18.5        | 0           | 0      |
| 43       | 2        | control | 1      | 64.8        | 0           | 0      |
| 44       | 2        | control | 1      | 111.1       | 0           | 0      |
| 45       | 2        | control | 1      | 0           | 0           | 0      |
| 46       | 2        | control | 1      | -18.5       | 0           | 0      |
| 47       | 2        | control | 1      | 64.8        | 0           | 0      |
| 48       | 2        | control | 1      | -           | -           | -      |
| 49       | 2        | control | 1      | 101.9       | 0           | 0      |
| 50       | 2        | control | 1      | 92.6        | 0           | 0      |
| 51       | 2        | vaccine | 1      | 74.1        | -           | -      |
| 52       | 2        | vaccine | 1      | 55.6        | -           | -      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 53       | 2        | vaccine | 1      | 0           | -           | -      |
| 54       | 2        | vaccine | 1      | 46.3        | -           | -      |
| 55       | 2        | vaccine | 1      | 64.8        | -           | -      |
| 56       | 2        | vaccine | 1      | 37.0        | -           | -      |
| 57       | 2        | vaccine | 1      | 0           | -           | -      |
| 58       | 2        | vaccine | 1      | 111.1       | -           | -      |
| 59       | 2        | vaccine | 1      | 74.1        | -           | -      |
| 60       | 2        | vaccine | 1      | 27.8        | -           | -      |
| 61       | 2        | vaccine | 1      | 37.0        | -           | -      |
| 62       | 2        | vaccine | 1      | 92.6        | -           | -      |
| 63       | 2        | vaccine | 1      | 83.3        | -           | -      |
| 64       | 2        | vaccine | 1      | 92.6        | -           | -      |
| 65       | 2        | vaccine | 1      | 92.6        | -           | -      |
| 66       | 2        | vaccine | 1      | 74.1        | -           | -      |
| 67       | 2        | vaccine | 1      | 120.4       | -           | -      |
| 68       | 2        | vaccine | 1      | 55.6        | -           | -      |
| 69       | 2        | vaccine | 1      | 111.1       | -           | -      |
| 70       | 2        | vaccine | 1      | 55.6        | -           | -      |
| 31       | 2        | control | 2      | 37.6        | 0           | 0      |
| 32       | 2        | control | 2      | 96.8        | 0           | 0      |
| 33       | 2        | control | 2      | 48.4        | 0           | 0      |
| 34       | 2        | control | 2      | 53.8        | 0           | 0      |
| 35       | 2        | control | 2      | 129.0       | 0           | 0      |
| 36       | 2        | control | 2      | 64.5        | 0           | 0      |
| 37       | 2        | control | 2      | 129.0       | 0           | 0      |
| 38       | 2        | control | 2      | 59.1        | 0           | 0      |
| 39       | 2        | control | 2      | 80.6        | 0           | 0      |
| 40       | 2        | control | 2      | 139.8       | 192         | 0      |
| 41       | 2        | control | 2      | 48.4        | 0           | 0      |
| 42       | 2        | control | 2      | 37.6        | 0           | 0      |
| 43       | 2        | control | 2      | 0.0         | 0           | 0      |
| 44       | 2        | control | 2      | 80.6        | 96          | 0      |
| 45       | 2        | control | 2      | 102.2       | 0           | 0      |
| 46       | 2        | control | 2      | 0           | -           | -      |
| 47       | 2        | control | 2      | 53.8        | 0           | 0      |
| 48       | 2        | control | 2      | -           | -           | -      |
| 49       | 2        | control | 2      | 59.1        | 0           | 0      |
| 50       | 2        | control | 2      | 53.8        | 0           | 0      |
| 51       | 2        | vaccine | 2      | 75.3        | -           | -      |
| 52       | 2        | vaccine | 2      | 75.3        | -           | -      |
| 53       | 2        | vaccine | 2      | 91.4        | -           | -      |
| 54       | 2        | vaccine | 2      | 48.4        | -           | -      |
| 55       | 2        | vaccine | 2      | 43.0        | -           | -      |
| 56       | 2        | vaccine | 2      | 53.8        | -           | -      |
| 57       | 2        | vaccine | 2      | -           | -           | -      |
| 58       | 2        | vaccine | 2      | 64.5        | -           | -      |
| 59       | 2        | vaccine | 2      | 60.2        | -           | -      |
| 60       | 2        | vaccine | 2      | 80.6        | -           | -      |
| 61       | 2        | vaccine | 2      | 145.2       | -           | -      |
| 62       | 2        | vaccine | 2      | 53.8        | -           | -      |
| 63       | 2        | vaccine | 2      | 64.5        | -           | -      |
| 64       | 2        | vaccine | 2      | 107.5       | -           | -      |
| 65       | 2        | vaccine | 2      | 91.4        | -           | -      |
| 66       | 2        | vaccine | 2      | 64.5        | -           | -      |
| 67       | 2        | vaccine | 2      | 37.6        | -           | -      |
| 68       | 2        | vaccine | 2      | 43.0        | -           | -      |
| 69       | 2        | vaccine | 2      | 91.4        | -           | -      |



| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 70       | 2        | vaccine | 2      | 69.9        | -           | -      |
| 31       | 2        | control | 3      | 89.9        | 0           | 0      |
| 32       | 2        | control | 3      | 44.9        | 0           | 0      |
| 33       | 2        | control | 3      | 118.0       | 0           | 0      |
| 34       | 2        | control | 3      | 146.1       | 0           | 0      |
| 35       | 2        | control | 3      | 185.4       | 0           | 0      |
| 36       | 2        | control | 3      | 118.0       | 0           | 0      |
| 37       | 2        | control | 3      | 224.7       | 0           | 0      |
| 38       | 2        | control | 3      | 207.9       | 0           | 0      |
| 39       | 2        | control | 3      | 179.8       | 0           | 0      |
| 40       | 2        | control | 3      | 146.1       | 48          | 0      |
| 41       | 2        | control | 3      | 213.5       | 0           | 0      |
| 42       | 2        | control | 3      | 202.2       | 0           | 0      |
| 43       | 2        | control | 3      | -           | 0           | 0      |
| 44       | 2        | control | 3      | 174.2       | 0           | 0      |
| 45       | 2        | control | 3      | 185.4       | 0           | 0      |
| 46       | 2        | control | 3      | -           | 0           | 0      |
| 47       | 2        | control | 3      | 174.2       | 0           | 0      |
| 48       | 2        | control | 3      | -           | 0           | 0      |
| 49       | 2        | control | 3      | 207.9       | 0           | 0      |
| 50       | 2        | control | 3      | 207.9       | 0           | 0      |
| 51       | 2        | vaccine | 3      | 112.4       | -           | -      |
| 52       | 2        | vaccine | 3      | 213.5       | -           | -      |
| 53       | 2        | vaccine | 3      | 179.8       | -           | -      |
| 54       | 2        | vaccine | 3      | 157.3       | -           | -      |
| 55       | 2        | vaccine | 3      | 191.0       | -           | -      |
| 56       | 2        | vaccine | 3      | -           | -           | -      |
| 57       | 2        | vaccine | 3      | -           | -           | -      |
| 58       | 2        | vaccine | 3      | 219.1       | -           | -      |
| 59       | 2        | vaccine | 3      | 189.9       | -           | -      |
| 60       | 2        | vaccine | 3      | 196.6       | -           | -      |
| 61       | 2        | vaccine | 3      | 174.2       | -           | -      |
| 62       | 2        | vaccine | 3      | 162.9       | -           | -      |
| 63       | 2        | vaccine | 3      | 157.3       | -           | -      |
| 64       | 2        | vaccine | 3      | 168.5       | -           | -      |
| 65       | 2        | vaccine | 3      | 241.6       | -           | -      |
| 66       | 2        | vaccine | 3      | 168.5       | -           | -      |
| 67       | 2        | vaccine | 3      | 196.6       | -           | -      |
| 68       | 2        | vaccine | 3      | 207.9       | -           | -      |
| 69       | 2        | vaccine | 3      | 191.0       | -           | -      |
| 70       | 2        | vaccine | 3      | 168.5       | -           | -      |
| 71       | 3        | control | 1      | 50.9        | 0           | 0      |
| 72       | 3        | control | 1      | 9.1         | 0           | 0      |
| 73       | 3        | control | 1      | 21.8        | 0           | 0      |
| 74       | 3        | control | 1      | 98.2        | 0           | 0      |
| 75       | 3        | control | 1      | 10.9        | 0           | 0      |
| 76       | 3        | control | 1      | 21.8        | 24          | 0      |
| 77       | 3        | control | 1      | 27.3        | 0           | 0      |
| 78       | 3        | control | 1      | 54.5        | 0           | 0      |
| 79       | 3        | control | 1      | 50.9        | 0           | 0      |
| 80       | 3        | control | 1      | 43.6        | 0           | 0      |
| 81       | 3        | control | 1      | -36.4       | 0           | 0      |
| 82       | 3        | control | 1      | 21.8        | 0           | 0      |
| 83       | 3        | control | 1      | 29.1        | 0           | 0      |
| 84       | 3        | control | 1      | 18.2        | 0           | 0      |
| 85       | 3        | control | 1      | 18.2        | 0           | 0      |
| 86       | 3        | control | 1      | 36.4        | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 87       | 3        | control | 1      | 45.5        | 0           | 0      |
| 88       | 3        | control | 1      | 43.6        | 0           | 0      |
| 89       | 3        | vaccine | 1      | 27.3        | -           | -      |
| 90       | 3        | vaccine | 1      | 43.6        | -           | -      |
| 91       | 3        | vaccine | 1      | 29.1        | -           | -      |
| 92       | 3        | vaccine | 1      | -10.9       | -           | -      |
| 93       | 3        | vaccine | 1      | 0           | -           | -      |
| 94       | 3        | vaccine | 1      | -21.8       | -           | -      |
| 95       | 3        | vaccine | 1      | -134.5      | -           | -      |
| 96       | 3        | vaccine | 1      | 18.2        | -           | -      |
| 97       | 3        | vaccine | 1      | -14.5       | -           | -      |
| 98       | 3        | vaccine | 1      | 87.3        | -           | -      |
| 99       | 3        | vaccine | 1      | -50.9       | -           | -      |
| 100      | 3        | vaccine | 1      | 18.2        | -           | -      |
| 101      | 3        | vaccine | 1      | -9.1        | -           | -      |
| 102      | 3        | vaccine | 1      | -50.9       | -           | -      |
| 103      | 3        | vaccine | 1      | 3.6         | -           | -      |
| 104      | 3        | vaccine | 1      | 36.4        | -           | -      |
| 105      | 3        | vaccine | 1      | 36.4        | -           | -      |
| 106      | 3        | vaccine | 1      | 27.3        | -           | -      |
| 107      | 3        | vaccine | 1      | -9.1        | -           | -      |
| 108      | 3        | vaccine | 1      | 43.6        | -           | -      |
| 109      | 3        | vaccine | 1      | 29.1        | -           | -      |
| 71       | 3        | control | 2      | -           | -           | -      |
| 72       | 3        | control | 2      | 0           | 0           | 0      |
| 73       | 3        | control | 2      | 28.9        | 0           | 0      |
| 74       | 3        | control | 2      | -26.8       | 0           | 0      |
| 75       | 3        | control | 2      | -           | -           | -      |
| 76       | 3        | control | 2      | -4.1        | 0           | 0      |
| 77       | 3        | control | 2      | -25.8       | 0           | 0      |
| 78       | 3        | control | 2      | -16.5       | 0           | 0      |
| 79       | 3        | control | 2      | -5.2        | 0           | 0      |
| 80       | 3        | control | 2      | 30.9        | 0           | 0      |
| 81       | 3        | control | 2      | -56.7       | 0           | 0      |
| 82       | 3        | control | 2      | 6.2         | 0           | 0      |
| 83       | 3        | control | 2      | -           | -           | -      |
| 84       | 3        | control | 2      | 67.0        | 0           | 0      |
| 85       | 3        | control | 2      | 61.9        | 0           | 0      |
| 86       | 3        | control | 2      | -28.9       | 0           | 0      |
| 87       | 3        | control | 2      | 5.2         | 0           | 0      |
| 88       | 3        | control | 2      | 0           | 0           | 0      |
| 89       | 3        | vaccine | 2      | -24.7       | -           | -      |
| 90       | 3        | vaccine | 2      | 10.3        | -           | -      |
| 91       | 3        | vaccine | 2      | 6.2         | -           | -      |
| 92       | 3        | vaccine | 2      | 28.9        | -           | -      |
| 93       | 3        | vaccine | 2      | 15.5        | -           | -      |
| 94       | 3        | vaccine | 2      | -6.2        | -           | -      |
| 95       | 3        | vaccine | 2      | 35.1        | -           | -      |
| 96       | 3        | vaccine | 2      | -           | -           | -      |
| 97       | 3        | vaccine | 2      | -8.2        | -           | -      |
| 98       | 3        | vaccine | 2      | 4.1         | -           | -      |
| 99       | 3        | vaccine | 2      | 2.1         | -           | -      |
| 100      | 3        | vaccine | 2      | -16.5       | -           | -      |
| 101      | 3        | vaccine | 2      | -32.0       | -           | -      |
| 102      | 3        | vaccine | 2      | -           | -           | -      |
| 103      | 3        | vaccine | 2      | 15.5        | -           | -      |
| 104      | 3        | vaccine | 2      | -           | -           | -      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 105      | 3        | vaccine | 2      | -6.2        | -           | -      |
| 106      | 3        | vaccine | 2      | -15.5       | -           | -      |
| 107      | 3        | vaccine | 2      | 56.7        | -           | -      |
| 108      | 3        | vaccine | 2      | 14.4        | -           | -      |
| 109      | 3        | vaccine | 2      | -16.5       | -           | -      |
| 71       | 3        | control | 3      | -           | -           | -      |
| 72       | 3        | control | 3      | 300.0       | 0           | 0      |
| 73       | 3        | control | 3      | 237.4       | 0           | 0      |
| 74       | 3        | control | 3      | 276.6       | 0           | 0      |
| 75       | 3        | control | 3      | -           | 0           | 0      |
| 76       | 3        | control | 3      | 65.4        | 0           | 0      |
| 77       | 3        | control | 3      | 287.9       | 48          | 0      |
| 78       | 3        | control | 3      | 39.3        | 0           | 0      |
| 79       | 3        | control | 3      | 172.9       | 0           | 0      |
| 80       | 3        | control | 3      | 246.7       | 0           | 0      |
| 81       | 3        | control | 3      | -33.6       | 0           | 0      |
| 82       | 3        | control | 3      | 175.7       | 24          | 0      |
| 83       | 3        | control | 3      | -           | -           | -      |
| 84       | 3        | control | 3      | 276.6       | 0           | 0      |
| 85       | 3        | control | 3      | 200.0       | 0           | 0      |
| 86       | 3        | control | 3      | 170.1       | 0           | 0      |
| 87       | 3        | control | 3      | 190.7       | 0           | 0      |
| 88       | 3        | control | 3      | -           | -           | -      |
| 89       | 3        | vaccine | 3      | 284.1       | -           | -      |
| 90       | 3        | vaccine | 3      | 375.7       | -           | -      |
| 91       | 3        | vaccine | 3      | 244.9       | -           | -      |
| 92       | 3        | vaccine | 3      | 250.5       | -           | -      |
| 93       | 3        | vaccine | 3      | 296.3       | -           | -      |
| 94       | 3        | vaccine | 3      | -           | -           | -      |
| 95       | 3        | vaccine | 3      | 373.8       | -           | -      |
| 96       | 3        | vaccine | 3      | -           | -           | -      |
| 97       | 3        | vaccine | 3      | 196.3       | -           | -      |
| 98       | 3        | vaccine | 3      | 207.5       | -           | -      |
| 99       | 3        | vaccine | 3      | 388.8       | -           | -      |
| 100      | 3        | vaccine | 3      | -           | -           | -      |
| 101      | 3        | vaccine | 3      | -           | -           | -      |
| 102      | 3        | vaccine | 3      | -           | -           | -      |
| 103      | 3        | vaccine | 3      | -           | -           | -      |
| 104      | 3        | vaccine | 3      | -           | -           | -      |
| 105      | 3        | vaccine | 3      | 343.9       | -           | -      |
| 106      | 3        | vaccine | 3      | 314.0       | -           | -      |
| 107      | 3        | vaccine | 3      | 94.4        | -           | -      |
| 108      | 3        | vaccine | 3      | 181.3       | -           | -      |
| 109      | 3        | vaccine | 3      | -           | -           | -      |
| 110      | 4        | control | 1      | 166.7       | 0           | 0      |
| 111      | 4        | control | 1      | 107.8       | 0           | 0      |
| 112      | 4        | control | 1      | 166.7       | 0           | 0      |
| 113      | 4        | control | 1      | 137.3       | 0           | 0      |
| 114      | 4        | control | 1      | 156.9       | 0           | 0      |
| 115      | 4        | control | 1      | 49.0        | 0           | 0      |
| 116      | 4        | control | 1      | 137.3       | 0           | 0      |
| 117      | 4        | control | 1      | -           | -           | -      |
| 118      | 4        | control | 1      | 166.7       | 0           | 0      |
| 119      | 4        | control | 1      | -117.6      | 0           | 0      |
| 120      | 4        | control | 1      | 107.8       | 0           | 0      |
| 121      | 4        | control | 1      | 127.5       | 0           | 0      |
| 122      | 4        | control | 1      | 186.3       | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 123      | 4        | control | 1      | 254.9       | 0           | 0      |
| 124      | 4        | control | 1      | 107.8       | 0           | 0      |
| 125      | 4        | control | 1      | 98.0        | 0           | 0      |
| 126      | 4        | control | 1      | 117.6       | 0           | 0      |
| 127      | 4        | control | 1      | 156.9       | 0           | 0      |
| 128      | 4        | vaccine | 1      | 156.9       | -           | -      |
| 129      | 4        | vaccine | 1      | 284.3       | -           | -      |
| 130      | 4        | vaccine | 1      | 88.2        | -           | -      |
| 131      | 4        | vaccine | 1      | 19.6        | -           | -      |
| 132      | 4        | vaccine | 1      | 98.0        | -           | -      |
| 133      | 4        | vaccine | 1      | 186.3       | -           | -      |
| 134      | 4        | vaccine | 1      | 215.7       | -           | -      |
| 135      | 4        | vaccine | 1      | 205.9       | -           | -      |
| 136      | 4        | vaccine | 1      | 39.2        | -           | -      |
| 137      | 4        | vaccine | 1      | 107.8       | -           | -      |
| 138      | 4        | vaccine | 1      | 127.5       | -           | -      |
| 139      | 4        | vaccine | 1      | 29.4        | -           | -      |
| 140      | 4        | vaccine | 1      | 147.1       | -           | -      |
| 141      | 4        | vaccine | 1      | 19.6        | -           | -      |
| 142      | 4        | vaccine | 1      | 205.9       | -           | -      |
| 143      | 4        | vaccine | 1      | -156.9      | -           | -      |
| 144      | 4        | vaccine | 1      | 58.8        | -           | -      |
| 145      | 4        | vaccine | 1      | 39.2        | -           | -      |
| 146      | 4        | vaccine | 1      | 88.2        | -           | -      |
| 147      | 4        | vaccine | 1      | 156.9       | -           | -      |
| 110      | 4        | control | 2      | 41.7        | 0           | 0      |
| 111      | 4        | control | 2      | 41.7        | 0           | 0      |
| 112      | 4        | control | 2      | 36.5        | 0           | 0      |
| 113      | 4        | control | 2      | -15.6       | 0           | 0      |
| 114      | 4        | control | 2      | -10.4       | 0           | 0      |
| 115      | 4        | control | 2      | 83.3        | 0           | 0      |
| 116      | 4        | control | 2      | 31.3        | 0           | 0      |
| 117      | 4        | control | 2      | -           | 0           | 0      |
| 118      | 4        | control | 2      | 0           | 0           | 0      |
| 119      | 4        | control | 2      | 36.5        | 0           | 0      |
| 120      | 4        | control | 2      | 78.1        | 0           | 0      |
| 121      | 4        | control | 2      | -10.4       | 0           | 0      |
| 122      | 4        | control | 2      | -62.5       | 0           | 0      |
| 123      | 4        | control | 2      | 10.4        | 0           | 0      |
| 124      | 4        | control | 2      | 78.1        | 0           | 0      |
| 125      | 4        | control | 2      | 104.2       | 0           | 0      |
| 126      | 4        | control | 2      | 99.0        | 0           | 0      |
| 127      | 4        | control | 2      | 46.9        | 0           | 0      |
| 128      | 4        | vaccine | 2      | 41.7        | -           | -      |
| 129      | 4        | vaccine | 2      | 41.7        | -           | -      |
| 130      | 4        | vaccine | 2      | -5.2        | -           | -      |
| 131      | 4        | vaccine | 2      | 161.5       | -           | -      |
| 132      | 4        | vaccine | 2      | 41.7        | -           | -      |
| 133      | 4        | vaccine | 2      | 72.9        | -           | -      |
| 134      | 4        | vaccine | 2      | 67.7        | -           | -      |
| 135      | 4        | vaccine | 2      | -57.3       | -           | -      |
| 136      | 4        | vaccine | 2      | 5.2         | -           | -      |
| 137      | 4        | vaccine | 2      | 72.9        | -           | -      |
| 138      | 4        | vaccine | 2      | 0           | -           | -      |
| 139      | 4        | vaccine | 2      | -           | -           | -      |
| 140      | 4        | vaccine | 2      | 62.5        | -           | -      |
| 141      | 4        | vaccine | 2      | 36.5        | -           | -      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 142      | 4        | vaccine | 2      | 52.1        | -           | -      |
| 143      | 4        | vaccine | 2      | 78.1        | -           | -      |
| 144      | 4        | vaccine | 2      | 72.9        | -           | -      |
| 145      | 4        | vaccine | 2      | 10.4        | -           | -      |
| 146      | 4        | vaccine | 2      | 72.9        | -           | -      |
| 147      | 4        | vaccine | 2      | 0           | -           | -      |
| 110      | 4        | control | 3      | 198.9       | 0           | 0      |
| 111      | 4        | control | 3      | 182.8       | 0           | 0      |
| 112      | 4        | control | 3      | 193.5       | 0           | 0      |
| 113      | 4        | control | 3      | 215.1       | 0           | 0      |
| 114      | 4        | control | 3      | 279.6       | 0           | 0      |
| 115      | 4        | control | 3      | 247.3       | 0           | 0      |
| 116      | 4        | control | 3      | 215.1       | 0           | 0      |
| 117      | 4        | control | 3      | -21.5       | 0           | 0      |
| 118      | 4        | control | 3      | 204.3       | 0           | 0      |
| 119      | 4        | control | 3      | 129.0       | 0           | 0      |
| 120      | 4        | control | 3      | 241.9       | 0           | 0      |
| 121      | 4        | control | 3      | 274.2       | 0           | 0      |
| 122      | 4        | control | 3      | 317.2       | 0           | 0      |
| 123      | 4        | control | 3      | 220.4       | 0           | 0      |
| 124      | 4        | control | 3      | 188.2       | 0           | 0      |
| 125      | 4        | control | 3      | 172.0       | 0           | 0      |
| 126      | 4        | control | 3      | 21.5        | 0           | 0      |
| 127      | 4        | control | 3      | 155.9       | 0           | 0      |
| 128      | 4        | vaccine | 3      | 209.7       | -           | -      |
| 129      | 4        | vaccine | 3      | 86.0        | -           | -      |
| 130      | 4        | vaccine | 3      | 182.8       | -           | -      |
| 131      | 4        | vaccine | 3      | 204.3       | -           | -      |
| 132      | 4        | vaccine | 3      | 166.7       | -           | -      |
| 133      | 4        | vaccine | 3      | 182.8       | -           | -      |
| 134      | 4        | vaccine | 3      | 134.4       | -           | -      |
| 135      | 4        | vaccine | 3      | 263.4       | -           | -      |
| 136      | 4        | vaccine | 3      | 166.7       | -           | -      |
| 137      | 4        | vaccine | 3      | 263.4       | -           | -      |
| 138      | 4        | vaccine | 3      | 220.4       | -           | -      |
| 139      | 4        | vaccine | 3      | -           | -           | -      |
| 140      | 4        | vaccine | 3      | 177.4       | -           | -      |
| 141      | 4        | vaccine | 3      | 215.1       | -           | -      |
| 142      | 4        | vaccine | 3      | 220.4       | -           | -      |
| 143      | 4        | vaccine | 3      | 177.4       | -           | -      |
| 144      | 4        | vaccine | 3      | 215.1       | -           | -      |
| 145      | 4        | vaccine | 3      | 274.2       | -           | -      |
| 146      | 4        | vaccine | 3      | 145.2       | -           | -      |
| 147      | 4        | vaccine | 3      | 21.5        | -           | -      |
| 148      | 5        | control | 1      | -18.5       | 0           | 0      |
| 149      | 5        | control | 1      | 18.5        | 0           | 0      |
| 150      | 5        | control | 1      | -           | 0           | 0      |
| 151      | 5        | control | 1      | 37.0        | 0           | 0      |
| 152      | 5        | control | 1      | 46.3        | 0           | 0      |
| 153      | 5        | control | 1      | 37.0        | 0           | 0      |
| 154      | 5        | control | 1      | -           | -           | -      |
| 155      | 5        | control | 1      | 64.8        | 0           | 0      |
| 156      | 5        | control | 1      | 64.8        | 0           | 0      |
| 157      | 5        | control | 1      | 74.1        | 0           | 0      |
| 158      | 5        | control | 1      | 64.8        | 0           | 0      |
| 159      | 5        | control | 1      | -9.3        | 0           | 0      |
| 160      | 5        | control | 1      | -           | -           | -      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 161      | 5        | control | 1      | 101.9       | 0           | 0      |
| 162      | 5        | control | 1      | 83.3        | 0           | 0      |
| 163      | 5        | control | 1      | -18.5       | 0           | 0      |
| 164      | 5        | control | 1      | 74.1        | 0           | 0      |
| 165      | 5        | control | 1      | 148.1       | 0           | 0      |
| 166      | 5        | control | 1      | 64.8        | 0           | 0      |
| 167      | 5        | control | 1      | 129.6       | -           | -      |
| 168      | 5        | control | 1      | 83.3        | 0           | 0      |
| 169      | 5        | control | 1      | 111.1       | 0           | 0      |
| 170      | 5        | control | 1      | 27.8        | 0           | 0      |
| 171      | 5        | control | 1      | 101.9       | 0           | 0      |
| 172      | 5        | vaccine | 1      | 37.0        | -           | -      |
| 173      | 5        | vaccine | 1      | 83.3        | -           | -      |
| 174      | 5        | vaccine | 1      | 74.1        | -           | -      |
| 175      | 5        | vaccine | 1      | -           | -           | -      |
| 176      | 5        | vaccine | 1      | 55.6        | -           | -      |
| 177      | 5        | vaccine | 1      | 129.6       | -           | -      |
| 178      | 5        | vaccine | 1      | -9.3        | -           | -      |
| 179      | 5        | vaccine | 1      | 9.3         | -           | -      |
| 180      | 5        | vaccine | 1      | 129.6       | -           | -      |
| 181      | 5        | vaccine | 1      | 120.4       | -           | -      |
| 182      | 5        | vaccine | 1      | 120.4       | -           | -      |
| 183      | 5        | vaccine | 1      | 55.6        | -           | -      |
| 184      | 5        | vaccine | 1      | -           | -           | -      |
| 185      | 5        | vaccine | 1      | 101.9       | -           | -      |
| 186      | 5        | vaccine | 1      | 64.8        | -           | -      |
| 187      | 5        | vaccine | 1      | 120.4       | -           | -      |
| 188      | 5        | vaccine | 1      | 64.8        | -           | -      |
| 189      | 5        | vaccine | 1      | 92.6        | -           | -      |
| 190      | 5        | vaccine | 1      | 55.6        | -           | -      |
| 191      | 5        | vaccine | 1      | 166.7       | -           | -      |
| 192      | 5        | vaccine | 1      | 0           | -           | -      |
| 193      | 5        | vaccine | 1      | 157.4       | -           | -      |
| 194      | 5        | vaccine | 1      | 92.6        | -           | -      |
| 195      | 5        | vaccine | 1      | 27.8        | -           | -      |
| 148      | 5        | control | 2      | 0           | 0           | 0      |
| 149      | 5        | control | 2      | -           | 0           | 0      |
| 150      | 5        | control | 2      | -           | -           | -      |
| 151      | 5        | control | 2      | 19.6        | 0           | 0      |
| 152      | 5        | control | 2      | 9.8         | 0           | 0      |
| 153      | 5        | control | 2      | 49.0        | 0           | 0      |
| 154      | 5        | control | 2      | -           | 0           | 0      |
| 155      | 5        | control | 2      | 49.0        | 0           | 0      |
| 156      | 5        | control | 2      | 24.5        | 0           | 0      |
| 157      | 5        | control | 2      | 93.1        | 0           | 0      |
| 158      | 5        | control | 2      | 19.6        | 0           | 0      |
| 159      | 5        | control | 2      | -24.5       | 0           | 0      |
| 160      | 5        | control | 2      | -           | 0           | 0      |
| 161      | 5        | control | 2      | 14.7        | 0           | 0      |
| 162      | 5        | control | 2      | 49.0        | 0           | 0      |
| 163      | 5        | control | 2      | 29.4        | 0           | 0      |
| 164      | 5        | control | 2      | -4.9        | 0           | 0      |
| 165      | 5        | control | 2      | 53.9        | 0           | 0      |
| 166      | 5        | control | 2      | -           | -           | -      |
| 167      | 5        | control | 2      | 19.6        | 0           | 0      |
| 168      | 5        | control | 2      | 53.9        | 0           | 0      |
| 169      | 5        | control | 2      | 53.9        | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 170      | 5        | control | 2      | 29.4        | 0           | 0      |
| 171      | 5        | control | 2      | 68.6        | 0           | 0      |
| 172      | 5        | vaccine | 2      | 29.4        | -           | -      |
| 173      | 5        | vaccine | 2      | 0           | -           | -      |
| 174      | 5        | vaccine | 2      | 88.2        | -           | -      |
| 175      | 5        | vaccine | 2      | -           | -           | -      |
| 176      | 5        | vaccine | 2      | 4.9         | -           | -      |
| 177      | 5        | vaccine | 2      | 0           | -           | -      |
| 178      | 5        | vaccine | 2      | -19.6       | -           | -      |
| 179      | 5        | vaccine | 2      | 49.0        | -           | -      |
| 180      | 5        | vaccine | 2      | 14.7        | -           | -      |
| 181      | 5        | vaccine | 2      | 34.3        | -           | -      |
| 182      | 5        | vaccine | 2      | 29.4        | -           | -      |
| 183      | 5        | vaccine | 2      | 44.1        | -           | -      |
| 184      | 5        | vaccine | 2      | -           | -           | -      |
| 185      | 5        | vaccine | 2      | 49.0        | -           | -      |
| 186      | 5        | vaccine | 2      | 58.8        | -           | -      |
| 187      | 5        | vaccine | 2      | -           | -           | -      |
| 188      | 5        | vaccine | 2      | 53.9        | -           | -      |
| 189      | 5        | vaccine | 2      | 34.3        | -           | -      |
| 190      | 5        | vaccine | 2      | 44.1        | -           | -      |
| 191      | 5        | vaccine | 2      | 9.8         | -           | -      |
| 192      | 5        | vaccine | 2      | 29.4        | -           | -      |
| 193      | 5        | vaccine | 2      | -29.4       | -           | -      |
| 194      | 5        | vaccine | 2      | -4.9        | -           | -      |
| 195      | 5        | vaccine | 2      | 29.4        | -           | -      |
| 148      | 5        | control | 3      | 260.6       | 0           | 0      |
| 149      | 5        | control | 3      | -           | 0           | 0      |
| 150      | 5        | control | 3      | -           | -           | -      |
| 151      | 5        | control | 3      | 271.3       | 0           | 0      |
| 152      | 5        | control | 3      | 223.4       | 0           | 0      |
| 153      | 5        | control | 3      | 271.3       | 0           | 0      |
| 154      | 5        | control | 3      | 297.9       | 0           | 0      |
| 155      | 5        | control | 3      | 292.6       | 0           | 0      |
| 156      | 5        | control | 3      | 271.3       | 0           | 0      |
| 157      | 5        | control | 3      | 308.5       | 0           | 0      |
| 158      | 5        | control | 3      | 218.1       | 0           | 0      |
| 159      | 5        | control | 3      | 106.4       | 0           | 0      |
| 160      | 5        | control | 3      | -           | -           | -      |
| 161      | 5        | control | 3      | 133.0       | 0           | 0      |
| 162      | 5        | control | 3      | 223.4       | 0           | 0      |
| 163      | 5        | control | 3      | 266.0       | 0           | 0      |
| 164      | 5        | control | 3      | 239.4       | 0           | 0      |
| 165      | 5        | control | 3      | 207.4       | 0           | 0      |
| 166      | 5        | control | 3      | -           | 0           | 0      |
| 167      | 5        | control | 3      | 239.4       | 0           | 0      |
| 168      | 5        | control | 3      | 260.6       | 0           | 0      |
| 169      | 5        | control | 3      | -           | -           | -      |
| 170      | 5        | control | 3      | -           | -           | -      |
| 171      | 5        | control | 3      | 143.6       | 0           | 0      |
| 172      | 5        | vaccine | 3      | 281.9       | -           | -      |
| 173      | 5        | vaccine | 3      | 186.2       | -           | -      |
| 174      | 5        | vaccine | 3      | 223.4       | -           | -      |
| 175      | 5        | vaccine | 3      | 170.2       | -           | -      |
| 176      | 5        | vaccine | 3      | 79.8        | -           | -      |
| 177      | 5        | vaccine | 3      | 223.4       | -           | -      |
| 178      | 5        | vaccine | 3      | 138.3       | -           | -      |

| Deer no. | Farm no. | Status  | Period | ADG (g/day) | Hardjobovis | Pomona |
|----------|----------|---------|--------|-------------|-------------|--------|
| 179      | 5        | vaccine | 3      | 260.6       | -           | -      |
| 180      | 5        | vaccine | 3      | 297.9       | -           | -      |
| 181      | 5        | vaccine | 3      | 287.2       | -           | -      |
| 182      | 5        | vaccine | 3      | 212.8       | -           | -      |
| 183      | 5        | vaccine | 3      | -           | -           | -      |
| 184      | 5        | vaccine | 3      | 159.6       | -           | -      |
| 185      | 5        | vaccine | 3      | 260.6       | -           | -      |
| 186      | 5        | vaccine | 3      | 239.4       | -           | -      |
| 187      | 5        | vaccine | 3      | -           | -           | -      |
| 188      | 5        | vaccine | 3      | -           | -           | -      |
| 189      | 5        | vaccine | 3      | 202.1       | -           | -      |
| 190      | 5        | vaccine | 3      | 276.6       | -           | -      |
| 191      | 5        | vaccine | 3      | 255.3       | -           | -      |
| 192      | 5        | vaccine | 3      | 191.5       | -           | -      |
| 193      | 5        | vaccine | 3      | 324.5       | -           | -      |
| 194      | 5        | vaccine | 3      | 271.3       | -           | -      |
| 195      | 5        | vaccine | 3      | 250.0       | -           | -      |



## Appendix 2b. Raw data of urine shedding and serology in female deer.

(Period: 1 = March, 2 = May, 3 = August, 4 = November)(Culture and PCR: 0 = negative, 1 = positive)

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 1        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 2        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 3        | 1        | control | 1      | 0       | 0   | 24          | 0      |
| 4        | 1        | control | 1      | 0       | 0   | 48          | 0      |
| 5        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 6        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 7        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 8        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 9        | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 10       | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 11       | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 12       | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 13       | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 14       | 1        | control | 1      | 0       | 0   | 0           | 0      |
| 15       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 16       | 1        | vaccine | 1      | 0       | 0   | 48          | 0      |
| 17       | 1        | vaccine | 1      | 0       | 0   | 48          | 0      |
| 18       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 19       | 1        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 20       | 1        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 21       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 22       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 23       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 24       | 1        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 25       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 26       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 27       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 28       | 1        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 1        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 2        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 3        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 4        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 5        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 6        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 7        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 8        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 9        | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 10       | 1        | control | 2      | -       | -   | 0           | 0      |
| 11       | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 12       | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 13       | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 14       | 1        | control | 2      | 0       | 0   | 0           | 0      |
| 15       | 1        | vaccine | 2      | 0       | 0   | 24          | 48     |
| 16       | 1        | vaccine | 2      | 0       | 0   | 48          | 48     |
| 17       | 1        | vaccine | 2      | 0       | 0   | 24          | 48     |
| 18       | 1        | vaccine | 2      | 0       | 0   | 24          | 768    |
| 19       | 1        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 20       | 1        | vaccine | 2      | 0       | 0   | 0           | 48     |
| 21       | 1        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 22       | 1        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 23       | 1        | vaccine | 2      | 0       | 0   | 96          | 192    |
| 24       | 1        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 25       | 1        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 26       | 1        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 27       | 1        | vaccine | 2      | 0       | 0   | 48          | 24     |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 28       | 1        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 1        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 2        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 3        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 4        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 5        | 1        | control | 3      | -       | -   | 0           | 0      |
| 6        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 7        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 8        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 9        | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 10       | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 11       | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 12       | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 13       | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 14       | 1        | control | 3      | 0       | 0   | 0           | 0      |
| 15       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 16       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 17       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 18       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 19       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 20       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 21       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 22       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 23       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 24       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 25       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 26       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 27       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 28       | 1        | vaccine | 3      | 0       | 0   | -           | -      |
| 1        | 1        | control | 4      | 0       | 1   | 96          | 0      |
| 2        | 1        | control | 4      | 0       | 0   | 24          | 0      |
| 3        | 1        | control | 4      | -       | -   | -           | -      |
| 4        | 1        | control | 4      | 0       | 0   | 96          | 0      |
| 5        | 1        | control | 4      | 0       | 0   | 24          | 0      |
| 6        | 1        | control | 4      | 0       | 1   | 192         | 0      |
| 7        | 1        | control | 4      | 1       | 1   | 48          | 0      |
| 8        | 1        | control | 4      | -       | -   | -           | -      |
| 9        | 1        | control | 4      | 0       | 0   | -           | -      |
| 10       | 1        | control | 4      | 0       | 1   | 48          | 0      |
| 11       | 1        | control | 4      | -       | -   | -           | -      |
| 12       | 1        | control | 4      | -       | -   | -           | -      |
| 13       | 1        | control | 4      | 0       | 1   | 48          | 0      |
| 14       | 1        | control | 4      | -       | -   | 96          | 0      |
| 15       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 16       | 1        | vaccine | 4      | -       | -   | -           | -      |
| 17       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 18       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 19       | 1        | vaccine | 4      | -       | -   | -           | -      |
| 20       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 21       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 22       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 23       | 1        | vaccine | 4      | -       | -   | -           | -      |
| 24       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 25       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 26       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 27       | 1        | vaccine | 4      | 0       | 0   | -           | -      |
| 28       | 1        | vaccine | 4      | 0       | 0   | -           | -      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 29       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 30       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 31       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 32       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 33       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 34       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 35       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 36       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 37       | 2        | control | 1      | 0       | 0   | 24          | 0      |
| 38       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 39       | 2        | control | 1      | 0       | 0   | 24          | 0      |
| 40       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 41       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 42       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 43       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 44       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 45       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 46       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 47       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 48       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 49       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 50       | 2        | control | 1      | 0       | 0   | 24          | 0      |
| 51       | 2        | control | 1      | 0       | 0   | 48          | 0      |
| 52       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 53       | 2        | control | 1      | 0       | 0   | 0           | 0      |
| 54       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 55       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 56       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 57       | 2        | vaccine | 1      | 0       | 0   | 48          | 0      |
| 58       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 59       | 2        | vaccine | 1      | 0       | 0   | 48          | 0      |
| 60       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 61       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 62       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 63       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 64       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 65       | 2        | vaccine | 1      | 0       | 0   | 48          | 0      |
| 66       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 67       | 2        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 68       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 69       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 70       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 71       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 72       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 73       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 74       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 75       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 76       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 77       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 78       | 2        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 79       | 2        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 29       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 30       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 31       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 32       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 33       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 34       | 2        | control | 2      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 35       | 2        | control | 2      | 0       | 0   | 24          | 0      |
| 36       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 37       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 38       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 39       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 40       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 41       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 42       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 43       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 44       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 45       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 46       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 47       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 48       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 49       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 50       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 51       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 52       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 53       | 2        | control | 2      | 0       | 0   | 0           | 0      |
| 54       | 2        | vaccine | 2      | 0       | 0   | 48          | 96     |
| 55       | 2        | vaccine | 2      | 0       | 0   | 768         | 192    |
| 56       | 2        | vaccine | 2      | 0       | 0   | 0           | 48     |
| 57       | 2        | vaccine | 2      | 0       | 0   | 0           | 48     |
| 58       | 2        | vaccine | 2      | 0       | 0   | 48          | 96     |
| 59       | 2        | vaccine | 2      | 0       | 0   | 24          | 384    |
| 60       | 2        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 61       | 2        | vaccine | 2      | 0       | 0   | 48          | 192    |
| 62       | 2        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 63       | 2        | vaccine | 2      | 0       | 0   | 48          | 384    |
| 64       | 2        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 65       | 2        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 66       | 2        | vaccine | 2      | 0       | 0   | 0           | 0      |
| 67       | 2        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 68       | 2        | vaccine | 2      | 0       | 0   | 96          | 1536   |
| 69       | 2        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 70       | 2        | vaccine | 2      | 0       | 0   | 0           | 48     |
| 71       | 2        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 72       | 2        | vaccine | 2      | 0       | 0   | 24          | 1536   |
| 73       | 2        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 74       | 2        | vaccine | 2      | 0       | 0   | 96          | 384    |
| 75       | 2        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 76       | 2        | vaccine | 2      | 0       | 0   | 48          | 192    |
| 77       | 2        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 78       | 2        | vaccine | 2      | 0       | 0   | 0           | 0      |
| 79       | 2        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 29       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 30       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 31       | 2        | control | 3      | -       | -   | -           | -      |
| 32       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 33       | 2        | control | 3      | 0       | 0   | 96          | 0      |
| 34       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 35       | 2        | control | 3      | 0       | 0   | 96          | 0      |
| 36       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 37       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 38       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 39       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 40       | 2        | control | 3      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 41       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 42       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 43       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 44       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 45       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 46       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 47       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 48       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 49       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 50       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 51       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 52       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 53       | 2        | control | 3      | 0       | 0   | 0           | 0      |
| 54       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 55       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 56       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 57       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 58       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 59       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 60       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 61       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 62       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 63       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 64       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 65       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 66       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 67       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 68       | 2        | vaccine | 3      | -       | -   | -           | -      |
| 69       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 70       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 71       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 72       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 73       | 2        | vaccine | 3      | -       | -   | -           | -      |
| 74       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 75       | 2        | vaccine | 3      | -       | -   | -           | -      |
| 76       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 77       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 78       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 79       | 2        | vaccine | 3      | 0       | 0   | -           | -      |
| 29       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 30       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 31       | 2        | control | 4      | 0       | 0   | -           | -      |
| 32       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 33       | 2        | control | 4      | 0       | 0   | 48          | 0      |
| 34       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 35       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 36       | 2        | control | 4      | -       | -   | -           | -      |
| 37       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 38       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 39       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 40       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 41       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 42       | 2        | control | 4      | 0       | 0   | 0           | 24     |
| 43       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 44       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 45       | 2        | control | 4      | 0       | 0   | 0           | 24     |
| 46       | 2        | control | 4      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 47       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 48       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 49       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 50       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 51       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 52       | 2        | control | 4      | 0       | 0   | 0           | 24     |
| 53       | 2        | control | 4      | 0       | 0   | 0           | 0      |
| 54       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 55       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 56       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 57       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 58       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 59       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 60       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 61       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 62       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 63       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 64       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 65       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 66       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 67       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 68       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 69       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 70       | 2        | vaccine | 4      | -       | -   | -           | -      |
| 71       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 72       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 73       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 74       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 75       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 76       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 77       | 2        | vaccine | 4      | -       | -   | -           | -      |
| 78       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 79       | 2        | vaccine | 4      | 0       | 0   | -           | -      |
| 80       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 81       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 82       | 3        | control | 1      | 0       | 0   | 24          | 0      |
| 83       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 84       | 3        | control | 1      | 0       | 0   | 24          | 0      |
| 85       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 86       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 87       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 88       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 89       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 90       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 91       | 3        | control | 1      | 0       | 0   | 24          | 0      |
| 92       | 3        | control | 1      | 0       | 0   | 24          | 0      |
| 93       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 94       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 95       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 96       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 97       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 98       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 99       | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 100      | 3        | control | 1      | 0       | 0   | 0           | 0      |
| 101      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 102      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 103      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 104      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 105      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 106      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 107      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 108      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 109      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 110      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 111      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 112      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 113      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 114      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 115      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 116      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 117      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 118      | 3        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 80       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 81       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 82       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 83       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 84       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 85       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 86       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 87       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 88       | 3        | control | 2      | -       | -   | 0           | 0      |
| 89       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 90       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 91       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 92       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 93       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 94       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 95       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 96       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 97       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 98       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 99       | 3        | control | 2      | 0       | 0   | 0           | 0      |
| 100      | 3        | control | 2      | -       | -   | 0           | 0      |
| 101      | 3        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 102      | 3        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 103      | 3        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 104      | 3        | vaccine | 2      | 0       | 0   | 24          | 48     |
| 105      | 3        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 106      | 3        | vaccine | 2      | 0       | 0   | 24          | 48     |
| 107      | 3        | vaccine | 2      | 0       | 0   | 0           | 48     |
| 108      | 3        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 109      | 3        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 110      | 3        | vaccine | 2      | 0       | 0   | 0           | 0      |
| 111      | 3        | vaccine | 2      | 0       | 0   | 48          | 0      |
| 112      | 3        | vaccine | 2      | 0       | 0   | 0           | 384    |
| 113      | 3        | vaccine | 2      | 0       | 0   | 24          | 0      |
| 114      | 3        | vaccine | 2      | 0       | 0   | 96          | 192    |
| 115      | 3        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 116      | 3        | vaccine | 2      | 0       | 0   | 0           | 0      |
| 117      | 3        | vaccine | 2      | 0       | 0   | 96          | 24     |
| 118      | 3        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 80       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 81       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 82       | 3        | control | 3      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 83       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 84       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 85       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 86       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 87       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 88       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 89       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 90       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 91       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 92       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 93       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 94       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 95       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 96       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 97       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 98       | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 99       | 3        | control | 3      | -       | -   | 0           | 0      |
| 100      | 3        | control | 3      | 0       | 0   | 0           | 0      |
| 101      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 102      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 103      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 104      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 105      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 106      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 107      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 108      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 109      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 110      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 111      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 112      | 3        | vaccine | 3      | -       | -   | -           | -      |
| 113      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 114      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 115      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 116      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 117      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 118      | 3        | vaccine | 3      | 0       | 0   | -           | -      |
| 80       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 81       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 82       | 3        | control | 4      | 0       | 0   | 24          | 0      |
| 83       | 3        | control | 4      | 0       | 0   | -           | -      |
| 84       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 85       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 86       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 87       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 88       | 3        | control | 4      | 0       | 0   | 48          | 0      |
| 89       | 3        | control | 4      | 0       | 0   | -           | -      |
| 90       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 91       | 3        | control | 4      | -       | -   | -           | -      |
| 92       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 93       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 94       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 95       | 3        | control | 4      | -       | -   | -           | -      |
| 96       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 97       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 98       | 3        | control | 4      | 0       | 0   | 0           | 0      |
| 99       | 3        | control | 4      | 0       | 0   | 96          | 0      |
| 100      | 3        | control | 4      | 0       | 0   | 0           | 0      |



| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 101      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 102      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 103      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 104      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 105      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 106      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 107      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 108      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 109      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 110      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 111      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 112      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 113      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 114      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 115      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 116      | 3        | vaccine | 4      | -       | -   | -           | -      |
| 117      | 3        | vaccine | 4      | -       | -   | -           | -      |
| 118      | 3        | vaccine | 4      | 0       | 0   | -           | -      |
| 119      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 120      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 121      | 4        | control | 1      | -       | -   | 0           | 0      |
| 122      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 123      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 124      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 125      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 126      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 127      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 128      | 4        | control | 1      | 0       | 0   | 24          | 0      |
| 129      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 130      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 131      | 4        | control | 1      | -       | -   | 0           | 0      |
| 132      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 133      | 4        | control | 1      | -       | -   | 0           | 0      |
| 134      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 135      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 136      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 137      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 138      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 139      | 4        | control | 1      | -       | -   | 0           | 0      |
| 140      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 141      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 142      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 143      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 144      | 4        | control | 1      | 0       | 0   | 0           | 0      |
| 145      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 146      | 4        | vaccine | 1      | -       | -   | 0           | 0      |
| 147      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 148      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 149      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 150      | 4        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 151      | 4        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 152      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 153      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 154      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 155      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 156      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 157      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 158      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 159      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 160      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 161      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 162      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 163      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 164      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 165      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 166      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 167      | 4        | vaccine | 1      | -       | -   | 0           | 0      |
| 168      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 169      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 170      | 4        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 171      | 4        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 119      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 120      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 121      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 122      | 4        | control | 2      | -       | -   | -           | -      |
| 123      | 4        | control | 2      | -       | -   | -           | -      |
| 124      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 125      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 126      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 127      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 128      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 129      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 130      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 131      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 132      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 133      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 134      | 4        | control | 2      | -       | -   | 0           | 0      |
| 135      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 136      | 4        | control | 2      | -       | -   | 0           | 0      |
| 137      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 138      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 139      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 140      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 141      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 142      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 143      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 144      | 4        | control | 2      | 0       | 0   | 0           | 0      |
| 145      | 4        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 146      | 4        | vaccine | 2      | 0       | 0   | 48          | 384    |
| 147      | 4        | vaccine | 2      | 0       | 0   | 48          | 192    |
| 148      | 4        | vaccine | 2      | 0       | 0   | 24          | 384    |
| 149      | 4        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 150      | 4        | vaccine | 2      | 0       | 0   | 0           | 384    |
| 151      | 4        | vaccine | 2      | 0       | 0   | 24          | 384    |
| 152      | 4        | vaccine | 2      | 0       | 0   | 96          | 384    |
| 153      | 4        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 154      | 4        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 155      | 4        | vaccine | 2      | 0       | 0   | -           | -      |
| 156      | 4        | vaccine | 2      | 0       | 0   | 0           | 0      |
| 157      | 4        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 158      | 4        | vaccine | 2      | 0       | 0   | 48          | 96     |
| 159      | 4        | vaccine | 2      | 0       | 0   | 96          | 384    |
| 160      | 4        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 161      | 4        | vaccine | 2      | 0       | 0   | 96          | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 162      | 4        | vaccine | 2      | 0       | 0   | 0           | 0      |
| 163      | 4        | vaccine | 2      | 0       | 0   | 24          | 48     |
| 164      | 4        | vaccine | 2      | 0       | 0   | 24          | 384    |
| 165      | 4        | vaccine | 2      | 0       | 0   | 24          | 384    |
| 166      | 4        | vaccine | 2      | 0       | 0   | 96          | 48     |
| 167      | 4        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 168      | 4        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 169      | 4        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 170      | 4        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 171      | 4        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 119      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 120      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 121      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 122      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 123      | 4        | control | 3      | -       | -   | -           | -      |
| 124      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 125      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 126      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 127      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 128      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 129      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 130      | 4        | control | 3      | 0       | 1   | 0           | 0      |
| 131      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 132      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 133      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 134      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 135      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 136      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 137      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 138      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 139      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 140      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 141      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 142      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 143      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 144      | 4        | control | 3      | 0       | 0   | 0           | 0      |
| 145      | 4        | vaccine | 3      | -       | -   | -           | -      |
| 146      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 147      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 148      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 149      | 4        | vaccine | 3      | -       | -   | -           | -      |
| 150      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 151      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 152      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 153      | 4        | vaccine | 3      | -       | -   | -           | -      |
| 154      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 155      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 156      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 157      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 158      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 159      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 160      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 161      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 162      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 163      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 164      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 165      | 4        | vaccine | 3      | 0       | 0   | -           | -      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 166      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 167      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 168      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 169      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 170      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 171      | 4        | vaccine | 3      | 0       | 0   | -           | -      |
| 119      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 120      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 121      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 122      | 4        | control | 4      | 0       | 1   | 192         | 0      |
| 123      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 124      | 4        | control | 4      | 0       | 0   | 768         | 0      |
| 125      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 126      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 127      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 128      | 4        | control | 4      | 0       | 1   | 0           | 0      |
| 129      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 130      | 4        | control | 4      | 0       | 1   | 0           | 0      |
| 131      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 132      | 4        | control | 4      | -       | -   | 0           | 0      |
| 133      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 134      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 135      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 136      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 137      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 138      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 139      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 140      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 141      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 142      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 143      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 144      | 4        | control | 4      | 0       | 0   | 0           | 0      |
| 145      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 146      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 147      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 148      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 149      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 150      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 151      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 152      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 153      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 154      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 155      | 4        | vaccine | 4      | 0       | -   | -           | -      |
| 156      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 157      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 158      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 159      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 160      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 161      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 162      | 4        | vaccine | 4      | -       | -   | -           | -      |
| 163      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 164      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 165      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 166      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 167      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 168      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 169      | 4        | vaccine | 4      | 0       | 0   | -           | -      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 170      | 4        | vaccine | 4      | 0       | 0   | -           | -      |
| 171      | 4        | vaccine | 4      | -       | -   | -           | -      |
| 172      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 173      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 174      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 175      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 176      | 5        | control | 1      | -       | -   | 0           | 0      |
| 177      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 178      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 179      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 180      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 181      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 182      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 183      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 184      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 185      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 186      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 187      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 188      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 189      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 190      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 191      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 192      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 193      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 194      | 5        | control | 1      | 0       | 0   | 0           | 0      |
| 195      | 5        | control | 1      | -       | -   | 0           | 0      |
| 196      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 197      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 198      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 199      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 200      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 201      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 202      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 203      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 204      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 205      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 206      | 5        | vaccine | 1      | -       | -   | 0           | 0      |
| 207      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 208      | 5        | vaccine | 1      | 0       | 0   | 24          | 0      |
| 209      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 210      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 211      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 212      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 213      | 5        | vaccine | 1      | -       | -   | 0           | 0      |
| 214      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 215      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 216      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 217      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 218      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 219      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 220      | 5        | vaccine | 1      | 0       | 0   | 0           | 0      |
| 172      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 173      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 174      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 175      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 176      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 177      | 5        | control | 2      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 178      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 179      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 180      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 181      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 182      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 183      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 184      | 5        | control | 2      | -       | -   | -           | -      |
| 185      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 186      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 187      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 188      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 189      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 190      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 191      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 192      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 193      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 194      | 5        | control | 2      | 0       | 0   | 0           | 0      |
| 195      | 5        | control | 2      | -       | -   | -           | -      |
| 196      | 5        | vaccine | 2      | 0       | 0   | 0           | 24     |
| 197      | 5        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 198      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 199      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 200      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 201      | 5        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 202      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 203      | 5        | vaccine | 2      | 0       | 0   | 0           | 48     |
| 204      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 205      | 5        | vaccine | 2      | 0       | 0   | 96          | 192    |
| 206      | 5        | vaccine | 2      | 0       | 0   | 24          | 48     |
| 207      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 208      | 5        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 209      | 5        | vaccine | 2      | 0       | 0   | 24          | 192    |
| 210      | 5        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 211      | 5        | vaccine | 2      | 0       | 0   | 0           | 192    |
| 212      | 5        | vaccine | 2      | 0       | 0   | 24          | 24     |
| 213      | 5        | vaccine | 2      | 0       | 0   | 24          | 24     |
| 214      | 5        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 215      | 5        | vaccine | 2      | -       | -   | -           | -      |
| 216      | 5        | vaccine | 2      | 0       | 0   | 24          | 96     |
| 217      | 5        | vaccine | 2      | -       | -   | -           | -      |
| 218      | 5        | vaccine | 2      | -       | -   | -           | -      |
| 219      | 5        | vaccine | 2      | 0       | 0   | 24          | 384    |
| 220      | 5        | vaccine | 2      | 0       | 0   | 0           | 96     |
| 172      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 173      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 174      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 175      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 176      | 5        | control | 3      | 0       | 0   | -           | -      |
| 177      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 178      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 179      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 180      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 181      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 182      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 183      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 184      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 185      | 5        | control | 3      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 186      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 187      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 188      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 189      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 190      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 191      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 192      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 193      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 194      | 5        | control | 3      | 0       | 0   | 0           | 0      |
| 195      | 5        | control | 3      | -       | -   | 0           | 0      |
| 196      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 197      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 198      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 199      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 200      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 201      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 202      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 203      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 204      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 205      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 206      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 207      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 208      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 209      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 210      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 211      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 212      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 213      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 214      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 215      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 216      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 217      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 218      | 5        | vaccine | 3      | -       | -   | -           | -      |
| 219      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 220      | 5        | vaccine | 3      | 0       | 0   | -           | -      |
| 172      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 173      | 5        | control | 4      | -       | -   | 0           | 0      |
| 174      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 175      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 176      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 177      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 178      | 5        | control | 4      | -       | -   | -           | -      |
| 179      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 180      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 181      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 182      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 183      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 184      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 185      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 186      | 5        | control | 4      | .       | .   | 0           | 0      |
| 187      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 188      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 189      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 190      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 191      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 192      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 193      | 5        | control | 4      | 0       | 0   | 0           | 0      |

| Deer no. | Farm no. | Status  | Period | Culture | PCR | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------|-----|-------------|--------|
| 194      | 5        | control | 4      | 0       | 0   | -           | -      |
| 195      | 5        | control | 4      | 0       | 0   | 0           | 0      |
| 196      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 197      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 198      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 199      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 200      | 5        | vaccine | 4      | -       | -   | -           | -      |
| 201      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 202      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 203      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 204      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 205      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 206      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 207      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 208      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 209      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 210      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 211      | 5        | vaccine | 4      | -       | -   | -           | -      |
| 212      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 213      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 214      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 215      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 216      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 217      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 218      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 219      | 5        | vaccine | 4      | 0       | 0   | -           | -      |
| 220      | 5        | vaccine | 4      | -       | -   | -           | -      |



**Appendix 2c.** Raw data of urine shedding in no-streptomycin control deer

(Period: 2 = May, 4 = November) (Culture and PCR: 0 = negative, 1 = positive)

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 1        | 1        | 2      | 0       | 0   |
| 2        | 1        | 2      | 0       | 0   |
| 3        | 1        | 2      | 0       | 0   |
| 4        | 1        | 2      | 0       | 0   |
| 5        | 1        | 2      | 0       | 0   |
| 6        | 1        | 2      | 0       | 0   |
| 7        | 1        | 2      | 0       | 0   |
| 8        | 1        | 2      | 0       | 0   |
| 9        | 1        | 2      | 0       | 0   |
| 10       | 1        | 2      | 0       | 0   |
| 11       | 1        | 2      | 0       | 0   |
| 12       | 1        | 2      | 0       | 0   |
| 13       | 1        | 2      | 0       | 0   |
| 14       | 1        | 2      | 0       | 0   |
| 15       | 1        | 2      | 0       | 0   |
| 16       | 1        | 2      | 0       | 0   |
| 17       | 1        | 2      | 0       | 0   |
| 18       | 1        | 2      | 0       | 0   |
| 19       | 1        | 2      | 0       | 0   |
| 20       | 1        | 4      | 1       | 1   |
| 21       | 1        | 4      | 1       | 1   |
| 22       | 1        | 4      | 1       | 1   |
| 23       | 1        | 4      | 0       | 1   |
| 24       | 1        | 4      | 0       | 1   |
| 25       | 1        | 4      | 0       | 1   |
| 26       | 1        | 4      | 0       | 0   |
| 27       | 1        | 4      | 0       | 1   |
| 28       | 1        | 4      | 0       | 1   |
| 29       | 1        | 4      | 1       | 1   |
| 30       | 1        | 4      | 0       | 1   |
| 31       | 1        | 4      | 0       | 1   |
| 32       | 1        | 4      | 0       | 1   |
| 33       | 1        | 4      | 0       | 1   |
| 34       | 1        | 4      | 1       | 1   |
| 35       | 1        | 4      | 0       | 0   |
| 36       | 1        | 4      | 0       | 0   |
| 37       | 1        | 4      | 1       | 1   |
| 38       | 2        | 2      | 0       | 0   |
| 39       | 2        | 2      | 0       | 0   |
| 40       | 2        | 2      | 0       | 0   |
| 41       | 2        | 2      | 0       | 0   |
| 42       | 2        | 2      | 0       | 0   |
| 43       | 2        | 2      | 0       | 0   |
| 44       | 2        | 2      | 0       | 0   |
| 45       | 2        | 2      | 0       | 0   |
| 46       | 2        | 2      | 0       | 0   |
| 47       | 2        | 2      | 0       | 0   |
| 48       | 2        | 2      | 0       | 0   |
| 49       | 2        | 2      | 0       | 0   |
| 50       | 2        | 2      | 0       | 0   |
| 51       | 2        | 2      | 0       | 0   |
| 52       | 2        | 2      | 0       | 0   |
| 53       | 2        | 2      | 0       | 0   |
| 54       | 2        | 2      | 0       | 0   |

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 55       | 2        | 2      | 0       | 0   |
| 56       | 2        | 2      | 0       | 0   |
| 57       | 2        | 2      | 0       | 0   |
| 58       | 2        | 4      | 0       | 0   |
| 59       | 2        | 4      | 0       | 0   |
| 60       | 2        | 4      | 0       | 0   |
| 61       | 2        | 4      | 0       | 0   |
| 62       | 2        | 4      | 0       | 0   |
| 63       | 2        | 4      | 0       | 0   |
| 64       | 2        | 4      | 0       | 0   |
| 65       | 2        | 4      | 0       | 0   |
| 66       | 2        | 4      | 0       | 0   |
| 67       | 2        | 4      | 0       | 0   |
| 68       | 2        | 4      | 0       | 0   |
| 69       | 2        | 4      | 0       | 0   |
| 70       | 2        | 4      | 0       | 0   |
| 71       | 2        | 4      | 0       | 0   |
| 72       | 2        | 4      | 0       | 0   |
| 73       | 2        | 4      | 0       | 0   |
| 74       | 2        | 4      | 0       | 0   |
| 75       | 2        | 4      | 0       | 0   |
| 76       | 2        | 4      | 0       | 0   |
| 77       | 2        | 4      | 0       | 0   |
| 78       | 3        | 2      | 0       | 0   |
| 79       | 3        | 2      | 0       | 0   |
| 80       | 3        | 2      | 0       | 0   |
| 81       | 3        | 2      | 0       | 0   |
| 82       | 3        | 2      | 0       | 0   |
| 83       | 3        | 2      | 0       | 0   |
| 84       | 3        | 2      | 0       | 0   |
| 85       | 3        | 2      | 0       | 0   |
| 86       | 3        | 2      | 0       | 0   |
| 87       | 3        | 2      | 0       | 0   |
| 88       | 3        | 2      | 0       | 0   |
| 89       | 3        | 2      | 0       | 0   |
| 90       | 3        | 2      | 0       | 0   |
| 91       | 3        | 2      | 0       | 0   |
| 92       | 3        | 2      | 0       | 0   |
| 93       | 3        | 2      | 0       | 0   |
| 94       | 3        | 2      | 0       | 0   |
| 95       | 3        | 2      | 0       | 0   |
| 96       | 3        | 2      | 0       | 0   |
| 97       | 3        | 2      | 0       | 0   |
| 98       | 3        | 4      | 0       | 0   |
| 99       | 3        | 4      | 0       | 0   |
| 100      | 3        | 4      | 0       | 0   |
| 101      | 3        | 4      | 0       | 0   |
| 102      | 3        | 4      | 0       | 0   |
| 103      | 3        | 4      | 0       | 0   |
| 104      | 3        | 4      | 0       | 0   |
| 105      | 3        | 4      | 0       | 0   |
| 106      | 3        | 4      | 0       | 0   |
| 107      | 3        | 4      | 0       | 0   |
| 108      | 3        | 4      | 0       | 0   |
| 109      | 3        | 4      | 0       | 0   |
| 110      | 3        | 4      | 0       | 0   |
| 111      | 3        | 4      | 0       | 0   |

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 112      | 3        | 4      | 0       | 0   |
| 113      | 3        | 4      | 0       | 0   |
| 114      | 3        | 4      | 0       | 0   |
| 115      | 3        | 4      | 0       | 0   |
| 116      | 3        | 4      | 0       | 0   |
| 117      | 4        | 2      | 0       | 0   |
| 118      | 4        | 2      | 0       | 0   |
| 119      | 4        | 2      | 0       | 0   |
| 120      | 4        | 2      | 0       | 0   |
| 121      | 4        | 2      | 0       | 0   |
| 122      | 4        | 2      | 0       | 0   |
| 123      | 4        | 2      | 0       | 0   |
| 124      | 4        | 2      | 0       | 0   |
| 125      | 4        | 2      | 0       | 0   |
| 126      | 4        | 2      | 0       | 0   |
| 127      | 4        | 2      | 0       | 0   |
| 128      | 4        | 2      | 0       | 0   |
| 129      | 4        | 2      | 0       | 0   |
| 130      | 4        | 2      | 0       | 0   |
| 131      | 4        | 2      | 0       | 0   |
| 132      | 4        | 2      | 0       | 0   |
| 133      | 4        | 2      | 0       | 0   |
| 134      | 4        | 2      | 0       | 0   |
| 135      | 4        | 2      | 0       | 0   |
| 136      | 4        | 4      | 0       | 0   |
| 137      | 4        | 4      | 0       | 0   |
| 138      | 4        | 4      | 0       | 0   |
| 139      | 4        | 4      | 0       | 0   |
| 140      | 4        | 4      | 0       | 0   |
| 141      | 4        | 4      | 0       | 0   |
| 142      | 4        | 4      | 0       | 0   |
| 143      | 4        | 4      | 0       | 0   |
| 144      | 4        | 4      | 0       | 0   |
| 145      | 4        | 4      | 0       | 0   |
| 146      | 4        | 4      | 0       | 0   |
| 147      | 4        | 4      | 0       | 0   |
| 148      | 4        | 4      | 0       | 1   |
| 149      | 4        | 4      | 0       | 0   |
| 150      | 4        | 4      | 0       | 0   |
| 151      | 4        | 4      | 0       | 0   |
| 152      | 4        | 4      | 0       | 1   |
| 153      | 4        | 4      | 0       | 1   |
| 154      | 4        | 4      | 0       | 1   |
| 155      | 4        | 4      | 0       | 1   |
| 156      | 5        | 2      | 0       | 0   |
| 157      | 5        | 2      | 0       | 0   |
| 158      | 5        | 2      | 0       | 0   |
| 159      | 5        | 2      | 0       | 0   |
| 160      | 5        | 2      | 0       | 0   |
| 161      | 5        | 2      | 0       | 0   |
| 162      | 5        | 2      | 0       | 0   |
| 163      | 5        | 2      | 0       | 0   |
| 164      | 5        | 2      | 0       | 0   |
| 165      | 5        | 2      | 0       | 0   |
| 166      | 5        | 2      | 0       | 0   |
| 167      | 5        | 2      | 0       | 0   |
| 168      | 5        | 2      | 0       | 0   |

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 169      | 5        | 2      | 0       | 0   |
| 170      | 5        | 2      | 0       | 0   |
| 171      | 5        | 2      | 0       | 0   |
| 172      | 5        | 2      | 0       | 0   |
| 173      | 5        | 2      | 0       | 0   |
| 174      | 5        | 2      | 0       | 0   |
| 175      | 5        | 2      | 0       | 0   |
| 176      | 5        | 4      | 0       | 0   |
| 177      | 5        | 4      | 0       | 0   |
| 178      | 5        | 4      | 0       | 0   |
| 179      | 5        | 4      | 0       | 0   |
| 180      | 5        | 4      | 0       | 0   |
| 181      | 5        | 4      | 0       | 0   |
| 182      | 5        | 4      | 0       | 0   |
| 183      | 5        | 4      | 0       | 0   |
| 184      | 5        | 4      | 0       | 0   |
| 185      | 5        | 4      | 0       | 0   |
| 186      | 5        | 4      | 0       | 0   |
| 187      | 5        | 4      | 0       | 0   |
| 188      | 5        | 4      | 0       | 0   |
| 189      | 5        | 4      | 0       | 0   |
| 190      | 5        | 4      | 0       | 0   |
| 191      | 5        | 4      | 0       | 0   |
| 192      | 5        | 4      | 0       | 0   |
| 193      | 5        | 4      | 0       | 0   |
| 194      | 5        | 4      | 0       | 0   |
| 195      | 5        | 4      | 0       | 0   |



### Appendix 3a. Raw data of maternal status and serology.

(Period: 1 = October'07 (carriage of foetus to term), 2 = March'08 (lactating at weaning)) (Mother status: 0 = No, 1 = yes)

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 1        | 1        | Control | 1      | 1             | 192         | 0      |
| 2        | 1        | Control | 1      | 1             | 96          | 0      |
| 3        | 1        | Control | 1      | 1             | 0           | 0      |
| 4        | 1        | Control | 1      | 1             | 0           | 0      |
| 5        | 1        | Control | 1      | 1             | 0           | 0      |
| 6        | 1        | Control | 1      | 1             | 0           | 0      |
| 7        | 1        | Control | 1      | 0             | 192         | 0      |
| 8        | 1        | Control | 1      | 1             | 384         | 0      |
| 9        | 1        | Control | 1      | 1             | 0           | 0      |
| 10       | 1        | Control | 1      | 1             | 96          | 0      |
| 11       | 1        | Control | 1      | 1             | 192         | 0      |
| 12       | 1        | Control | 1      | 1             | 0           | 0      |
| 13       | 1        | Control | 1      | 1             | -           | -      |
| 14       | 1        | Control | 1      | 1             | 0           | 0      |
| 15       | 1        | Control | 1      | 1             | 192         | 0      |
| 16       | 1        | Control | 1      | 1             | 0           | 0      |
| 17       | 1        | Control | 1      | 1             | 0           | 0      |
| 18       | 1        | Vaccine | 1      | 1             | -           | -      |
| 19       | 1        | Vaccine | 1      | 1             | -           | -      |
| 20       | 1        | Vaccine | 1      | 1             | -           | -      |
| 21       | 1        | Vaccine | 1      | 1             | -           | -      |
| 22       | 1        | Vaccine | 1      | 1             | -           | -      |
| 23       | 1        | Vaccine | 1      | 1             | -           | -      |
| 24       | 1        | Vaccine | 1      | 1             | -           | -      |
| 25       | 1        | Vaccine | 1      | 1             | -           | -      |
| 26       | 1        | Vaccine | 1      | 1             | -           | -      |
| 27       | 1        | Vaccine | 1      | 1             | -           | -      |
| 28       | 1        | Vaccine | 1      | 1             | -           | -      |
| 29       | 1        | Vaccine | 1      | 1             | -           | -      |
| 30       | 1        | Vaccine | 1      | 1             | -           | -      |
| 31       | 1        | Vaccine | 1      | 1             | -           | -      |
| 32       | 1        | Vaccine | 1      | 1             | -           | -      |
| 33       | 1        | Vaccine | 1      | 1             | -           | -      |
| 34       | 1        | Vaccine | 1      | 1             | -           | -      |
| 35       | 1        | Vaccine | 1      | 1             | -           | -      |
| 1        | 1        | Control | 2      | 1             | 96          | 0      |
| 2        | 1        | Control | 2      | 0             | 48          | 0      |
| 3        | 1        | Control | 2      | 1             | 0           | 0      |
| 4        | 1        | Control | 2      | 1             | 0           | 0      |
| 5        | 1        | Control | 2      | 0             | 0           | 0      |
| 6        | 1        | Control | 2      | 1             | 0           | 0      |
| 7        | 1        | Control | 2      | 0             | -           | -      |
| 8        | 1        | Control | 2      | 1             | 384         | 48     |
| 9        | 1        | Control | 2      | 0             | 0           | 0      |
| 10       | 1        | Control | 2      | 1             | 192         | 0      |
| 11       | 1        | Control | 2      | 1             | 192         | 0      |
| 12       | 1        | Control | 2      | 1             | 0           | 0      |
| 13       | 1        | Control | 2      | 1             | 48          | 0      |
| 14       | 1        | Control | 2      | 1             | 96          | 0      |
| 15       | 1        | Control | 2      | 1             | 0           | 0      |
| 16       | 1        | Control | 2      | 1             | 0           | 0      |
| 17       | 1        | Control | 2      | 1             | -           | -      |
| 18       | 1        | Vaccine | 2      | 1             | -           | -      |
| 19       | 1        | Vaccine | 2      | 1             | -           | -      |
| 20       | 1        | Vaccine | 2      | 0             | -           | -      |

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 21       | 1        | Vaccine | 2      | 1             | -           | -      |
| 22       | 1        | Vaccine | 2      | 0             | -           | -      |
| 23       | 1        | Vaccine | 2      | 1             | -           | -      |
| 24       | 1        | Vaccine | 2      | 1             | -           | -      |
| 25       | 1        | Vaccine | 2      | 1             | -           | -      |
| 26       | 1        | Vaccine | 2      | 1             | -           | -      |
| 27       | 1        | Vaccine | 2      | 0             | -           | -      |
| 28       | 1        | Vaccine | 2      | 1             | -           | -      |
| 29       | 1        | Vaccine | 2      | 1             | -           | -      |
| 30       | 1        | Vaccine | 2      | 1             | -           | -      |
| 31       | 1        | Vaccine | 2      | 0             | -           | -      |
| 32       | 1        | Vaccine | 2      | 1             | -           | -      |
| 33       | 1        | Vaccine | 2      | 1             | -           | -      |
| 34       | 1        | Vaccine | 2      | 1             | -           | -      |
| 35       | 1        | Vaccine | 2      | 1             | -           | -      |
| 36       | 2        | Control | 1      | 1             | 24          | 0      |
| 37       | 2        | Control | 1      | 1             | 24          | 0      |
| 38       | 2        | Control | 1      | 1             | 24          | 0      |
| 39       | 2        | Control | 1      | 1             | 24          | 0      |
| 40       | 2        | Control | 1      | 1             | 24          | 0      |
| 41       | 2        | Control | 1      | 1             | 24          | 0      |
| 42       | 2        | Control | 1      | 1             | 24          | 0      |
| 43       | 2        | Control | 1      | 1             | 48          | 0      |
| 44       | 2        | Control | 1      | 1             | 24          | 0      |
| 45       | 2        | Control | 1      | 1             | 24          | 0      |
| 46       | 2        | Control | 1      | 1             | -           | -      |
| 47       | 2        | Control | 1      | 1             | 0           | 0      |
| 48       | 2        | Control | 1      | 1             | 0           | 0      |
| 49       | 2        | Control | 1      | 1             | 48          | 0      |
| 50       | 2        | Control | 1      | 1             | -           | -      |
| 51       | 2        | Control | 1      | 1             | -           | -      |
| 52       | 2        | Control | 1      | 1             | 24          | 0      |
| 53       | 2        | Control | 1      | 1             | 24          | 0      |
| 54       | 2        | Control | 1      | 1             | 0           | 0      |
| 55       | 2        | Control | 1      | 1             | 24          | 0      |
| 56       | 2        | Control | 1      | 1             | 24          | 0      |
| 57       | 2        | Control | 1      | 1             | 0           | 0      |
| 58       | 2        | Control | 1      | 1             | 96          | 0      |
| 59       | 2        | Control | 1      | 1             | 0           | 0      |
| 60       | 2        | Vaccine | 1      | 1             | -           | -      |
| 61       | 2        | Vaccine | 1      | 1             | -           | -      |
| 62       | 2        | Vaccine | 1      | 1             | -           | -      |
| 63       | 2        | Vaccine | 1      | 1             | -           | -      |
| 64       | 2        | Vaccine | 1      | 1             | -           | -      |
| 65       | 2        | Vaccine | 1      | 1             | -           | -      |
| 66       | 2        | Vaccine | 1      | 1             | -           | -      |
| 67       | 2        | Vaccine | 1      | 1             | -           | -      |
| 68       | 2        | Vaccine | 1      | 1             | -           | -      |
| 69       | 2        | Vaccine | 1      | 1             | -           | -      |
| 70       | 2        | Vaccine | 1      | 1             | -           | -      |
| 71       | 2        | Vaccine | 1      | 1             | -           | -      |
| 72       | 2        | Vaccine | 1      | 1             | -           | -      |
| 73       | 2        | Vaccine | 1      | 1             | -           | -      |
| 74       | 2        | Vaccine | 1      | 1             | -           | -      |
| 75       | 2        | Vaccine | 1      | 1             | -           | -      |
| 76       | 2        | Vaccine | 1      | 1             | -           | -      |
| 77       | 2        | Vaccine | 1      | 1             | -           | -      |

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 78       | 2        | Vaccine | 1      | 1             | -           | -      |
| 36       | 2        | Control | 2      | 0             | -           | -      |
| 37       | 2        | Control | 2      | 1             | -           | -      |
| 38       | 2        | Control | 2      | 1             | 24          | 0      |
| 39       | 2        | Control | 2      | 1             | -           | -      |
| 40       | 2        | Control | 2      | 1             | -           | -      |
| 41       | 2        | Control | 2      | 1             | 24          | 0      |
| 42       | 2        | Control | 2      | 1             | 24          | 0      |
| 43       | 2        | Control | 2      | 1             | -           | -      |
| 44       | 2        | Control | 2      | 0             | -           | -      |
| 45       | 2        | Control | 2      | 1             | -           | -      |
| 46       | 2        | Control | 2      | 0             | 0           | 0      |
| 47       | 2        | Control | 2      | 1             | 24          | 0      |
| 48       | 2        | Control | 2      | 1             | -           | -      |
| 49       | 2        | Control | 2      | 1             | -           | -      |
| 50       | 2        | Control | 2      | 1             | -           | -      |
| 51       | 2        | Control | 2      | 1             | 48          | 0      |
| 52       | 2        | Control | 2      | 1             | -           | -      |
| 53       | 2        | Control | 2      | 1             | -           | -      |
| 54       | 2        | Control | 2      | 1             | -           | -      |
| 55       | 2        | Control | 2      | 1             | -           | -      |
| 56       | 2        | Control | 2      | 1             | -           | -      |
| 57       | 2        | Control | 2      | 1             | -           | -      |
| 58       | 2        | Control | 2      | 1             | 48          | 0      |
| 59       | 2        | Control | 2      | 1             | -           | -      |
| 60       | 2        | Vaccine | 2      | 1             | -           | -      |
| 61       | 2        | Vaccine | 2      | 1             | -           | -      |
| 62       | 2        | Vaccine | 2      | 1             | -           | -      |
| 63       | 2        | Vaccine | 2      | 1             | -           | -      |
| 64       | 2        | Vaccine | 2      | 1             | -           | -      |
| 65       | 2        | Vaccine | 2      | 1             | -           | -      |
| 66       | 2        | Vaccine | 2      | 1             | -           | -      |
| 67       | 2        | Vaccine | 2      | 0             | -           | -      |
| 68       | 2        | Vaccine | 2      | 1             | -           | -      |
| 69       | 2        | Vaccine | 2      | 1             | -           | -      |
| 70       | 2        | Vaccine | 2      | 1             | -           | -      |
| 71       | 2        | Vaccine | 2      | 1             | -           | -      |
| 72       | 2        | Vaccine | 2      | 1             | -           | -      |
| 73       | 2        | Vaccine | 2      | 1             | -           | -      |
| 74       | 2        | Vaccine | 2      | 1             | -           | -      |
| 75       | 2        | Vaccine | 2      | 1             | -           | -      |
| 76       | 2        | Vaccine | 2      | 1             | -           | -      |
| 77       | 2        | Vaccine | 2      | 1             | -           | -      |
| 78       | 2        | Vaccine | 2      | 1             | -           | -      |
| 79       | 3        | Control | 1      | 1             | 24          | 0      |
| 80       | 3        | Control | 1      | 0             | 0           | 0      |
| 81       | 3        | Control | 1      | 1             | 48          | 0      |
| 82       | 3        | Control | 1      | 1             | 24          | 0      |
| 83       | 3        | Control | 1      | 1             | 48          | 0      |
| 84       | 3        | Control | 1      | 1             | 24          | 0      |
| 85       | 3        | Control | 1      | 1             | 48          | 0      |
| 86       | 3        | Control | 1      | 1             | 24          | 0      |
| 87       | 3        | Control | 1      | 1             | -           | -      |
| 88       | 3        | Control | 1      | 1             | 0           | 0      |
| 89       | 3        | Control | 1      | 1             | 48          | 0      |
| 90       | 3        | Control | 1      | 1             | 96          | 0      |
| 91       | 3        | Control | 1      | 1             | 0           | 0      |



| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 92       | 3        | Control | 1      | 1             | 0           | 0      |
| 93       | 3        | Control | 1      | 1             | -           | -      |
| 94       | 3        | Control | 1      | 1             | 24          | 0      |
| 95       | 3        | Control | 1      | 1             | 24          | 0      |
| 96       | 3        | Control | 1      | 1             | 48          | 0      |
| 97       | 3        | Control | 1      | 1             | -           | -      |
| 98       | 3        | Control | 1      | 1             | 48          | 0      |
| 99       | 3        | Control | 1      | 1             | 24          | 0      |
| 100      | 3        | Control | 1      | 1             | 48          | 0      |
| 101      | 3        | Control | 1      | 1             | 24          | 0      |
| 102      | 3        | Control | 1      | 1             | 24          | 0      |
| 103      | 3        | Control | 1      | 1             | 24          | 0      |
| 104      | 3        | Control | 1      | 1             | 24          | 0      |
| 105      | 3        | Control | 1      | 1             | 48          | 0      |
| 106      | 3        | Control | 1      | 1             | 24          | 0      |
| 107      | 3        | Control | 1      | 1             | 48          | 0      |
| 108      | 3        | Control | 1      | 1             | 48          | 0      |
| 109      | 3        | Control | 1      | 1             | 48          | 0      |
| 110      | 3        | Control | 1      | 1             | 24          | 0      |
| 111      | 3        | Vaccine | 1      | 1             | -           | -      |
| 112      | 3        | Vaccine | 1      | 1             | -           | -      |
| 113      | 3        | Vaccine | 1      | 1             | -           | -      |
| 114      | 3        | Vaccine | 1      | 1             | -           | -      |
| 115      | 3        | Vaccine | 1      | 1             | -           | -      |
| 116      | 3        | Vaccine | 1      | 1             | -           | -      |
| 117      | 3        | Vaccine | 1      | 1             | -           | -      |
| 118      | 3        | Vaccine | 1      | 1             | -           | -      |
| 119      | 3        | Vaccine | 1      | 1             | -           | -      |
| 120      | 3        | Vaccine | 1      | 1             | -           | -      |
| 121      | 3        | Vaccine | 1      | 1             | -           | -      |
| 122      | 3        | Vaccine | 1      | 1             | -           | -      |
| 123      | 3        | Vaccine | 1      | 1             | -           | -      |
| 124      | 3        | Vaccine | 1      | 1             | -           | -      |
| 125      | 3        | Vaccine | 1      | 1             | -           | -      |
| 126      | 3        | Vaccine | 1      | 1             | -           | -      |
| 127      | 3        | Vaccine | 1      | 1             | -           | -      |
| 128      | 3        | Vaccine | 1      | 1             | -           | -      |
| 129      | 3        | Vaccine | 1      | 1             | -           | -      |
| 130      | 3        | Vaccine | 1      | 1             | -           | -      |
| 131      | 3        | Vaccine | 1      | 1             | -           | -      |
| 132      | 3        | Vaccine | 1      | 1             | -           | -      |
| 133      | 3        | Vaccine | 1      | 1             | -           | -      |
| 134      | 3        | Vaccine | 1      | 1             | -           | -      |
| 135      | 3        | Vaccine | 1      | 1             | -           | -      |
| 136      | 3        | Vaccine | 1      | 1             | -           | -      |
| 137      | 3        | Vaccine | 1      | 1             | -           | -      |
| 138      | 3        | Vaccine | 1      | 1             | -           | -      |
| 139      | 3        | Vaccine | 1      | 1             | -           | -      |
| 140      | 3        | Vaccine | 1      | 1             | -           | -      |
| 141      | 3        | Vaccine | 1      | 1             | -           | -      |
| 79       | 3        | Control | 2      | 1             | 24          | 0      |
| 80       | 3        | Control | 2      | 0             | -           | -      |
| 81       | 3        | Control | 2      | 1             | 24          | 0      |
| 82       | 3        | Control | 2      | 1             | 24          | 0      |
| 83       | 3        | Control | 2      | 1             | 48          | 0      |
| 84       | 3        | Control | 2      | 1             | 48          | 0      |
| 85       | 3        | Control | 2      | 1             | 24          | 0      |

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 86       | 3        | Control | 2      | 1             | 48          | 0      |
| 87       | 3        | Control | 2      | 1             | 48          | 0      |
| 88       | 3        | Control | 2      | 1             | 24          | 0      |
| 89       | 3        | Control | 2      | 1             | 48          | 0      |
| 90       | 3        | Control | 2      | 1             | 48          | 0      |
| 91       | 3        | Control | 2      | 1             | 0           | 0      |
| 92       | 3        | Control | 2      | 1             | 0           | 0      |
| 93       | 3        | Control | 2      | 0             | -           | -      |
| 94       | 3        | Control | 2      | 1             | 24          | 0      |
| 95       | 3        | Control | 2      | 1             | 24          | 0      |
| 96       | 3        | Control | 2      | 1             | 48          | 0      |
| 97       | 3        | Control | 2      | 1             | 0           | 0      |
| 98       | 3        | Control | 2      | 1             | 48          | 0      |
| 99       | 3        | Control | 2      | 1             | 0           | 0      |
| 100      | 3        | Control | 2      | 0             | -           | -      |
| 101      | 3        | Control | 2      | 1             | 48          | 0      |
| 102      | 3        | Control | 2      | 1             | 24          | 0      |
| 103      | 3        | Control | 2      | 0             | 24          | 0      |
| 104      | 3        | Control | 2      | 1             | 0           | 0      |
| 105      | 3        | Control | 2      | 1             | 48          | 0      |
| 106      | 3        | Control | 2      | 1             | 24          | 0      |
| 107      | 3        | Control | 2      | 1             | 48          | 0      |
| 108      | 3        | Control | 2      | 0             | 48          | 0      |
| 109      | 3        | Control | 2      | 1             | 24          | 0      |
| 110      | 3        | Control | 2      | 1             | 24          | 0      |
| 111      | 3        | Vaccine | 2      | 1             | -           | -      |
| 112      | 3        | Vaccine | 2      | 1             | -           | -      |
| 113      | 3        | Vaccine | 2      | 0             | -           | -      |
| 114      | 3        | Vaccine | 2      | 1             | -           | -      |
| 115      | 3        | Vaccine | 2      | 1             | -           | -      |
| 116      | 3        | Vaccine | 2      | 1             | -           | -      |
| 117      | 3        | Vaccine | 2      | 1             | -           | -      |
| 118      | 3        | Vaccine | 2      | 1             | -           | -      |
| 119      | 3        | Vaccine | 2      | 1             | -           | -      |
| 120      | 3        | Vaccine | 2      | 1             | -           | -      |
| 121      | 3        | Vaccine | 2      | 1             | -           | -      |
| 122      | 3        | Vaccine | 2      | 1             | -           | -      |
| 123      | 3        | Vaccine | 2      | 1             | -           | -      |
| 124      | 3        | Vaccine | 2      | 1             | -           | -      |
| 125      | 3        | Vaccine | 2      | 0             | -           | -      |
| 126      | 3        | Vaccine | 2      | 1             | -           | -      |
| 127      | 3        | Vaccine | 2      | 1             | -           | -      |
| 128      | 3        | Vaccine | 2      | 1             | -           | -      |
| 129      | 3        | Vaccine | 2      | 1             | -           | -      |
| 130      | 3        | Vaccine | 2      | 1             | -           | -      |
| 131      | 3        | Vaccine | 2      | 1             | -           | -      |
| 132      | 3        | Vaccine | 2      | 1             | -           | -      |
| 133      | 3        | Vaccine | 2      | 1             | -           | -      |
| 134      | 3        | Vaccine | 2      | 1             | -           | -      |
| 135      | 3        | Vaccine | 2      | 1             | -           | -      |
| 136      | 3        | Vaccine | 2      | 1             | -           | -      |
| 137      | 3        | Vaccine | 2      | 1             | -           | -      |
| 138      | 3        | Vaccine | 2      | 1             | -           | -      |
| 139      | 3        | Vaccine | 2      | 1             | -           | -      |
| 140      | 3        | Vaccine | 2      | 1             | -           | -      |
| 141      | 3        | Vaccine | 2      | 1             | -           | -      |
| 142      | 4        | Control | 1      | 1             | 24          | 0      |

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 143      | 4        | Control | 1      | 1             | 24          | 0      |
| 144      | 4        | Control | 1      | 1             | 0           | 0      |
| 145      | 4        | Control | 1      | 1             | 48          | 0      |
| 146      | 4        | Control | 1      | 1             | 0           | 0      |
| 147      | 4        | Control | 1      | 1             | 0           | 0      |
| 148      | 4        | Control | 1      | 1             | 0           | 0      |
| 149      | 4        | Control | 1      | 1             | 0           | 0      |
| 150      | 4        | Control | 1      | 1             | 0           | 0      |
| 151      | 4        | Control | 1      | 1             | 0           | 0      |
| 152      | 4        | Control | 1      | 1             | 0           | 0      |
| 153      | 4        | Control | 1      | 1             | 0           | 0      |
| 154      | 4        | Control | 1      | 1             | 0           | 0      |
| 155      | 4        | Control | 1      | 1             | 24          | 0      |
| 156      | 4        | Control | 1      | 1             | 0           | 0      |
| 157      | 4        | Control | 1      | 1             | 24          | 0      |
| 158      | 4        | Control | 1      | 1             | 24          | 0      |
| 159      | 4        | Control | 1      | 1             | 192         | 0      |
| 160      | 4        | Control | 1      | 1             | 0           | 0      |
| 161      | 4        | Control | 1      | 1             | 48          | 0      |
| 162      | 4        | Control | 1      | 1             | 0           | 0      |
| 163      | 4        | Control | 1      | 0             | 48          | 0      |
| 164      | 4        | Control | 1      | 1             | -           | -      |
| 165      | 4        | Control | 1      | 1             | 0           | 0      |
| 166      | 4        | Control | 1      | 1             | 24          | 0      |
| 167      | 4        | Control | 1      | 1             | -           | -      |
| 168      | 4        | Control | 1      | 1             | 24          | 0      |
| 169      | 4        | Control | 1      | 1             | 24          | 0      |
| 170      | 4        | Control | 1      | 1             | 0           | 0      |
| 171      | 4        | Control | 1      | 1             | 0           | 0      |
| 172      | 4        | Control | 1      | 1             | 0           | 0      |
| 173      | 4        | Control | 1      | 1             | 0           | 0      |
| 174      | 4        | Control | 1      | 1             | 24          | 0      |
| 175      | 4        | Vaccine | 1      | 1             | -           | -      |
| 176      | 4        | Vaccine | 1      | 1             | -           | -      |
| 177      | 4        | Vaccine | 1      | 1             | -           | -      |
| 178      | 4        | Vaccine | 1      | 1             | -           | -      |
| 179      | 4        | Vaccine | 1      | 1             | -           | -      |
| 180      | 4        | Vaccine | 1      | 1             | -           | -      |
| 181      | 4        | Vaccine | 1      | 1             | -           | -      |
| 182      | 4        | Vaccine | 1      | 1             | -           | -      |
| 183      | 4        | Vaccine | 1      | 1             | -           | -      |
| 184      | 4        | Vaccine | 1      | 1             | -           | -      |
| 185      | 4        | Vaccine | 1      | 1             | -           | -      |
| 186      | 4        | Vaccine | 1      | 1             | -           | -      |
| 187      | 4        | Vaccine | 1      | 1             | -           | -      |
| 188      | 4        | Vaccine | 1      | 1             | -           | -      |
| 189      | 4        | Vaccine | 1      | 1             | -           | -      |
| 190      | 4        | Vaccine | 1      | 1             | -           | -      |
| 191      | 4        | Vaccine | 1      | 1             | -           | -      |
| 192      | 4        | Vaccine | 1      | 1             | -           | -      |
| 193      | 4        | Vaccine | 1      | 1             | -           | -      |
| 194      | 4        | Vaccine | 1      | 1             | -           | -      |
| 195      | 4        | Vaccine | 1      | 1             | -           | -      |
| 196      | 4        | Vaccine | 1      | 1             | -           | -      |
| 197      | 4        | Vaccine | 1      | 1             | -           | -      |
| 198      | 4        | Vaccine | 1      | 1             | -           | -      |
| 199      | 4        | Vaccine | 1      | 1             | -           | -      |

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjibovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 200      | 4        | Vaccine | 1      | 1             | -           | -      |
| 201      | 4        | Vaccine | 1      | 1             | -           | -      |
| 202      | 4        | Vaccine | 1      | 1             | -           | -      |
| 203      | 4        | Vaccine | 1      | 1             | -           | -      |
| 142      | 4        | Control | 2      | 0             | 0           | 0      |
| 143      | 4        | Control | 2      | 1             | 24          | 0      |
| 144      | 4        | Control | 2      | 1             | 0           | 0      |
| 145      | 4        | Control | 2      | 1             | 24          | 0      |
| 146      | 4        | Control | 2      | 0             | 0           | 0      |
| 147      | 4        | Control | 2      | 1             | 0           | 0      |
| 148      | 4        | Control | 2      | 1             | 24          | 0      |
| 149      | 4        | Control | 2      | 1             | 0           | 0      |
| 150      | 4        | Control | 2      | 1             | 0           | 0      |
| 151      | 4        | Control | 2      | 1             | 24          | 0      |
| 152      | 4        | Control | 2      | 1             | 48          | 0      |
| 153      | 4        | Control | 2      | 1             | 0           | 0      |
| 154      | 4        | Control | 2      | 1             | 24          | 0      |
| 155      | 4        | Control | 2      | 1             | 48          | 0      |
| 156      | 4        | Control | 2      | 1             | 24          | 0      |
| 157      | 4        | Control | 2      | 1             | 24          | 0      |
| 158      | 4        | Control | 2      | 1             | 0           | 0      |
| 159      | 4        | Control | 2      | 1             | 48          | 0      |
| 160      | 4        | Control | 2      | 1             | 0           | 0      |
| 161      | 4        | Control | 2      | 1             | 48          | 0      |
| 162      | 4        | Control | 2      | 1             | 0           | 0      |
| 163      | 4        | Control | 2      | 0             | 0           | 0      |
| 164      | 4        | Control | 2      | 0             | 48          | 0      |
| 165      | 4        | Control | 2      | 1             | 0           | 0      |
| 166      | 4        | Control | 2      | 1             | 48          | 0      |
| 167      | 4        | Control | 2      | 0             | 96          | 0      |
| 168      | 4        | Control | 2      | 0             | 24          | 0      |
| 169      | 4        | Control | 2      | 1             | 0           | 0      |
| 170      | 4        | Control | 2      | 1             | 0           | 0      |
| 171      | 4        | Control | 2      | 1             | 0           | 0      |
| 172      | 4        | Control | 2      | 1             | 0           | 0      |
| 173      | 4        | Control | 2      | 1             | 0           | 0      |
| 174      | 4        | Control | 2      | 1             | 0           | 0      |
| 175      | 4        | Vaccine | 2      | 1             | -           | -      |
| 176      | 4        | Vaccine | 2      | 1             | -           | -      |
| 177      | 4        | Vaccine | 2      | 1             | -           | -      |
| 178      | 4        | Vaccine | 2      | 0             | -           | -      |
| 179      | 4        | Vaccine | 2      | 1             | -           | -      |
| 180      | 4        | Vaccine | 2      | 1             | -           | -      |
| 181      | 4        | Vaccine | 2      | 1             | -           | -      |
| 182      | 4        | Vaccine | 2      | 1             | -           | -      |
| 183      | 4        | Vaccine | 2      | 1             | -           | -      |
| 184      | 4        | Vaccine | 2      | 1             | -           | -      |
| 185      | 4        | Vaccine | 2      | 1             | -           | -      |
| 186      | 4        | Vaccine | 2      | 1             | -           | -      |
| 187      | 4        | Vaccine | 2      | 1             | -           | -      |
| 188      | 4        | Vaccine | 2      | 1             | -           | -      |
| 189      | 4        | Vaccine | 2      | 1             | -           | -      |
| 190      | 4        | Vaccine | 2      | 1             | -           | -      |
| 191      | 4        | Vaccine | 2      | 0             | -           | -      |
| 192      | 4        | Vaccine | 2      | 1             | -           | -      |
| 193      | 4        | Vaccine | 2      | 1             | -           | -      |
| 194      | 4        | Vaccine | 2      | 1             | -           | -      |

| Deer no. | Farm no. | Status  | Period | Mother status | Hardjobovis | Pomona |
|----------|----------|---------|--------|---------------|-------------|--------|
| 195      | 4        | Vaccine | 2      | 1             | -           | -      |
| 196      | 4        | Vaccine | 2      | 0             | -           | -      |
| 197      | 4        | Vaccine | 2      | 1             | -           | -      |
| 198      | 4        | Vaccine | 2      | 0             | -           | -      |
| 199      | 4        | Vaccine | 2      | 1             | -           | -      |
| 200      | 4        | Vaccine | 2      | 1             | -           | -      |
| 201      | 4        | Vaccine | 2      | 1             | -           | -      |
| 202      | 4        | Vaccine | 2      | 1             | -           | -      |
| 203      | 4        | Vaccine | 2      | 1             | -           | -      |

**Appendix 3b.** Raw data of urine shedding in no-streptomycin control deer.

(Period: 1 = June'07, 2 = March'08) (Culture and PCR: 0 = negative, 1 = positive)

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 1        | 1        | 1      | 0       | 0   |
| 2        | 1        | 1      | 0       | 0   |
| 3        | 1        | 1      | 0       | 0   |
| 4        | 1        | 1      | 0       | 0   |
| 5        | 1        | 1      | 0       | 1   |
| 6        | 1        | 1      | 0       | 0   |
| 7        | 1        | 1      | 0       | 0   |
| 8        | 1        | 1      | 0       | 1   |
| 9        | 1        | 1      | 0       | 0   |
| 10       | 1        | 1      | 0       | 0   |
| 11       | 1        | 1      | 0       | 0   |
| 12       | 1        | 1      | 0       | 1   |
| 13       | 1        | 1      | 0       | 0   |
| 14       | 1        | 1      | 0       | 0   |
| 15       | 1        | 1      | 0       | 0   |
| 16       | 1        | 1      | 0       | 0   |
| 17       | 1        | 1      | 0       | 0   |
| 18       | 1        | 1      | 0       | 1   |
| 19       | 1        | 1      | 0       | 0   |
| 20       | 1        | 2      | 0       | 0   |
| 21       | 1        | 2      | 0       | 0   |
| 22       | 1        | 2      | 0       | 1   |
| 23       | 1        | 2      | 0       | 0   |
| 24       | 1        | 2      | 0       | 0   |
| 25       | 1        | 2      | 0       | 0   |
| 26       | 1        | 2      | 0       | 0   |
| 27       | 1        | 2      | 0       | 0   |
| 28       | 1        | 2      | 0       | 0   |
| 29       | 1        | 2      | 0       | 0   |
| 30       | 1        | 2      | 0       | 0   |
| 31       | 1        | 2      | 0       | 0   |
| 32       | 1        | 2      | 0       | 1   |
| 33       | 1        | 2      | 0       | 0   |
| 34       | 1        | 2      | 0       | 0   |
| 35       | 1        | 2      | 0       | 0   |
| 36       | 1        | 2      | 0       | 0   |
| 37       | 1        | 2      | 0       | 0   |
| 38       | 1        | 2      | 0       | 0   |
| 39       | 1        | 2      | 0       | 0   |
| 40       | 1        | 2      | 0       | 0   |
| 41       | 2        | 1      | 0       | 0   |
| 42       | 2        | 1      | 0       | 0   |
| 43       | 2        | 1      | 0       | 0   |
| 44       | 2        | 1      | 0       | 0   |
| 45       | 2        | 1      | 0       | 0   |
| 46       | 2        | 1      | 0       | 0   |
| 47       | 2        | 1      | 0       | 0   |
| 48       | 2        | 1      | 0       | 0   |
| 49       | 2        | 1      | 0       | 0   |
| 50       | 2        | 1      | 0       | 0   |
| 51       | 2        | 1      | 0       | 0   |
| 52       | 2        | 1      | 0       | 0   |
| 53       | 2        | 1      | 0       | 0   |
| 54       | 2        | 1      | 0       | 0   |

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 55       | 2        | 1      | 0       | 0   |
| 56       | 2        | 1      | 0       | 0   |
| 57       | 2        | 1      | 0       | 0   |
| 58       | 2        | 1      | 0       | 0   |
| 59       | 2        | 1      | 0       | 0   |
| 60       | 2        | 1      | 0       | 0   |
| 61       | 2        | 2      | 0       | 0   |
| 62       | 2        | 2      | 0       | 0   |
| 63       | 2        | 2      | 0       | 0   |
| 64       | 2        | 2      | 0       | 0   |
| 65       | 2        | 2      | 0       | 0   |
| 66       | 2        | 2      | 0       | 0   |
| 67       | 2        | 2      | 0       | 0   |
| 68       | 2        | 2      | 0       | 0   |
| 69       | 2        | 2      | 0       | 0   |
| 70       | 2        | 2      | 0       | 0   |
| 71       | 2        | 2      | 0       | 0   |
| 72       | 2        | 2      | 0       | 0   |
| 73       | 2        | 2      | 0       | 0   |
| 74       | 2        | 2      | 0       | 0   |
| 75       | 2        | 2      | 0       | 0   |
| 76       | 2        | 2      | 0       | 0   |
| 77       | 2        | 2      | 0       | 0   |
| 78       | 2        | 2      | 0       | 0   |
| 79       | 2        | 2      | 0       | 0   |
| 80       | 2        | 2      | 0       | 0   |
| 81       | 3        | 1      | 0       | 0   |
| 82       | 3        | 1      | 0       | 0   |
| 83       | 3        | 1      | 0       | 0   |
| 84       | 3        | 1      | 0       | 0   |
| 85       | 3        | 1      | 0       | 0   |
| 86       | 3        | 1      | 0       | 0   |
| 87       | 3        | 1      | 0       | 0   |
| 88       | 3        | 1      | 0       | 0   |
| 89       | 3        | 1      | 0       | 0   |
| 90       | 3        | 1      | 0       | 0   |
| 91       | 3        | 1      | 0       | 0   |
| 92       | 3        | 1      | 0       | 0   |
| 93       | 3        | 1      | 0       | 0   |
| 94       | 3        | 1      | 0       | 0   |
| 95       | 3        | 1      | 0       | 0   |
| 96       | 3        | 1      | 0       | 0   |
| 97       | 3        | 1      | 0       | 0   |
| 98       | 3        | 1      | 0       | 0   |
| 99       | 3        | 1      | 0       | 0   |
| 100      | 3        | 1      | 0       | 0   |
| 101      | 3        | 2      | 0       | 0   |
| 102      | 3        | 2      | 0       | 0   |
| 103      | 3        | 2      | 0       | 0   |
| 104      | 3        | 2      | 0       | 0   |
| 105      | 3        | 2      | 0       | 0   |
| 106      | 3        | 2      | 0       | 0   |
| 107      | 3        | 2      | 0       | 0   |
| 108      | 3        | 2      | 0       | 0   |
| 109      | 3        | 2      | 0       | 0   |
| 110      | 3        | 2      | 0       | 0   |
| 111      | 3        | 2      | 0       | 0   |

| Deer no. | Farm no. | Period | Culture | PCR |
|----------|----------|--------|---------|-----|
| 112      | 3        | 2      | 0       | 0   |
| 113      | 3        | 2      | 0       | 0   |
| 114      | 3        | 2      | 0       | 0   |
| 115      | 3        | 2      | 0       | 0   |
| 116      | 3        | 2      | 0       | 0   |
| 117      | 3        | 2      | 0       | 0   |
| 118      | 3        | 2      | 0       | 0   |
| 119      | 3        | 2      | 0       | 0   |
| 120      | 3        | 2      | 0       | 0   |
| 121      | 4        | 1      | 0       | 0   |
| 122      | 4        | 1      | 0       | 0   |
| 123      | 4        | 1      | 0       | 0   |
| 124      | 4        | 1      | 0       | 0   |
| 125      | 4        | 1      | 0       | 0   |
| 126      | 4        | 1      | 0       | 0   |
| 127      | 4        | 1      | 0       | 0   |
| 128      | 4        | 1      | 0       | 0   |
| 129      | 4        | 1      | 0       | 0   |
| 130      | 4        | 1      | 0       | 0   |
| 131      | 4        | 1      | 0       | 0   |
| 132      | 4        | 1      | 0       | 0   |
| 133      | 4        | 1      | 0       | 0   |
| 134      | 4        | 1      | 0       | 0   |
| 135      | 4        | 1      | 0       | 0   |
| 136      | 4        | 1      | 0       | 0   |
| 137      | 4        | 1      | 0       | 0   |
| 138      | 4        | 1      | 0       | 0   |
| 139      | 4        | 1      | 0       | 0   |
| 140      | 4        | 1      | 0       | 0   |
| 141      | 4        | 2      | 0       | 0   |
| 142      | 4        | 2      | 0       | 1   |
| 143      | 4        | 2      | 0       | 0   |
| 144      | 4        | 2      | 0       | 0   |
| 145      | 4        | 2      | 0       | 0   |
| 146      | 4        | 2      | 0       | 0   |
| 147      | 4        | 2      | 0       | 0   |
| 148      | 4        | 2      | 0       | 1   |
| 149      | 4        | 2      | 0       | 0   |
| 150      | 4        | 2      | 0       | 0   |
| 151      | 4        | 2      | 0       | 0   |
| 152      | 4        | 2      | 0       | 1   |
| 153      | 4        | 2      | 0       | 1   |
| 154      | 4        | 2      | 0       | 1   |
| 155      | 4        | 2      | 0       | 1   |
| 156      | 5        | 2      | 0       | 0   |
| 157      | 5        | 2      | 0       | 0   |
| 158      | 5        | 2      | 0       | 0   |
| 159      | 5        | 2      | 0       | 0   |
| 160      | 5        | 2      | 0       | 0   |





## Appendix 4a. Farm questionnaire.



### Mixed-species farm leptospirosis survey project Farm owner/manager questionnaire

#### Section A: General information

Farm name:

.....

Contact person (owner or manager):

.....

Postal address:

.....

Farm location:

.....

Tel: ..... Mobile: ..... Email: .....

#### Section B: Stock number/Breed

Please fill in your current number and breed of the animals in each species/class in the following table.

| Deer                  | Number |   | Breed | Cattle                | Number |   | Breed | Sheep               | Number |   | Breed |
|-----------------------|--------|---|-------|-----------------------|--------|---|-------|---------------------|--------|---|-------|
|                       | M      | F |       |                       | M      | F |       |                     | M      | F |       |
| Weaner<br>(0-12 M)    |        |   |       | Calf<br>(0-12 M)      |        |   |       | Lamb<br>(0-12 M)    |        |   |       |
| Yearling<br>(12-24 M) |        |   |       | Yearling<br>(12-24 M) |        |   |       | Hogget<br>(12-24 M) |        |   |       |
| Hinds<br>(>24 M)      |        |   |       | Cow<br>(>24 M)        |        |   |       | Ewe<br>(>24 M)      |        |   |       |
| Stags<br>(>24 M)      |        |   |       | Bull<br>(>24 M)       |        |   |       | Ram<br>(>24 M)      |        |   |       |

Other species (please describe)..... Total number.....

#### Section C: Farm geography

Total area of farm: ..... acre/ha

Effective area of farm: ..... acre/ha

Deer fenced area of farm: ..... acre/ha

Number of Paddocks.....

Number of paddocks with deer fenced.....

Source of water: Troughs ☐ Dams ☐ Streams ☐ Irrigation ditches ☐

Others ☐ (please describe).....

Please obtain your farm map (print or photo). Describe and estimate (%) the land type (e.g. hill country, downland, flat), areas that would collect drainage (ponds, streams, irrigation ditches, water wells) and identify animal species/class usually grazing in each area.

## Section D: Purpose of farms

Please cross (x) your purposes of each livestock on farm as appropriate (can be > 1)

| Deer                     |  | Cattle                   |  | Sheep                    |  |
|--------------------------|--|--------------------------|--|--------------------------|--|
| Breeding                 |  | Breeding                 |  | Breeding                 |  |
| Finishing                |  | Finishing                |  | Finishing                |  |
| Velvet                   |  | Dairy                    |  | Wool                     |  |
| Venison                  |  | Beef                     |  | Mutton                   |  |
| Other(describe)<br>..... |  | Other(describe)<br>..... |  | Other(describe)<br>..... |  |

If you are a finishing farm, do you finish your own-bred stock only or buy in animals?

Own stock ☐ Buy in ☐

If you buy in, have you brought in any stock to finish during the past year (Aug 07- Jul 08)? Yes ☐ No ☐

If yes, please fill in the details in the table below as appropriate.

| Mob number | Buy in animals |       |               | Time period (Months) | Animal source |
|------------|----------------|-------|---------------|----------------------|---------------|
|            | Species        | Class | No. of animal |                      |               |
|            |                |       |               |                      |               |
|            |                |       |               |                      |               |
|            |                |       |               |                      |               |
|            |                |       |               |                      |               |
|            |                |       |               |                      |               |
|            |                |       |               |                      |               |

## Section E: Grazing management

Did either of your animal species cross-graze (same paddock, different time) or co-graze (same paddock, same time) during the past year (Aug 07- Jul 08)? Yes ☐ No ☐

If yes, please cross (×) the type of animal grazing in the table below as appropriate (leave it blank if there are no cross/co-graze).

| Animal Species        | Winter |    | Spring |    | Summer |    | Autumn |    |
|-----------------------|--------|----|--------|----|--------|----|--------|----|
|                       | Cross  | Co | Cross  | Co | Cross  | Co | Cross  | Co |
| Deer + Sheep          |        |    |        |    |        |    |        |    |
| Deer + Cattle         |        |    |        |    |        |    |        |    |
| Sheep + Cattle        |        |    |        |    |        |    |        |    |
| Deer + Sheep + Cattle |        |    |        |    |        |    |        |    |

Did any of your animals graze off-farm during the past year (Aug 07- Jul 08)?

Yes ☐ No ☐

If yes, please fill in the detail in the table below.

| Your livestock |                 | Species your livestock graze after or with |        |       |      |
|----------------|-----------------|--------------------------------------------|--------|-------|------|
| No. /          | Detail          | Deer                                       | Cattle | Sheep | None |
| <b>1</b>       | Animal species  |                                            |        |       |      |
|                | Number          |                                            |        |       |      |
|                | Period (months) |                                            |        |       |      |
| <b>2</b>       | Animal species  |                                            |        |       |      |
|                | Number          |                                            |        |       |      |
|                | Period (months) |                                            |        |       |      |
| <b>3</b>       | Animal species  |                                            |        |       |      |
|                | Number          |                                            |        |       |      |
|                | Period (months) |                                            |        |       |      |

Do you share-graze any stock not belonging to you on your paddocks? Yes ☐ No ☐

If yes, please fill in the details in **section I**.

## Section F: Herd health

Please indicate mortality rate in each animal species/class during the past year (Aug 07- Jul 08) in the following table.

| Species | Class    | Mortality Rate (%) |
|---------|----------|--------------------|
| Deer    | Weaner   |                    |
|         | Yearling |                    |
|         | Hinds    |                    |
|         | Stags    |                    |
| Cattle  | Calf     |                    |
|         | Yearling |                    |
|         | Cow      |                    |
|         | Bull     |                    |
| Sheep   | Lamb     |                    |
|         | Hogget   |                    |
|         | Ewe      |                    |
|         | Ram      |                    |

Please cross (x) the type of vaccine administered to your animal species/class during the past year (Aug 07- Jul 08).

| Vaccination regime        | Deer   |          |       |       | Cattle |          |      |       | Sheep |        |      |      |
|---------------------------|--------|----------|-------|-------|--------|----------|------|-------|-------|--------|------|------|
|                           | Weaner | Yearling | Hinds | Stags | Calf   | Yearling | Cows | Bulls | Lamb  | Hogget | Ewes | Rams |
| No Vaccine                |        |          |       |       |        |          |      |       |       |        |      |      |
| 5 in 1 (Clostridia)       |        |          |       |       |        |          |      |       |       |        |      |      |
| 7 in 1 (Clostridia,Lepto) |        |          |       |       |        |          |      |       |       |        |      |      |
| Yersinia                  |        |          |       |       |        |          |      |       |       |        |      |      |
| Lepto                     |        |          |       |       |        |          |      |       |       |        |      |      |
| Tetanus                   |        |          |       |       |        |          |      |       |       |        |      |      |
| Salmonella                |        |          |       |       |        |          |      |       |       |        |      |      |
| BVD                       |        |          |       |       |        |          |      |       |       |        |      |      |
| Toxoplasma                |        |          |       |       |        |          |      |       |       |        |      |      |
| Campylobacter             |        |          |       |       |        |          |      |       |       |        |      |      |
| Other(describe)           |        |          |       |       |        |          |      |       |       |        |      |      |
| *****                     |        |          |       |       |        |          |      |       |       |        |      |      |

Have your animals being diagnosed or suspected with any of the following conditions during the past year (Aug 07- Jul 08)? If yes, please cross (x) in the box as appropriate. If no, please leave it blank.

| Condition                 | Deer   |          |       |       | Cattle |          |      |       | Sheep |        |      |      |
|---------------------------|--------|----------|-------|-------|--------|----------|------|-------|-------|--------|------|------|
|                           | Weaner | Yearling | Hinds | Stags | Calf   | Yearling | Cows | Bulls | Lamb  | Hogget | Ewes | Rams |
| Yersiniosis               |        |          |       |       |        |          |      |       |       |        |      |      |
| Leptospirosis             |        |          |       |       |        |          |      |       |       |        |      |      |
| Salmonellosis             |        |          |       |       |        |          |      |       |       |        |      |      |
| Malignant Catarrhal Fever |        |          |       |       |        |          |      |       |       |        |      |      |
| E. coli diarrhoea         |        |          |       |       |        |          |      |       |       |        |      |      |
| Bovine Viral Diarrhoea    |        |          |       |       |        |          |      |       |       |        |      |      |
| Cryptosporidiosis         |        |          |       |       |        |          |      |       |       |        |      |      |
| Foot lameness             |        |          |       |       |        |          |      |       |       |        |      |      |
| Pneumonia                 |        |          |       |       |        |          |      |       |       |        |      |      |
| Parapox                   |        |          |       |       |        |          |      |       |       |        |      |      |
| Internal parasitism       |        |          |       |       |        |          |      |       |       |        |      |      |
| Black leg                 |        |          |       |       |        |          |      |       |       |        |      |      |
| Black disease             |        |          |       |       |        |          |      |       |       |        |      |      |
| Malignant oedema          |        |          |       |       |        |          |      |       |       |        |      |      |
| Pulpy kidney              |        |          |       |       |        |          |      |       |       |        |      |      |
| Tetanus                   |        |          |       |       |        |          |      |       |       |        |      |      |
| Other(describe)<br>.....  |        |          |       |       |        |          |      |       |       |        |      |      |

## Section G: Replacement policy

Is your farm open or closed? Open (buy in animals on regular basis) ☐ Closed ☐

Do you quarantine replacement animals? Yes ☐ No ☐

If yes, do you have a quarantine paddock for replacement animals? Yes ☐ No ☐

If no, do you keep replacement animals separated from your other livestock?

Yes ☐ No ☐

Have you brought in any animals for replacement during the past year (Aug 07- Jul 08)? Yes ☐ No ☐

If yes, please fill in the details in the table below as appropriate.

| Mob no. | Replacement |       |               | Time period (months) | Quarantine Length (days) |
|---------|-------------|-------|---------------|----------------------|--------------------------|
|         | Species     | Class | No. of animal |                      |                          |
|         |             |       |               |                      |                          |
|         |             |       |               |                      |                          |
|         |             |       |               |                      |                          |
|         |             |       |               |                      |                          |
|         |             |       |               |                      |                          |
|         |             |       |               |                      |                          |

## Section H: Leptospirosis

Has leptospirosis ever been diagnosed by a veterinarian in your animals? Yes ☐ No ☐

If yes, in what kind and class of animals?

.....

If yes, when did it happen?

.....

If yes, what were the signs? (e.g. death, anaemia, red water, abortions, jaundice)

.....

If yes, what tests were done for diagnosis? (can be > 1)

Serological test ☐ Bacteria culture test ☐ No test were done ☐

Have you ever seen animals with signs such as anaemia/ red water/ jaundice on your property? Yes ☐ No ☐

Have you ever discussed leptospirosis issues with your veterinarian? Yes ☐ No ☐

What are the sources of your knowledge of leptospirosis? (can be > 1)

Veterinarian ☐ Other farmers ☐ Magazines ☐ Drug company ☐

ACC/OSH ☐ Internet ☐ Others ☐ (please describe).....

How do you rate leptospirosis as a threat to your animals?

Significant risk ☐ Minor risk ☐ No risk at all ☐

Have you or your workers ever been diagnosed with leptospirosis? Yes ☐ No ☐

If yes, when did it happen?

.....

If yes, what were the signs? (e.g. fever, headache, myalgia, photophobia)

.....

How do you rate leptospirosis as far as your and your family's health is concerned?

Significant risk ☐ Minor risk ☐ No risk at all ☐

## Section I: Neighbour

Please indicate animal species and time period of neighbour livestock share-graze with your deer, cattle and sheep (leave it blank if there is no share-graze between your livestock and neighbour)

| Neighbour<br>No. / Detail |                 | Your livestock property |        |       |
|---------------------------|-----------------|-------------------------|--------|-------|
|                           |                 | Deer                    | Cattle | Sheep |
| 1                         | Animal species  |                         |        |       |
|                           | Period (months) |                         |        |       |
| 2                         | Animal species  |                         |        |       |
|                           | Period (months) |                         |        |       |
| 3                         | Animal species  |                         |        |       |
|                           | Period (months) |                         |        |       |
| 4                         | Animal species  |                         |        |       |
|                           | Period (months) |                         |        |       |

Is there any water flow from neighbours onto your property? Yes ☐ No ☐

If yes, please indicate animal species on neighbour's farms (can be > 1).

Deer ☐ Beef Cattle ☐ Dairy Cattle ☐ Sheep ☐ Goat ☐

Other ☐ (please describe) .....

## Section J: Veterinarians

Your current veterinarian(s):

.....

Clinic:

.....

How frequently do you seek a veterinary service?

Once a month ☐ Once in 3 months ☐ Once in 6 months ☐ Once a year ☐

What is your main purpose of seeking a veterinary service?



Animal health problems ☐ Pregnancy scanning ☐ Management consult ☐  
Other ☐ (Please describe).....

Will you agree if we send your farm's leptospirosis test results to your veterinarian?

Yes ☐ No ☐

### **Section K: Signature of farm owner/manager**

I understand that all information contained with in this form is totally confidential to the researchers or where agreed, your veterinary practitioners. No individual farmer or property will be identifiable to other parties when this data is used for any purpose.

.....  
Signature of farm owner/manager

.....  
Date

## Appendix 4b. Raw data of serology for deer, cattle and sheep from 2006 to 2008.

\*Farm 8 was lost to follow-up in 2007 and 2008

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1      | 1    | 2006 | Deer    | 0           | 0      |
| 2      | 1    | 2006 | Deer    | 0           | 0      |
| 3      | 1    | 2006 | Deer    | 0           | 0      |
| 4      | 1    | 2006 | Deer    | 0           | 0      |
| 5      | 1    | 2006 | Deer    | 0           | 0      |
| 6      | 1    | 2006 | Deer    | 0           | 0      |
| 7      | 1    | 2006 | Deer    | 0           | 0      |
| 8      | 1    | 2006 | Deer    | 0           | 0      |
| 9      | 1    | 2006 | Deer    | 0           | 0      |
| 10     | 1    | 2006 | Deer    | 0           | 0      |
| 11     | 1    | 2006 | Deer    | 0           | 0      |
| 12     | 1    | 2006 | Deer    | 0           | 0      |
| 13     | 1    | 2006 | Deer    | 48          | 0      |
| 14     | 1    | 2006 | Deer    | 0           | 0      |
| 15     | 1    | 2006 | Deer    | 0           | 0      |
| 16     | 1    | 2006 | Deer    | 0           | 0      |
| 17     | 1    | 2006 | Deer    | 0           | 0      |
| 18     | 1    | 2006 | Deer    | 0           | 0      |
| 19     | 1    | 2006 | Deer    | 24          | 0      |
| 20     | 2    | 2006 | Deer    | 0           | 0      |
| 21     | 2    | 2006 | Deer    | 0           | 0      |
| 22     | 2    | 2006 | Deer    | 0           | 0      |
| 23     | 2    | 2006 | Deer    | 0           | 0      |
| 24     | 2    | 2006 | Deer    | 0           | 0      |
| 25     | 2    | 2006 | Deer    | 0           | 0      |
| 26     | 2    | 2006 | Deer    | 0           | 0      |
| 27     | 2    | 2006 | Deer    | 0           | 0      |
| 28     | 2    | 2006 | Deer    | 0           | 0      |
| 29     | 2    | 2006 | Deer    | 0           | 0      |
| 30     | 2    | 2006 | Deer    | 0           | 0      |
| 31     | 2    | 2006 | Deer    | 0           | 0      |
| 32     | 2    | 2006 | Deer    | 0           | 0      |
| 33     | 2    | 2006 | Deer    | 0           | 0      |
| 34     | 2    | 2006 | Deer    | 24          | 0      |
| 35     | 2    | 2006 | Deer    | 0           | 0      |
| 36     | 2    | 2006 | Deer    | 0           | 0      |
| 37     | 2    | 2006 | Deer    | 0           | 0      |
| 38     | 2    | 2006 | Deer    | 0           | 0      |
| 39     | 2    | 2006 | Deer    | 0           | 0      |
| 40     | 3    | 2006 | Deer    | 48          | 0      |
| 41     | 3    | 2006 | Deer    | 0           | 0      |
| 42     | 3    | 2006 | Deer    | 24          | 0      |
| 43     | 3    | 2006 | Deer    | 0           | 0      |
| 44     | 3    | 2006 | Deer    | 0           | 0      |
| 45     | 3    | 2006 | Deer    | 0           | 0      |
| 46     | 3    | 2006 | Deer    | 0           | 0      |
| 47     | 3    | 2006 | Deer    | 0           | 0      |
| 48     | 3    | 2006 | Deer    | 0           | 0      |
| 49     | 3    | 2006 | Deer    | 0           | 0      |
| 50     | 3    | 2006 | Deer    | 0           | 0      |
| 51     | 3    | 2006 | Deer    | 0           | 0      |
| 52     | 3    | 2006 | Deer    | 0           | 0      |
| 53     | 3    | 2006 | Deer    | 0           | 0      |
| 54     | 3    | 2006 | Deer    | 24          | 0      |
| 55     | 3    | 2006 | Deer    | 0           | 0      |
| 56     | 3    | 2006 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 57     | 3    | 2006 | Deer    | 0           | 0      |
| 58     | 3    | 2006 | Deer    | 0           | 0      |
| 59     | 3    | 2006 | Deer    | 0           | 0      |
| 60     | 4    | 2006 | Deer    | 0           | 0      |
| 61     | 4    | 2006 | Deer    | 24          | 0      |
| 62     | 4    | 2006 | Deer    | 24          | 96     |
| 63     | 4    | 2006 | Deer    | 0           | 0      |
| 64     | 4    | 2006 | Deer    | 0           | 0      |
| 65     | 4    | 2006 | Deer    | 0           | 192    |
| 66     | 4    | 2006 | Deer    | 0           | 384    |
| 67     | 4    | 2006 | Deer    | 0           | 0      |
| 68     | 4    | 2006 | Deer    | 96          | 0      |
| 69     | 4    | 2006 | Deer    | 0           | 0      |
| 70     | 4    | 2006 | Deer    | 0           | 24     |
| 71     | 4    | 2006 | Deer    | 0           | 192    |
| 72     | 4    | 2006 | Deer    | 0           | 96     |
| 73     | 4    | 2006 | Deer    | 0           | 0      |
| 74     | 4    | 2006 | Deer    | 0           | 0      |
| 75     | 4    | 2006 | Deer    | 0           | 24     |
| 76     | 4    | 2006 | Deer    | 0           | 0      |
| 77     | 4    | 2006 | Deer    | 0           | 192    |
| 78     | 4    | 2006 | Deer    | 0           | 24     |
| 79     | 4    | 2006 | Deer    | 0           | 192    |
| 80     | 4    | 2006 | Deer    | 0           | 0      |
| 81     | 4    | 2006 | Cattle  | 0           | 96     |
| 82     | 4    | 2006 | Cattle  | 96          | 0      |
| 83     | 4    | 2006 | Cattle  | 192         | 0      |
| 84     | 4    | 2006 | Cattle  | 96          | 48     |
| 85     | 4    | 2006 | Cattle  | 0           | 0      |
| 86     | 5    | 2006 | Deer    | 0           | 0      |
| 87     | 5    | 2006 | Deer    | 96          | 0      |
| 88     | 5    | 2006 | Deer    | 0           | 0      |
| 89     | 5    | 2006 | Deer    | 0           | 0      |
| 90     | 5    | 2006 | Deer    | 24          | 96     |
| 91     | 5    | 2006 | Deer    | 0           | 48     |
| 92     | 5    | 2006 | Deer    | 0           | 48     |
| 93     | 5    | 2006 | Deer    | 0           | 0      |
| 94     | 5    | 2006 | Deer    | 0           | 96     |
| 95     | 5    | 2006 | Deer    | 0           | 0      |
| 96     | 5    | 2006 | Deer    | 0           | 96     |
| 97     | 5    | 2006 | Deer    | 0           | 0      |
| 98     | 5    | 2006 | Deer    | 48          | 384    |
| 99     | 5    | 2006 | Deer    | 0           | 48     |
| 100    | 5    | 2006 | Deer    | 0           | 0      |
| 101    | 5    | 2006 | Deer    | 48          | 0      |
| 102    | 5    | 2006 | Deer    | 0           | 0      |
| 103    | 5    | 2006 | Deer    | 0           | 96     |
| 104    | 5    | 2006 | Deer    | 0           | 192    |
| 105    | 5    | 2006 | Deer    | 0           | 96     |
| 106    | 5    | 2006 | Deer    | 0           | 0      |
| 107    | 5    | 2006 | Cattle  | 48          | 0      |
| 108    | 5    | 2006 | Cattle  | 0           | 0      |
| 109    | 5    | 2006 | Cattle  | 96          | 0      |
| 110    | 5    | 2006 | Cattle  | 48          | 0      |
| 111    | 5    | 2006 | Cattle  | 24          | 0      |
| 112    | 5    | 2006 | Cattle  | 384         | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 113    | 5    | 2006 | Cattle  | 96          | 0      |
| 114    | 5    | 2006 | Cattle  | 24          | 0      |
| 115    | 5    | 2006 | Cattle  | 48          | 0      |
| 116    | 5    | 2006 | Cattle  | 192         | 0      |
| 117    | 5    | 2006 | Cattle  | 48          | 0      |
| 118    | 5    | 2006 | Cattle  | 24          | 0      |
| 119    | 5    | 2006 | Cattle  | 24          | 0      |
| 120    | 5    | 2006 | Cattle  | 48          | 0      |
| 121    | 5    | 2006 | Cattle  | 48          | 0      |
| 122    | 5    | 2006 | Cattle  | 48          | 0      |
| 123    | 5    | 2006 | Cattle  | 24          | 0      |
| 124    | 5    | 2006 | Cattle  | 24          | 0      |
| 125    | 5    | 2006 | Cattle  | 48          | 0      |
| 126    | 5    | 2006 | Cattle  | 96          | 0      |
| 127    | 6    | 2006 | Deer    | 48          | 0      |
| 128    | 6    | 2006 | Deer    | 48          | 0      |
| 129    | 6    | 2006 | Deer    | 0           | 0      |
| 130    | 6    | 2006 | Deer    | 48          | 0      |
| 131    | 6    | 2006 | Deer    | 0           | 0      |
| 132    | 6    | 2006 | Deer    | 0           | 0      |
| 133    | 6    | 2006 | Deer    | 0           | 0      |
| 134    | 6    | 2006 | Deer    | 0           | 0      |
| 135    | 6    | 2006 | Deer    | 0           | 0      |
| 136    | 6    | 2006 | Deer    | 0           | 0      |
| 137    | 6    | 2006 | Deer    | 0           | 0      |
| 138    | 6    | 2006 | Deer    | 0           | 0      |
| 139    | 6    | 2006 | Deer    | 0           | 0      |
| 140    | 6    | 2006 | Deer    | 48          | 192    |
| 141    | 6    | 2006 | Deer    | 0           | 0      |
| 142    | 6    | 2006 | Deer    | 48          | 0      |
| 143    | 6    | 2006 | Deer    | 0           | 0      |
| 144    | 6    | 2006 | Deer    | 96          | 0      |
| 145    | 6    | 2006 | Deer    | 48          | 0      |
| 146    | 6    | 2006 | Deer    | 48          | 0      |
| 147    | 6    | 2006 | Cattle  | 48          | 0      |
| 148    | 6    | 2006 | Cattle  | 96          | 0      |
| 149    | 6    | 2006 | Cattle  | 48          | 0      |
| 150    | 6    | 2006 | Cattle  | 96          | 24     |
| 151    | 6    | 2006 | Cattle  | 192         | 0      |
| 152    | 6    | 2006 | Cattle  | 384         | 0      |
| 153    | 6    | 2006 | Cattle  | 0           | 0      |
| 154    | 6    | 2006 | Cattle  | 192         | 0      |
| 155    | 6    | 2006 | Cattle  | 24          | 0      |
| 156    | 6    | 2006 | Cattle  | 48          | 0      |
| 157    | 6    | 2006 | Cattle  | 96          | 0      |
| 158    | 6    | 2006 | Cattle  | 192         | 0      |
| 159    | 6    | 2006 | Cattle  | 96          | 48     |
| 160    | 6    | 2006 | Cattle  | 0           | 24     |
| 161    | 6    | 2006 | Cattle  | 24          | 0      |
| 162    | 6    | 2006 | Cattle  | 96          | 0      |
| 163    | 6    | 2006 | Cattle  | 384         | 0      |
| 164    | 6    | 2006 | Cattle  | 384         | 0      |
| 165    | 6    | 2006 | Cattle  | 192         | 0      |
| 166    | 6    | 2006 | Cattle  | 384         | 0      |
| 167    | 7    | 2006 | Deer    | 0           | 0      |
| 168    | 7    | 2006 | Deer    | 48          | 0      |
| 169    | 7    | 2006 | Deer    | 24          | 0      |
| 170    | 7    | 2006 | Deer    | 48          | 192    |
| 171    | 7    | 2006 | Deer    | 48          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 172    | 7    | 2006 | Deer    | 0           | 0      |
| 173    | 7    | 2006 | Deer    | 96          | 0      |
| 174    | 7    | 2006 | Deer    | 0           | 0      |
| 175    | 7    | 2006 | Deer    | 96          | 0      |
| 176    | 7    | 2006 | Deer    | 0           | 0      |
| 177    | 7    | 2006 | Deer    | 24          | 0      |
| 178    | 7    | 2006 | Deer    | 0           | 0      |
| 179    | 7    | 2006 | Deer    | 24          | 0      |
| 180    | 7    | 2006 | Deer    | 48          | 0      |
| 181    | 7    | 2006 | Deer    | 0           | 0      |
| 182    | 7    | 2006 | Deer    | 0           | 0      |
| 183    | 7    | 2006 | Deer    | 0           | 0      |
| 184    | 7    | 2006 | Deer    | 0           | 0      |
| 185    | 7    | 2006 | Deer    | 24          | 0      |
| 186    | 7    | 2006 | Deer    | 0           | 0      |
| 187    | 7    | 2006 | Sheep   | 0           | 0      |
| 188    | 7    | 2006 | Sheep   | 0           | 0      |
| 189    | 7    | 2006 | Sheep   | 0           | 0      |
| 190    | 7    | 2006 | Sheep   | 0           | 0      |
| 191    | 7    | 2006 | Sheep   | 0           | 0      |
| 192    | 7    | 2006 | Sheep   | 0           | 0      |
| 193    | 7    | 2006 | Sheep   | 0           | 0      |
| 194    | 7    | 2006 | Sheep   | 0           | 0      |
| 195    | 7    | 2006 | Sheep   | 0           | 0      |
| 196    | 7    | 2006 | Sheep   | 0           | 0      |
| 197    | 7    | 2006 | Sheep   | 0           | 0      |
| 198    | 7    | 2006 | Sheep   | 0           | 0      |
| 199    | 7    | 2006 | Sheep   | 0           | 0      |
| 200    | 7    | 2006 | Sheep   | 0           | 0      |
| 201    | 7    | 2006 | Sheep   | 0           | 0      |
| 202    | 7    | 2006 | Sheep   | 0           | 0      |
| 203    | 7    | 2006 | Sheep   | 0           | 0      |
| 204    | 7    | 2006 | Sheep   | 0           | 0      |
| 205    | 7    | 2006 | Sheep   | 0           | 0      |
| 206    | 7    | 2006 | Sheep   | 0           | 0      |
| 207    | 7    | 2006 | Sheep   | 0           | 0      |
| 208    | 7    | 2006 | Sheep   | 0           | 0      |
| 209    | 7    | 2006 | Sheep   | 0           | 0      |
| 210    | 7    | 2006 | Sheep   | 0           | 0      |
| 211    | 7    | 2006 | Sheep   | 0           | 0      |
| 212    | 7    | 2006 | Sheep   | 0           | 0      |
| 213    | 7    | 2006 | Sheep   | 0           | 0      |
| 214    | 7    | 2006 | Sheep   | 0           | 0      |
| 215    | 7    | 2006 | Sheep   | 0           | 0      |
| 216    | 7    | 2006 | Sheep   | 0           | 0      |
| 217    | 8*   | 2006 | Deer    | 0           | 0      |
| 218    | 8*   | 2006 | Deer    | 0           | 48     |
| 219    | 8*   | 2006 | Deer    | 0           | 0      |
| 220    | 8*   | 2006 | Deer    | 0           | 0      |
| 221    | 8*   | 2006 | Deer    | 48          | 0      |
| 222    | 8*   | 2006 | Deer    | 0           | 0      |
| 223    | 8*   | 2006 | Deer    | 0           | 0      |
| 224    | 8*   | 2006 | Deer    | 0           | 0      |
| 225    | 8*   | 2006 | Deer    | 0           | 0      |
| 226    | 8*   | 2006 | Deer    | 0           | 0      |
| 227    | 8*   | 2006 | Deer    | 0           | 0      |
| 228    | 8*   | 2006 | Deer    | 0           | 48     |
| 229    | 8*   | 2006 | Deer    | 0           | 0      |
| 230    | 8*   | 2006 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 231    | 8*   | 2006 | Deer    | 0           | 0      |
| 232    | 8*   | 2006 | Deer    | 0           | 0      |
| 233    | 8*   | 2006 | Deer    | 0           | 0      |
| 234    | 8*   | 2006 | Deer    | 0           | 0      |
| 235    | 8*   | 2006 | Deer    | 0           | 0      |
| 236    | 8*   | 2006 | Sheep   | 192         | 0      |
| 237    | 8*   | 2006 | Sheep   | 0           | 0      |
| 238    | 8*   | 2006 | Sheep   | 0           | 0      |
| 239    | 8*   | 2006 | Sheep   | 96          | 0      |
| 240    | 8*   | 2006 | Sheep   | 384         | 0      |
| 241    | 8*   | 2006 | Sheep   | 0           | 0      |
| 242    | 8*   | 2006 | Sheep   | 0           | 0      |
| 243    | 8*   | 2006 | Sheep   | 0           | 96     |
| 244    | 8*   | 2006 | Sheep   | 768         | 0      |
| 245    | 8*   | 2006 | Sheep   | 0           | 0      |
| 246    | 8*   | 2006 | Sheep   | 0           | 0      |
| 247    | 8*   | 2006 | Sheep   | 0           | 0      |
| 248    | 8*   | 2006 | Sheep   | 192         | 0      |
| 249    | 8*   | 2006 | Sheep   | 0           | 0      |
| 250    | 8*   | 2006 | Sheep   | 0           | 0      |
| 251    | 8*   | 2006 | Sheep   | 0           | 0      |
| 252    | 8*   | 2006 | Sheep   | 1536        | 0      |
| 253    | 8*   | 2006 | Sheep   | 0           | 0      |
| 254    | 8*   | 2006 | Sheep   | 192         | 0      |
| 255    | 8*   | 2006 | Sheep   | 384         | 0      |
| 256    | 8*   | 2006 | Sheep   | 0           | 0      |
| 257    | 8*   | 2006 | Sheep   | 0           | 0      |
| 258    | 8*   | 2006 | Sheep   | 96          | 0      |
| 259    | 8*   | 2006 | Sheep   | 96          | 0      |
| 260    | 8*   | 2006 | Sheep   | 0           | 0      |
| 261    | 8*   | 2006 | Sheep   | 0           | 0      |
| 262    | 8*   | 2006 | Sheep   | 384         | 0      |
| 263    | 8*   | 2006 | Sheep   | 96          | 0      |
| 264    | 8*   | 2006 | Sheep   | 0           | 0      |
| 265    | 8*   | 2006 | Sheep   | 0           | 0      |
| 266    | 8*   | 2006 | Sheep   | 768         | 0      |
| 267    | 8*   | 2006 | Sheep   | 0           | 0      |
| 268    | 8*   | 2006 | Sheep   | 384         | 0      |
| 269    | 8*   | 2006 | Sheep   | 0           | 0      |
| 270    | 8*   | 2006 | Sheep   | 0           | 0      |
| 271    | 8*   | 2006 | Sheep   | 0           | 0      |
| 272    | 8*   | 2006 | Sheep   | 0           | 0      |
| 273    | 8*   | 2006 | Sheep   | 0           | 0      |
| 274    | 8*   | 2006 | Sheep   | 284         | 0      |
| 275    | 8*   | 2006 | Sheep   | 24          | 0      |
| 276    | 8*   | 2006 | Sheep   | 192         | 0      |
| 277    | 8*   | 2006 | Sheep   | 0           | 0      |
| 278    | 8*   | 2006 | Sheep   | 0           | 0      |
| 279    | 8*   | 2006 | Sheep   | 0           | 0      |
| 280    | 8*   | 2006 | Sheep   | 0           | 0      |
| 281    | 8*   | 2006 | Sheep   | 384         | 0      |
| 282    | 8*   | 2006 | Sheep   | 0           | 0      |
| 283    | 8*   | 2006 | Sheep   | 768         | 0      |
| 284    | 8*   | 2006 | Sheep   | 96          | 0      |
| 285    | 8*   | 2006 | Sheep   | 24          | 0      |
| 286    | 8*   | 2006 | Sheep   | 768         | 0      |
| 287    | 8*   | 2006 | Sheep   | 0           | 0      |
| 288    | 8*   | 2006 | Sheep   | 0           | 0      |
| 289    | 8*   | 2006 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 290    | 8*   | 2006 | Sheep   | 48          | 0      |
| 291    | 8*   | 2006 | Sheep   | 0           | 0      |
| 292    | 9    | 2006 | Deer    | 48          | 0      |
| 293    | 9    | 2006 | Deer    | 24          | 0      |
| 294    | 9    | 2006 | Deer    | 0           | 0      |
| 295    | 9    | 2006 | Deer    | 48          | 0      |
| 296    | 9    | 2006 | Deer    | 24          | 0      |
| 297    | 9    | 2006 | Deer    | 0           | 0      |
| 298    | 9    | 2006 | Deer    | 0           | 0      |
| 299    | 9    | 2006 | Deer    | 0           | 0      |
| 300    | 9    | 2006 | Deer    | 48          | 48     |
| 301    | 9    | 2006 | Deer    | 24          | 0      |
| 302    | 9    | 2006 | Deer    | 0           | 0      |
| 303    | 9    | 2006 | Deer    | 24          | 0      |
| 304    | 9    | 2006 | Deer    | 96          | 0      |
| 305    | 9    | 2006 | Deer    | 24          | 0      |
| 306    | 9    | 2006 | Deer    | 24          | 0      |
| 307    | 9    | 2006 | Deer    | 48          | 0      |
| 308    | 9    | 2006 | Deer    | 48          | 0      |
| 309    | 9    | 2006 | Deer    | 0           | 0      |
| 310    | 9    | 2006 | Deer    | 0           | 192    |
| 311    | 9    | 2006 | Deer    | 0           | 0      |
| 312    | 9    | 2006 | Cattle  | 48          | 0      |
| 313    | 9    | 2006 | Cattle  | 48          | 48     |
| 314    | 9    | 2006 | Cattle  | 24          | 0      |
| 315    | 9    | 2006 | Cattle  | 0           | 0      |
| 316    | 9    | 2006 | Cattle  | 96          | 0      |
| 317    | 9    | 2006 | Cattle  | 48          | 0      |
| 318    | 9    | 2006 | Cattle  | 24          | 0      |
| 319    | 9    | 2006 | Cattle  | 24          | 192    |
| 320    | 9    | 2006 | Cattle  | 0           | 0      |
| 321    | 9    | 2006 | Cattle  | 48          | 0      |
| 322    | 9    | 2006 | Cattle  | 48          | 0      |
| 323    | 9    | 2006 | Cattle  | 96          | 0      |
| 324    | 9    | 2006 | Cattle  | 48          | 0      |
| 325    | 9    | 2006 | Cattle  | 48          | 0      |
| 326    | 9    | 2006 | Cattle  | 24          | 0      |
| 327    | 9    | 2006 | Cattle  | 96          | 0      |
| 328    | 9    | 2006 | Cattle  | 24          | 0      |
| 329    | 9    | 2006 | Sheep   | 24          | 0      |
| 330    | 9    | 2006 | Sheep   | 96          | 0      |
| 331    | 9    | 2006 | Sheep   | 48          | 0      |
| 332    | 9    | 2006 | Sheep   | 48          | 0      |
| 333    | 9    | 2006 | Sheep   | 24          | 0      |
| 334    | 9    | 2006 | Sheep   | 24          | 0      |
| 335    | 9    | 2006 | Sheep   | 24          | 0      |
| 336    | 9    | 2006 | Sheep   | 48          | 0      |
| 337    | 9    | 2006 | Sheep   | 48          | 0      |
| 338    | 9    | 2006 | Sheep   | 24          | 0      |
| 339    | 9    | 2006 | Sheep   | 48          | 0      |
| 340    | 9    | 2006 | Sheep   | 384         | 0      |
| 341    | 9    | 2006 | Sheep   | 24          | 0      |
| 342    | 9    | 2006 | Sheep   | 0           | 0      |
| 343    | 9    | 2006 | Sheep   | 24          | 0      |
| 344    | 9    | 2006 | Sheep   | 0           | 0      |
| 345    | 9    | 2006 | Sheep   | 24          | 0      |
| 346    | 9    | 2006 | Sheep   | 192         | 0      |
| 347    | 9    | 2006 | Sheep   | 0           | 0      |
| 348    | 9    | 2006 | Sheep   | 24          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 349    | 9    | 2006 | Sheep   | 48          | 0      |
| 350    | 9    | 2006 | Sheep   | 48          | 96     |
| 351    | 9    | 2006 | Sheep   | 48          | 0      |
| 352    | 9    | 2006 | Sheep   | 96          | 0      |
| 353    | 9    | 2006 | Sheep   | 384         | 384    |
| 354    | 9    | 2006 | Sheep   | 48          | 0      |
| 355    | 9    | 2006 | Sheep   | 24          | 0      |
| 356    | 9    | 2006 | Sheep   | 48          | 0      |
| 357    | 9    | 2006 | Sheep   | 24          | 0      |
| 358    | 9    | 2006 | Sheep   | 48          | 0      |
| 359    | 9    | 2006 | Sheep   | 24          | 0      |
| 360    | 9    | 2006 | Sheep   | 24          | 0      |
| 361    | 9    | 2006 | Sheep   | 24          | 0      |
| 362    | 9    | 2006 | Sheep   | 24          | 0      |
| 363    | 9    | 2006 | Sheep   | 48          | 0      |
| 364    | 9    | 2006 | Sheep   | 48          | 0      |
| 365    | 9    | 2006 | Sheep   | 24          | 0      |
| 366    | 9    | 2006 | Sheep   | 24          | 0      |
| 367    | 9    | 2006 | Sheep   | 24          | 0      |
| 368    | 9    | 2006 | Sheep   | 24          | 0      |
| 369    | 9    | 2006 | Sheep   | 24          | 0      |
| 370    | 9    | 2006 | Sheep   | 0           | 0      |
| 371    | 9    | 2006 | Sheep   | 24          | 0      |
| 372    | 9    | 2006 | Sheep   | 24          | 0      |
| 373    | 9    | 2006 | Sheep   | 0           | 0      |
| 374    | 9    | 2006 | Sheep   | 24          | 0      |
| 375    | 9    | 2006 | Sheep   | 24          | 0      |
| 376    | 9    | 2006 | Sheep   | 24          | 0      |
| 377    | 9    | 2006 | Sheep   | 24          | 0      |
| 378    | 9    | 2006 | Sheep   | 24          | 0      |
| 379    | 9    | 2006 | Sheep   | 24          | 0      |
| 380    | 9    | 2006 | Sheep   | 24          | 0      |
| 381    | 9    | 2006 | Sheep   | 0           | 0      |
| 382    | 9    | 2006 | Sheep   | 0           | 0      |
| 383    | 9    | 2006 | Sheep   | 0           | 0      |
| 384    | 9    | 2006 | Sheep   | 24          | 0      |
| 385    | 9    | 2006 | Sheep   | 24          | 0      |
| 386    | 9    | 2006 | Sheep   | 24          | 0      |
| 387    | 9    | 2006 | Sheep   | 0           | 0      |
| 388    | 9    | 2006 | Sheep   | 24          | 0      |
| 389    | 10   | 2006 | Deer    | 0           | 0      |
| 390    | 10   | 2006 | Deer    | 0           | 0      |
| 391    | 10   | 2006 | Deer    | 0           | 0      |
| 392    | 10   | 2006 | Deer    | 0           | 0      |
| 393    | 10   | 2006 | Deer    | 0           | 0      |
| 394    | 10   | 2006 | Deer    | 0           | 0      |
| 395    | 10   | 2006 | Deer    | 0           | 0      |
| 396    | 10   | 2006 | Deer    | 24          | 0      |
| 397    | 10   | 2006 | Deer    | 0           | 0      |
| 398    | 10   | 2006 | Deer    | 0           | 0      |
| 399    | 10   | 2006 | Deer    | 0           | 0      |
| 400    | 10   | 2006 | Deer    | 0           | 0      |
| 401    | 10   | 2006 | Deer    | 0           | 0      |
| 402    | 10   | 2006 | Deer    | 0           | 0      |
| 403    | 10   | 2006 | Deer    | 0           | 0      |
| 404    | 10   | 2006 | Deer    | 0           | 0      |
| 405    | 10   | 2006 | Deer    | 0           | 0      |
| 406    | 10   | 2006 | Deer    | 0           | 192    |
| 407    | 10   | 2006 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 408    | 10   | 2006 | Deer    | 0           | 0      |
| 409    | 10   | 2006 | Cattle  | 96          | 0      |
| 410    | 10   | 2006 | Cattle  | 24          | 0      |
| 411    | 10   | 2006 | Cattle  | 96          | 0      |
| 412    | 10   | 2006 | Cattle  | 96          | 0      |
| 413    | 10   | 2006 | Cattle  | 48          | 0      |
| 414    | 10   | 2006 | Cattle  | 0           | 0      |
| 415    | 10   | 2006 | Cattle  | 96          | 24     |
| 416    | 10   | 2006 | Cattle  | 192         | 0      |
| 417    | 10   | 2006 | Cattle  | 48          | 192    |
| 418    | 10   | 2006 | Cattle  | 48          | 24     |
| 419    | 10   | 2006 | Cattle  | 0           | 24     |
| 420    | 10   | 2006 | Cattle  | 48          | 48     |
| 421    | 10   | 2006 | Cattle  | 48          | 0      |
| 422    | 10   | 2006 | Cattle  | 48          | 24     |
| 423    | 10   | 2006 | Cattle  | 96          | 0      |
| 424    | 10   | 2006 | Cattle  | 96          | 0      |
| 425    | 10   | 2006 | Cattle  | 96          | 0      |
| 426    | 10   | 2006 | Cattle  | 48          | 0      |
| 427    | 10   | 2006 | Cattle  | 0           | 0      |
| 428    | 10   | 2006 | Cattle  | 96          | 0      |
| 429    | 10   | 2006 | Sheep   | 0           | 0      |
| 430    | 10   | 2006 | Sheep   | 0           | 0      |
| 431    | 10   | 2006 | Sheep   | 0           | 0      |
| 432    | 10   | 2006 | Sheep   | 0           | 0      |
| 433    | 10   | 2006 | Sheep   | 0           | 0      |
| 434    | 10   | 2006 | Sheep   | 0           | 0      |
| 435    | 10   | 2006 | Sheep   | 0           | 0      |
| 436    | 10   | 2006 | Sheep   | 0           | 0      |
| 437    | 10   | 2006 | Sheep   | 0           | 0      |
| 438    | 10   | 2006 | Sheep   | 0           | 0      |
| 439    | 10   | 2006 | Sheep   | 24          | 0      |
| 440    | 10   | 2006 | Sheep   | 0           | 0      |
| 441    | 10   | 2006 | Sheep   | 24          | 0      |
| 442    | 10   | 2006 | Sheep   | 0           | 0      |
| 443    | 10   | 2006 | Sheep   | 0           | 0      |
| 444    | 10   | 2006 | Sheep   | 0           | 0      |
| 445    | 10   | 2006 | Sheep   | 0           | 0      |
| 446    | 10   | 2006 | Sheep   | 0           | 0      |
| 447    | 10   | 2006 | Sheep   | 0           | 0      |
| 448    | 10   | 2006 | Sheep   | 0           | 0      |
| 449    | 10   | 2006 | Sheep   | 0           | 0      |
| 450    | 10   | 2006 | Sheep   | 24          | 0      |
| 451    | 10   | 2006 | Sheep   | 0           | 0      |
| 452    | 10   | 2006 | Sheep   | 0           | 0      |
| 453    | 10   | 2006 | Sheep   | 0           | 0      |
| 454    | 10   | 2006 | Sheep   | 24          | 0      |
| 455    | 10   | 2006 | Sheep   | 0           | 0      |
| 456    | 10   | 2006 | Sheep   | 0           | 0      |
| 457    | 10   | 2006 | Sheep   | 24          | 0      |
| 458    | 10   | 2006 | Sheep   | 48          | 0      |
| 459    | 10   | 2006 | Sheep   | 24          | 0      |
| 460    | 10   | 2006 | Sheep   | 0           | 0      |
| 461    | 10   | 2006 | Sheep   | 24          | 0      |
| 462    | 10   | 2006 | Sheep   | 24          | 0      |
| 463    | 10   | 2006 | Sheep   | 0           | 0      |
| 464    | 10   | 2006 | Sheep   | 0           | 0      |
| 465    | 10   | 2006 | Sheep   | 0           | 0      |
| 466    | 10   | 2006 | Sheep   | 96          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 467    | 10   | 2006 | Sheep   | 192         | 0      |
| 468    | 10   | 2006 | Sheep   | 0           | 0      |
| 469    | 10   | 2006 | Sheep   | 24          | 192    |
| 470    | 10   | 2006 | Sheep   | 0           | 0      |
| 471    | 10   | 2006 | Sheep   | 24          | 0      |
| 472    | 10   | 2006 | Sheep   | 0           | 0      |
| 473    | 10   | 2006 | Sheep   | 24          | 0      |
| 474    | 10   | 2006 | Sheep   | 0           | 0      |
| 475    | 10   | 2006 | Sheep   | 24          | 0      |
| 476    | 10   | 2006 | Sheep   | 0           | 0      |
| 477    | 10   | 2006 | Sheep   | 0           | 0      |
| 478    | 10   | 2006 | Sheep   | 0           | 0      |
| 479    | 10   | 2006 | Sheep   | 0           | 0      |
| 480    | 10   | 2006 | Sheep   | 0           | 0      |
| 481    | 10   | 2006 | Sheep   | 0           | 0      |
| 482    | 10   | 2006 | Sheep   | 0           | 0      |
| 483    | 10   | 2006 | Sheep   | 0           | 0      |
| 484    | 10   | 2006 | Sheep   | 0           | 0      |
| 485    | 10   | 2006 | Sheep   | 0           | 0      |
| 486    | 10   | 2006 | Sheep   | 0           | 0      |
| 487    | 10   | 2006 | Sheep   | 0           | 0      |
| 488    | 10   | 2006 | Sheep   | 0           | 0      |
| 489    | 11   | 2006 | Deer    | 0           | 0      |
| 490    | 11   | 2006 | Deer    | 0           | 0      |
| 491    | 11   | 2006 | Deer    | 0           | 0      |
| 492    | 11   | 2006 | Deer    | 0           | 0      |
| 493    | 11   | 2006 | Deer    | 0           | 0      |
| 494    | 11   | 2006 | Deer    | 24          | 0      |
| 495    | 11   | 2006 | Deer    | 0           | 0      |
| 496    | 11   | 2006 | Deer    | 24          | 0      |
| 497    | 11   | 2006 | Deer    | 48          | 24     |
| 498    | 11   | 2006 | Deer    | 0           | 0      |
| 499    | 11   | 2006 | Deer    | 0           | 0      |
| 500    | 11   | 2006 | Deer    | 48          | 0      |
| 501    | 11   | 2006 | Deer    | 0           | 0      |
| 502    | 11   | 2006 | Deer    | 0           | 0      |
| 503    | 11   | 2006 | Deer    | 0           | 0      |
| 504    | 11   | 2006 | Deer    | 0           | 0      |
| 505    | 11   | 2006 | Deer    | 0           | 0      |
| 506    | 11   | 2006 | Deer    | 96          | 0      |
| 507    | 11   | 2006 | Deer    | 0           | 0      |
| 508    | 11   | 2006 | Cattle  | 0           | 0      |
| 509    | 11   | 2006 | Cattle  | 0           | 24     |
| 510    | 11   | 2006 | Cattle  | 24          | 0      |
| 511    | 11   | 2006 | Cattle  | 24          | 0      |
| 512    | 11   | 2006 | Cattle  | 0           | 0      |
| 513    | 11   | 2006 | Cattle  | 0           | 0      |
| 514    | 11   | 2006 | Cattle  | 0           | 0      |
| 515    | 11   | 2006 | Cattle  | 0           | 0      |
| 516    | 11   | 2006 | Cattle  | 24          | 0      |
| 517    | 11   | 2006 | Cattle  | 24          | 0      |
| 518    | 11   | 2006 | Cattle  | 0           | 0      |
| 519    | 11   | 2006 | Cattle  | 0           | 0      |
| 520    | 11   | 2006 | Cattle  | 0           | 0      |
| 521    | 11   | 2006 | Cattle  | 0           | 0      |
| 522    | 11   | 2006 | Cattle  | 0           | 0      |
| 523    | 11   | 2006 | Cattle  | 0           | 0      |
| 524    | 11   | 2006 | Cattle  | 0           | 0      |
| 525    | 11   | 2006 | Cattle  | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 526    | 11   | 2006 | Cattle  | 0           | 0      |
| 527    | 11   | 2006 | Cattle  | 0           | 0      |
| 528    | 11   | 2006 | Sheep   | 96          | 0      |
| 529    | 11   | 2006 | Sheep   | 0           | 0      |
| 530    | 11   | 2006 | Sheep   | 48          | 0      |
| 531    | 11   | 2006 | Sheep   | 24          | 0      |
| 532    | 11   | 2006 | Sheep   | 0           | 0      |
| 533    | 11   | 2006 | Sheep   | 0           | 0      |
| 534    | 11   | 2006 | Sheep   | 0           | 0      |
| 535    | 11   | 2006 | Sheep   | 0           | 0      |
| 536    | 11   | 2006 | Sheep   | 96          | 0      |
| 537    | 11   | 2006 | Sheep   | 0           | 0      |
| 538    | 11   | 2006 | Sheep   | 0           | 0      |
| 539    | 11   | 2006 | Sheep   | 0           | 0      |
| 540    | 11   | 2006 | Sheep   | 192         | 0      |
| 541    | 11   | 2006 | Sheep   | 0           | 0      |
| 542    | 11   | 2006 | Sheep   | 0           | 0      |
| 543    | 11   | 2006 | Sheep   | 0           | 0      |
| 544    | 11   | 2006 | Sheep   | 0           | 0      |
| 545    | 11   | 2006 | Sheep   | 48          | 0      |
| 546    | 11   | 2006 | Sheep   | 48          | 0      |
| 547    | 11   | 2006 | Sheep   | 48          | 0      |
| 548    | 11   | 2006 | Sheep   | 96          | 0      |
| 549    | 11   | 2006 | Sheep   | 0           | 0      |
| 550    | 11   | 2006 | Sheep   | 0           | 0      |
| 551    | 11   | 2006 | Sheep   | 0           | 0      |
| 552    | 11   | 2006 | Sheep   | 0           | 0      |
| 553    | 11   | 2006 | Sheep   | 0           | 0      |
| 554    | 11   | 2006 | Sheep   | 0           | 0      |
| 555    | 11   | 2006 | Sheep   | 0           | 0      |
| 556    | 11   | 2006 | Sheep   | 0           | 0      |
| 557    | 11   | 2006 | Sheep   | 0           | 0      |
| 558    | 11   | 2006 | Sheep   | 0           | 0      |
| 559    | 11   | 2006 | Sheep   | 0           | 0      |
| 560    | 11   | 2006 | Sheep   | 0           | 0      |
| 561    | 11   | 2006 | Sheep   | 48          | 0      |
| 562    | 11   | 2006 | Sheep   | 0           | 0      |
| 563    | 11   | 2006 | Sheep   | 0           | 0      |
| 564    | 11   | 2006 | Sheep   | 48          | 0      |
| 565    | 11   | 2006 | Sheep   | 0           | 0      |
| 566    | 11   | 2006 | Sheep   | 0           | 0      |
| 567    | 11   | 2006 | Sheep   | 96          | 0      |
| 568    | 11   | 2006 | Sheep   | 0           | 0      |
| 569    | 11   | 2006 | Sheep   | 0           | 0      |
| 570    | 11   | 2006 | Sheep   | 24          | 0      |
| 571    | 11   | 2006 | Sheep   | 0           | 0      |
| 572    | 11   | 2006 | Sheep   | 96          | 0      |
| 573    | 11   | 2006 | Sheep   | 48          | 0      |
| 574    | 11   | 2006 | Sheep   | 0           | 0      |
| 575    | 11   | 2006 | Sheep   | 384         | 0      |
| 576    | 11   | 2006 | Sheep   | 0           | 0      |
| 577    | 11   | 2006 | Sheep   | 0           | 0      |
| 578    | 11   | 2006 | Sheep   | 48          | 0      |
| 579    | 11   | 2006 | Sheep   | 0           | 0      |
| 580    | 11   | 2006 | Sheep   | 192         | 0      |
| 581    | 11   | 2006 | Sheep   | 96          | 0      |
| 582    | 11   | 2006 | Sheep   | 96          | 0      |
| 583    | 11   | 2006 | Sheep   | 0           | 0      |
| 584    | 11   | 2006 | Sheep   | 96          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 585    | 11   | 2006 | Sheep   | 96          | 0      |
| 586    | 11   | 2006 | Sheep   | 0           | 0      |
| 587    | 11   | 2006 | Sheep   | 0           | 0      |
| 588    | 12   | 2006 | Deer    | 48          | 0      |
| 589    | 12   | 2006 | Deer    | 0           | 0      |
| 590    | 12   | 2006 | Deer    | 0           | 0      |
| 591    | 12   | 2006 | Deer    | 0           | 0      |
| 592    | 12   | 2006 | Deer    | 0           | 0      |
| 593    | 12   | 2006 | Deer    | 24          | 0      |
| 594    | 12   | 2006 | Deer    | 0           | 0      |
| 595    | 12   | 2006 | Deer    | 0           | 0      |
| 596    | 12   | 2006 | Deer    | 0           | 0      |
| 597    | 12   | 2006 | Deer    | 0           | 0      |
| 598    | 12   | 2006 | Deer    | 24          | 0      |
| 599    | 12   | 2006 | Deer    | 48          | 0      |
| 600    | 12   | 2006 | Deer    | 0           | 0      |
| 601    | 12   | 2006 | Deer    | 0           | 0      |
| 602    | 12   | 2006 | Deer    | 0           | 0      |
| 603    | 12   | 2006 | Deer    | 0           | 0      |
| 604    | 12   | 2006 | Deer    | 24          | 0      |
| 605    | 12   | 2006 | Deer    | 0           | 0      |
| 606    | 12   | 2006 | Deer    | 24          | 0      |
| 607    | 12   | 2006 | Deer    | 48          | 0      |
| 608    | 12   | 2006 | Cattle  | 0           | 0      |
| 609    | 12   | 2006 | Cattle  | 0           | 0      |
| 610    | 12   | 2006 | Cattle  | 384         | 0      |
| 611    | 12   | 2006 | Cattle  | 0           | 0      |
| 612    | 12   | 2006 | Cattle  | 0           | 0      |
| 613    | 12   | 2006 | Cattle  | 24          | 0      |
| 614    | 12   | 2006 | Cattle  | 0           | 0      |
| 615    | 12   | 2006 | Cattle  | 48          | 0      |
| 616    | 12   | 2006 | Cattle  | 0           | 0      |
| 617    | 12   | 2006 | Cattle  | 0           | 0      |
| 618    | 12   | 2006 | Cattle  | 0           | 0      |
| 619    | 12   | 2006 | Cattle  | 0           | 0      |
| 620    | 12   | 2006 | Cattle  | 0           | 0      |
| 621    | 12   | 2006 | Cattle  | 0           | 0      |
| 622    | 12   | 2006 | Cattle  | 0           | 0      |
| 623    | 12   | 2006 | Cattle  | 0           | 0      |
| 624    | 12   | 2006 | Cattle  | 0           | 0      |
| 625    | 12   | 2006 | Cattle  | 0           | 0      |
| 626    | 12   | 2006 | Cattle  | 0           | 0      |
| 627    | 12   | 2006 | Cattle  | 48          | 0      |
| 628    | 12   | 2006 | Sheep   | 0           | 0      |
| 629    | 12   | 2006 | Sheep   | 0           | 0      |
| 630    | 12   | 2006 | Sheep   | 0           | 0      |
| 631    | 12   | 2006 | Sheep   | 96          | 0      |
| 632    | 12   | 2006 | Sheep   | 0           | 0      |
| 633    | 12   | 2006 | Sheep   | 0           | 0      |
| 634    | 12   | 2006 | Sheep   | 0           | 0      |
| 635    | 12   | 2006 | Sheep   | 24          | 0      |
| 636    | 12   | 2006 | Sheep   | 0           | 0      |
| 637    | 12   | 2006 | Sheep   | 24          | 0      |
| 638    | 12   | 2006 | Sheep   | 0           | 0      |
| 639    | 12   | 2006 | Sheep   | 0           | 0      |
| 640    | 12   | 2006 | Sheep   | 0           | 0      |
| 641    | 12   | 2006 | Sheep   | 0           | 0      |
| 642    | 12   | 2006 | Sheep   | 0           | 0      |
| 643    | 12   | 2006 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 644    | 12   | 2006 | Sheep   | 0           | 0      |
| 645    | 12   | 2006 | Sheep   | 0           | 0      |
| 646    | 12   | 2006 | Sheep   | 0           | 0      |
| 647    | 12   | 2006 | Sheep   | 0           | 0      |
| 648    | 12   | 2006 | Sheep   | 0           | 0      |
| 649    | 12   | 2006 | Sheep   | 0           | 0      |
| 650    | 12   | 2006 | Sheep   | 0           | 0      |
| 651    | 12   | 2006 | Sheep   | 0           | 0      |
| 652    | 12   | 2006 | Sheep   | 0           | 0      |
| 653    | 12   | 2006 | Sheep   | 0           | 0      |
| 654    | 12   | 2006 | Sheep   | 0           | 0      |
| 655    | 12   | 2006 | Sheep   | 0           | 0      |
| 656    | 12   | 2006 | Sheep   | 0           | 0      |
| 657    | 12   | 2006 | Sheep   | 0           | 0      |
| 658    | 12   | 2006 | Sheep   | 0           | 0      |
| 659    | 12   | 2006 | Sheep   | 0           | 0      |
| 660    | 12   | 2006 | Sheep   | 0           | 0      |
| 661    | 12   | 2006 | Sheep   | 0           | 0      |
| 662    | 12   | 2006 | Sheep   | 0           | 0      |
| 663    | 12   | 2006 | Sheep   | 0           | 0      |
| 664    | 12   | 2006 | Sheep   | 0           | 0      |
| 665    | 12   | 2006 | Sheep   | 0           | 0      |
| 666    | 12   | 2006 | Sheep   | 0           | 0      |
| 667    | 12   | 2006 | Sheep   | 0           | 0      |
| 668    | 12   | 2006 | Sheep   | 0           | 0      |
| 669    | 12   | 2006 | Sheep   | 0           | 0      |
| 670    | 12   | 2006 | Sheep   | 0           | 0      |
| 671    | 12   | 2006 | Sheep   | 0           | 0      |
| 672    | 12   | 2006 | Sheep   | 0           | 0      |
| 673    | 12   | 2006 | Sheep   | 0           | 0      |
| 674    | 12   | 2006 | Sheep   | 0           | 0      |
| 675    | 12   | 2006 | Sheep   | 0           | 0      |
| 676    | 12   | 2006 | Sheep   | 0           | 0      |
| 677    | 12   | 2006 | Sheep   | 0           | 0      |
| 678    | 12   | 2006 | Sheep   | 0           | 0      |
| 679    | 12   | 2006 | Sheep   | 0           | 0      |
| 680    | 12   | 2006 | Sheep   | 0           | 0      |
| 681    | 12   | 2006 | Sheep   | 0           | 0      |
| 682    | 12   | 2006 | Sheep   | 0           | 0      |
| 683    | 12   | 2006 | Sheep   | 0           | 0      |
| 684    | 12   | 2006 | Sheep   | 0           | 0      |
| 685    | 12   | 2006 | Sheep   | 0           | 0      |
| 686    | 12   | 2006 | Sheep   | 0           | 0      |
| 687    | 12   | 2006 | Sheep   | 0           | 0      |
| 688    | 13   | 2006 | Deer    | 48          | 0      |
| 689    | 13   | 2006 | Deer    | 48          | 0      |
| 690    | 13   | 2006 | Deer    | 48          | 0      |
| 691    | 13   | 2006 | Deer    | 48          | 0      |
| 692    | 13   | 2006 | Deer    | 0           | 0      |
| 693    | 13   | 2006 | Deer    | 96          | 0      |
| 694    | 13   | 2006 | Deer    | 48          | 0      |
| 695    | 13   | 2006 | Deer    | 48          | 0      |
| 696    | 13   | 2006 | Deer    | 24          | 0      |
| 697    | 13   | 2006 | Deer    | 48          | 0      |
| 698    | 13   | 2006 | Deer    | 48          | 0      |
| 699    | 13   | 2006 | Deer    | 24          | 0      |
| 700    | 13   | 2006 | Deer    | 24          | 0      |
| 701    | 13   | 2006 | Deer    | 0           | 0      |
| 702    | 13   | 2006 | Cattle  | 96          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 703    | 13   | 2006 | Cattle  | 48          | 0      |
| 704    | 13   | 2006 | Cattle  | 0           | 0      |
| 705    | 13   | 2006 | Cattle  | 0           | 0      |
| 706    | 13   | 2006 | Cattle  | 48          | 0      |
| 707    | 13   | 2006 | Cattle  | 384         | 0      |
| 708    | 13   | 2006 | Cattle  | 48          | 0      |
| 709    | 13   | 2006 | Cattle  | 48          | 0      |
| 710    | 13   | 2006 | Cattle  | 192         | 0      |
| 711    | 13   | 2006 | Cattle  | 48          | 0      |
| 712    | 13   | 2006 | Cattle  | 192         | 0      |
| 713    | 13   | 2006 | Cattle  | 192         | 0      |
| 714    | 13   | 2006 | Cattle  | 192         | 0      |
| 715    | 13   | 2006 | Cattle  | 48          | 0      |
| 716    | 13   | 2006 | Cattle  | 48          | 0      |
| 717    | 13   | 2006 | Cattle  | 384         | 0      |
| 718    | 13   | 2006 | Cattle  | 96          | 0      |
| 719    | 13   | 2006 | Cattle  | 96          | 0      |
| 720    | 13   | 2006 | Cattle  | 384         | 0      |
| 721    | 13   | 2006 | Sheep   | 0           | 0      |
| 722    | 13   | 2006 | Sheep   | 0           | 0      |
| 723    | 13   | 2006 | Sheep   | 0           | 0      |
| 724    | 13   | 2006 | Sheep   | 0           | 0      |
| 725    | 13   | 2006 | Sheep   | 0           | 0      |
| 726    | 13   | 2006 | Sheep   | 0           | 0      |
| 727    | 13   | 2006 | Sheep   | 0           | 0      |
| 728    | 13   | 2006 | Sheep   | 0           | 0      |
| 729    | 13   | 2006 | Sheep   | 0           | 0      |
| 730    | 13   | 2006 | Sheep   | 0           | 0      |
| 731    | 13   | 2006 | Sheep   | 0           | 0      |
| 732    | 13   | 2006 | Sheep   | 0           | 0      |
| 733    | 13   | 2006 | Sheep   | 0           | 0      |
| 734    | 13   | 2006 | Sheep   | 0           | 0      |
| 735    | 13   | 2006 | Sheep   | 0           | 0      |
| 736    | 13   | 2006 | Sheep   | 0           | 0      |
| 737    | 13   | 2006 | Sheep   | 0           | 0      |
| 738    | 13   | 2006 | Sheep   | 0           | 0      |
| 739    | 13   | 2006 | Sheep   | 0           | 0      |
| 740    | 13   | 2006 | Sheep   | 0           | 0      |
| 741    | 13   | 2006 | Sheep   | 0           | 0      |
| 742    | 13   | 2006 | Sheep   | 0           | 0      |
| 743    | 13   | 2006 | Sheep   | 0           | 0      |
| 744    | 13   | 2006 | Sheep   | 0           | 0      |
| 745    | 13   | 2006 | Sheep   | 0           | 0      |
| 746    | 13   | 2006 | Sheep   | 0           | 0      |
| 747    | 13   | 2006 | Sheep   | 0           | 0      |
| 748    | 13   | 2006 | Sheep   | 0           | 0      |
| 749    | 13   | 2006 | Sheep   | 0           | 0      |
| 750    | 13   | 2006 | Sheep   | 0           | 0      |
| 751    | 13   | 2006 | Sheep   | 0           | 0      |
| 752    | 13   | 2006 | Sheep   | 0           | 0      |
| 753    | 13   | 2006 | Sheep   | 0           | 0      |
| 754    | 13   | 2006 | Sheep   | 0           | 0      |
| 755    | 13   | 2006 | Sheep   | 0           | 0      |
| 756    | 13   | 2006 | Sheep   | 0           | 0      |
| 757    | 13   | 2006 | Sheep   | 0           | 0      |
| 758    | 13   | 2006 | Sheep   | 0           | 0      |
| 759    | 13   | 2006 | Sheep   | 0           | 0      |
| 760    | 13   | 2006 | Sheep   | 0           | 0      |
| 761    | 13   | 2006 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 762    | 13   | 2006 | Sheep   | 0           | 0      |
| 763    | 13   | 2006 | Sheep   | 0           | 0      |
| 764    | 13   | 2006 | Sheep   | 0           | 0      |
| 765    | 13   | 2006 | Sheep   | 0           | 0      |
| 766    | 13   | 2006 | Sheep   | 0           | 0      |
| 767    | 13   | 2006 | Sheep   | 0           | 0      |
| 768    | 13   | 2006 | Sheep   | 0           | 0      |
| 769    | 13   | 2006 | Sheep   | 0           | 0      |
| 770    | 13   | 2006 | Sheep   | 0           | 0      |
| 771    | 13   | 2006 | Sheep   | 0           | 0      |
| 772    | 13   | 2006 | Sheep   | 0           | 0      |
| 773    | 13   | 2006 | Sheep   | 0           | 0      |
| 774    | 13   | 2006 | Sheep   | 0           | 0      |
| 775    | 13   | 2006 | Sheep   | 0           | 0      |
| 776    | 13   | 2006 | Sheep   | 0           | 0      |
| 777    | 13   | 2006 | Sheep   | 0           | 0      |
| 778    | 13   | 2006 | Sheep   | 0           | 0      |
| 779    | 13   | 2006 | Sheep   | 0           | 0      |
| 780    | 13   | 2006 | Sheep   | 0           | 0      |
| 781    | 14   | 2006 | Deer    | 192         | 0      |
| 782    | 14   | 2006 | Deer    | 96          | 96     |
| 783    | 14   | 2006 | Deer    | 24          | 24     |
| 784    | 14   | 2006 | Deer    | 192         | 0      |
| 785    | 14   | 2006 | Deer    | 24          | 0      |
| 786    | 14   | 2006 | Deer    | 48          | 0      |
| 787    | 14   | 2006 | Deer    | 24          | 48     |
| 788    | 14   | 2006 | Deer    | 48          | 96     |
| 789    | 14   | 2006 | Deer    | 48          | 192    |
| 790    | 14   | 2006 | Deer    | 384         | 0      |
| 791    | 14   | 2006 | Deer    | 0           | 0      |
| 792    | 14   | 2006 | Deer    | 0           | 24     |
| 793    | 14   | 2006 | Deer    | 192         | 384    |
| 794    | 14   | 2006 | Deer    | 0           | 24     |
| 795    | 14   | 2006 | Deer    | 192         | 192    |
| 796    | 14   | 2006 | Deer    | 0           | 0      |
| 797    | 14   | 2006 | Deer    | 24          | 0      |
| 798    | 14   | 2006 | Deer    | 0           | 0      |
| 799    | 14   | 2006 | Cattle  | 48          | 192    |
| 800    | 14   | 2006 | Cattle  | 384         | 0      |
| 801    | 14   | 2006 | Cattle  | 384         | 192    |
| 802    | 14   | 2006 | Cattle  | 192         | 0      |
| 803    | 14   | 2006 | Cattle  | 768         | 0      |
| 804    | 14   | 2006 | Cattle  | 96          | 0      |
| 805    | 14   | 2006 | Cattle  | 384         | 96     |
| 806    | 14   | 2006 | Cattle  | 192         | 0      |
| 807    | 14   | 2006 | Cattle  | 192         | 384    |
| 808    | 14   | 2006 | Cattle  | 48          | 0      |
| 809    | 14   | 2006 | Cattle  | 96          | 0      |
| 810    | 14   | 2006 | Cattle  | 96          | 0      |
| 811    | 14   | 2006 | Cattle  | 96          | 0      |
| 812    | 14   | 2006 | Cattle  | 384         | 0      |
| 813    | 14   | 2006 | Cattle  | 192         | 0      |
| 814    | 14   | 2006 | Cattle  | 192         | 0      |
| 815    | 14   | 2006 | Cattle  | 96          | 96     |
| 816    | 14   | 2006 | Cattle  | 192         | 0      |
| 817    | 14   | 2006 | Cattle  | 192         | 0      |
| 818    | 14   | 2006 | Cattle  | 48          | 0      |
| 819    | 14   | 2006 | Sheep   | 384         | 0      |
| 820    | 14   | 2006 | Sheep   | 768         | 0      |



| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 821    | 14   | 2006 | Sheep   | 384         | 0      |
| 822    | 14   | 2006 | Sheep   | 384         | 0      |
| 823    | 14   | 2006 | Sheep   | 384         | 0      |
| 824    | 14   | 2006 | Sheep   | 192         | 0      |
| 825    | 14   | 2006 | Sheep   | 384         | 0      |
| 826    | 14   | 2006 | Sheep   | 384         | 0      |
| 827    | 14   | 2006 | Sheep   | 0           | 0      |
| 828    | 14   | 2006 | Sheep   | 192         | 0      |
| 829    | 14   | 2006 | Sheep   | 96          | 0      |
| 830    | 14   | 2006 | Sheep   | 0           | 0      |
| 831    | 14   | 2006 | Sheep   | 192         | 0      |
| 832    | 14   | 2006 | Sheep   | 384         | 0      |
| 833    | 14   | 2006 | Sheep   | 24          | 0      |
| 834    | 14   | 2006 | Sheep   | 192         | 0      |
| 835    | 14   | 2006 | Sheep   | 192         | 0      |
| 836    | 14   | 2006 | Sheep   | 0           | 0      |
| 837    | 14   | 2006 | Sheep   | 24          | 0      |
| 838    | 14   | 2006 | Sheep   | 24          | 0      |
| 839    | 14   | 2006 | Sheep   | 192         | 0      |
| 840    | 14   | 2006 | Sheep   | 0           | 0      |
| 841    | 14   | 2006 | Sheep   | 96          | 0      |
| 842    | 14   | 2006 | Sheep   | 24          | 0      |
| 843    | 14   | 2006 | Sheep   | 24          | 0      |
| 844    | 14   | 2006 | Sheep   | 48          | 0      |
| 845    | 14   | 2006 | Sheep   | 384         | 0      |
| 846    | 14   | 2006 | Sheep   | 0           | 0      |
| 847    | 14   | 2006 | Sheep   | 0           | 0      |
| 848    | 14   | 2006 | Sheep   | 0           | 0      |
| 849    | 14   | 2006 | Sheep   | 24          | 0      |
| 850    | 14   | 2006 | Sheep   | 0           | 0      |
| 851    | 14   | 2006 | Sheep   | 24          | 0      |
| 852    | 14   | 2006 | Sheep   | 192         | 96     |
| 853    | 14   | 2006 | Sheep   | 384         | 0      |
| 854    | 14   | 2006 | Sheep   | 192         | 0      |
| 855    | 14   | 2006 | Sheep   | 192         | 0      |
| 856    | 14   | 2006 | Sheep   | 96          | 0      |
| 857    | 14   | 2006 | Sheep   | 0           | 0      |
| 858    | 14   | 2006 | Sheep   | 96          | 0      |
| 859    | 14   | 2006 | Sheep   | 192         | 0      |
| 860    | 14   | 2006 | Sheep   | 96          | 0      |
| 861    | 14   | 2006 | Sheep   | 192         | 0      |
| 862    | 14   | 2006 | Sheep   | 96          | 0      |
| 863    | 14   | 2006 | Sheep   | 384         | 0      |
| 864    | 14   | 2006 | Sheep   | 96          | 0      |
| 865    | 14   | 2006 | Sheep   | 384         | 0      |
| 866    | 14   | 2006 | Sheep   | 96          | 0      |
| 867    | 14   | 2006 | Sheep   | 0           | 0      |
| 868    | 14   | 2006 | Sheep   | 24          | 0      |
| 869    | 14   | 2006 | Sheep   | 384         | 0      |
| 870    | 14   | 2006 | Sheep   | 384         | 0      |
| 871    | 14   | 2006 | Sheep   | 384         | 0      |
| 872    | 14   | 2006 | Sheep   | 192         | 0      |
| 873    | 14   | 2006 | Sheep   | 192         | 0      |
| 874    | 14   | 2006 | Sheep   | 192         | 768    |
| 875    | 14   | 2006 | Sheep   | 96          | 0      |
| 876    | 14   | 2006 | Sheep   | 96          | 0      |
| 877    | 14   | 2006 | Sheep   | 384         | 0      |
| 878    | 14   | 2006 | Sheep   | 0           | 0      |
| 879    | 15   | 2006 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 880    | 15   | 2006 | Deer    | 24          | 0      |
| 881    | 15   | 2006 | Deer    | 48          | 0      |
| 882    | 15   | 2006 | Deer    | 0           | 0      |
| 883    | 15   | 2006 | Deer    | 24          | 0      |
| 884    | 15   | 2006 | Deer    | 24          | 0      |
| 885    | 15   | 2006 | Deer    | 0           | 0      |
| 886    | 15   | 2006 | Deer    | 96          | 0      |
| 887    | 15   | 2006 | Deer    | 0           | 0      |
| 888    | 15   | 2006 | Deer    | 24          | 0      |
| 889    | 15   | 2006 | Deer    | 48          | 0      |
| 890    | 15   | 2006 | Deer    | 0           | 0      |
| 891    | 15   | 2006 | Deer    | 24          | 0      |
| 892    | 15   | 2006 | Deer    | 24          | 0      |
| 893    | 15   | 2006 | Deer    | 0           | 0      |
| 894    | 15   | 2006 | Deer    | 0           | 0      |
| 895    | 15   | 2006 | Deer    | 24          | 0      |
| 896    | 15   | 2006 | Deer    | 48          | 0      |
| 897    | 15   | 2006 | Deer    | 48          | 0      |
| 898    | 15   | 2006 | Cattle  | 96          | 0      |
| 899    | 15   | 2006 | Cattle  | 24          | 0      |
| 900    | 15   | 2006 | Cattle  | 0           | 0      |
| 901    | 15   | 2006 | Cattle  | 192         | 0      |
| 902    | 15   | 2006 | Cattle  | 24          | 0      |
| 903    | 15   | 2006 | Cattle  | 96          | 0      |
| 904    | 15   | 2006 | Cattle  | 96          | 0      |
| 905    | 15   | 2006 | Cattle  | 192         | 0      |
| 906    | 15   | 2006 | Cattle  | 96          | 0      |
| 907    | 15   | 2006 | Cattle  | 24          | 0      |
| 908    | 15   | 2006 | Cattle  | 192         | 0      |
| 909    | 15   | 2006 | Cattle  | 192         | 0      |
| 910    | 15   | 2006 | Cattle  | 96          | 0      |
| 911    | 15   | 2006 | Cattle  | 0           | 96     |
| 912    | 15   | 2006 | Cattle  | 24          | 0      |
| 913    | 15   | 2006 | Cattle  | 192         | 0      |
| 914    | 15   | 2006 | Cattle  | 48          | 0      |
| 915    | 15   | 2006 | Cattle  | 96          | 0      |
| 916    | 15   | 2006 | Cattle  | 96          | 0      |
| 917    | 15   | 2006 | Sheep   | 192         | 0      |
| 918    | 15   | 2006 | Sheep   | 384         | 0      |
| 919    | 15   | 2006 | Sheep   | 96          | 0      |
| 920    | 15   | 2006 | Sheep   | 192         | 0      |
| 921    | 15   | 2006 | Sheep   | 96          | 0      |
| 922    | 15   | 2006 | Sheep   | 384         | 0      |
| 923    | 15   | 2006 | Sheep   | 192         | 0      |
| 924    | 15   | 2006 | Sheep   | 384         | 0      |
| 925    | 15   | 2006 | Sheep   | 384         | 0      |
| 926    | 15   | 2006 | Sheep   | 0           | 0      |
| 927    | 15   | 2006 | Sheep   | 0           | 0      |
| 928    | 15   | 2006 | Sheep   | 384         | 0      |
| 929    | 15   | 2006 | Sheep   | 96          | 0      |
| 930    | 15   | 2006 | Sheep   | 96          | 0      |
| 931    | 15   | 2006 | Sheep   | 192         | 0      |
| 932    | 15   | 2006 | Sheep   | 0           | 0      |
| 933    | 15   | 2006 | Sheep   | 96          | 0      |
| 934    | 15   | 2006 | Sheep   | 384         | 0      |
| 935    | 15   | 2006 | Sheep   | 96          | 0      |
| 936    | 15   | 2006 | Sheep   | 96          | 0      |
| 937    | 15   | 2006 | Sheep   | 192         | 0      |
| 938    | 15   | 2006 | Sheep   | 192         | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 939    | 15   | 2006 | Sheep   | 384         | 0      |
| 940    | 15   | 2006 | Sheep   | 96          | 0      |
| 941    | 15   | 2006 | Sheep   | 192         | 0      |
| 942    | 15   | 2006 | Sheep   | 48          | 0      |
| 943    | 15   | 2006 | Sheep   | 96          | 0      |
| 944    | 15   | 2006 | Sheep   | 768         | 0      |
| 945    | 15   | 2006 | Sheep   | 96          | 0      |
| 946    | 15   | 2006 | Sheep   | 48          | 0      |
| 947    | 15   | 2006 | Sheep   | 192         | 0      |
| 948    | 15   | 2006 | Sheep   | 96          | 0      |
| 949    | 15   | 2006 | Sheep   | 384         | 0      |
| 950    | 15   | 2006 | Sheep   | 96          | 0      |
| 951    | 15   | 2006 | Sheep   | 48          | 0      |
| 952    | 15   | 2006 | Sheep   | 384         | 0      |
| 953    | 15   | 2006 | Sheep   | 384         | 0      |
| 954    | 15   | 2006 | Sheep   | 192         | 0      |
| 955    | 15   | 2006 | Sheep   | 192         | 0      |
| 956    | 15   | 2006 | Sheep   | 192         | 0      |
| 957    | 15   | 2006 | Sheep   | 96          | 0      |
| 958    | 15   | 2006 | Sheep   | 192         | 0      |
| 959    | 15   | 2006 | Sheep   | 96          | 0      |
| 960    | 15   | 2006 | Sheep   | 96          | 0      |
| 961    | 15   | 2006 | Sheep   | 24          | 0      |
| 962    | 15   | 2006 | Sheep   | 96          | 0      |
| 963    | 15   | 2006 | Sheep   | 96          | 0      |
| 964    | 15   | 2006 | Sheep   | 192         | 0      |
| 965    | 15   | 2006 | Sheep   | 48          | 0      |
| 966    | 15   | 2006 | Sheep   | 384         | 0      |
| 967    | 15   | 2006 | Sheep   | 96          | 0      |
| 968    | 15   | 2006 | Sheep   | 96          | 0      |
| 969    | 15   | 2006 | Sheep   | 48          | 0      |
| 970    | 15   | 2006 | Sheep   | 96          | 0      |
| 971    | 15   | 2006 | Sheep   | 96          | 0      |
| 972    | 15   | 2006 | Sheep   | 96          | 0      |
| 973    | 15   | 2006 | Sheep   | 192         | 0      |
| 974    | 15   | 2006 | Sheep   | 192         | 0      |
| 975    | 15   | 2006 | Sheep   | 192         | 0      |
| 976    | 16   | 2006 | Deer    | 96          | 0      |
| 977    | 16   | 2006 | Deer    | 24          | 0      |
| 978    | 16   | 2006 | Deer    | 48          | 0      |
| 979    | 16   | 2006 | Deer    | 0           | 0      |
| 980    | 16   | 2006 | Deer    | 96          | 0      |
| 981    | 16   | 2006 | Deer    | 24          | 0      |
| 982    | 16   | 2006 | Deer    | 24          | 0      |
| 983    | 16   | 2006 | Deer    | 48          | 0      |
| 984    | 16   | 2006 | Deer    | 48          | 0      |
| 985    | 16   | 2006 | Deer    | 0           | 0      |
| 986    | 16   | 2006 | Deer    | 0           | 0      |
| 987    | 16   | 2006 | Deer    | 24          | 0      |
| 988    | 16   | 2006 | Deer    | 24          | 0      |
| 989    | 16   | 2006 | Deer    | 24          | 0      |
| 990    | 16   | 2006 | Deer    | 0           | 0      |
| 991    | 16   | 2006 | Deer    | 24          | 0      |
| 992    | 16   | 2006 | Deer    | 48          | 0      |
| 993    | 16   | 2006 | Deer    | 24          | 0      |
| 994    | 16   | 2006 | Deer    | 48          | 0      |
| 995    | 16   | 2006 | Deer    | 192         | 0      |
| 996    | 16   | 2006 | Cattle  | 0           | 0      |
| 997    | 16   | 2006 | Cattle  | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 998    | 16   | 2006 | Cattle  | 192         | 0      |
| 999    | 16   | 2006 | Cattle  | 192         | 0      |
| 1000   | 16   | 2006 | Cattle  | 48          | 0      |
| 1001   | 16   | 2006 | Cattle  | 24          | 0      |
| 1002   | 16   | 2006 | Cattle  | 384         | 0      |
| 1003   | 16   | 2006 | Cattle  | 96          | 0      |
| 1004   | 16   | 2006 | Cattle  | 96          | 0      |
| 1005   | 16   | 2006 | Cattle  | 192         | 0      |
| 1006   | 16   | 2006 | Cattle  | 24          | 0      |
| 1007   | 16   | 2006 | Cattle  | 0           | 0      |
| 1008   | 16   | 2006 | Cattle  | 384         | 0      |
| 1009   | 16   | 2006 | Cattle  | 0           | 0      |
| 1010   | 16   | 2006 | Cattle  | 96          | 0      |
| 1011   | 16   | 2006 | Cattle  | 192         | 0      |
| 1012   | 16   | 2006 | Cattle  | 192         | 0      |
| 1013   | 16   | 2006 | Cattle  | 48          | 0      |
| 1014   | 16   | 2006 | Cattle  | 48          | 0      |
| 1015   | 16   | 2006 | Cattle  | 24          | 0      |
| 1016   | 16   | 2006 | Sheep   | 0           | 0      |
| 1017   | 16   | 2006 | Sheep   | 0           | 0      |
| 1018   | 16   | 2006 | Sheep   | 0           | 0      |
| 1019   | 16   | 2006 | Sheep   | 0           | 0      |
| 1020   | 16   | 2006 | Sheep   | 0           | 0      |
| 1021   | 16   | 2006 | Sheep   | 0           | 0      |
| 1022   | 16   | 2006 | Sheep   | 0           | 0      |
| 1023   | 16   | 2006 | Sheep   | 0           | 0      |
| 1024   | 16   | 2006 | Sheep   | 0           | 0      |
| 1025   | 16   | 2006 | Sheep   | 0           | 0      |
| 1026   | 16   | 2006 | Sheep   | 0           | 0      |
| 1027   | 16   | 2006 | Sheep   | 0           | 0      |
| 1028   | 16   | 2006 | Sheep   | 0           | 0      |
| 1029   | 16   | 2006 | Sheep   | 0           | 0      |
| 1030   | 16   | 2006 | Sheep   | 0           | 0      |
| 1031   | 16   | 2006 | Sheep   | 0           | 0      |
| 1032   | 16   | 2006 | Sheep   | 0           | 0      |
| 1033   | 16   | 2006 | Sheep   | 0           | 0      |
| 1034   | 16   | 2006 | Sheep   | 0           | 0      |
| 1035   | 16   | 2006 | Sheep   | 0           | 0      |
| 1036   | 16   | 2006 | Sheep   | 0           | 0      |
| 1037   | 16   | 2006 | Sheep   | 0           | 0      |
| 1038   | 16   | 2006 | Sheep   | 0           | 0      |
| 1039   | 16   | 2006 | Sheep   | 0           | 0      |
| 1040   | 16   | 2006 | Sheep   | 192         | 0      |
| 1041   | 16   | 2006 | Sheep   | 0           | 0      |
| 1042   | 16   | 2006 | Sheep   | 0           | 0      |
| 1043   | 16   | 2006 | Sheep   | 0           | 0      |
| 1044   | 16   | 2006 | Sheep   | 0           | 0      |
| 1045   | 16   | 2006 | Sheep   | 0           | 0      |
| 1046   | 16   | 2006 | Sheep   | 0           | 0      |
| 1047   | 16   | 2006 | Sheep   | 0           | 0      |
| 1048   | 16   | 2006 | Sheep   | 0           | 0      |
| 1049   | 16   | 2006 | Sheep   | 0           | 0      |
| 1050   | 16   | 2006 | Sheep   | 384         | 0      |
| 1051   | 16   | 2006 | Sheep   | 0           | 0      |
| 1052   | 16   | 2006 | Sheep   | 0           | 0      |
| 1053   | 16   | 2006 | Sheep   | 0           | 0      |
| 1054   | 16   | 2006 | Sheep   | 0           | 0      |
| 1055   | 16   | 2006 | Sheep   | 0           | 0      |
| 1056   | 16   | 2006 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1057   | 16   | 2006 | Sheep   | 0           | 0      |
| 1058   | 16   | 2006 | Sheep   | 0           | 0      |
| 1059   | 16   | 2006 | Sheep   | 0           | 0      |
| 1060   | 16   | 2006 | Sheep   | 192         | 0      |
| 1061   | 16   | 2006 | Sheep   | 384         | 0      |
| 1062   | 16   | 2006 | Sheep   | 0           | 0      |
| 1063   | 16   | 2006 | Sheep   | 0           | 0      |
| 1064   | 16   | 2006 | Sheep   | 0           | 0      |
| 1065   | 16   | 2006 | Sheep   | 192         | 0      |
| 1066   | 16   | 2006 | Sheep   | 0           | 0      |
| 1067   | 16   | 2006 | Sheep   | 0           | 0      |
| 1068   | 16   | 2006 | Sheep   | 0           | 0      |
| 1069   | 16   | 2006 | Sheep   | 0           | 0      |
| 1070   | 16   | 2006 | Sheep   | 0           | 0      |
| 1071   | 16   | 2006 | Sheep   | 0           | 0      |
| 1072   | 16   | 2006 | Sheep   | 0           | 0      |
| 1073   | 16   | 2006 | Sheep   | 0           | 0      |
| 1074   | 16   | 2006 | Sheep   | 0           | 0      |
| 1075   | 16   | 2006 | Sheep   | 0           | 0      |
| 1076   | 17   | 2006 | Deer    | 0           | 0      |
| 1077   | 17   | 2006 | Deer    | 0           | 0      |
| 1078   | 17   | 2006 | Deer    | 0           | 0      |
| 1079   | 17   | 2006 | Deer    | 0           | 0      |
| 1080   | 17   | 2006 | Deer    | 24          | 0      |
| 1081   | 17   | 2006 | Deer    | 0           | 0      |
| 1082   | 17   | 2006 | Deer    | 0           | 0      |
| 1083   | 17   | 2006 | Deer    | 0           | 0      |
| 1084   | 17   | 2006 | Deer    | 0           | 0      |
| 1085   | 17   | 2006 | Deer    | 0           | 0      |
| 1086   | 17   | 2006 | Deer    | 0           | 0      |
| 1087   | 17   | 2006 | Deer    | 0           | 0      |
| 1088   | 17   | 2006 | Deer    | 0           | 0      |
| 1089   | 17   | 2006 | Deer    | 0           | 0      |
| 1090   | 17   | 2006 | Deer    | 0           | 0      |
| 1091   | 17   | 2006 | Deer    | 0           | 0      |
| 1092   | 17   | 2006 | Deer    | 0           | 0      |
| 1093   | 17   | 2006 | Deer    | 0           | 0      |
| 1094   | 17   | 2006 | Deer    | 0           | 0      |
| 1095   | 17   | 2006 | Deer    | 0           | 0      |
| 1096   | 17   | 2006 | Cattle  | 0           | 0      |
| 1097   | 17   | 2006 | Cattle  | 0           | 0      |
| 1098   | 17   | 2006 | Cattle  | 48          | 0      |
| 1099   | 17   | 2006 | Cattle  | 24          | 0      |
| 1100   | 17   | 2006 | Cattle  | 24          | 0      |
| 1101   | 17   | 2006 | Cattle  | 0           | 0      |
| 1102   | 17   | 2006 | Cattle  | 48          | 0      |
| 1103   | 17   | 2006 | Cattle  | 0           | 0      |
| 1104   | 17   | 2006 | Cattle  | 0           | 0      |
| 1105   | 17   | 2006 | Cattle  | 384         | 0      |
| 1106   | 17   | 2006 | Cattle  | 0           | 0      |
| 1107   | 17   | 2006 | Cattle  | 0           | 0      |
| 1108   | 17   | 2006 | Cattle  | 48          | 0      |
| 1109   | 17   | 2006 | Cattle  | 48          | 0      |
| 1110   | 17   | 2006 | Cattle  | 48          | 0      |
| 1111   | 17   | 2006 | Sheep   | 0           | 0      |
| 1112   | 17   | 2006 | Sheep   | 192         | 0      |
| 1113   | 17   | 2006 | Sheep   | 0           | 0      |
| 1114   | 17   | 2006 | Sheep   | 24          | 0      |
| 1115   | 17   | 2006 | Sheep   | 384         | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1116   | 17   | 2006 | Sheep   | 192         | 0      |
| 1117   | 17   | 2006 | Sheep   | 48          | 0      |
| 1118   | 17   | 2006 | Sheep   | 0           | 0      |
| 1119   | 17   | 2006 | Sheep   | 96          | 0      |
| 1120   | 17   | 2006 | Sheep   | 0           | 0      |
| 1121   | 17   | 2006 | Sheep   | 0           | 0      |
| 1122   | 17   | 2006 | Sheep   | 192         | 0      |
| 1123   | 17   | 2006 | Sheep   | 0           | 0      |
| 1124   | 17   | 2006 | Sheep   | 192         | 0      |
| 1125   | 17   | 2006 | Sheep   | 96          | 24     |
| 1126   | 17   | 2006 | Sheep   | 0           | 0      |
| 1127   | 17   | 2006 | Sheep   | 96          | 0      |
| 1128   | 17   | 2006 | Sheep   | 0           | 0      |
| 1129   | 17   | 2006 | Sheep   | 96          | 0      |
| 1130   | 17   | 2006 | Sheep   | 0           | 0      |
| 1131   | 17   | 2006 | Sheep   | 96          | 0      |
| 1132   | 17   | 2006 | Sheep   | 0           | 0      |
| 1133   | 17   | 2006 | Sheep   | 192         | 0      |
| 1134   | 17   | 2006 | Sheep   | 96          | 0      |
| 1135   | 17   | 2006 | Sheep   | 96          | 0      |
| 1136   | 17   | 2006 | Sheep   | 192         | 0      |
| 1137   | 17   | 2006 | Sheep   | 0           | 0      |
| 1138   | 17   | 2006 | Sheep   | 96          | 0      |
| 1139   | 17   | 2006 | Sheep   | 192         | 0      |
| 1140   | 17   | 2006 | Sheep   | 0           | 0      |
| 1141   | 17   | 2006 | Sheep   | 48          | 0      |
| 1142   | 17   | 2006 | Sheep   | 192         | 0      |
| 1143   | 17   | 2006 | Sheep   | 96          | 0      |
| 1144   | 17   | 2006 | Sheep   | 384         | 0      |
| 1145   | 17   | 2006 | Sheep   | 0           | 0      |
| 1146   | 17   | 2006 | Sheep   | 768         | 0      |
| 1147   | 17   | 2006 | Sheep   | 96          | 48     |
| 1148   | 17   | 2006 | Sheep   | 768         | 0      |
| 1149   | 17   | 2006 | Sheep   | 96          | 0      |
| 1150   | 17   | 2006 | Sheep   | 24          | 0      |
| 1151   | 17   | 2006 | Sheep   | 96          | 0      |
| 1152   | 17   | 2006 | Sheep   | 384         | 0      |
| 1153   | 17   | 2006 | Sheep   | 0           | 0      |
| 1154   | 17   | 2006 | Sheep   | 192         | 0      |
| 1155   | 17   | 2006 | Sheep   | 0           | 0      |
| 1156   | 17   | 2006 | Sheep   | 0           | 0      |
| 1157   | 17   | 2006 | Sheep   | 96          | 0      |
| 1158   | 17   | 2006 | Sheep   | 0           | 0      |
| 1159   | 17   | 2006 | Sheep   | 96          | 0      |
| 1160   | 17   | 2006 | Sheep   | 96          | 0      |
| 1161   | 17   | 2006 | Sheep   | 96          | 0      |
| 1162   | 17   | 2006 | Sheep   | 48          | 0      |
| 1163   | 17   | 2006 | Sheep   | 96          | 0      |
| 1164   | 17   | 2006 | Sheep   | 24          | 0      |
| 1165   | 17   | 2006 | Sheep   | 96          | 0      |
| 1166   | 17   | 2006 | Sheep   | 192         | 0      |
| 1167   | 17   | 2006 | Sheep   | 48          | 0      |
| 1168   | 17   | 2006 | Sheep   | 0           | 0      |
| 1169   | 17   | 2006 | Sheep   | 384         | 0      |
| 1170   | 17   | 2006 | Sheep   | 0           | 0      |
| 1171   | 18   | 2006 | Deer    | 0           | 0      |
| 1172   | 18   | 2006 | Deer    | 0           | 0      |
| 1173   | 18   | 2006 | Deer    | 0           | 24     |
| 1174   | 18   | 2006 | Deer    | 48          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1175   | 18   | 2006 | Deer    | 0           | 768    |
| 1176   | 18   | 2006 | Deer    | 0           | 0      |
| 1177   | 18   | 2006 | Deer    | 0           | 384    |
| 1178   | 18   | 2006 | Deer    | 0           | 192    |
| 1179   | 18   | 2006 | Deer    | 0           | 0      |
| 1180   | 18   | 2006 | Deer    | 0           | 192    |
| 1181   | 18   | 2006 | Deer    | 96          | 0      |
| 1182   | 18   | 2006 | Deer    | 0           | 48     |
| 1183   | 18   | 2006 | Deer    | 0           | 0      |
| 1184   | 18   | 2006 | Deer    | 0           | 0      |
| 1185   | 18   | 2006 | Deer    | 0           | 0      |
| 1186   | 18   | 2006 | Deer    | 0           | 0      |
| 1187   | 18   | 2006 | Deer    | 48          | 192    |
| 1188   | 18   | 2006 | Deer    | 96          | 1536   |
| 1189   | 18   | 2006 | Deer    | 0           | 0      |
| 1190   | 18   | 2006 | Deer    | 0           | 0      |
| 1191   | 18   | 2006 | Cattle  | 768         | 0      |
| 1192   | 18   | 2006 | Cattle  | 384         | 0      |
| 1193   | 18   | 2006 | Cattle  | 0           | 0      |
| 1194   | 18   | 2006 | Cattle  | 192         | 0      |
| 1195   | 18   | 2006 | Cattle  | 0           | 0      |
| 1196   | 18   | 2006 | Cattle  | 192         | 0      |
| 1197   | 18   | 2006 | Cattle  | 0           | 0      |
| 1198   | 18   | 2006 | Cattle  | 0           | 0      |
| 1199   | 18   | 2006 | Cattle  | 0           | 0      |
| 1200   | 18   | 2006 | Cattle  | 0           | 0      |
| 1201   | 18   | 2006 | Cattle  | 0           | 96     |
| 1202   | 18   | 2006 | Cattle  | 384         | 192    |
| 1203   | 18   | 2006 | Cattle  | 0           | 0      |
| 1204   | 18   | 2006 | Cattle  | 0           | 0      |
| 1205   | 18   | 2006 | Cattle  | 384         | 0      |
| 1206   | 18   | 2006 | Cattle  | 0           | 0      |
| 1207   | 18   | 2006 | Cattle  | 0           | 0      |
| 1208   | 18   | 2006 | Cattle  | 384         | 0      |
| 1209   | 18   | 2006 | Cattle  | 0           | 0      |
| 1210   | 18   | 2006 | Cattle  | 0           | 0      |
| 1211   | 18   | 2006 | Sheep   | 0           | 0      |
| 1212   | 18   | 2006 | Sheep   | 0           | 0      |
| 1213   | 18   | 2006 | Sheep   | 0           | 0      |
| 1214   | 18   | 2006 | Sheep   | 0           | 0      |
| 1215   | 18   | 2006 | Sheep   | 0           | 0      |
| 1216   | 18   | 2006 | Sheep   | 768         | 0      |
| 1217   | 18   | 2006 | Sheep   | 0           | 0      |
| 1218   | 18   | 2006 | Sheep   | 0           | 0      |
| 1219   | 18   | 2006 | Sheep   | 0           | 0      |
| 1220   | 18   | 2006 | Sheep   | 0           | 0      |
| 1221   | 18   | 2006 | Sheep   | 0           | 0      |
| 1222   | 18   | 2006 | Sheep   | 0           | 0      |
| 1223   | 18   | 2006 | Sheep   | 0           | 0      |
| 1224   | 18   | 2006 | Sheep   | 0           | 0      |
| 1225   | 18   | 2006 | Sheep   | 0           | 0      |
| 1226   | 18   | 2006 | Sheep   | 0           | 0      |
| 1227   | 18   | 2006 | Sheep   | 0           | 0      |
| 1228   | 18   | 2006 | Sheep   | 0           | 0      |
| 1229   | 18   | 2006 | Sheep   | 0           | 0      |
| 1230   | 18   | 2006 | Sheep   | 0           | 0      |
| 1231   | 18   | 2006 | Sheep   | 0           | 0      |
| 1232   | 18   | 2006 | Sheep   | 0           | 0      |
| 1233   | 18   | 2006 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1234   | 18   | 2006 | Sheep   | 0           | 0      |
| 1235   | 18   | 2006 | Sheep   | 768         | 0      |
| 1236   | 18   | 2006 | Sheep   | 0           | 0      |
| 1237   | 18   | 2006 | Sheep   | 0           | 0      |
| 1238   | 18   | 2006 | Sheep   | 0           | 0      |
| 1239   | 18   | 2006 | Sheep   | 0           | 0      |
| 1240   | 18   | 2006 | Sheep   | 0           | 0      |
| 1241   | 18   | 2006 | Sheep   | 0           | 0      |
| 1242   | 18   | 2006 | Sheep   | 0           | 0      |
| 1243   | 18   | 2006 | Sheep   | 0           | 0      |
| 1244   | 18   | 2006 | Sheep   | 384         | 0      |
| 1245   | 18   | 2006 | Sheep   | 0           | 0      |
| 1246   | 18   | 2006 | Sheep   | 0           | 0      |
| 1247   | 18   | 2006 | Sheep   | 0           | 0      |
| 1248   | 18   | 2006 | Sheep   | 0           | 0      |
| 1249   | 18   | 2006 | Sheep   | 768         | 0      |
| 1250   | 18   | 2006 | Sheep   | 384         | 0      |
| 1251   | 18   | 2006 | Sheep   | 0           | 0      |
| 1252   | 18   | 2006 | Sheep   | 0           | 0      |
| 1253   | 18   | 2006 | Sheep   | 0           | 0      |
| 1254   | 18   | 2006 | Sheep   | 0           | 0      |
| 1255   | 18   | 2006 | Sheep   | 768         | 0      |
| 1256   | 18   | 2006 | Sheep   | 0           | 0      |
| 1257   | 18   | 2006 | Sheep   | 0           | 0      |
| 1258   | 18   | 2006 | Sheep   | 0           | 0      |
| 1259   | 18   | 2006 | Sheep   | 0           | 0      |
| 1260   | 18   | 2006 | Sheep   | 0           | 0      |
| 1261   | 18   | 2006 | Sheep   | 0           | 0      |
| 1262   | 18   | 2006 | Sheep   | 0           | 0      |
| 1263   | 18   | 2006 | Sheep   | 0           | 0      |
| 1264   | 18   | 2006 | Sheep   | 0           | 0      |
| 1265   | 18   | 2006 | Sheep   | 1536        | 0      |
| 1266   | 18   | 2006 | Sheep   | 0           | 0      |
| 1267   | 18   | 2006 | Sheep   | 768         | 0      |
| 1268   | 18   | 2006 | Sheep   | 24          | 0      |
| 1269   | 18   | 2006 | Sheep   | 0           | 0      |
| 1270   | 18   | 2006 | Sheep   | 0           | 0      |
| 1271   | 19   | 2006 | Deer    | 24          | 0      |
| 1272   | 19   | 2006 | Deer    | 0           | 0      |
| 1273   | 19   | 2006 | Deer    | 48          | 0      |
| 1274   | 19   | 2006 | Deer    | 0           | 0      |
| 1275   | 19   | 2006 | Deer    | 0           | 0      |
| 1276   | 19   | 2006 | Deer    | 384         | 0      |
| 1277   | 19   | 2006 | Deer    | 384         | 0      |
| 1278   | 19   | 2006 | Deer    | 48          | 0      |
| 1279   | 19   | 2006 | Deer    | 0           | 0      |
| 1280   | 19   | 2006 | Deer    | 24          | 0      |
| 1281   | 19   | 2006 | Deer    | 0           | 0      |
| 1282   | 19   | 2006 | Deer    | 24          | 0      |
| 1283   | 19   | 2006 | Deer    | 24          | 0      |
| 1284   | 19   | 2006 | Deer    | 24          | 0      |
| 1285   | 19   | 2006 | Deer    | 0           | 0      |
| 1286   | 19   | 2006 | Deer    | 192         | 0      |
| 1287   | 19   | 2006 | Deer    | 0           | 0      |
| 1288   | 19   | 2006 | Deer    | 48          | 0      |
| 1289   | 19   | 2006 | Deer    | 0           | 0      |
| 1290   | 19   | 2006 | Deer    | 0           | 0      |
| 1291   | 19   | 2006 | Cattle  | 384         | 0      |
| 1292   | 19   | 2006 | Cattle  | 384         | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1293   | 19   | 2006 | Cattle  | 192         | 0      |
| 1294   | 19   | 2006 | Cattle  | 192         | 0      |
| 1295   | 19   | 2006 | Cattle  | 768         | 0      |
| 1296   | 19   | 2006 | Cattle  | 768         | 0      |
| 1297   | 19   | 2006 | Cattle  | 384         | 0      |
| 1298   | 19   | 2006 | Cattle  | 384         | 0      |
| 1299   | 19   | 2006 | Cattle  | 768         | 0      |
| 1300   | 19   | 2006 | Cattle  | 96          | 0      |
| 1301   | 19   | 2006 | Cattle  | 96          | 0      |
| 1302   | 19   | 2006 | Cattle  | 384         | 0      |
| 1303   | 19   | 2006 | Cattle  | 192         | 0      |
| 1304   | 19   | 2006 | Cattle  | 384         | 0      |
| 1305   | 19   | 2006 | Cattle  | 384         | 0      |
| 1306   | 19   | 2006 | Cattle  | 384         | 0      |
| 1307   | 19   | 2006 | Cattle  | 384         | 0      |
| 1308   | 19   | 2006 | Cattle  | 384         | 0      |
| 1309   | 19   | 2006 | Cattle  | 384         | 0      |
| 1310   | 19   | 2006 | Cattle  | 192         | 0      |
| 1311   | 19   | 2006 | Sheep   | 48          | 0      |
| 1312   | 19   | 2006 | Sheep   | 192         | 0      |
| 1313   | 19   | 2006 | Sheep   | 384         | 0      |
| 1314   | 19   | 2006 | Sheep   | 48          | 0      |
| 1315   | 19   | 2006 | Sheep   | 24          | 0      |
| 1316   | 19   | 2006 | Sheep   | 24          | 0      |
| 1317   | 19   | 2006 | Sheep   | 0           | 0      |
| 1318   | 19   | 2006 | Sheep   | 0           | 0      |
| 1319   | 19   | 2006 | Sheep   | 96          | 0      |
| 1320   | 19   | 2006 | Sheep   | 0           | 0      |
| 1321   | 19   | 2006 | Sheep   | 0           | 0      |
| 1322   | 19   | 2006 | Sheep   | 48          | 0      |
| 1323   | 19   | 2006 | Sheep   | 48          | 0      |
| 1324   | 19   | 2006 | Sheep   | 48          | 0      |
| 1325   | 19   | 2006 | Sheep   | 24          | 0      |
| 1326   | 19   | 2006 | Sheep   | 384         | 0      |
| 1327   | 19   | 2006 | Sheep   | 24          | 0      |
| 1328   | 19   | 2006 | Sheep   | 48          | 0      |
| 1329   | 19   | 2006 | Sheep   | 768         | 0      |
| 1330   | 19   | 2006 | Sheep   | 768         | 0      |
| 1331   | 19   | 2006 | Sheep   | 0           | 0      |
| 1332   | 19   | 2006 | Sheep   | 48          | 0      |
| 1333   | 19   | 2006 | Sheep   | 24          | 0      |
| 1334   | 19   | 2006 | Sheep   | 768         | 0      |
| 1335   | 19   | 2006 | Sheep   | 0           | 0      |
| 1336   | 19   | 2006 | Sheep   | 24          | 0      |
| 1337   | 19   | 2006 | Sheep   | 192         | 0      |
| 1338   | 19   | 2006 | Sheep   | 24          | 0      |
| 1339   | 19   | 2006 | Sheep   | 384         | 0      |
| 1340   | 19   | 2006 | Sheep   | 192         | 0      |
| 1341   | 19   | 2006 | Sheep   | 768         | 0      |
| 1342   | 19   | 2006 | Sheep   | 384         | 0      |
| 1343   | 19   | 2006 | Sheep   | 0           | 0      |
| 1344   | 19   | 2006 | Sheep   | 96          | 0      |
| 1345   | 19   | 2006 | Sheep   | 48          | 0      |
| 1346   | 19   | 2006 | Sheep   | 0           | 0      |
| 1347   | 19   | 2006 | Sheep   | 384         | 0      |
| 1348   | 19   | 2006 | Sheep   | 96          | 0      |
| 1349   | 19   | 2006 | Sheep   | 0           | 0      |
| 1350   | 19   | 2006 | Sheep   | 0           | 0      |
| 1351   | 19   | 2006 | Sheep   | 48          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1352   | 19   | 2006 | Sheep   | 192         | 0      |
| 1353   | 19   | 2006 | Sheep   | 96          | 0      |
| 1354   | 19   | 2006 | Sheep   | 24          | 0      |
| 1355   | 19   | 2006 | Sheep   | 24          | 0      |
| 1356   | 19   | 2006 | Sheep   | 0           | 0      |
| 1357   | 19   | 2006 | Sheep   | 24          | 0      |
| 1358   | 19   | 2006 | Sheep   | 24          | 0      |
| 1359   | 19   | 2006 | Sheep   | 768         | 0      |
| 1360   | 19   | 2006 | Sheep   | 48          | 0      |
| 1361   | 19   | 2006 | Sheep   | 24          | 0      |
| 1362   | 19   | 2006 | Sheep   | 384         | 0      |
| 1363   | 19   | 2006 | Sheep   | 96          | 0      |
| 1364   | 19   | 2006 | Sheep   | 384         | 0      |
| 1365   | 19   | 2006 | Sheep   | 96          | 0      |
| 1366   | 19   | 2006 | Sheep   | 192         | 0      |
| 1367   | 19   | 2006 | Sheep   | 48          | 0      |
| 1368   | 19   | 2006 | Sheep   | 48          | 0      |
| 1369   | 19   | 2006 | Sheep   | 192         | 0      |
| 1370   | 19   | 2006 | Sheep   | 384         | 0      |
| 1371   | 20   | 2006 | Deer    | 24          | 48     |
| 1372   | 20   | 2006 | Deer    | 0           | 0      |
| 1373   | 20   | 2006 | Deer    | 24          | 96     |
| 1374   | 20   | 2006 | Deer    | 0           | 24     |
| 1375   | 20   | 2006 | Deer    | 24          | 0      |
| 1376   | 20   | 2006 | Deer    | 0           | 0      |
| 1377   | 20   | 2006 | Deer    | 0           | 192    |
| 1378   | 20   | 2006 | Deer    | 96          | 0      |
| 1379   | 20   | 2006 | Deer    | 24          | 48     |
| 1380   | 20   | 2006 | Deer    | 24          | 0      |
| 1381   | 20   | 2006 | Deer    | 96          | 0      |
| 1382   | 20   | 2006 | Deer    | 24          | 96     |
| 1383   | 20   | 2006 | Deer    | 0           | 192    |
| 1384   | 20   | 2006 | Deer    | 192         | 48     |
| 1385   | 20   | 2006 | Deer    | 0           | 24     |
| 1386   | 20   | 2006 | Deer    | 192         | 0      |
| 1387   | 20   | 2006 | Deer    | 0           | 0      |
| 1388   | 20   | 2006 | Deer    | 96          | 0      |
| 1389   | 20   | 2006 | Deer    | 48          | 48     |
| 1390   | 20   | 2006 | Deer    | 0           | 0      |
| 1391   | 20   | 2006 | Deer    | 192         | 0      |
| 1392   | 20   | 2006 | Cattle  | 0           | 0      |
| 1393   | 20   | 2006 | Cattle  | 0           | 0      |
| 1394   | 20   | 2006 | Cattle  | 0           | 0      |
| 1395   | 20   | 2006 | Cattle  | 0           | 0      |
| 1396   | 20   | 2006 | Cattle  | 48          | 0      |
| 1397   | 20   | 2006 | Cattle  | 0           | 0      |
| 1398   | 20   | 2006 | Cattle  | 0           | 0      |
| 1399   | 20   | 2006 | Cattle  | 24          | 0      |
| 1400   | 20   | 2006 | Cattle  | 0           | 0      |
| 1401   | 20   | 2006 | Cattle  | 0           | 0      |
| 1402   | 20   | 2006 | Cattle  | 0           | 0      |
| 1403   | 20   | 2006 | Cattle  | 48          | 0      |
| 1404   | 20   | 2006 | Cattle  | 384         | 0      |
| 1405   | 20   | 2006 | Cattle  | 0           | 0      |
| 1406   | 20   | 2006 | Cattle  | 0           | 0      |
| 1407   | 20   | 2006 | Cattle  | 192         | 0      |
| 1408   | 20   | 2006 | Cattle  | 768         | 0      |
| 1409   | 20   | 2006 | Cattle  | 0           | 0      |
| 1410   | 20   | 2006 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1411   | 20   | 2006 | Sheep   | 0           | 0      |
| 1412   | 20   | 2006 | Sheep   | 384         | 0      |
| 1413   | 20   | 2006 | Sheep   | 0           | 0      |
| 1414   | 20   | 2006 | Sheep   | 0           | 0      |
| 1415   | 20   | 2006 | Sheep   | 0           | 0      |
| 1416   | 20   | 2006 | Sheep   | 0           | 0      |
| 1417   | 20   | 2006 | Sheep   | 0           | 0      |
| 1418   | 20   | 2006 | Sheep   | 48          | 0      |
| 1419   | 20   | 2006 | Sheep   | 0           | 0      |
| 1420   | 20   | 2006 | Sheep   | 0           | 0      |
| 1421   | 20   | 2006 | Sheep   | 0           | 0      |
| 1422   | 20   | 2006 | Sheep   | 0           | 0      |
| 1423   | 20   | 2006 | Sheep   | 0           | 0      |
| 1424   | 20   | 2006 | Sheep   | 0           | 0      |
| 1425   | 20   | 2006 | Sheep   | 0           | 0      |
| 1426   | 20   | 2006 | Sheep   | 0           | 0      |
| 1427   | 20   | 2006 | Sheep   | 0           | 0      |
| 1428   | 20   | 2006 | Sheep   | 0           | 0      |
| 1429   | 20   | 2006 | Sheep   | 0           | 0      |
| 1430   | 20   | 2006 | Sheep   | 0           | 0      |
| 1431   | 20   | 2006 | Sheep   | 0           | 0      |
| 1432   | 20   | 2006 | Sheep   | 0           | 0      |
| 1433   | 20   | 2006 | Sheep   | 0           | 0      |
| 1434   | 20   | 2006 | Sheep   | 0           | 0      |
| 1435   | 20   | 2006 | Sheep   | 0           | 0      |
| 1436   | 20   | 2006 | Sheep   | 24          | 0      |
| 1437   | 20   | 2006 | Sheep   | 384         | 0      |
| 1438   | 20   | 2006 | Sheep   | 0           | 0      |
| 1439   | 20   | 2006 | Sheep   | 0           | 0      |
| 1440   | 20   | 2006 | Sheep   | 0           | 0      |
| 1441   | 20   | 2006 | Sheep   | 0           | 0      |
| 1442   | 20   | 2006 | Sheep   | 0           | 0      |
| 1443   | 20   | 2006 | Sheep   | 96          | 0      |
| 1444   | 20   | 2006 | Sheep   | 0           | 0      |
| 1445   | 20   | 2006 | Sheep   | 0           | 0      |
| 1446   | 20   | 2006 | Sheep   | 0           | 0      |
| 1447   | 20   | 2006 | Sheep   | 0           | 0      |
| 1448   | 20   | 2006 | Sheep   | 0           | 0      |
| 1449   | 20   | 2006 | Sheep   | 0           | 0      |
| 1450   | 20   | 2006 | Sheep   | 0           | 0      |
| 1451   | 20   | 2006 | Sheep   | 0           | 0      |
| 1452   | 20   | 2006 | Sheep   | 0           | 0      |
| 1453   | 20   | 2006 | Sheep   | 0           | 0      |
| 1454   | 20   | 2006 | Sheep   | 0           | 0      |
| 1455   | 20   | 2006 | Sheep   | 0           | 0      |
| 1456   | 1    | 2007 | Deer    | 0           | 0      |
| 1457   | 1    | 2007 | Deer    | 0           | 0      |
| 1458   | 1    | 2007 | Deer    | 96          | 0      |
| 1459   | 1    | 2007 | Deer    | 0           | 0      |
| 1460   | 1    | 2007 | Deer    | 0           | 0      |
| 1461   | 1    | 2007 | Deer    | 0           | 0      |
| 1462   | 1    | 2007 | Deer    | 0           | 0      |
| 1463   | 1    | 2007 | Deer    | 0           | 0      |
| 1464   | 1    | 2007 | Deer    | 0           | 0      |
| 1465   | 1    | 2007 | Deer    | 0           | 0      |
| 1466   | 1    | 2007 | Deer    | 0           | 0      |
| 1467   | 1    | 2007 | Deer    | 0           | 0      |
| 1468   | 1    | 2007 | Deer    | 0           | 0      |
| 1469   | 1    | 2007 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1470   | 1    | 2007 | Deer    | 0           | 0      |
| 1471   | 1    | 2007 | Deer    | 0           | 0      |
| 1472   | 1    | 2007 | Deer    | 0           | 0      |
| 1473   | 1    | 2007 | Deer    | 0           | 0      |
| 1474   | 1    | 2007 | Deer    | 0           | 0      |
| 1475   | 2    | 2007 | Deer    | 48          | 0      |
| 1476   | 2    | 2007 | Deer    | 0           | 0      |
| 1477   | 2    | 2007 | Deer    | 24          | 0      |
| 1478   | 2    | 2007 | Deer    | 0           | 0      |
| 1479   | 2    | 2007 | Deer    | 24          | 0      |
| 1480   | 2    | 2007 | Deer    | 24          | 0      |
| 1481   | 2    | 2007 | Deer    | 24          | 0      |
| 1482   | 2    | 2007 | Deer    | 48          | 0      |
| 1483   | 2    | 2007 | Deer    | 0           | 0      |
| 1484   | 2    | 2007 | Deer    | 48          | 0      |
| 1485   | 2    | 2007 | Deer    | 48          | 0      |
| 1486   | 2    | 2007 | Deer    | 0           | 0      |
| 1487   | 2    | 2007 | Deer    | 24          | 0      |
| 1488   | 2    | 2007 | Deer    | 0           | 0      |
| 1489   | 2    | 2007 | Deer    | 24          | 0      |
| 1490   | 2    | 2007 | Deer    | 24          | 0      |
| 1491   | 2    | 2007 | Deer    | 48          | 0      |
| 1492   | 2    | 2007 | Deer    | 24          | 0      |
| 1493   | 2    | 2007 | Deer    | 0           | 0      |
| 1494   | 3    | 2007 | Deer    | 0           | 0      |
| 1495   | 3    | 2007 | Deer    | 0           | 0      |
| 1496   | 3    | 2007 | Deer    | 0           | 0      |
| 1497   | 3    | 2007 | Deer    | 0           | 0      |
| 1498   | 3    | 2007 | Deer    | 0           | 0      |
| 1499   | 3    | 2007 | Deer    | 0           | 0      |
| 1500   | 3    | 2007 | Deer    | 0           | 0      |
| 1501   | 3    | 2007 | Deer    | 0           | 0      |
| 1502   | 3    | 2007 | Deer    | 0           | 0      |
| 1503   | 3    | 2007 | Deer    | 0           | 0      |
| 1504   | 3    | 2007 | Deer    | 0           | 0      |
| 1505   | 3    | 2007 | Deer    | 0           | 0      |
| 1506   | 3    | 2007 | Deer    | 0           | 0      |
| 1507   | 3    | 2007 | Deer    | 0           | 0      |
| 1508   | 3    | 2007 | Deer    | 0           | 0      |
| 1509   | 3    | 2007 | Deer    | 0           | 0      |
| 1510   | 3    | 2007 | Deer    | 0           | 0      |
| 1511   | 3    | 2007 | Deer    | 0           | 0      |
| 1512   | 3    | 2007 | Deer    | 0           | 0      |
| 1513   | 3    | 2007 | Deer    | 0           | 0      |
| 1514   | 4    | 2007 | Deer    | 48          | 0      |
| 1515   | 4    | 2007 | Deer    | 0           | 48     |
| 1516   | 4    | 2007 | Deer    | 24          | 192    |
| 1517   | 4    | 2007 | Deer    | 0           | 24     |
| 1518   | 4    | 2007 | Deer    | 0           | 24     |
| 1519   | 4    | 2007 | Deer    | 48          | 0      |
| 1520   | 4    | 2007 | Deer    | 48          | 0      |
| 1521   | 4    | 2007 | Deer    | 0           | 0      |
| 1522   | 4    | 2007 | Deer    | 48          | 48     |
| 1523   | 4    | 2007 | Deer    | 0           | 48     |
| 1524   | 4    | 2007 | Deer    | 0           | 0      |
| 1525   | 4    | 2007 | Deer    | 0           | 0      |
| 1526   | 4    | 2007 | Deer    | 0           | 0      |
| 1527   | 4    | 2007 | Deer    | 24          | 0      |
| 1528   | 4    | 2007 | Deer    | 24          | 24     |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1529   | 4    | 2007 | Deer    | 0           | 24     |
| 1530   | 4    | 2007 | Cattle  | 0           | 0      |
| 1531   | 4    | 2007 | Cattle  | 0           | 0      |
| 1532   | 4    | 2007 | Cattle  | 0           | 0      |
| 1533   | 4    | 2007 | Cattle  | 0           | 0      |
| 1534   | 4    | 2007 | Cattle  | 0           | 0      |
| 1535   | 4    | 2007 | Cattle  | 0           | 0      |
| 1536   | 4    | 2007 | Cattle  | 0           | 0      |
| 1537   | 4    | 2007 | Cattle  | 0           | 0      |
| 1538   | 5    | 2007 | Deer    | 0           | 0      |
| 1539   | 5    | 2007 | Deer    | 0           | 0      |
| 1540   | 5    | 2007 | Deer    | 0           | 0      |
| 1541   | 5    | 2007 | Deer    | 0           | 0      |
| 1542   | 5    | 2007 | Deer    | 0           | 0      |
| 1543   | 5    | 2007 | Deer    | 0           | 0      |
| 1544   | 5    | 2007 | Deer    | 384         | 0      |
| 1545   | 5    | 2007 | Deer    | 0           | 0      |
| 1546   | 5    | 2007 | Deer    | 0           | 0      |
| 1547   | 5    | 2007 | Deer    | 0           | 0      |
| 1548   | 5    | 2007 | Deer    | 0           | 0      |
| 1549   | 5    | 2007 | Deer    | 0           | 0      |
| 1550   | 5    | 2007 | Deer    | 0           | 0      |
| 1551   | 5    | 2007 | Deer    | 0           | 0      |
| 1552   | 5    | 2007 | Deer    | 0           | 0      |
| 1553   | 5    | 2007 | Deer    | 0           | 0      |
| 1554   | 5    | 2007 | Deer    | 0           | 0      |
| 1555   | 5    | 2007 | Deer    | 0           | 0      |
| 1556   | 5    | 2007 | Deer    | 0           | 0      |
| 1557   | 5    | 2007 | Deer    | 0           | 0      |
| 1558   | 5    | 2007 | Cattle  | 0           | 0      |
| 1559   | 5    | 2007 | Cattle  | 0           | 0      |
| 1560   | 5    | 2007 | Cattle  | 0           | 0      |
| 1561   | 5    | 2007 | Cattle  | 0           | 0      |
| 1562   | 5    | 2007 | Cattle  | 0           | 0      |
| 1563   | 5    | 2007 | Cattle  | 0           | 0      |
| 1564   | 5    | 2007 | Cattle  | 0           | 0      |
| 1565   | 5    | 2007 | Cattle  | 0           | 0      |
| 1566   | 5    | 2007 | Cattle  | 0           | 0      |
| 1567   | 5    | 2007 | Cattle  | 0           | 0      |
| 1568   | 5    | 2007 | Cattle  | 0           | 24     |
| 1569   | 5    | 2007 | Cattle  | 0           | 0      |
| 1570   | 5    | 2007 | Cattle  | 0           | 0      |
| 1571   | 5    | 2007 | Cattle  | 0           | 0      |
| 1572   | 5    | 2007 | Cattle  | 0           | 0      |
| 1573   | 5    | 2007 | Cattle  | 0           | 0      |
| 1574   | 5    | 2007 | Cattle  | 0           | 0      |
| 1575   | 5    | 2007 | Cattle  | 0           | 0      |
| 1576   | 5    | 2007 | Cattle  | 0           | 0      |
| 1577   | 6    | 2007 | Deer    | 24          | 0      |
| 1578   | 6    | 2007 | Deer    | 192         | 0      |
| 1579   | 6    | 2007 | Deer    | 48          | 0      |
| 1580   | 6    | 2007 | Deer    | 192         | 0      |
| 1581   | 6    | 2007 | Deer    | 0           | 0      |
| 1582   | 6    | 2007 | Deer    | 0           | 0      |
| 1583   | 6    | 2007 | Deer    | 192         | 0      |
| 1584   | 6    | 2007 | Deer    | 96          | 0      |
| 1585   | 6    | 2007 | Deer    | 48          | 0      |
| 1586   | 6    | 2007 | Deer    | 48          | 0      |
| 1587   | 6    | 2007 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1588   | 6    | 2007 | Deer    | 48          | 0      |
| 1589   | 6    | 2007 | Deer    | 96          | 0      |
| 1590   | 6    | 2007 | Deer    | 96          | 0      |
| 1591   | 6    | 2007 | Deer    | 48          | 0      |
| 1592   | 6    | 2007 | Deer    | 24          | 0      |
| 1593   | 6    | 2007 | Deer    | 0           | 0      |
| 1594   | 6    | 2007 | Deer    | 24          | 0      |
| 1595   | 6    | 2007 | Deer    | 192         | 0      |
| 1596   | 6    | 2007 | Deer    | 24          | 0      |
| 1597   | 6    | 2007 | Cattle  | 192         | 0      |
| 1598   | 6    | 2007 | Cattle  | 96          | 0      |
| 1599   | 6    | 2007 | Cattle  | 192         | 0      |
| 1600   | 6    | 2007 | Cattle  | 192         | 0      |
| 1601   | 6    | 2007 | Cattle  | 48          | 0      |
| 1602   | 6    | 2007 | Cattle  | 48          | 0      |
| 1603   | 6    | 2007 | Cattle  | 96          | 0      |
| 1604   | 6    | 2007 | Cattle  | 96          | 0      |
| 1605   | 6    | 2007 | Cattle  | 96          | 0      |
| 1606   | 6    | 2007 | Cattle  | 96          | 0      |
| 1607   | 6    | 2007 | Cattle  | 192         | 0      |
| 1608   | 6    | 2007 | Cattle  | 384         | 0      |
| 1609   | 6    | 2007 | Cattle  | 96          | 0      |
| 1610   | 6    | 2007 | Cattle  | 48          | 0      |
| 1611   | 6    | 2007 | Cattle  | 96          | 0      |
| 1612   | 6    | 2007 | Cattle  | 0           | 24     |
| 1613   | 6    | 2007 | Cattle  | 48          | 0      |
| 1614   | 6    | 2007 | Cattle  | 96          | 0      |
| 1615   | 6    | 2007 | Cattle  | 0           | 0      |
| 1616   | 6    | 2007 | Cattle  | 0           | 0      |
| 1617   | 7    | 2007 | Deer    | 0           | 0      |
| 1618   | 7    | 2007 | Deer    | 0           | 0      |
| 1619   | 7    | 2007 | Deer    | 0           | 0      |
| 1620   | 7    | 2007 | Deer    | 24          | 0      |
| 1621   | 7    | 2007 | Deer    | 0           | 0      |
| 1622   | 7    | 2007 | Deer    | 48          | 0      |
| 1623   | 7    | 2007 | Deer    | 24          | 0      |
| 1624   | 7    | 2007 | Deer    | 48          | 0      |
| 1625   | 7    | 2007 | Deer    | 48          | 0      |
| 1626   | 7    | 2007 | Deer    | 0           | 0      |
| 1627   | 7    | 2007 | Deer    | 0           | 0      |
| 1628   | 7    | 2007 | Deer    | 96          | 96     |
| 1629   | 7    | 2007 | Deer    | 0           | 0      |
| 1630   | 7    | 2007 | Deer    | 0           | 0      |
| 1631   | 7    | 2007 | Deer    | 96          | 0      |
| 1632   | 7    | 2007 | Deer    | 0           | 0      |
| 1633   | 7    | 2007 | Deer    | 0           | 0      |
| 1634   | 7    | 2007 | Deer    | 48          | 0      |
| 1635   | 7    | 2007 | Deer    | 0           | 0      |
| 1636   | 7    | 2007 | Deer    | 0           | 0      |
| 1637   | 7    | 2007 | Sheep   | 0           | 0      |
| 1638   | 7    | 2007 | Sheep   | 0           | 0      |
| 1639   | 7    | 2007 | Sheep   | 0           | 0      |
| 1640   | 7    | 2007 | Sheep   | 0           | 0      |
| 1641   | 7    | 2007 | Sheep   | 0           | 0      |
| 1642   | 7    | 2007 | Sheep   | 0           | 0      |
| 1643   | 7    | 2007 | Sheep   | 0           | 0      |
| 1644   | 7    | 2007 | Sheep   | 0           | 0      |
| 1645   | 7    | 2007 | Sheep   | 0           | 0      |
| 1646   | 7    | 2007 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1647   | 7    | 2007 | Sheep   | 0           | 0      |
| 1648   | 7    | 2007 | Sheep   | 0           | 0      |
| 1649   | 7    | 2007 | Sheep   | 0           | 0      |
| 1650   | 7    | 2007 | Sheep   | 0           | 0      |
| 1651   | 7    | 2007 | Sheep   | 0           | 0      |
| 1652   | 7    | 2007 | Sheep   | 0           | 0      |
| 1653   | 7    | 2007 | Sheep   | 0           | 0      |
| 1654   | 7    | 2007 | Sheep   | 0           | 0      |
| 1655   | 7    | 2007 | Sheep   | 0           | 0      |
| 1656   | 9    | 2007 | Deer    | 24          | 192    |
| 1657   | 9    | 2007 | Deer    | 0           | 0      |
| 1658   | 9    | 2007 | Deer    | 0           | 24     |
| 1659   | 9    | 2007 | Deer    | 0           | 0      |
| 1660   | 9    | 2007 | Deer    | 0           | 0      |
| 1661   | 9    | 2007 | Deer    | 0           | 24     |
| 1662   | 9    | 2007 | Deer    | 0           | 0      |
| 1663   | 9    | 2007 | Deer    | 0           | 0      |
| 1664   | 9    | 2007 | Deer    | 0           | 0      |
| 1665   | 9    | 2007 | Deer    | 0           | 24     |
| 1666   | 9    | 2007 | Deer    | 24          | 0      |
| 1667   | 9    | 2007 | Deer    | 0           | 24     |
| 1668   | 9    | 2007 | Deer    | 0           | 0      |
| 1669   | 9    | 2007 | Deer    | 24          | 0      |
| 1670   | 9    | 2007 | Deer    | 0           | 0      |
| 1671   | 9    | 2007 | Deer    | 0           | 0      |
| 1672   | 9    | 2007 | Deer    | 0           | 0      |
| 1673   | 9    | 2007 | Deer    | 24          | 96     |
| 1674   | 9    | 2007 | Deer    | 0           | 48     |
| 1675   | 9    | 2007 | Cattle  | 96          | 48     |
| 1676   | 9    | 2007 | Cattle  | 96          | 0      |
| 1677   | 9    | 2007 | Cattle  | 48          | 0      |
| 1678   | 9    | 2007 | Cattle  | 24          | 0      |
| 1679   | 9    | 2007 | Cattle  | 24          | 48     |
| 1680   | 9    | 2007 | Cattle  | 0           | 96     |
| 1681   | 9    | 2007 | Cattle  | 24          | 24     |
| 1682   | 9    | 2007 | Cattle  | 24          | 0      |
| 1683   | 9    | 2007 | Cattle  | 96          | 48     |
| 1684   | 9    | 2007 | Cattle  | 0           | 0      |
| 1685   | 9    | 2007 | Cattle  | 96          | 0      |
| 1686   | 9    | 2007 | Cattle  | 0           | 0      |
| 1687   | 9    | 2007 | Cattle  | 0           | 96     |
| 1688   | 9    | 2007 | Cattle  | 192         | 24     |
| 1689   | 9    | 2007 | Cattle  | 0           | 48     |
| 1690   | 9    | 2007 | Cattle  | 24          | 48     |
| 1691   | 9    | 2007 | Cattle  | 0           | 48     |
| 1692   | 9    | 2007 | Cattle  | 96          | 0      |
| 1693   | 9    | 2007 | Cattle  | 0           | 768    |
| 1694   | 9    | 2007 | Sheep   | 0           | 48     |
| 1695   | 9    | 2007 | Sheep   | 96          | 0      |
| 1696   | 9    | 2007 | Sheep   | 0           | 0      |
| 1697   | 9    | 2007 | Sheep   | 0           | 0      |
| 1698   | 9    | 2007 | Sheep   | 0           | 0      |
| 1699   | 9    | 2007 | Sheep   | 0           | 0      |
| 1700   | 9    | 2007 | Sheep   | 24          | 0      |
| 1701   | 9    | 2007 | Sheep   | 48          | 0      |
| 1702   | 9    | 2007 | Sheep   | 96          | 0      |
| 1703   | 9    | 2007 | Sheep   | 24          | 0      |
| 1704   | 9    | 2007 | Sheep   | 48          | 0      |
| 1705   | 9    | 2007 | Sheep   | 48          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1706   | 9    | 2007 | Sheep   | 48          | 0      |
| 1707   | 9    | 2007 | Sheep   | 48          | 0      |
| 1708   | 9    | 2007 | Sheep   | 96          | 0      |
| 1709   | 9    | 2007 | Sheep   | 96          | 0      |
| 1710   | 9    | 2007 | Sheep   | 48          | 0      |
| 1711   | 10   | 2007 | Deer    | 24          | 0      |
| 1712   | 10   | 2007 | Deer    | 0           | 0      |
| 1713   | 10   | 2007 | Deer    | 96          | 24     |
| 1714   | 10   | 2007 | Deer    | 24          | 0      |
| 1715   | 10   | 2007 | Deer    | 96          | 96     |
| 1716   | 10   | 2007 | Deer    | 48          | 0      |
| 1717   | 10   | 2007 | Deer    | 0           | 96     |
| 1718   | 10   | 2007 | Deer    | 0           | 96     |
| 1719   | 10   | 2007 | Deer    | 0           | 96     |
| 1720   | 10   | 2007 | Deer    | 48          | 96     |
| 1721   | 10   | 2007 | Deer    | 48          | 48     |
| 1722   | 10   | 2007 | Deer    | 0           | 192    |
| 1723   | 10   | 2007 | Deer    | 0           | 48     |
| 1724   | 10   | 2007 | Deer    | 0           | 192    |
| 1725   | 10   | 2007 | Deer    | 0           | 48     |
| 1726   | 10   | 2007 | Deer    | 48          | 384    |
| 1727   | 10   | 2007 | Deer    | 0           | 96     |
| 1728   | 10   | 2007 | Deer    | 0           | 0      |
| 1729   | 10   | 2007 | Deer    | 24          | 0      |
| 1730   | 10   | 2007 | Cattle  | 0           | 0      |
| 1731   | 10   | 2007 | Cattle  | 24          | 0      |
| 1732   | 10   | 2007 | Cattle  | 0           | 96     |
| 1733   | 10   | 2007 | Cattle  | 0           | 0      |
| 1734   | 10   | 2007 | Cattle  | 24          | 0      |
| 1735   | 10   | 2007 | Cattle  | 0           | 0      |
| 1736   | 10   | 2007 | Cattle  | 0           | 0      |
| 1737   | 10   | 2007 | Cattle  | 48          | 0      |
| 1738   | 10   | 2007 | Cattle  | 24          | 48     |
| 1739   | 10   | 2007 | Cattle  | 192         | 0      |
| 1740   | 10   | 2007 | Cattle  | 24          | 0      |
| 1741   | 10   | 2007 | Cattle  | 0           | 0      |
| 1742   | 10   | 2007 | Cattle  | 48          | 24     |
| 1743   | 10   | 2007 | Cattle  | 0           | 0      |
| 1744   | 10   | 2007 | Cattle  | 0           | 0      |
| 1745   | 10   | 2007 | Cattle  | 48          | 0      |
| 1746   | 10   | 2007 | Cattle  | 24          | 0      |
| 1747   | 10   | 2007 | Cattle  | 48          | 0      |
| 1748   | 10   | 2007 | Sheep   | 48          | 0      |
| 1749   | 10   | 2007 | Sheep   | 96          | 0      |
| 1750   | 10   | 2007 | Sheep   | 0           | 24     |
| 1751   | 10   | 2007 | Sheep   | 48          | 96     |
| 1752   | 10   | 2007 | Sheep   | 0           | 0      |
| 1753   | 10   | 2007 | Sheep   | 96          | 0      |
| 1754   | 10   | 2007 | Sheep   | 0           | 0      |
| 1755   | 10   | 2007 | Sheep   | 0           | 0      |
| 1756   | 10   | 2007 | Sheep   | 0           | 0      |
| 1757   | 10   | 2007 | Sheep   | 0           | 0      |
| 1758   | 10   | 2007 | Sheep   | 48          | 0      |
| 1759   | 10   | 2007 | Sheep   | 48          | 0      |
| 1760   | 10   | 2007 | Sheep   | 0           | 48     |
| 1761   | 10   | 2007 | Sheep   | 0           | 0      |
| 1762   | 10   | 2007 | Sheep   | 0           | 96     |
| 1763   | 10   | 2007 | Sheep   | 192         | 0      |
| 1764   | 10   | 2007 | Sheep   | 24          | 0      |



| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1765   | 10   | 2007 | Sheep   | 24          | 0      |
| 1766   | 10   | 2007 | Sheep   | 0           | 0      |
| 1767   | 10   | 2007 | Sheep   | 0           | 0      |
| 1768   | 11   | 2007 | Deer    | 48          | 0      |
| 1769   | 11   | 2007 | Deer    | 48          | 0      |
| 1770   | 11   | 2007 | Deer    | 24          | 0      |
| 1771   | 11   | 2007 | Deer    | 24          | 0      |
| 1772   | 11   | 2007 | Deer    | 96          | 0      |
| 1773   | 11   | 2007 | Deer    | 96          | 0      |
| 1774   | 11   | 2007 | Deer    | 48          | 0      |
| 1775   | 11   | 2007 | Deer    | 48          | 0      |
| 1776   | 11   | 2007 | Deer    | 96          | 0      |
| 1777   | 11   | 2007 | Deer    | 48          | 0      |
| 1778   | 11   | 2007 | Deer    | 48          | 0      |
| 1779   | 11   | 2007 | Deer    | 48          | 0      |
| 1780   | 11   | 2007 | Deer    | 96          | 0      |
| 1781   | 11   | 2007 | Deer    | 24          | 0      |
| 1782   | 11   | 2007 | Deer    | 48          | 0      |
| 1783   | 11   | 2007 | Deer    | 96          | 0      |
| 1784   | 11   | 2007 | Deer    | 96          | 0      |
| 1785   | 11   | 2007 | Deer    | 96          | 0      |
| 1786   | 11   | 2007 | Deer    | 96          | 0      |
| 1787   | 11   | 2007 | Deer    | 96          | 0      |
| 1788   | 11   | 2007 | Cattle  | 48          | 24     |
| 1789   | 11   | 2007 | Cattle  | 0           | 0      |
| 1790   | 11   | 2007 | Cattle  | 24          | 24     |
| 1791   | 11   | 2007 | Cattle  | 0           | 0      |
| 1792   | 11   | 2007 | Cattle  | 24          | 0      |
| 1793   | 11   | 2007 | Cattle  | 0           | 0      |
| 1794   | 11   | 2007 | Cattle  | 0           | 0      |
| 1795   | 11   | 2007 | Cattle  | 0           | 0      |
| 1796   | 11   | 2007 | Cattle  | 0           | 0      |
| 1797   | 11   | 2007 | Cattle  | 192         | 0      |
| 1798   | 11   | 2007 | Cattle  | 48          | 0      |
| 1799   | 11   | 2007 | Cattle  | 0           | 0      |
| 1800   | 11   | 2007 | Cattle  | 0           | 0      |
| 1801   | 11   | 2007 | Cattle  | 0           | 0      |
| 1802   | 11   | 2007 | Cattle  | 24          | 0      |
| 1803   | 11   | 2007 | Cattle  | 0           | 0      |
| 1804   | 11   | 2007 | Cattle  | 48          | 0      |
| 1805   | 11   | 2007 | Cattle  | 24          | 0      |
| 1806   | 11   | 2007 | Cattle  | 192         | 0      |
| 1807   | 11   | 2007 | Cattle  | 24          | 24     |
| 1808   | 11   | 2007 | Sheep   | 96          | 0      |
| 1809   | 11   | 2007 | Sheep   | 0           | 0      |
| 1810   | 11   | 2007 | Sheep   | 192         | 0      |
| 1811   | 11   | 2007 | Sheep   | 0           | 0      |
| 1812   | 11   | 2007 | Sheep   | 192         | 0      |
| 1813   | 11   | 2007 | Sheep   | 0           | 0      |
| 1814   | 11   | 2007 | Sheep   | 192         | 0      |
| 1815   | 11   | 2007 | Sheep   | 0           | 0      |
| 1816   | 11   | 2007 | Sheep   | 192         | 0      |
| 1817   | 11   | 2007 | Sheep   | 768         | 0      |
| 1818   | 11   | 2007 | Sheep   | 96          | 0      |
| 1819   | 11   | 2007 | Sheep   | 0           | 0      |
| 1820   | 11   | 2007 | Sheep   | 0           | 0      |
| 1821   | 11   | 2007 | Sheep   | 0           | 0      |
| 1822   | 11   | 2007 | Sheep   | 0           | 0      |
| 1823   | 11   | 2007 | Sheep   | 192         | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1824   | 11   | 2007 | Sheep   | 0           | 0      |
| 1825   | 11   | 2007 | Sheep   | 0           | 0      |
| 1826   | 11   | 2007 | Sheep   | 0           | 0      |
| 1827   | 11   | 2007 | Sheep   | 96          | 0      |
| 1828   | 12   | 2007 | Deer    | 0           | 0      |
| 1829   | 12   | 2007 | Deer    | 0           | 0      |
| 1830   | 12   | 2007 | Deer    | 0           | 0      |
| 1831   | 12   | 2007 | Deer    | 0           | 0      |
| 1832   | 12   | 2007 | Deer    | 0           | 0      |
| 1833   | 12   | 2007 | Deer    | 24          | 0      |
| 1834   | 12   | 2007 | Deer    | 96          | 0      |
| 1835   | 12   | 2007 | Deer    | 24          | 0      |
| 1836   | 12   | 2007 | Deer    | 96          | 0      |
| 1837   | 12   | 2007 | Deer    | 0           | 0      |
| 1838   | 12   | 2007 | Deer    | 0           | 0      |
| 1839   | 12   | 2007 | Deer    | 0           | 0      |
| 1840   | 12   | 2007 | Deer    | 0           | 0      |
| 1841   | 12   | 2007 | Deer    | 0           | 0      |
| 1842   | 12   | 2007 | Deer    | 0           | 0      |
| 1843   | 12   | 2007 | Deer    | 0           | 0      |
| 1844   | 12   | 2007 | Deer    | 0           | 0      |
| 1845   | 12   | 2007 | Deer    | 48          | 0      |
| 1846   | 12   | 2007 | Deer    | 0           | 0      |
| 1847   | 12   | 2007 | Deer    | 96          | 0      |
| 1848   | 12   | 2007 | Cattle  | 0           | 0      |
| 1849   | 12   | 2007 | Cattle  | 0           | 0      |
| 1850   | 12   | 2007 | Cattle  | 0           | 0      |
| 1851   | 12   | 2007 | Cattle  | 48          | 0      |
| 1852   | 12   | 2007 | Cattle  | 0           | 0      |
| 1853   | 12   | 2007 | Cattle  | 0           | 0      |
| 1854   | 12   | 2007 | Cattle  | 96          | 0      |
| 1855   | 12   | 2007 | Cattle  | 0           | 0      |
| 1856   | 12   | 2007 | Cattle  | 0           | 0      |
| 1857   | 12   | 2007 | Cattle  | 0           | 0      |
| 1858   | 12   | 2007 | Cattle  | 96          | 0      |
| 1859   | 12   | 2007 | Cattle  | 0           | 0      |
| 1860   | 12   | 2007 | Cattle  | 48          | 0      |
| 1861   | 12   | 2007 | Cattle  | 192         | 0      |
| 1862   | 12   | 2007 | Cattle  | 96          | 0      |
| 1863   | 12   | 2007 | Cattle  | 96          | 0      |
| 1864   | 12   | 2007 | Cattle  | 0           | 0      |
| 1865   | 12   | 2007 | Cattle  | 0           | 0      |
| 1866   | 12   | 2007 | Cattle  | 0           | 0      |
| 1867   | 12   | 2007 | Cattle  | 0           | 0      |
| 1868   | 12   | 2007 | Sheep   | 24          | 96     |
| 1869   | 12   | 2007 | Sheep   | 96          | 0      |
| 1870   | 12   | 2007 | Sheep   | 96          | 0      |
| 1871   | 12   | 2007 | Sheep   | 24          | 0      |
| 1872   | 12   | 2007 | Sheep   | 0           | 0      |
| 1873   | 12   | 2007 | Sheep   | 48          | 0      |
| 1874   | 12   | 2007 | Sheep   | 48          | 0      |
| 1875   | 12   | 2007 | Sheep   | 0           | 0      |
| 1876   | 12   | 2007 | Sheep   | 48          | 0      |
| 1877   | 12   | 2007 | Sheep   | 48          | 0      |
| 1878   | 12   | 2007 | Sheep   | 24          | 0      |
| 1879   | 12   | 2007 | Sheep   | 0           | 0      |
| 1880   | 12   | 2007 | Sheep   | 96          | 0      |
| 1881   | 12   | 2007 | Sheep   | 48          | 0      |
| 1882   | 12   | 2007 | Sheep   | 48          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1883   | 12   | 2007 | Sheep   | 48          | 0      |
| 1884   | 12   | 2007 | Sheep   | 48          | 0      |
| 1885   | 12   | 2007 | Sheep   | 24          | 0      |
| 1886   | 12   | 2007 | Sheep   | 48          | 0      |
| 1887   | 12   | 2007 | Sheep   | 96          | 0      |
| 1888   | 13   | 2007 | Deer    | 48          | 0      |
| 1889   | 13   | 2007 | Deer    | 0           | 0      |
| 1890   | 13   | 2007 | Deer    | 24          | 0      |
| 1891   | 13   | 2007 | Deer    | 24          | 0      |
| 1892   | 13   | 2007 | Deer    | 24          | 0      |
| 1893   | 13   | 2007 | Deer    | 48          | 0      |
| 1894   | 13   | 2007 | Deer    | 24          | 0      |
| 1895   | 13   | 2007 | Deer    | 24          | 0      |
| 1896   | 13   | 2007 | Deer    | 48          | 24     |
| 1897   | 13   | 2007 | Deer    | 0           | 0      |
| 1898   | 13   | 2007 | Deer    | 48          | 0      |
| 1899   | 13   | 2007 | Deer    | 96          | 0      |
| 1900   | 13   | 2007 | Deer    | 96          | 0      |
| 1901   | 13   | 2007 | Deer    | 24          | 0      |
| 1902   | 13   | 2007 | Deer    | 0           | 0      |
| 1903   | 13   | 2007 | Deer    | 24          | 0      |
| 1904   | 13   | 2007 | Deer    | 48          | 0      |
| 1905   | 13   | 2007 | Deer    | 96          | 0      |
| 1906   | 13   | 2007 | Deer    | 48          | 0      |
| 1907   | 13   | 2007 | Deer    | 24          | 0      |
| 1908   | 13   | 2007 | Sheep   | 0           | 0      |
| 1909   | 13   | 2007 | Sheep   | 0           | 0      |
| 1910   | 13   | 2007 | Sheep   | 0           | 0      |
| 1911   | 13   | 2007 | Sheep   | 0           | 0      |
| 1912   | 13   | 2007 | Sheep   | 0           | 0      |
| 1913   | 13   | 2007 | Sheep   | 0           | 0      |
| 1914   | 13   | 2007 | Sheep   | 0           | 0      |
| 1915   | 13   | 2007 | Sheep   | 0           | 0      |
| 1916   | 13   | 2007 | Sheep   | 0           | 0      |
| 1917   | 13   | 2007 | Sheep   | 0           | 0      |
| 1918   | 13   | 2007 | Sheep   | 0           | 0      |
| 1919   | 13   | 2007 | Sheep   | 0           | 0      |
| 1920   | 13   | 2007 | Sheep   | 0           | 0      |
| 1921   | 13   | 2007 | Sheep   | 0           | 0      |
| 1922   | 13   | 2007 | Sheep   | 0           | 0      |
| 1923   | 13   | 2007 | Sheep   | 0           | 0      |
| 1924   | 13   | 2007 | Sheep   | 0           | 0      |
| 1925   | 13   | 2007 | Sheep   | 0           | 0      |
| 1926   | 13   | 2007 | Sheep   | 0           | 0      |
| 1927   | 13   | 2007 | Sheep   | 0           | 0      |
| 1928   | 14   | 2007 | Deer    | 0           | 96     |
| 1929   | 14   | 2007 | Deer    | 0           | 192    |
| 1930   | 14   | 2007 | Deer    | 0           | 0      |
| 1931   | 14   | 2007 | Deer    | 0           | 96     |
| 1932   | 14   | 2007 | Deer    | 0           | 0      |
| 1933   | 14   | 2007 | Deer    | 24          | 0      |
| 1934   | 14   | 2007 | Deer    | 96          | 48     |
| 1935   | 14   | 2007 | Deer    | 0           | 0      |
| 1936   | 14   | 2007 | Deer    | 96          | 96     |
| 1937   | 14   | 2007 | Deer    | 0           | 96     |
| 1938   | 14   | 2007 | Deer    | 0           | 0      |
| 1939   | 14   | 2007 | Deer    | 0           | 48     |
| 1940   | 14   | 2007 | Deer    | 0           | 0      |
| 1941   | 14   | 2007 | Deer    | 0           | 96     |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 1942   | 14   | 2007 | Deer    | 0           | 0      |
| 1943   | 14   | 2007 | Deer    | 24          | 0      |
| 1944   | 14   | 2007 | Deer    | 96          | 0      |
| 1945   | 14   | 2007 | Deer    | 48          | 48     |
| 1946   | 14   | 2007 | Deer    | 0           | 384    |
| 1947   | 14   | 2007 | Deer    | 0           | 0      |
| 1948   | 14   | 2007 | Cattle  | 0           | 0      |
| 1949   | 14   | 2007 | Cattle  | 0           | 0      |
| 1950   | 14   | 2007 | Cattle  | 0           | 0      |
| 1951   | 14   | 2007 | Cattle  | 0           | 0      |
| 1952   | 14   | 2007 | Cattle  | 0           | 0      |
| 1953   | 14   | 2007 | Cattle  | 0           | 0      |
| 1954   | 14   | 2007 | Cattle  | 0           | 0      |
| 1955   | 14   | 2007 | Cattle  | 0           | 0      |
| 1956   | 14   | 2007 | Cattle  | 0           | 0      |
| 1957   | 14   | 2007 | Cattle  | 0           | 0      |
| 1958   | 14   | 2007 | Cattle  | 0           | 0      |
| 1959   | 14   | 2007 | Cattle  | 0           | 0      |
| 1960   | 14   | 2007 | Cattle  | 0           | 96     |
| 1961   | 14   | 2007 | Cattle  | 0           | 0      |
| 1962   | 14   | 2007 | Cattle  | 0           | 0      |
| 1963   | 14   | 2007 | Cattle  | 0           | 0      |
| 1964   | 14   | 2007 | Cattle  | 0           | 0      |
| 1965   | 14   | 2007 | Cattle  | 0           | 0      |
| 1966   | 14   | 2007 | Cattle  | 0           | 0      |
| 1967   | 14   | 2007 | Cattle  | 0           | 0      |
| 1968   | 14   | 2007 | Sheep   | 0           | 0      |
| 1969   | 14   | 2007 | Sheep   | 24          | 0      |
| 1970   | 14   | 2007 | Sheep   | 48          | 0      |
| 1971   | 14   | 2007 | Sheep   | 0           | 0      |
| 1972   | 14   | 2007 | Sheep   | 24          | 0      |
| 1973   | 14   | 2007 | Sheep   | 24          | 0      |
| 1974   | 14   | 2007 | Sheep   | 48          | 0      |
| 1975   | 14   | 2007 | Sheep   | 0           | 0      |
| 1976   | 14   | 2007 | Sheep   | 0           | 0      |
| 1977   | 14   | 2007 | Sheep   | 0           | 0      |
| 1978   | 14   | 2007 | Sheep   | 0           | 0      |
| 1979   | 14   | 2007 | Sheep   | 48          | 0      |
| 1980   | 14   | 2007 | Sheep   | 0           | 0      |
| 1981   | 14   | 2007 | Sheep   | 0           | 0      |
| 1982   | 14   | 2007 | Sheep   | 192         | 0      |
| 1983   | 14   | 2007 | Sheep   | 0           | 0      |
| 1984   | 14   | 2007 | Sheep   | 96          | 0      |
| 1985   | 14   | 2007 | Sheep   | 96          | 48     |
| 1986   | 14   | 2007 | Sheep   | 0           | 0      |
| 1987   | 14   | 2007 | Sheep   | 0           | 0      |
| 1988   | 15   | 2007 | Deer    | 0           | 0      |
| 1989   | 15   | 2007 | Deer    | 0           | 0      |
| 1990   | 15   | 2007 | Deer    | 0           | 0      |
| 1991   | 15   | 2007 | Deer    | 0           | 0      |
| 1992   | 15   | 2007 | Deer    | 0           | 0      |
| 1993   | 15   | 2007 | Deer    | 0           | 0      |
| 1994   | 15   | 2007 | Deer    | 0           | 0      |
| 1995   | 15   | 2007 | Deer    | 0           | 0      |
| 1996   | 15   | 2007 | Deer    | 0           | 0      |
| 1997   | 15   | 2007 | Deer    | 0           | 0      |
| 1998   | 15   | 2007 | Deer    | 0           | 0      |
| 1999   | 15   | 2007 | Deer    | 0           | 0      |
| 2000   | 15   | 2007 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2001   | 15   | 2007 | Deer    | 0           | 0      |
| 2002   | 15   | 2007 | Deer    | 0           | 0      |
| 2003   | 15   | 2007 | Deer    | 0           | 0      |
| 2004   | 15   | 2007 | Deer    | 384         | 0      |
| 2005   | 15   | 2007 | Deer    | 48          | 0      |
| 2006   | 15   | 2007 | Deer    | 0           | 0      |
| 2007   | 15   | 2007 | Cattle  | 192         | 0      |
| 2008   | 15   | 2007 | Cattle  | 0           | 0      |
| 2009   | 15   | 2007 | Cattle  | 0           | 0      |
| 2010   | 15   | 2007 | Cattle  | 24          | 0      |
| 2011   | 15   | 2007 | Cattle  | 192         | 0      |
| 2012   | 15   | 2007 | Cattle  | 96          | 0      |
| 2013   | 15   | 2007 | Cattle  | 48          | 0      |
| 2014   | 15   | 2007 | Cattle  | 0           | 0      |
| 2015   | 15   | 2007 | Cattle  | 0           | 0      |
| 2016   | 15   | 2007 | Cattle  | 384         | 0      |
| 2017   | 15   | 2007 | Cattle  | 48          | 0      |
| 2018   | 15   | 2007 | Cattle  | 48          | 0      |
| 2019   | 15   | 2007 | Cattle  | 48          | 0      |
| 2020   | 15   | 2007 | Cattle  | 24          | 0      |
| 2021   | 15   | 2007 | Cattle  | 48          | 0      |
| 2022   | 15   | 2007 | Cattle  | 192         | 0      |
| 2023   | 15   | 2007 | Cattle  | 0           | 0      |
| 2024   | 15   | 2007 | Cattle  | 96          | 0      |
| 2025   | 15   | 2007 | Cattle  | 0           | 0      |
| 2026   | 15   | 2007 | Cattle  | 0           | 0      |
| 2027   | 15   | 2007 | Sheep   | 0           | 0      |
| 2028   | 15   | 2007 | Sheep   | 48          | 0      |
| 2029   | 15   | 2007 | Sheep   | 0           | 0      |
| 2030   | 15   | 2007 | Sheep   | 96          | 0      |
| 2031   | 15   | 2007 | Sheep   | 48          | 0      |
| 2032   | 15   | 2007 | Sheep   | 48          | 0      |
| 2033   | 15   | 2007 | Sheep   | 0           | 0      |
| 2034   | 15   | 2007 | Sheep   | 96          | 0      |
| 2035   | 15   | 2007 | Sheep   | 96          | 0      |
| 2036   | 15   | 2007 | Sheep   | 0           | 0      |
| 2037   | 15   | 2007 | Sheep   | 96          | 0      |
| 2038   | 15   | 2007 | Sheep   | 96          | 0      |
| 2039   | 15   | 2007 | Sheep   | 96          | 0      |
| 2040   | 15   | 2007 | Sheep   | 48          | 0      |
| 2041   | 15   | 2007 | Sheep   | 96          | 0      |
| 2042   | 15   | 2007 | Sheep   | 24          | 0      |
| 2043   | 15   | 2007 | Sheep   | 48          | 0      |
| 2044   | 15   | 2007 | Sheep   | 0           | 0      |
| 2045   | 15   | 2007 | Sheep   | 96          | 0      |
| 2046   | 16   | 2007 | Deer    | 0           | 0      |
| 2047   | 16   | 2007 | Deer    | 0           | 0      |
| 2048   | 16   | 2007 | Deer    | 0           | 0      |
| 2049   | 16   | 2007 | Deer    | 0           | 0      |
| 2050   | 16   | 2007 | Deer    | 0           | 0      |
| 2051   | 16   | 2007 | Deer    | 0           | 0      |
| 2052   | 16   | 2007 | Deer    | 0           | 0      |
| 2053   | 16   | 2007 | Deer    | 0           | 0      |
| 2054   | 16   | 2007 | Deer    | 0           | 0      |
| 2055   | 16   | 2007 | Deer    | 0           | 0      |
| 2056   | 16   | 2007 | Deer    | 0           | 0      |
| 2057   | 16   | 2007 | Deer    | 0           | 0      |
| 2058   | 16   | 2007 | Deer    | 48          | 0      |
| 2059   | 16   | 2007 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2060   | 16   | 2007 | Deer    | 48          | 0      |
| 2061   | 16   | 2007 | Deer    | 0           | 0      |
| 2062   | 16   | 2007 | Deer    | 0           | 0      |
| 2063   | 16   | 2007 | Deer    | 0           | 0      |
| 2064   | 16   | 2007 | Deer    | 48          | 0      |
| 2065   | 16   | 2007 | Deer    | 24          | 0      |
| 2066   | 16   | 2007 | Cattle  | 0           | 0      |
| 2067   | 16   | 2007 | Cattle  | 0           | 0      |
| 2068   | 16   | 2007 | Cattle  | 0           | 0      |
| 2069   | 16   | 2007 | Cattle  | 0           | 0      |
| 2070   | 16   | 2007 | Cattle  | 0           | 0      |
| 2071   | 16   | 2007 | Cattle  | 0           | 0      |
| 2072   | 16   | 2007 | Cattle  | 0           | 0      |
| 2073   | 16   | 2007 | Cattle  | 0           | 0      |
| 2074   | 16   | 2007 | Cattle  | 0           | 0      |
| 2075   | 16   | 2007 | Cattle  | 0           | 0      |
| 2076   | 16   | 2007 | Cattle  | 0           | 0      |
| 2077   | 16   | 2007 | Cattle  | 0           | 24     |
| 2078   | 16   | 2007 | Cattle  | 0           | 0      |
| 2079   | 16   | 2007 | Cattle  | 0           | 0      |
| 2080   | 16   | 2007 | Cattle  | 0           | 0      |
| 2081   | 16   | 2007 | Cattle  | 0           | 0      |
| 2082   | 16   | 2007 | Cattle  | 0           | 0      |
| 2083   | 16   | 2007 | Cattle  | 0           | 0      |
| 2084   | 16   | 2007 | Cattle  | 0           | 0      |
| 2085   | 16   | 2007 | Cattle  | 0           | 0      |
| 2086   | 16   | 2007 | Sheep   | 0           | 0      |
| 2087   | 16   | 2007 | Sheep   | 0           | 0      |
| 2088   | 16   | 2007 | Sheep   | 0           | 0      |
| 2089   | 16   | 2007 | Sheep   | 0           | 0      |
| 2090   | 16   | 2007 | Sheep   | 0           | 0      |
| 2091   | 16   | 2007 | Sheep   | 0           | 0      |
| 2092   | 16   | 2007 | Sheep   | 0           | 0      |
| 2093   | 16   | 2007 | Sheep   | 0           | 0      |
| 2094   | 16   | 2007 | Sheep   | 0           | 0      |
| 2095   | 16   | 2007 | Sheep   | 0           | 0      |
| 2096   | 16   | 2007 | Sheep   | 0           | 0      |
| 2097   | 16   | 2007 | Sheep   | 0           | 0      |
| 2098   | 16   | 2007 | Sheep   | 0           | 0      |
| 2099   | 16   | 2007 | Sheep   | 0           | 0      |
| 2100   | 16   | 2007 | Sheep   | 0           | 0      |
| 2101   | 16   | 2007 | Sheep   | 0           | 0      |
| 2102   | 16   | 2007 | Sheep   | 0           | 0      |
| 2103   | 16   | 2007 | Sheep   | 0           | 0      |
| 2104   | 16   | 2007 | Sheep   | 0           | 0      |
| 2105   | 16   | 2007 | Sheep   | 0           | 0      |
| 2106   | 17   | 2007 | Deer    | 48          | 0      |
| 2107   | 17   | 2007 | Deer    | 48          | 0      |
| 2108   | 17   | 2007 | Deer    | 96          | 0      |
| 2109   | 17   | 2007 | Deer    | 0           | 0      |
| 2110   | 17   | 2007 | Deer    | 48          | 0      |
| 2111   | 17   | 2007 | Deer    | 96          | 0      |
| 2112   | 17   | 2007 | Deer    | 96          | 0      |
| 2113   | 17   | 2007 | Deer    | 0           | 0      |
| 2114   | 17   | 2007 | Deer    | 48          | 24     |
| 2115   | 17   | 2007 | Deer    | 48          | 24     |
| 2116   | 17   | 2007 | Deer    | 48          | 0      |
| 2117   | 17   | 2007 | Deer    | 48          | 0      |
| 2118   | 17   | 2007 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2119   | 17   | 2007 | Deer    | 0           | 0      |
| 2120   | 17   | 2007 | Deer    | 96          | 0      |
| 2121   | 17   | 2007 | Deer    | 192         | 48     |
| 2122   | 17   | 2007 | Deer    | 0           | 24     |
| 2123   | 17   | 2007 | Deer    | 0           | 0      |
| 2124   | 17   | 2007 | Deer    | 48          | 0      |
| 2125   | 17   | 2007 | Cattle  | 48          | 0      |
| 2126   | 17   | 2007 | Cattle  | 96          | 0      |
| 2127   | 17   | 2007 | Cattle  | 384         | 0      |
| 2128   | 17   | 2007 | Cattle  | 768         | 0      |
| 2129   | 17   | 2007 | Cattle  | 768         | 0      |
| 2130   | 17   | 2007 | Cattle  | 384         | 0      |
| 2131   | 17   | 2007 | Cattle  | 384         | 0      |
| 2132   | 17   | 2007 | Sheep   | 0           | 0      |
| 2133   | 17   | 2007 | Sheep   | 0           | 0      |
| 2134   | 17   | 2007 | Sheep   | 0           | 0      |
| 2135   | 17   | 2007 | Sheep   | 0           | 0      |
| 2136   | 17   | 2007 | Sheep   | 0           | 0      |
| 2137   | 17   | 2007 | Sheep   | 0           | 0      |
| 2138   | 17   | 2007 | Sheep   | 48          | 0      |
| 2139   | 17   | 2007 | Sheep   | 0           | 0      |
| 2140   | 17   | 2007 | Sheep   | 0           | 0      |
| 2141   | 17   | 2007 | Sheep   | 0           | 0      |
| 2142   | 17   | 2007 | Sheep   | 24          | 0      |
| 2143   | 17   | 2007 | Sheep   | 0           | 0      |
| 2144   | 17   | 2007 | Sheep   | 0           | 0      |
| 2145   | 17   | 2007 | Sheep   | 0           | 0      |
| 2146   | 17   | 2007 | Sheep   | 96          | 0      |
| 2147   | 17   | 2007 | Sheep   | 48          | 0      |
| 2148   | 17   | 2007 | Sheep   | 0           | 0      |
| 2149   | 17   | 2007 | Sheep   | 48          | 0      |
| 2150   | 17   | 2007 | Sheep   | 0           | 0      |
| 2151   | 17   | 2007 | Sheep   | 0           | 0      |
| 2152   | 18   | 2007 | Deer    | 24          | 48     |
| 2153   | 18   | 2007 | Deer    | 48          | 192    |
| 2154   | 18   | 2007 | Deer    | 0           | 96     |
| 2155   | 18   | 2007 | Deer    | 24          | 48     |
| 2156   | 18   | 2007 | Deer    | 0           | 0      |
| 2157   | 18   | 2007 | Deer    | 0           | 0      |
| 2158   | 18   | 2007 | Deer    | 0           | 48     |
| 2159   | 18   | 2007 | Deer    | 24          | 48     |
| 2160   | 18   | 2007 | Deer    | 192         | 96     |
| 2161   | 18   | 2007 | Deer    | 96          | 0      |
| 2162   | 18   | 2007 | Deer    | 192         | 0      |
| 2163   | 18   | 2007 | Deer    | 0           | 0      |
| 2164   | 18   | 2007 | Deer    | 0           | 0      |
| 2165   | 18   | 2007 | Deer    | 48          | 96     |
| 2166   | 18   | 2007 | Deer    | 0           | 192    |
| 2167   | 18   | 2007 | Deer    | 0           | 192    |
| 2168   | 18   | 2007 | Deer    | 96          | 48     |
| 2169   | 18   | 2007 | Deer    | 96          | 384    |
| 2170   | 18   | 2007 | Deer    | 48          | 0      |
| 2171   | 18   | 2007 | Deer    | 0           | 0      |
| 2172   | 18   | 2007 | Cattle  | 192         | 0      |
| 2173   | 18   | 2007 | Cattle  | 48          | 0      |
| 2174   | 18   | 2007 | Cattle  | 48          | 0      |
| 2175   | 18   | 2007 | Cattle  | 192         | 0      |
| 2176   | 18   | 2007 | Cattle  | 96          | 0      |
| 2177   | 18   | 2007 | Cattle  | 96          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2178   | 18   | 2007 | Cattle  | 192         | 0      |
| 2179   | 18   | 2007 | Cattle  | 192         | 0      |
| 2180   | 18   | 2007 | Cattle  | 0           | 0      |
| 2181   | 18   | 2007 | Cattle  | 96          | 0      |
| 2182   | 18   | 2007 | Cattle  | 0           | 0      |
| 2183   | 18   | 2007 | Cattle  | 96          | 0      |
| 2184   | 18   | 2007 | Cattle  | 48          | 0      |
| 2185   | 18   | 2007 | Cattle  | 96          | 0      |
| 2186   | 18   | 2007 | Cattle  | 48          | 0      |
| 2187   | 18   | 2007 | Cattle  | 48          | 0      |
| 2188   | 18   | 2007 | Cattle  | 24          | 0      |
| 2189   | 18   | 2007 | Cattle  | 192         | 0      |
| 2190   | 18   | 2007 | Cattle  | 48          | 24     |
| 2191   | 18   | 2007 | Cattle  | 96          | 0      |
| 2192   | 18   | 2007 | Sheep   | 0           | 0      |
| 2193   | 18   | 2007 | Sheep   | 0           | 0      |
| 2194   | 18   | 2007 | Sheep   | 0           | 0      |
| 2195   | 18   | 2007 | Sheep   | 0           | 0      |
| 2196   | 18   | 2007 | Sheep   | 0           | 0      |
| 2197   | 18   | 2007 | Sheep   | 0           | 0      |
| 2198   | 18   | 2007 | Sheep   | 384         | 0      |
| 2199   | 18   | 2007 | Sheep   | 0           | 0      |
| 2200   | 18   | 2007 | Sheep   | 0           | 0      |
| 2201   | 18   | 2007 | Sheep   | 0           | 0      |
| 2202   | 18   | 2007 | Sheep   | 0           | 0      |
| 2203   | 18   | 2007 | Sheep   | 0           | 0      |
| 2204   | 18   | 2007 | Sheep   | 0           | 0      |
| 2205   | 18   | 2007 | Sheep   | 0           | 0      |
| 2206   | 18   | 2007 | Sheep   | 0           | 0      |
| 2207   | 18   | 2007 | Sheep   | 0           | 0      |
| 2208   | 18   | 2007 | Sheep   | 0           | 0      |
| 2209   | 18   | 2007 | Sheep   | 0           | 0      |
| 2210   | 18   | 2007 | Sheep   | 0           | 0      |
| 2211   | 19   | 2007 | Deer    | 24          | 0      |
| 2212   | 19   | 2007 | Deer    | 24          | 0      |
| 2213   | 19   | 2007 | Deer    | 0           | 0      |
| 2214   | 19   | 2007 | Deer    | 24          | 0      |
| 2215   | 19   | 2007 | Deer    | 24          | 24     |
| 2216   | 19   | 2007 | Deer    | 0           | 0      |
| 2217   | 19   | 2007 | Deer    | 0           | 0      |
| 2218   | 19   | 2007 | Deer    | 0           | 0      |
| 2219   | 19   | 2007 | Deer    | 0           | 0      |
| 2220   | 19   | 2007 | Deer    | 0           | 0      |
| 2221   | 19   | 2007 | Deer    | 48          | 0      |
| 2222   | 19   | 2007 | Deer    | 0           | 0      |
| 2223   | 19   | 2007 | Deer    | 48          | 0      |
| 2224   | 19   | 2007 | Deer    | 0           | 0      |
| 2225   | 19   | 2007 | Deer    | 96          | 0      |
| 2226   | 19   | 2007 | Deer    | 24          | 0      |
| 2227   | 19   | 2007 | Deer    | 24          | 0      |
| 2228   | 19   | 2007 | Deer    | 0           | 0      |
| 2229   | 19   | 2007 | Deer    | 0           | 0      |
| 2230   | 19   | 2007 | Deer    | 0           | 0      |
| 2231   | 19   | 2007 | Deer    | 24          | 48     |
| 2232   | 19   | 2007 | Deer    | 0           | 0      |
| 2233   | 19   | 2007 | Deer    | 0           | 96     |
| 2234   | 19   | 2007 | Deer    | 48          | 48     |
| 2235   | 19   | 2007 | Deer    | 192         | 0      |
| 2236   | 19   | 2007 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2237   | 19   | 2007 | Deer    | 96          | 0      |
| 2238   | 19   | 2007 | Deer    | 0           | 0      |
| 2239   | 19   | 2007 | Deer    | 0           | 0      |
| 2240   | 19   | 2007 | Deer    | 0           | 0      |
| 2241   | 19   | 2007 | Deer    | 0           | 48     |
| 2242   | 19   | 2007 | Deer    | 0           | 0      |
| 2243   | 19   | 2007 | Deer    | 192         | 0      |
| 2244   | 19   | 2007 | Deer    | 0           | 0      |
| 2245   | 19   | 2007 | Deer    | 0           | 0      |
| 2246   | 19   | 2007 | Deer    | 0           | 0      |
| 2247   | 19   | 2007 | Deer    | 0           | 0      |
| 2248   | 19   | 2007 | Deer    | 0           | 0      |
| 2249   | 19   | 2007 | Deer    | 48          | 24     |
| 2250   | 19   | 2007 | Deer    | 192         | 0      |
| 2251   | 19   | 2007 | Cattle  | 0           | 0      |
| 2252   | 19   | 2007 | Cattle  | 24          | 0      |
| 2253   | 19   | 2007 | Cattle  | 0           | 0      |
| 2254   | 19   | 2007 | Cattle  | 24          | 0      |
| 2255   | 19   | 2007 | Cattle  | 96          | 0      |
| 2256   | 19   | 2007 | Cattle  | 96          | 0      |
| 2257   | 19   | 2007 | Cattle  | 24          | 0      |
| 2258   | 19   | 2007 | Cattle  | 96          | 0      |
| 2259   | 19   | 2007 | Cattle  | 48          | 0      |
| 2260   | 19   | 2007 | Cattle  | 48          | 0      |
| 2261   | 19   | 2007 | Cattle  | 24          | 0      |
| 2262   | 19   | 2007 | Cattle  | 48          | 0      |
| 2263   | 19   | 2007 | Cattle  | 48          | 0      |
| 2264   | 19   | 2007 | Cattle  | 48          | 0      |
| 2265   | 19   | 2007 | Cattle  | 48          | 0      |
| 2266   | 19   | 2007 | Cattle  | 0           | 0      |
| 2267   | 19   | 2007 | Cattle  | 48          | 0      |
| 2268   | 19   | 2007 | Cattle  | 96          | 0      |
| 2269   | 19   | 2007 | Cattle  | 96          | 0      |
| 2270   | 19   | 2007 | Cattle  | 0           | 0      |
| 2271   | 19   | 2007 | Cattle  | 0           | 0      |
| 2272   | 19   | 2007 | Cattle  | 0           | 0      |
| 2273   | 19   | 2007 | Cattle  | 0           | 0      |
| 2274   | 19   | 2007 | Cattle  | 0           | 0      |
| 2275   | 19   | 2007 | Cattle  | 0           | 0      |
| 2276   | 19   | 2007 | Cattle  | 0           | 0      |
| 2277   | 19   | 2007 | Cattle  | 0           | 0      |
| 2278   | 19   | 2007 | Cattle  | 0           | 0      |
| 2279   | 19   | 2007 | Cattle  | 0           | 0      |
| 2280   | 19   | 2007 | Cattle  | 0           | 0      |
| 2281   | 19   | 2007 | Cattle  | 0           | 0      |
| 2282   | 19   | 2007 | Cattle  | 0           | 0      |
| 2283   | 19   | 2007 | Cattle  | 0           | 0      |
| 2284   | 19   | 2007 | Cattle  | 0           | 0      |
| 2285   | 19   | 2007 | Cattle  | 0           | 0      |
| 2286   | 19   | 2007 | Cattle  | 0           | 0      |
| 2287   | 19   | 2007 | Cattle  | 0           | 0      |
| 2288   | 19   | 2007 | Cattle  | 0           | 0      |
| 2289   | 19   | 2007 | Cattle  | 0           | 0      |
| 2290   | 19   | 2007 | Cattle  | 0           | 0      |
| 2291   | 19   | 2007 | Sheep   | 0           | 384    |
| 2292   | 19   | 2007 | Sheep   | 0           | 192    |
| 2293   | 19   | 2007 | Sheep   | 0           | 0      |
| 2294   | 19   | 2007 | Sheep   | 768         | 0      |
| 2295   | 19   | 2007 | Sheep   | 192         | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2296   | 19   | 2007 | Sheep   | 768         | 0      |
| 2297   | 19   | 2007 | Sheep   | 0           | 96     |
| 2298   | 19   | 2007 | Sheep   | 0           | 192    |
| 2299   | 19   | 2007 | Sheep   | 0           | 192    |
| 2300   | 19   | 2007 | Sheep   | 0           | 384    |
| 2301   | 19   | 2007 | Sheep   | 0           | 0      |
| 2302   | 19   | 2007 | Sheep   | 48          | 192    |
| 2303   | 19   | 2007 | Sheep   | 0           | 192    |
| 2304   | 19   | 2007 | Sheep   | 0           | 192    |
| 2305   | 19   | 2007 | Sheep   | 0           | 192    |
| 2306   | 19   | 2007 | Sheep   | 96          | 192    |
| 2307   | 19   | 2007 | Sheep   | 48          | 96     |
| 2308   | 19   | 2007 | Sheep   | 96          | 384    |
| 2309   | 19   | 2007 | Sheep   | 48          | 48     |
| 2310   | 19   | 2007 | Sheep   | 384         | 192    |
| 2311   | 20   | 2007 | Deer    | 48          | 48     |
| 2312   | 20   | 2007 | Deer    | 48          | 192    |
| 2313   | 20   | 2007 | Deer    | 0           | 0      |
| 2314   | 20   | 2007 | Deer    | 0           | 48     |
| 2315   | 20   | 2007 | Deer    | 24          | 0      |
| 2316   | 20   | 2007 | Deer    | 48          | 96     |
| 2317   | 20   | 2007 | Deer    | 0           | 96     |
| 2318   | 20   | 2007 | Deer    | 0           | 48     |
| 2319   | 20   | 2007 | Deer    | 0           | 0      |
| 2320   | 20   | 2007 | Deer    | 48          | 96     |
| 2321   | 20   | 2007 | Deer    | 96          | 48     |
| 2322   | 20   | 2007 | Deer    | 24          | 24     |
| 2323   | 20   | 2007 | Deer    | 0           | 192    |
| 2324   | 20   | 2007 | Deer    | 96          | 0      |
| 2325   | 20   | 2007 | Deer    | 48          | 0      |
| 2326   | 20   | 2007 | Deer    | 48          | 96     |
| 2327   | 20   | 2007 | Deer    | 96          | 96     |
| 2328   | 20   | 2007 | Deer    | 0           | 0      |
| 2329   | 20   | 2007 | Deer    | 96          | 0      |
| 2330   | 20   | 2007 | Deer    | 0           | 0      |
| 2331   | 20   | 2007 | Cattle  | 0           | 0      |
| 2332   | 20   | 2007 | Cattle  | 0           | 0      |
| 2333   | 20   | 2007 | Cattle  | 96          | 96     |
| 2334   | 20   | 2007 | Cattle  | 24          | 192    |
| 2335   | 20   | 2007 | Cattle  | 0           | 0      |
| 2336   | 20   | 2007 | Cattle  | 0           | 0      |
| 2337   | 20   | 2007 | Cattle  | 0           | 0      |
| 2338   | 20   | 2007 | Cattle  | 0           | 0      |
| 2339   | 20   | 2007 | Cattle  | 0           | 0      |
| 2340   | 20   | 2007 | Cattle  | 96          | 0      |
| 2341   | 20   | 2007 | Cattle  | 0           | 0      |
| 2342   | 20   | 2007 | Cattle  | 96          | 0      |
| 2343   | 20   | 2007 | Cattle  | 0           | 0      |
| 2344   | 20   | 2007 | Cattle  | 48          | 0      |
| 2345   | 20   | 2007 | Cattle  | 0           | 0      |
| 2346   | 20   | 2007 | Cattle  | 0           | 0      |
| 2347   | 20   | 2007 | Cattle  | 0           | 96     |
| 2348   | 20   | 2007 | Cattle  | 0           | 0      |
| 2349   | 20   | 2007 | Cattle  | 0           | 0      |
| 2350   | 20   | 2007 | Cattle  | 0           | 0      |
| 2351   | 20   | 2007 | Sheep   | 0           | 0      |
| 2352   | 20   | 2007 | Sheep   | 0           | 96     |
| 2353   | 20   | 2007 | Sheep   | 24          | 0      |
| 2354   | 20   | 2007 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2355   | 20   | 2007 | Sheep   | 0           | 0      |
| 2356   | 20   | 2007 | Sheep   | 0           | 0      |
| 2357   | 20   | 2007 | Sheep   | 0           | 0      |
| 2358   | 20   | 2007 | Sheep   | 0           | 0      |
| 2359   | 20   | 2007 | Sheep   | 0           | 0      |
| 2360   | 20   | 2007 | Sheep   | 0           | 0      |
| 2361   | 20   | 2007 | Sheep   | 0           | 0      |
| 2362   | 20   | 2007 | Sheep   | 0           | 24     |
| 2363   | 20   | 2007 | Sheep   | 0           | 0      |
| 2364   | 20   | 2007 | Sheep   | 0           | 0      |
| 2365   | 20   | 2007 | Sheep   | 0           | 0      |
| 2366   | 20   | 2007 | Sheep   | 0           | 0      |
| 2367   | 20   | 2007 | Sheep   | 24          | 0      |
| 2368   | 20   | 2007 | Sheep   | 0           | 0      |
| 2369   | 20   | 2007 | Sheep   | 0           | 0      |
| 2370   | 1    | 2008 | Deer    | 0           | 0      |
| 2371   | 1    | 2008 | Deer    | 0           | 0      |
| 2372   | 1    | 2008 | Deer    | 0           | 0      |
| 2373   | 1    | 2008 | Deer    | 0           | 0      |
| 2374   | 1    | 2008 | Deer    | 0           | 0      |
| 2375   | 1    | 2008 | Deer    | 0           | 0      |
| 2376   | 1    | 2008 | Deer    | 96          | 0      |
| 2377   | 1    | 2008 | Deer    | 24          | 0      |
| 2378   | 1    | 2008 | Deer    | 0           | 0      |
| 2379   | 1    | 2008 | Deer    | 0           | 0      |
| 2380   | 1    | 2008 | Deer    | 0           | 0      |
| 2370   | 1    | 2008 | Deer    | 0           | 0      |
| 2371   | 1    | 2008 | Deer    | 0           | 0      |
| 2372   | 1    | 2008 | Deer    | 0           | 0      |
| 2373   | 1    | 2008 | Deer    | 0           | 0      |
| 2374   | 1    | 2008 | Deer    | 0           | 0      |
| 2375   | 1    | 2008 | Deer    | 0           | 0      |
| 2376   | 1    | 2008 | Deer    | 96          | 0      |
| 2377   | 1    | 2008 | Deer    | 24          | 0      |
| 2378   | 1    | 2008 | Deer    | 0           | 0      |
| 2379   | 1    | 2008 | Deer    | 0           | 0      |
| 2380   | 1    | 2008 | Deer    | 0           | 0      |
| 2381   | 1    | 2008 | Deer    | 0           | 0      |
| 2382   | 1    | 2008 | Deer    | 0           | 0      |
| 2383   | 1    | 2008 | Deer    | 0           | 0      |
| 2384   | 1    | 2008 | Deer    | 0           | 0      |
| 2385   | 1    | 2008 | Deer    | 0           | 0      |
| 2386   | 1    | 2008 | Deer    | 0           | 0      |
| 2387   | 1    | 2008 | Deer    | 0           | 0      |
| 2388   | 1    | 2008 | Deer    | 0           | 0      |
| 2389   | 1    | 2008 | Deer    | 0           | 0      |
| 2390   | 2    | 2008 | Deer    | 0           | 0      |
| 2391   | 2    | 2008 | Deer    | 24          | 0      |
| 2392   | 2    | 2008 | Deer    | 0           | 0      |
| 2393   | 2    | 2008 | Deer    | 24          | 0      |
| 2394   | 2    | 2008 | Deer    | 0           | 0      |
| 2395   | 2    | 2008 | Deer    | 0           | 0      |
| 2396   | 2    | 2008 | Deer    | 0           | 0      |
| 2397   | 2    | 2008 | Deer    | 48          | 0      |
| 2398   | 2    | 2008 | Deer    | 0           | 0      |
| 2399   | 2    | 2008 | Deer    | 0           | 0      |
| 2400   | 2    | 2008 | Deer    | 0           | 0      |
| 2401   | 2    | 2008 | Deer    | 0           | 0      |
| 2402   | 2    | 2008 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2403   | 2    | 2008 | Deer    | 0           | 0      |
| 2404   | 2    | 2008 | Deer    | 0           | 0      |
| 2405   | 2    | 2008 | Deer    | 48          | 0      |
| 2406   | 2    | 2008 | Deer    | 0           | 0      |
| 2407   | 2    | 2008 | Deer    | 0           | 0      |
| 2408   | 2    | 2008 | Deer    | 0           | 0      |
| 2409   | 3    | 2008 | Deer    | 0           | 0      |
| 2410   | 3    | 2008 | Deer    | 0           | 0      |
| 2411   | 3    | 2008 | Deer    | 0           | 0      |
| 2412   | 3    | 2008 | Deer    | 0           | 0      |
| 2413   | 3    | 2008 | Deer    | 0           | 0      |
| 2414   | 3    | 2008 | Deer    | 0           | 0      |
| 2415   | 3    | 2008 | Deer    | 0           | 0      |
| 2416   | 3    | 2008 | Deer    | 0           | 0      |
| 2417   | 3    | 2008 | Deer    | 0           | 0      |
| 2418   | 3    | 2008 | Deer    | 0           | 0      |
| 2419   | 3    | 2008 | Deer    | 24          | 0      |
| 2420   | 3    | 2008 | Deer    | 0           | 0      |
| 2421   | 3    | 2008 | Deer    | 48          | 0      |
| 2422   | 3    | 2008 | Deer    | 0           | 0      |
| 2423   | 3    | 2008 | Deer    | 0           | 0      |
| 2424   | 3    | 2008 | Deer    | 0           | 0      |
| 2425   | 3    | 2008 | Deer    | 0           | 0      |
| 2426   | 3    | 2008 | Deer    | 0           | 0      |
| 2427   | 3    | 2008 | Deer    | 0           | 0      |
| 2428   | 3    | 2008 | Deer    | 0           | 0      |
| 2429   | 4    | 2008 | Deer    | 0           | 0      |
| 2430   | 4    | 2008 | Deer    | 0           | 384    |
| 2431   | 4    | 2008 | Deer    | 0           | 0      |
| 2432   | 4    | 2008 | Deer    | 0           | 0      |
| 2433   | 4    | 2008 | Deer    | 0           | 96     |
| 2434   | 4    | 2008 | Deer    | 0           | 0      |
| 2435   | 4    | 2008 | Deer    | 0           | 1536   |
| 2436   | 4    | 2008 | Deer    | 0           | 0      |
| 2437   | 4    | 2008 | Deer    | 0           | 0      |
| 2438   | 4    | 2008 | Deer    | 0           | 768    |
| 2439   | 4    | 2008 | Deer    | 0           | 0      |
| 2440   | 4    | 2008 | Deer    | 0           | 0      |
| 2441   | 4    | 2008 | Deer    | 0           | 96     |
| 2442   | 4    | 2008 | Deer    | 0           | 0      |
| 2443   | 4    | 2008 | Deer    | 0           | 384    |
| 2444   | 4    | 2008 | Deer    | 0           | 0      |
| 2445   | 4    | 2008 | Deer    | 0           | 96     |
| 2446   | 4    | 2008 | Deer    | 0           | 0      |
| 2447   | 4    | 2008 | Deer    | 0           | 48     |
| 2448   | 4    | 2008 | Deer    | 0           | 768    |
| 2449   | 4    | 2008 | Cattle  | 0           | 0      |
| 2450   | 4    | 2008 | Cattle  | 0           | 0      |
| 2451   | 4    | 2008 | Cattle  | 0           | 0      |
| 2452   | 4    | 2008 | Cattle  | 0           | 0      |
| 2453   | 4    | 2008 | Cattle  | 0           | 0      |
| 2454   | 4    | 2008 | Cattle  | 0           | 0      |
| 2455   | 4    | 2008 | Cattle  | 0           | 0      |
| 2456   | 5    | 2008 | Deer    | 0           | 0      |
| 2457   | 5    | 2008 | Deer    | 0           | 0      |
| 2458   | 5    | 2008 | Deer    | 0           | 0      |
| 2459   | 5    | 2008 | Deer    | 0           | 0      |
| 2460   | 5    | 2008 | Deer    | 0           | 0      |
| 2461   | 5    | 2008 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2462   | 5    | 2008 | Deer    | 0           | 0      |
| 2463   | 5    | 2008 | Deer    | 0           | 0      |
| 2464   | 5    | 2008 | Deer    | 0           | 0      |
| 2465   | 5    | 2008 | Deer    | 0           | 0      |
| 2466   | 5    | 2008 | Deer    | 0           | 0      |
| 2467   | 5    | 2008 | Deer    | 0           | 0      |
| 2468   | 5    | 2008 | Deer    | 0           | 0      |
| 2469   | 5    | 2008 | Deer    | 24          | 0      |
| 2470   | 5    | 2008 | Deer    | 0           | 0      |
| 2471   | 5    | 2008 | Deer    | 0           | 0      |
| 2472   | 5    | 2008 | Deer    | 0           | 0      |
| 2473   | 5    | 2008 | Deer    | 0           | 0      |
| 2474   | 5    | 2008 | Deer    | 0           | 24     |
| 2475   | 5    | 2008 | Deer    | 0           | 24     |
| 2476   | 5    | 2008 | Cattle  | 48          | 1536   |
| 2477   | 5    | 2008 | Cattle  | 0           | 384    |
| 2478   | 5    | 2008 | Cattle  | 0           | 768    |
| 2479   | 5    | 2008 | Cattle  | 0           | 768    |
| 2480   | 5    | 2008 | Cattle  | 0           | 768    |
| 2481   | 5    | 2008 | Cattle  | 0           | 96     |
| 2482   | 5    | 2008 | Cattle  | 0           | 768    |
| 2483   | 5    | 2008 | Cattle  | 192         | 768    |
| 2484   | 5    | 2008 | Cattle  | 0           | 768    |
| 2485   | 5    | 2008 | Cattle  | 0           | 768    |
| 2486   | 5    | 2008 | Cattle  | 0           | 384    |
| 2487   | 5    | 2008 | Cattle  | 24          | 768    |
| 2488   | 5    | 2008 | Cattle  | 0           | 384    |
| 2489   | 5    | 2008 | Cattle  | 0           | 96     |
| 2490   | 5    | 2008 | Cattle  | 0           | 3072   |
| 2491   | 5    | 2008 | Cattle  | 0           | 768    |
| 2492   | 5    | 2008 | Cattle  | 0           | 1536   |
| 2493   | 5    | 2008 | Cattle  | 0           | 384    |
| 2494   | 5    | 2008 | Cattle  | 0           | 24     |
| 2495   | 5    | 2008 | Cattle  | 0           | 768    |
| 2496   | 6    | 2008 | Deer    | 0           | 0      |
| 2497   | 6    | 2008 | Deer    | 48          | 0      |
| 2498   | 6    | 2008 | Deer    | 192         | 0      |
| 2499   | 6    | 2008 | Deer    | 0           | 0      |
| 2500   | 6    | 2008 | Deer    | 48          | 0      |
| 2501   | 6    | 2008 | Deer    | 96          | 0      |
| 2502   | 6    | 2008 | Deer    | 0           | 0      |
| 2503   | 6    | 2008 | Deer    | 48          | 0      |
| 2504   | 6    | 2008 | Deer    | 192         | 0      |
| 2505   | 6    | 2008 | Deer    | 0           | 0      |
| 2506   | 6    | 2008 | Deer    | 48          | 0      |
| 2507   | 6    | 2008 | Deer    | 48          | 0      |
| 2508   | 6    | 2008 | Deer    | 96          | 0      |
| 2509   | 6    | 2008 | Deer    | 48          | 0      |
| 2510   | 6    | 2008 | Deer    | 192         | 0      |
| 2511   | 6    | 2008 | Deer    | 0           | 0      |
| 2512   | 6    | 2008 | Deer    | 24          | 0      |
| 2513   | 6    | 2008 | Deer    | 768         | 0      |
| 2514   | 6    | 2008 | Deer    | 96          | 0      |
| 2515   | 6    | 2008 | Deer    | 24          | 0      |
| 2516   | 6    | 2008 | Cattle  | 0           | 0      |
| 2517   | 6    | 2008 | Cattle  | 24          | 0      |
| 2518   | 6    | 2008 | Cattle  | 0           | 0      |
| 2519   | 6    | 2008 | Cattle  | 0           | 0      |
| 2520   | 6    | 2008 | Cattle  | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2521   | 6    | 2008 | Cattle  | 0           | 0      |
| 2522   | 6    | 2008 | Cattle  | 0           | 0      |
| 2523   | 6    | 2008 | Cattle  | 24          | 0      |
| 2524   | 6    | 2008 | Cattle  | 0           | 0      |
| 2525   | 6    | 2008 | Cattle  | 0           | 0      |
| 2526   | 6    | 2008 | Cattle  | 0           | 0      |
| 2527   | 6    | 2008 | Cattle  | 192         | 0      |
| 2528   | 6    | 2008 | Cattle  | 0           | 0      |
| 2529   | 6    | 2008 | Cattle  | 0           | 0      |
| 2530   | 6    | 2008 | Cattle  | 0           | 0      |
| 2531   | 6    | 2008 | Cattle  | 24          | 0      |
| 2532   | 6    | 2008 | Cattle  | 0           | 0      |
| 2533   | 6    | 2008 | Cattle  | 0           | 0      |
| 2534   | 6    | 2008 | Cattle  | 24          | 0      |
| 2535   | 6    | 2008 | Cattle  | 0           | 0      |
| 2536   | 7    | 2008 | Deer    | 384         | 0      |
| 2537   | 7    | 2008 | Deer    | 24          | 0      |
| 2538   | 7    | 2008 | Deer    | 24          | 0      |
| 2539   | 7    | 2008 | Deer    | 24          | 0      |
| 2540   | 7    | 2008 | Deer    | 24          | 0      |
| 2541   | 7    | 2008 | Deer    | 24          | 0      |
| 2542   | 7    | 2008 | Deer    | 192         | 0      |
| 2543   | 7    | 2008 | Deer    | 24          | 0      |
| 2544   | 7    | 2008 | Deer    | 48          | 0      |
| 2545   | 7    | 2008 | Deer    | 24          | 0      |
| 2546   | 7    | 2008 | Deer    | 48          | 0      |
| 2547   | 7    | 2008 | Deer    | 48          | 48     |
| 2548   | 7    | 2008 | Deer    | 24          | 0      |
| 2549   | 7    | 2008 | Deer    | 24          | 0      |
| 2550   | 7    | 2008 | Deer    | 48          | 0      |
| 2551   | 7    | 2008 | Sheep   | 0           | 0      |
| 2552   | 7    | 2008 | Sheep   | 0           | 0      |
| 2553   | 7    | 2008 | Sheep   | 0           | 0      |
| 2554   | 7    | 2008 | Sheep   | 0           | 0      |
| 2555   | 7    | 2008 | Sheep   | 0           | 0      |
| 2556   | 7    | 2008 | Sheep   | 0           | 0      |
| 2557   | 7    | 2008 | Sheep   | 0           | 0      |
| 2558   | 7    | 2008 | Sheep   | 0           | 0      |
| 2559   | 7    | 2008 | Sheep   | 0           | 0      |
| 2560   | 7    | 2008 | Sheep   | 0           | 0      |
| 2561   | 7    | 2008 | Sheep   | 0           | 24     |
| 2562   | 7    | 2008 | Sheep   | 0           | 0      |
| 2563   | 7    | 2008 | Sheep   | 0           | 0      |
| 2564   | 7    | 2008 | Sheep   | 0           | 0      |
| 2565   | 7    | 2008 | Sheep   | 0           | 0      |
| 2566   | 7    | 2008 | Sheep   | 0           | 0      |
| 2567   | 7    | 2008 | Sheep   | 0           | 0      |
| 2568   | 7    | 2008 | Sheep   | 0           | 0      |
| 2569   | 7    | 2008 | Sheep   | 0           | 0      |
| 2570   | 9    | 2008 | Deer    | 0           | 0      |
| 2571   | 9    | 2008 | Deer    | 48          | 96     |
| 2572   | 9    | 2008 | Deer    | 0           | 48     |
| 2573   | 9    | 2008 | Deer    | 192         | 48     |
| 2574   | 9    | 2008 | Deer    | 0           | 96     |
| 2575   | 9    | 2008 | Deer    | 0           | 192    |
| 2576   | 9    | 2008 | Deer    | 0           | 0      |
| 2577   | 9    | 2008 | Deer    | 48          | 0      |
| 2578   | 9    | 2008 | Deer    | 48          | 96     |
| 2579   | 9    | 2008 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2580   | 9    | 2008 | Deer    | 0           | 96     |
| 2581   | 9    | 2008 | Deer    | 0           | 0      |
| 2582   | 9    | 2008 | Deer    | 0           | 192    |
| 2583   | 9    | 2008 | Deer    | 0           | 0      |
| 2584   | 9    | 2008 | Deer    | 0           | 0      |
| 2585   | 9    | 2008 | Deer    | 24          | 384    |
| 2586   | 9    | 2008 | Deer    | 0           | 48     |
| 2587   | 9    | 2008 | Deer    | 24          | 96     |
| 2588   | 9    | 2008 | Cattle  | 0           | 24     |
| 2589   | 9    | 2008 | Cattle  | 0           | 192    |
| 2590   | 9    | 2008 | Cattle  | 0           | 48     |
| 2591   | 9    | 2008 | Cattle  | 24          | 192    |
| 2592   | 9    | 2008 | Cattle  | 0           | 0      |
| 2593   | 9    | 2008 | Cattle  | 0           | 96     |
| 2594   | 9    | 2008 | Cattle  | 0           | 0      |
| 2595   | 9    | 2008 | Cattle  | 0           | 0      |
| 2596   | 9    | 2008 | Cattle  | 0           | 0      |
| 2597   | 9    | 2008 | Cattle  | 48          | 0      |
| 2598   | 9    | 2008 | Cattle  | 96          | 0      |
| 2599   | 9    | 2008 | Cattle  | 96          | 24     |
| 2600   | 9    | 2008 | Cattle  | 96          | 0      |
| 2601   | 9    | 2008 | Cattle  | 0           | 0      |
| 2602   | 9    | 2008 | Cattle  | 0           | 0      |
| 2603   | 9    | 2008 | Cattle  | 0           | 192    |
| 2604   | 9    | 2008 | Cattle  | 48          | 0      |
| 2605   | 9    | 2008 | Cattle  | 0           | 384    |
| 2606   | 9    | 2008 | Cattle  | 0           | 1536   |
| 2607   | 9    | 2008 | Cattle  | 0           | 0      |
| 2608   | 9    | 2008 | Sheep   | 24          | 0      |
| 2609   | 9    | 2008 | Sheep   | 24          | 24     |
| 2610   | 9    | 2008 | Sheep   | 48          | 48     |
| 2611   | 9    | 2008 | Sheep   | 96          | 24     |
| 2612   | 9    | 2008 | Sheep   | 48          | 0      |
| 2613   | 9    | 2008 | Sheep   | 96          | 384    |
| 2614   | 9    | 2008 | Sheep   | 24          | 0      |
| 2615   | 9    | 2008 | Sheep   | 96          | 24     |
| 2616   | 9    | 2008 | Sheep   | 24          | 0      |
| 2617   | 9    | 2008 | Sheep   | 96          | 384    |
| 2618   | 9    | 2008 | Sheep   | 0           | 24     |
| 2619   | 9    | 2008 | Sheep   | 24          | 192    |
| 2620   | 9    | 2008 | Sheep   | 192         | 0      |
| 2621   | 9    | 2008 | Sheep   | 96          | 0      |
| 2622   | 9    | 2008 | Sheep   | 48          | 0      |
| 2623   | 9    | 2008 | Sheep   | 24          | 0      |
| 2624   | 9    | 2008 | Sheep   | 96          | 384    |
| 2625   | 9    | 2008 | Sheep   | 48          | 96     |
| 2626   | 9    | 2008 | Sheep   | 24          | 0      |
| 2627   | 9    | 2008 | Sheep   | 96          | 0      |
| 2628   | 10   | 2008 | Deer    | 0           | 0      |
| 2629   | 10   | 2008 | Deer    | 0           | 96     |
| 2630   | 10   | 2008 | Deer    | 0           | 0      |
| 2631   | 10   | 2008 | Deer    | 0           | 48     |
| 2632   | 10   | 2008 | Deer    | 96          | 0      |
| 2633   | 10   | 2008 | Deer    | 48          | 48     |
| 2634   | 10   | 2008 | Deer    | 0           | 0      |
| 2635   | 10   | 2008 | Deer    | 48          | 96     |
| 2636   | 10   | 2008 | Deer    | 96          | 0      |
| 2637   | 10   | 2008 | Deer    | 96          | 0      |
| 2638   | 10   | 2008 | Deer    | 24          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2639   | 10   | 2008 | Deer    | 96          | 0      |
| 2640   | 10   | 2008 | Deer    | 48          | 0      |
| 2641   | 10   | 2008 | Deer    | 24          | 0      |
| 2642   | 10   | 2008 | Deer    | 96          | 24     |
| 2643   | 10   | 2008 | Deer    | 48          | 0      |
| 2644   | 10   | 2008 | Deer    | 0           | 0      |
| 2645   | 10   | 2008 | Deer    | 24          | 24     |
| 2646   | 10   | 2008 | Deer    | 96          | 0      |
| 2647   | 10   | 2008 | Deer    | 24          | 0      |
| 2648   | 10   | 2008 | Cattle  | 0           | 0      |
| 2649   | 10   | 2008 | Cattle  | 192         | 0      |
| 2650   | 10   | 2008 | Cattle  | 0           | 0      |
| 2651   | 10   | 2008 | Cattle  | 192         | 0      |
| 2652   | 10   | 2008 | Cattle  | 96          | 0      |
| 2653   | 10   | 2008 | Cattle  | 0           | 0      |
| 2654   | 10   | 2008 | Cattle  | 0           | 0      |
| 2655   | 10   | 2008 | Cattle  | 192         | 0      |
| 2656   | 10   | 2008 | Cattle  | 0           | 0      |
| 2657   | 10   | 2008 | Cattle  | 0           | 0      |
| 2658   | 10   | 2008 | Cattle  | 0           | 96     |
| 2659   | 10   | 2008 | Cattle  | 192         | 0      |
| 2660   | 10   | 2008 | Cattle  | 96          | 0      |
| 2661   | 10   | 2008 | Cattle  | 192         | 0      |
| 2662   | 10   | 2008 | Cattle  | 768         | 0      |
| 2663   | 10   | 2008 | Cattle  | 48          | 24     |
| 2664   | 10   | 2008 | Cattle  | 192         | 0      |
| 2665   | 10   | 2008 | Cattle  | 24          | 96     |
| 2666   | 10   | 2008 | Cattle  | 192         | 0      |
| 2667   | 10   | 2008 | Cattle  | 0           | 0      |
| 2668   | 10   | 2008 | Sheep   | 192         | 0      |
| 2669   | 10   | 2008 | Sheep   | 192         | 0      |
| 2670   | 10   | 2008 | Sheep   | 768         | 0      |
| 2671   | 10   | 2008 | Sheep   | 96          | 0      |
| 2672   | 10   | 2008 | Sheep   | 384         | 0      |
| 2673   | 10   | 2008 | Sheep   | 96          | 0      |
| 2674   | 10   | 2008 | Sheep   | 0           | 0      |
| 2675   | 10   | 2008 | Sheep   | 0           | 0      |
| 2676   | 10   | 2008 | Sheep   | 192         | 24     |
| 2677   | 10   | 2008 | Sheep   | 192         | 0      |
| 2678   | 10   | 2008 | Sheep   | 0           | 0      |
| 2679   | 10   | 2008 | Sheep   | 0           | 0      |
| 2680   | 10   | 2008 | Sheep   | 0           | 0      |
| 2681   | 10   | 2008 | Sheep   | 0           | 0      |
| 2682   | 10   | 2008 | Sheep   | 192         | 0      |
| 2683   | 10   | 2008 | Sheep   | 192         | 0      |
| 2684   | 10   | 2008 | Sheep   | 192         | 0      |
| 2685   | 10   | 2008 | Sheep   | 192         | 0      |
| 2686   | 10   | 2008 | Sheep   | 0           | 0      |
| 2687   | 10   | 2008 | Sheep   | 0           | 0      |
| 2688   | 11   | 2008 | Deer    | 48          | 0      |
| 2689   | 11   | 2008 | Deer    | 96          | 0      |
| 2690   | 11   | 2008 | Deer    | 0           | 0      |
| 2691   | 11   | 2008 | Deer    | 24          | 0      |
| 2692   | 11   | 2008 | Deer    | 0           | 0      |
| 2693   | 11   | 2008 | Deer    | 0           | 0      |
| 2694   | 11   | 2008 | Deer    | 48          | 0      |
| 2695   | 11   | 2008 | Deer    | 0           | 0      |
| 2696   | 11   | 2008 | Deer    | 48          | 0      |
| 2697   | 11   | 2008 | Deer    | 0           | 0      |



| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2698   | 11   | 2008 | Deer    | 0           | 0      |
| 2699   | 11   | 2008 | Deer    | 0           | 0      |
| 2700   | 11   | 2008 | Deer    | 48          | 0      |
| 2701   | 11   | 2008 | Deer    | 48          | 0      |
| 2702   | 11   | 2008 | Deer    | 48          | 0      |
| 2703   | 11   | 2008 | Deer    | 0           | 0      |
| 2704   | 11   | 2008 | Deer    | 48          | 0      |
| 2705   | 11   | 2008 | Deer    | 48          | 0      |
| 2706   | 11   | 2008 | Deer    | 0           | 0      |
| 2707   | 11   | 2008 | Deer    | 24          | 0      |
| 2708   | 11   | 2008 | Cattle  | 0           | 0      |
| 2709   | 11   | 2008 | Cattle  | 0           | 0      |
| 2710   | 11   | 2008 | Cattle  | 0           | 0      |
| 2711   | 11   | 2008 | Cattle  | 48          | 0      |
| 2712   | 11   | 2008 | Cattle  | 0           | 0      |
| 2713   | 11   | 2008 | Cattle  | 0           | 0      |
| 2714   | 11   | 2008 | Cattle  | 0           | 0      |
| 2715   | 11   | 2008 | Cattle  | 0           | 0      |
| 2716   | 11   | 2008 | Cattle  | 0           | 0      |
| 2717   | 11   | 2008 | Cattle  | 0           | 0      |
| 2718   | 11   | 2008 | Cattle  | 0           | 0      |
| 2719   | 11   | 2008 | Cattle  | 0           | 0      |
| 2720   | 11   | 2008 | Cattle  | 0           | 24     |
| 2721   | 11   | 2008 | Cattle  | 24          | 192    |
| 2722   | 11   | 2008 | Cattle  | 0           | 0      |
| 2723   | 11   | 2008 | Cattle  | 0           | 0      |
| 2724   | 11   | 2008 | Cattle  | 0           | 24     |
| 2725   | 11   | 2008 | Cattle  | 0           | 0      |
| 2726   | 11   | 2008 | Cattle  | 0           | 0      |
| 2727   | 11   | 2008 | Cattle  | 0           | 384    |
| 2728   | 11   | 2008 | Sheep   | 0           | 0      |
| 2729   | 11   | 2008 | Sheep   | 0           | 24     |
| 2730   | 11   | 2008 | Sheep   | 48          | 24     |
| 2731   | 11   | 2008 | Sheep   | 96          | 0      |
| 2732   | 11   | 2008 | Sheep   | 0           | 0      |
| 2733   | 11   | 2008 | Sheep   | 0           | 0      |
| 2734   | 11   | 2008 | Sheep   | 0           | 0      |
| 2735   | 11   | 2008 | Sheep   | 0           | 0      |
| 2736   | 11   | 2008 | Sheep   | 0           | 0      |
| 2737   | 11   | 2008 | Sheep   | 0           | 24     |
| 2738   | 11   | 2008 | Sheep   | 48          | 0      |
| 2739   | 11   | 2008 | Sheep   | 0           | 24     |
| 2740   | 11   | 2008 | Sheep   | 96          | 0      |
| 2741   | 11   | 2008 | Sheep   | 192         | 0      |
| 2742   | 11   | 2008 | Sheep   | 0           | 0      |
| 2743   | 11   | 2008 | Sheep   | 96          | 0      |
| 2744   | 11   | 2008 | Sheep   | 96          | 0      |
| 2745   | 11   | 2008 | Sheep   | 0           | 24     |
| 2746   | 11   | 2008 | Sheep   | 0           | 0      |
| 2747   | 11   | 2008 | Sheep   | 0           | 0      |
| 2748   | 12   | 2008 | Deer    | 48          | 0      |
| 2749   | 12   | 2008 | Deer    | 96          | 0      |
| 2750   | 12   | 2008 | Deer    | 96          | 0      |
| 2751   | 12   | 2008 | Deer    | 48          | 0      |
| 2752   | 12   | 2008 | Deer    | 0           | 0      |
| 2753   | 12   | 2008 | Deer    | 48          | 0      |
| 2754   | 12   | 2008 | Deer    | 0           | 0      |
| 2755   | 12   | 2008 | Deer    | 48          | 0      |
| 2756   | 12   | 2008 | Deer    | 96          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 2757   | 12   | 2008 | Deer    | 48          | 0      |
| 2758   | 12   | 2008 | Deer    | 24          | 0      |
| 2759   | 12   | 2008 | Deer    | 48          | 0      |
| 2760   | 12   | 2008 | Deer    | 48          | 0      |
| 2761   | 12   | 2008 | Deer    | 24          | 0      |
| 2762   | 12   | 2008 | Deer    | 96          | 0      |
| 2763   | 12   | 2008 | Cattle  | 0           | 0      |
| 2764   | 12   | 2008 | Cattle  | 384         | 0      |
| 2765   | 12   | 2008 | Cattle  | 192         | 0      |
| 2766   | 12   | 2008 | Cattle  | 192         | 0      |
| 2767   | 12   | 2008 | Cattle  | 768         | 0      |
| 2768   | 12   | 2008 | Cattle  | 192         | 0      |
| 2769   | 12   | 2008 | Cattle  | 0           | 0      |
| 2770   | 12   | 2008 | Cattle  | 384         | 0      |
| 2771   | 12   | 2008 | Cattle  | 0           | 0      |
| 2772   | 12   | 2008 | Cattle  | 0           | 0      |
| 2773   | 12   | 2008 | Cattle  | 0           | 0      |
| 2774   | 12   | 2008 | Cattle  | 1536        | 0      |
| 2775   | 12   | 2008 | Cattle  | 384         | 0      |
| 2776   | 12   | 2008 | Cattle  | 192         | 0      |
| 2777   | 12   | 2008 | Cattle  | 192         | 0      |
| 2778   | 12   | 2008 | Cattle  | 192         | 0      |
| 2779   | 12   | 2008 | Cattle  | 0           | 0      |
| 2780   | 12   | 2008 | Cattle  | 0           | 0      |
| 2781   | 12   | 2008 | Cattle  | 48          | 0      |
| 2782   | 12   | 2008 | Cattle  | 384         | 0      |
| 2783   | 12   | 2008 | Sheep   | 384         | 0      |
| 2784   | 12   | 2008 | Sheep   | 768         | 0      |
| 2785   | 12   | 2008 | Sheep   | 192         | 0      |
| 2786   | 12   | 2008 | Sheep   | 192         | 0      |
| 2787   | 12   | 2008 | Sheep   | 0           | 0      |
| 2788   | 12   | 2008 | Sheep   | 384         | 0      |
| 2789   | 12   | 2008 | Sheep   | 384         | 0      |
| 2790   | 12   | 2008 | Sheep   | 192         | 0      |
| 2791   | 12   | 2008 | Sheep   | 192         | 0      |
| 2792   | 12   | 2008 | Sheep   | 384         | 0      |
| 2793   | 12   | 2008 | Sheep   | 384         | 0      |
| 2794   | 12   | 2008 | Sheep   | 192         | 0      |
| 2795   | 12   | 2008 | Sheep   | 96          | 0      |
| 2796   | 12   | 2008 | Sheep   | 192         | 0      |
| 2797   | 12   | 2008 | Sheep   | 192         | 0      |
| 2798   | 12   | 2008 | Sheep   | 384         | 0      |
| 2799   | 12   | 2008 | Sheep   | 192         | 0      |
| 2800   | 12   | 2008 | Sheep   | 0           | 0      |
| 2801   | 12   | 2008 | Sheep   | 192         | 0      |
| 2802   | 13   | 2008 | Deer    | 0           | 48     |
| 2803   | 13   | 2008 | Deer    | 0           | 0      |
| 2804   | 13   | 2008 | Deer    | 0           | 0      |
| 2805   | 13   | 2008 | Deer    | 0           | 0      |
| 2806   | 13   | 2008 | Deer    | 0           | 0      |
| 2807   | 13   | 2008 | Deer    | 0           | 0      |
| 2808   | 13   | 2008 | Deer    | 0           | 0      |
| 2809   | 13   | 2008 | Deer    | 0           | 0      |
| 2810   | 13   | 2008 | Deer    | 0           | 0      |
| 2811   | 13   | 2008 | Deer    | 0           | 0      |
| 2812   | 13   | 2008 | Deer    | 0           | 0      |
| 2813   | 13   | 2008 | Deer    | 0           | 0      |
| 2814   | 13   | 2008 | Deer    | 0           | 0      |
| 2815   | 13   | 2008 | Deer    | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 3111   | 18   | 2008 | Cattle  | 0           | 48     |
| 3112   | 18   | 2008 | Cattle  | 48          | 0      |
| 3113   | 18   | 2008 | Cattle  | 0           | 0      |
| 3114   | 18   | 2008 | Cattle  | 0           | 0      |
| 3115   | 18   | 2008 | Sheep   | 0           | 0      |
| 3116   | 18   | 2008 | Sheep   | 0           | 0      |
| 3117   | 18   | 2008 | Sheep   | 0           | 0      |
| 3118   | 18   | 2008 | Sheep   | 0           | 0      |
| 3119   | 18   | 2008 | Sheep   | 0           | 0      |
| 3120   | 18   | 2008 | Sheep   | 0           | 0      |
| 3121   | 18   | 2008 | Sheep   | 0           | 0      |
| 3122   | 18   | 2008 | Sheep   | 0           | 0      |
| 3123   | 18   | 2008 | Sheep   | 0           | 0      |
| 3124   | 18   | 2008 | Sheep   | 0           | 0      |
| 3125   | 18   | 2008 | Sheep   | 0           | 0      |
| 3126   | 18   | 2008 | Sheep   | 0           | 0      |
| 3127   | 18   | 2008 | Sheep   | 0           | 0      |
| 3128   | 18   | 2008 | Sheep   | 0           | 0      |
| 3129   | 18   | 2008 | Sheep   | 0           | 0      |
| 3130   | 18   | 2008 | Sheep   | 0           | 0      |
| 3131   | 18   | 2008 | Sheep   | 0           | 0      |
| 3132   | 18   | 2008 | Sheep   | 0           | 0      |
| 3133   | 18   | 2008 | Sheep   | 0           | 0      |
| 3134   | 18   | 2008 | Sheep   | 0           | 0      |
| 3135   | 19   | 2008 | Deer    | 0           | 0      |
| 3136   | 19   | 2008 | Deer    | 0           | 0      |
| 3137   | 19   | 2008 | Deer    | 0           | 0      |
| 3138   | 19   | 2008 | Deer    | 0           | 0      |
| 3139   | 19   | 2008 | Deer    | 0           | 0      |
| 3140   | 19   | 2008 | Deer    | 0           | 0      |
| 3141   | 19   | 2008 | Deer    | 0           | 0      |
| 3142   | 19   | 2008 | Deer    | 0           | 0      |
| 3143   | 19   | 2008 | Deer    | 0           | 0      |
| 3144   | 19   | 2008 | Deer    | 0           | 0      |
| 3145   | 19   | 2008 | Deer    | 0           | 0      |
| 3146   | 19   | 2008 | Deer    | 0           | 0      |
| 3147   | 19   | 2008 | Deer    | 0           | 0      |
| 3148   | 19   | 2008 | Deer    | 0           | 0      |
| 3149   | 19   | 2008 | Deer    | 0           | 0      |
| 3150   | 19   | 2008 | Deer    | 0           | 0      |
| 3151   | 19   | 2008 | Deer    | 0           | 0      |
| 3152   | 19   | 2008 | Deer    | 0           | 0      |
| 3153   | 19   | 2008 | Deer    | 0           | 0      |
| 3154   | 19   | 2008 | Deer    | 0           | 0      |
| 3155   | 19   | 2008 | Deer    | 48          | 0      |
| 3156   | 19   | 2008 | Deer    | 24          | 0      |
| 3157   | 19   | 2008 | Deer    | 48          | 0      |
| 3158   | 19   | 2008 | Deer    | 48          | 0      |
| 3159   | 19   | 2008 | Deer    | 24          | 0      |
| 3160   | 19   | 2008 | Deer    | 24          | 0      |
| 3161   | 19   | 2008 | Deer    | 0           | 0      |
| 3162   | 19   | 2008 | Deer    | 0           | 0      |
| 3163   | 19   | 2008 | Deer    | 24          | 0      |
| 3164   | 19   | 2008 | Deer    | 0           | 0      |
| 3165   | 19   | 2008 | Deer    | 48          | 0      |
| 3166   | 19   | 2008 | Deer    | 24          | 0      |
| 3167   | 19   | 2008 | Deer    | 24          | 0      |
| 3168   | 19   | 2008 | Deer    | 0           | 0      |
| 3169   | 19   | 2008 | Deer    | 24          | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 3170   | 19   | 2008 | Deer    | 0           | 0      |
| 3171   | 19   | 2008 | Deer    | 0           | 0      |
| 3172   | 19   | 2008 | Deer    | 0           | 0      |
| 3173   | 19   | 2008 | Deer    | 48          | 0      |
| 3174   | 19   | 2008 | Cattle  | 96          | 0      |
| 3175   | 19   | 2008 | Cattle  | 96          | 0      |
| 3176   | 19   | 2008 | Cattle  | 96          | 0      |
| 3177   | 19   | 2008 | Cattle  | 24          | 0      |
| 3178   | 19   | 2008 | Cattle  | 96          | 0      |
| 3179   | 19   | 2008 | Cattle  | 48          | 0      |
| 3180   | 19   | 2008 | Cattle  | 48          | 0      |
| 3181   | 19   | 2008 | Cattle  | 192         | 0      |
| 3182   | 19   | 2008 | Cattle  | 0           | 0      |
| 3183   | 19   | 2008 | Cattle  | 48          | 0      |
| 3184   | 19   | 2008 | Cattle  | 96          | 0      |
| 3185   | 19   | 2008 | Cattle  | 768         | 0      |
| 3186   | 19   | 2008 | Cattle  | 96          | 0      |
| 3187   | 19   | 2008 | Cattle  | 0           | 0      |
| 3188   | 19   | 2008 | Cattle  | 192         | 0      |
| 3189   | 19   | 2008 | Cattle  | 96          | 0      |
| 3190   | 19   | 2008 | Cattle  | 0           | 0      |
| 3191   | 19   | 2008 | Cattle  | 384         | 0      |
| 3192   | 19   | 2008 | Cattle  | 24          | 0      |
| 3193   | 19   | 2008 | Cattle  | 96          | 192    |
| 3194   | 19   | 2008 | Cattle  | 96          | 48     |
| 3195   | 19   | 2008 | Cattle  | 96          | 0      |
| 3196   | 19   | 2008 | Cattle  | 384         | 24     |
| 3197   | 19   | 2008 | Cattle  | 96          | 96     |
| 3198   | 19   | 2008 | Cattle  | 96          | 192    |
| 3199   | 19   | 2008 | Cattle  | 192         | 192    |
| 3200   | 19   | 2008 | Cattle  | 96          | 0      |
| 3201   | 19   | 2008 | Cattle  | 192         | 384    |
| 3202   | 19   | 2008 | Cattle  | 192         | 48     |
| 3203   | 19   | 2008 | Cattle  | 192         | 0      |
| 3204   | 19   | 2008 | Cattle  | 192         | 0      |
| 3205   | 19   | 2008 | Cattle  | 96          | 48     |
| 3206   | 19   | 2008 | Cattle  | 48          | 48     |
| 3207   | 19   | 2008 | Cattle  | 96          | 0      |
| 3208   | 19   | 2008 | Cattle  | 192         | 0      |
| 3209   | 19   | 2008 | Cattle  | 192         | 0      |
| 3210   | 19   | 2008 | Cattle  | 96          | 384    |
| 3211   | 19   | 2008 | Cattle  | 192         | 0      |
| 3212   | 19   | 2008 | Cattle  | 48          | 24     |
| 3213   | 19   | 2008 | Sheep   | 96          | 0      |
| 3214   | 19   | 2008 | Sheep   | 192         | 0      |
| 3215   | 19   | 2008 | Sheep   | 192         | 0      |
| 3216   | 19   | 2008 | Sheep   | 0           | 96     |
| 3217   | 19   | 2008 | Sheep   | 0           | 192    |
| 3218   | 19   | 2008 | Sheep   | 0           | 0      |
| 3219   | 19   | 2008 | Sheep   | 0           | 0      |
| 3220   | 19   | 2008 | Sheep   | 0           | 192    |
| 3221   | 19   | 2008 | Sheep   | 192         | 0      |
| 3222   | 19   | 2008 | Sheep   | 24          | 48     |
| 3223   | 19   | 2008 | Sheep   | 96          | 0      |
| 3224   | 19   | 2008 | Sheep   | 96          | 0      |
| 3225   | 19   | 2008 | Sheep   | 0           | 0      |
| 3226   | 19   | 2008 | Sheep   | 96          | 0      |
| 3227   | 19   | 2008 | Sheep   | 48          | 0      |
| 3228   | 19   | 2008 | Sheep   | 0           | 0      |

| Animal | Farm | Year | Species | Hardjobovis | Pomona |
|--------|------|------|---------|-------------|--------|
| 3229   | 19   | 2008 | Sheep   | 0           | 48     |
| 3230   | 19   | 2008 | Sheep   | 0           | 0      |
| 3231   | 19   | 2008 | Sheep   | 0           | 0      |
| 3232   | 19   | 2008 | Sheep   | 96          | 0      |
| 3233   | 20   | 2008 | Deer    | 0           | 0      |
| 3234   | 20   | 2008 | Deer    | 48          | 24     |
| 3235   | 20   | 2008 | Deer    | 0           | 384    |
| 3236   | 20   | 2008 | Deer    | 96          | 96     |
| 3237   | 20   | 2008 | Deer    | 0           | 192    |
| 3238   | 20   | 2008 | Deer    | 48          | 24     |
| 3239   | 20   | 2008 | Deer    | 96          | 0      |
| 3240   | 20   | 2008 | Deer    | 384         | 24     |
| 3241   | 20   | 2008 | Deer    | 48          | 96     |
| 3242   | 20   | 2008 | Deer    | 0           | 0      |
| 3243   | 20   | 2008 | Deer    | 0           | 24     |
| 3244   | 20   | 2008 | Deer    | 0           | 0      |
| 3245   | 20   | 2008 | Deer    | 192         | 96     |
| 3246   | 20   | 2008 | Deer    | 24          | 384    |
| 3247   | 20   | 2008 | Deer    | 48          | 48     |
| 3248   | 20   | 2008 | Deer    | 48          | 96     |
| 3249   | 20   | 2008 | Deer    | 24          | 0      |
| 3250   | 20   | 2008 | Deer    | 24          | 0      |
| 3251   | 20   | 2008 | Deer    | 24          | 24     |
| 3252   | 20   | 2008 | Deer    | 48          | 96     |
| 3253   | 20   | 2008 | Cattle  | 0           | 0      |
| 3254   | 20   | 2008 | Cattle  | 0           | 0      |
| 3255   | 20   | 2008 | Cattle  | 0           | 0      |
| 3256   | 20   | 2008 | Cattle  | 0           | 0      |
| 3257   | 20   | 2008 | Cattle  | 0           | 0      |
| 3258   | 20   | 2008 | Cattle  | 0           | 0      |
| 3259   | 20   | 2008 | Cattle  | 0           | 0      |
| 3260   | 20   | 2008 | Cattle  | 0           | 0      |
| 3261   | 20   | 2008 | Cattle  | 0           | 0      |
| 3262   | 20   | 2008 | Cattle  | 0           | 0      |
| 3263   | 20   | 2008 | Sheep   | 192         | 0      |
| 3264   | 20   | 2008 | Sheep   | 0           | 0      |
| 3265   | 20   | 2008 | Sheep   | 0           | 384    |
| 3266   | 20   | 2008 | Sheep   | 0           | 0      |
| 3267   | 20   | 2008 | Sheep   | 0           | 0      |
| 3268   | 20   | 2008 | Sheep   | 0           | 0      |
| 3269   | 20   | 2008 | Sheep   | 0           | 0      |
| 3270   | 20   | 2008 | Sheep   | 0           | 0      |
| 3271   | 20   | 2008 | Sheep   | 0           | 0      |
| 3272   | 20   | 2008 | Sheep   | 96          | 0      |
| 3273   | 20   | 2008 | Sheep   | 0           | 0      |
| 3274   | 20   | 2008 | Sheep   | 0           | 0      |
| 3275   | 20   | 2008 | Sheep   | 0           | 0      |
| 3276   | 20   | 2008 | Sheep   | 0           | 0      |
| 3277   | 20   | 2008 | Sheep   | 0           | 0      |
| 3278   | 20   | 2008 | Sheep   | 0           | 0      |
| 3279   | 20   | 2008 | Sheep   | 0           | 0      |
| 3280   | 20   | 2008 | Sheep   | 0           | 0      |
| 3281   | 20   | 2008 | Sheep   | 24          | 48     |
| 3282   | 20   | 2008 | Sheep   | 0           | 0      |

### Appendix 6a. Raw data of serology against 23 reference panel serovars.

[illegible]

254

[illegible]







[illegible]

[illegible]



| Serum number | Farm number | Region        | Pomona | Hardjo | Tarassovi | Grippotyphosa | Celledoni | Copenhageni | Australis | Zanoni | Robinsoni | Canicola | Kremastos | Szwajzak | Medanensis | Bulgarica | Cynopteri | Arborea | Ballum | Bataviae | Djasiman | Javanica | Panama | Shermani | Topaz |
|--------------|-------------|---------------|--------|--------|-----------|---------------|-----------|-------------|-----------|--------|-----------|----------|-----------|----------|------------|-----------|-----------|---------|--------|----------|----------|----------|--------|----------|-------|
| 239          | 48          | Bay of Plenty | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 240          | 48          | Bay of Plenty | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 241          | 49          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 242          | 49          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 243          | 49          | Southland     | <50    | 100    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | 50       | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 244          | 49          | Southland     | <50    | 50     | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 245          | 49          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 246          | 50          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 247          | 50          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 248          | 50          | Southland     | <50    | 50     | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | 100      | 50         | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 249          | 50          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | 50      | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 250          | 50          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 251          | 51          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | 50      | <50    | <50      | <50      | <50      | 50     | <50      | <50   |
| 252          | 51          | Southland     | <50    | 100    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | 50        | 200      | 100        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 253          | 51          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | 100     | <50    | <50      | <50      | <50      | 50     | <50      | <50   |
| 254          | 51          | Southland     | <50    | 100    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | 50       | <50        | <50       | <50       | 50      | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 255          | 51          | Southland     | <50    | 200    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | 50       | 50         | <50       | <50       | <50     | <50    | <50      | 100      | <50      | <50    | <50      | <50   |
| 256          | 52          | Manawatu      | 400    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | 100   |
| 257          | 52          | Manawatu      | 100    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | 100     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 258          | 52          | Manawatu      | 400    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 259          | 52          | Manawatu      | 1600   | <50    | <50       | 50            | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | 50      | <50    | <50      | <50      | <50      | <50    | <50      | 200   |
| 260          | 52          | Manawatu      | 100    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | 100   |
| 261          | 53          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 262          | 53          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 263          | 53          | Southland     | <50    | 100    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 264          | 53          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 265          | 53          | Southland     | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 266          | 54          | Bay of Plenty | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 267          | 54          | Bay of Plenty | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |
| 268          | 54          | Bay of Plenty | <50    | <50    | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50      | <50        | <50       | <50       | <50     | <50    | <50      | <50      | <50      | <50    | <50      | <50   |





| Serum number | Farm number | Region     | Pomona     | Hardjo     | Tarassovi | Grippotyphosa | Celledoni | Copenhageni | Australis | Zanoni | Robinsoni | Canicola | Kremastos | Szwajizak | Medanensis | Bulgarica | Cynopteri  | Arborea   | Ballum | Bataviae | Djasiman   | Javanica | Panama | Shermani | Topaz |
|--------------|-------------|------------|------------|------------|-----------|---------------|-----------|-------------|-----------|--------|-----------|----------|-----------|-----------|------------|-----------|------------|-----------|--------|----------|------------|----------|--------|----------|-------|
| 329          | 66          | Hawkes Bay | <b>200</b> | <b>50</b>  | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <b>100</b> | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 330          | 66          | Hawkes Bay | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 331          | 67          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 332          | 67          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 333          | 67          | Manawatu   | <b>100</b> | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 334          | 67          | Manawatu   | <b>200</b> | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <b>100</b> | <50      | <50    | <50      | <50   |
| 335          | 67          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 336          | 68          | Manawatu   | <50        | <b>100</b> | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 337          | 68          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 338          | 68          | Manawatu   | <50        | <b>50</b>  | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <b>50</b> | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 339          | 68          | Manawatu   | <50        | <b>100</b> | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 340          | 68          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 341          | 69          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 342          | 69          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 343          | 69          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 344          | 69          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 345          | 69          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 346          | 70          | Manawatu   | <50        | <50        | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 347          | 70          | Manawatu   | <50        | <b>100</b> | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 348          | 70          | Manawatu   | <50        | <b>100</b> | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 349          | 70          | Manawatu   | <50        | <b>50</b>  | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <50       | <50        | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |
| 350          | 70          | Manawatu   | <50        | <b>400</b> | <50       | <50           | <50       | <50         | <50       | <50    | <50       | <50      | <50       | <b>50</b> | <b>50</b>  | <50       | <50        | <50       | <50    | <50      | <50        | <50      | <50    | <50      | <50   |

**Appendix 6b.** Raw data of follow-up serology (additional serum bank samples) for Arborea and Ballum and farm region.

| Sample no. | Farm no. | Region        | Arborea   | Ballum |
|------------|----------|---------------|-----------|--------|
| 1          | 14       | Canterbury    | <50       | <50    |
| 2          | 14       | Canterbury    | <50       | <50    |
| 3          | 14       | Canterbury    | <50       | <50    |
| 4          | 14       | Canterbury    | <50       | <50    |
| 5          | 14       | Canterbury    | <50       | <50    |
| 6          | 14       | Canterbury    | <50       | <50    |
| 7          | 14       | Canterbury    | <50       | <50    |
| 8          | 14       | Canterbury    | <50       | <50    |
| 9          | 14       | Canterbury    | <50       | <50    |
| 10         | 14       | Canterbury    | <50       | <50    |
| 11         | 14       | Canterbury    | <50       | <50    |
| 12         | 14       | Canterbury    | <50       | <50    |
| 13         | 14       | Canterbury    | <50       | <50    |
| 14         | 14       | Canterbury    | <50       | <50    |
| 15         | 14       | Canterbury    | <50       | <50    |
| 16         | 16       | Bay of Plenty | <50       | <50    |
| 17         | 16       | Bay of Plenty | <50       | <50    |
| 18         | 16       | Bay of Plenty | <50       | <50    |
| 19         | 16       | Bay of Plenty | <50       | <50    |
| 20         | 16       | Bay of Plenty | <50       | <50    |
| 21         | 16       | Bay of Plenty | <50       | <50    |
| 22         | 16       | Bay of Plenty | <50       | <50    |
| 23         | 16       | Bay of Plenty | <50       | <50    |
| 24         | 16       | Bay of Plenty | <50       | <50    |
| 25         | 31       | Southland     | <50       | <50    |
| 26         | 31       | Southland     | <50       | <50    |
| 27         | 31       | Southland     | <50       | <50    |
| 28         | 31       | Southland     | <50       | <50    |
| 29         | 31       | Southland     | <50       | <50    |
| 30         | 31       | Southland     | <50       | <50    |
| 31         | 31       | Southland     | <50       | <50    |
| 32         | 31       | Southland     | <50       | <50    |
| 33         | 31       | Southland     | <50       | <50    |
| 34         | 31       | Southland     | <50       | <50    |
| 35         | 31       | Southland     | <50       | <50    |
| 36         | 31       | Southland     | <50       | <50    |
| 37         | 31       | Southland     | <50       | <50    |
| 38         | 31       | Southland     | <50       | <50    |
| 39         | 31       | Southland     | <50       | <50    |
| 40         | 50       | Southland     | <50       | <50    |
| 41         | 50       | Southland     | <50       | <50    |
| 42         | 50       | Southland     | <50       | <50    |
| 43         | 50       | Southland     | <50       | <50    |
| 44         | 50       | Southland     | <50       | <50    |
| 45         | 50       | Southland     | <b>50</b> | <50    |
| 46         | 50       | Southland     | <50       | <50    |
| 47         | 50       | Southland     | <50       | <50    |
| 48         | 51       | Southland     | <b>50</b> | <50    |
| 49         | 51       | Southland     | <50       | <50    |
| 50         | 51       | Southland     | <50       | <50    |
| 51         | 51       | Southland     | <50       | <50    |
| 52         | 51       | Southland     | <50       | <50    |
| 53         | 51       | Southland     | <b>50</b> | <50    |
| 54         | 51       | Southland     | <50       | <50    |



| Sample no. | Farm no. | Region     | Arborea   | Ballum    |
|------------|----------|------------|-----------|-----------|
| 55         | 51       | Southland  | <50       | <50       |
| 56         | 51       | Southland  | <50       | <50       |
| 57         | 51       | Southland  | <50       | <50       |
| 58         | 51       | Southland  | <50       | <50       |
| 59         | 51       | Southland  | <50       | <50       |
| 60         | 51       | Southland  | <b>50</b> | <50       |
| 61         | 51       | Southland  | <50       | <50       |
| 62         | 51       | Southland  | <50       | <50       |
| 63         | 52       | Manawatu   | <50       | <50       |
| 64         | 52       | Manawatu   | <50       | <50       |
| 65         | 52       | Manawatu   | <50       | <50       |
| 66         | 52       | Manawatu   | <50       | <50       |
| 67         | 52       | Manawatu   | <50       | <50       |
| 68         | 52       | Manawatu   | <50       | <50       |
| 69         | 52       | Manawatu   | <50       | <50       |
| 70         | 52       | Manawatu   | <50       | <50       |
| 71         | 52       | Manawatu   | <50       | <50       |
| 72         | 52       | Manawatu   | <b>50</b> | <b>50</b> |
| 73         | 52       | Manawatu   | <50       | <50       |
| 74         | 52       | Manawatu   | <50       | <50       |
| 75         | 52       | Manawatu   | <50       | <50       |
| 76         | 52       | Manawatu   | <50       | <50       |
| 77         | 52       | Manawatu   | <50       | <50       |
| 78         | 52       | Manawatu   | <50       | <50       |
| 79         | 52       | Manawatu   | <50       | <50       |
| 80         | 52       | Manawatu   | <50       | <50       |
| 81         | 56       | Southland  | <50       | <50       |
| 82         | 56       | Southland  | <50       | <50       |
| 83         | 56       | Southland  | <50       | <50       |
| 84         | 56       | Southland  | <50       | <50       |
| 85         | 56       | Southland  | <50       | <50       |
| 86         | 56       | Southland  | <50       | <50       |
| 87         | 56       | Southland  | <50       | <50       |
| 88         | 56       | Southland  | <50       | <50       |
| 89         | 56       | Southland  | <50       | <50       |
| 90         | 56       | Southland  | <50       | <50       |
| 91         | 56       | Southland  | <50       | <50       |
| 92         | 57       | Canterbury | <50       | <50       |
| 93         | 57       | Canterbury | <50       | <50       |
| 94         | 57       | Canterbury | <50       | <50       |
| 95         | 57       | Canterbury | <50       | <50       |
| 96         | 57       | Canterbury | <50       | <50       |
| 97         | 57       | Canterbury | <50       | <50       |
| 98         | 57       | Canterbury | <50       | <50       |
| 99         | 57       | Canterbury | <50       | <50       |
| 100        | 57       | Canterbury | <50       | <50       |
| 101        | 57       | Canterbury | <50       | <50       |
| 102        | 57       | Canterbury | <50       | <50       |
| 103        | 57       | Canterbury | <50       | <50       |
| 104        | 57       | Canterbury | <50       | <50       |
| 105        | 57       | Canterbury | <50       | <50       |
| 106        | 57       | Canterbury | <50       | <50       |
| 107        | 57       | Canterbury | <50       | <50       |
| 108        | 57       | Canterbury | <50       | <50       |
| 109        | 57       | Canterbury | <50       | <50       |
| 110        | 57       | Canterbury | <50       | <50       |
| 111        | 57       | Canterbury | <50       | <50       |

| Sample no. | Farm no. | Region    | Arborea | Ballum |
|------------|----------|-----------|---------|--------|
| 112        | 60       | Southland | <50     | <50    |
| 113        | 60       | Southland | <50     | <50    |
| 114        | 60       | Southland | <50     | <50    |
| 115        | 60       | Southland | <50     | <50    |
| 116        | 60       | Southland | <50     | <50    |
| 117        | 60       | Southland | <50     | <50    |
| 118        | 60       | Southland | <50     | <50    |
| 119        | 60       | Southland | <50     | <50    |
| 120        | 60       | Southland | <50     | <50    |
| 121        | 60       | Southland | <50     | <50    |
| 122        | 60       | Southland | <50     | <50    |
| 123        | 60       | Southland | <50     | <50    |
| 124        | 60       | Southland | <50     | <50    |
| 125        | 60       | Southland | <50     | <50    |
| 126        | 60       | Southland | <50     | <50    |

**Appendix 6c.** Raw data of serology for additional blood samples against Arborea and Ballum and Farm region.

| Sample no. | Farm no. | Region    | Arborea   | Ballum    |
|------------|----------|-----------|-----------|-----------|
| 1          | 50       | Southland | <50       | <50       |
| 2          | 50       | Southland | <50       | <50       |
| 3          | 50       | Southland | <50       | <50       |
| 4          | 50       | Southland | <50       | <50       |
| 5          | 50       | Southland | <50       | <50       |
| 6          | 50       | Southland | <50       | <50       |
| 7          | 50       | Southland | <50       | <50       |
| 8          | 50       | Southland | <50       | <50       |
| 9          | 51       | Southland | <50       | <50       |
| 10         | 51       | Southland | <50       | <50       |
| 11         | 51       | Southland | <50       | <50       |
| 12         | 51       | Southland | <50       | <50       |
| 13         | 51       | Southland | <50       | <50       |
| 14         | 51       | Southland | <50       | <50       |
| 15         | 51       | Southland | <50       | <50       |
| 16         | 51       | Southland | <50       | <50       |
| 17         | 51       | Southland | <50       | <50       |
| 18         | 51       | Southland | <50       | <50       |
| 19         | 51       | Southland | <50       | <50       |
| 20         | 51       | Southland | <50       | <50       |
| 21         | 51       | Southland | <50       | <50       |
| 22         | 51       | Southland | <50       | <50       |
| 23         | 51       | Southland | <50       | <50       |
| 24         | 51       | Southland | <50       | <50       |
| 25         | 51       | Southland | <50       | <50       |
| 26         | 51       | Southland | <50       | <50       |
| 27         | 51       | Southland | <50       | <50       |
| 28         | 51       | Southland | <50       | <50       |
| 29         | 51       | Southland | <50       | <50       |
| 30         | 51       | Southland | <50       | <50       |
| 31         | 51       | Southland | <50       | <50       |
| 32         | 51       | Southland | <50       | <50       |
| 33         | 51       | Southland | <50       | <50       |
| 34         | 51       | Southland | <50       | <50       |
| 35         | 51       | Southland | <50       | <50       |
| 36         | 51       | Southland | <50       | <50       |
| 37         | 51       | Southland | <50       | <50       |
| 38         | 51       | Southland | <50       | <50       |
| 39         | 51       | Southland | <50       | <50       |
| 40         | 51       | Southland | <50       | <50       |
| 41         | 51       | Southland | <b>50</b> | <b>50</b> |
| 42         | 51       | Southland | <50       | <50       |
| 43         | 51       | Southland | <50       | <50       |

# **Appendix 7.** Raw data of culture and PCR for kidney, uterus and foetus (if available) and serology.

(PCR and culture: 0 = negative, 1 = positive, n/a = not available)

| Sample no. | Line no. | Kidney culture | Kidney PCR | Uterus culture | Uterus PCR | Hardjobovis | Pomona | Foetus culture | Foetus PCR |
|------------|----------|----------------|------------|----------------|------------|-------------|--------|----------------|------------|
| 1          | 1        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 2          | 1        | 1              | 0          | 0              | 0          | 96          | 192    | n/a            | n/a        |
| 3          | 1        | 0              | 0          | 0              | 0          | 96          | 0      | n/a            | n/a        |
| 4          | 1        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 5          | 1        | 1              | 0          | 0              | 0          | 48          | 192    | n/a            | n/a        |
| 6          | 1        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 7          | 1        | 0              | 0          | 0              | 0          | 0           | 96     | n/a            | n/a        |
| 8          | 1        | 0              | 0          | 0              | 0          | 0           | 96     | n/a            | n/a        |
| 9          | 1        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 10         | 1        | 0              | 0          | 0              | 0          | 192         | 0      | n/a            | n/a        |
| 11         | 1        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 12         | 1        | 1              | 1          | 0              | 0          | 96          | 0      | n/a            | n/a        |
| 13         | 1        | 1              | 1          | 0              | 0          | 96          | 0      | n/a            | n/a        |
| 14         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 15         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 16         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 17         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 18         | 2        | 0              | 0          | 0              | 0          | 0           | 48     | n/a            | n/a        |
| 19         | 2        | 0              | 0          | 0              | 0          | 0           | 768    | n/a            | n/a        |
| 20         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 21         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 22         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 23         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 24         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 25         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 26         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 27         | 2        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 28         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 29         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 30         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 31         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 32         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 33         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 34         | 3        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 35         | 3        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 36         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 37         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 38         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 39         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 40         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 41         | 3        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 42         | 4        | 0              | 0          | 0              | 0          | 96          | 0      | n/a            | n/a        |
| 43         | 4        | 0              | 0          | 0              | 0          | 48          | 96     | n/a            | n/a        |
| 44         | 4        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 45         | 4        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 46         | 4        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 47         | 4        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 48         | 4        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 49         | 4        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 50         | 4        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 51         | 4        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 52         | 4        | 0              | 0          | 0              | 0          | 48          | 48     | n/a            | n/a        |
| 53         | 4        | 1              | 1          | 0              | 0          | 24          | 0      | n/a            | n/a        |
| 54         | 4        | 0              | 0          | 0              | 0          | 0           | 24     | n/a            | n/a        |

| Sample no. | Line no. | Kidney culture | Kidney PCR | Uterus culture | Uterus PCR | Hardjobovis | Pomona | Foetus culture | Foetus PCR |
|------------|----------|----------------|------------|----------------|------------|-------------|--------|----------------|------------|
| 55         | 4        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 56         | 4        | 1              | 1          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 57         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 58         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 59         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 60         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 61         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 62         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 63         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 64         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 65         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 66         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 67         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 68         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 69         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 70         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 71         | 5        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 72         | 6        | 0              | 0          | 0              | 0          | n/a         | n/a    | n/a            | n/a        |
| 73         | 6        | 0              | 0          | 0              | 0          | n/a         | n/a    | 0              | 0          |
| 74         | 6        | 0              | 0          | 0              | 0          | n/a         | n/a    | 0              | 0          |
| 75         | 6        | 0              | 0          | 0              | 0          | n/a         | n/a    | n/a            | n/a        |
| 76         | 6        | 0              | 0          | 0              | 0          | 48          | 48     | 0              | 0          |
| 77         | 6        | 0              | 0          | 0              | 0          | 192         | 0      | 0              | 0          |
| 78         | 6        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 79         | 6        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 80         | 6        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 81         | 6        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 82         | 6        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 83         | 6        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 84         | 7        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 85         | 7        | 0              | 0          | 0              | 0          | 192         | 0      | 0              | 1          |
| 86         | 7        | 0              | 0          | 0              | 0          | 48          | 0      | 0              | 0          |
| 87         | 7        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 88         | 7        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 89         | 7        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 90         | 7        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 91         | 7        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 92         | 7        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 93         | 7        | 0              | 0          | 0              | 0          | 0           | 384    | 0              | 0          |
| 94         | 7        | 0              | 0          | 0              | 0          | 96          | 0      | 0              | 0          |
| 95         | 7        | 1              | 1          | 0              | 0          | 192         | 384    | 0              | 0          |
| 96         | 7        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 97         | 7        | 0              | 0          | 0              | 0          | 24          | 48     | 0              | 0          |
| 98         | 8        | 0              | 0          | 0              | 0          | 24          | 0      | n/a            | n/a        |
| 99         | 8        | 0              | 0          | 0              | 0          | 24          | 0      | n/a            | n/a        |
| 100        | 8        | 0              | 0          | 0              | 0          | 24          | 0      | n/a            | n/a        |
| 101        | 8        | 0              | 0          | 0              | 0          | 192         | 0      | n/a            | n/a        |
| 102        | 8        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 103        | 8        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 104        | 8        | 0              | 0          | 0              | 0          | 24          | 0      | n/a            | n/a        |
| 105        | 8        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 106        | 8        | 0              | 0          | 0              | 0          | 48          | 48     | n/a            | n/a        |
| 107        | 8        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 108        | 9        | 0              | 0          | 0              | 0          | 48          | 0      | 0              | 0          |
| 109        | 9        | 0              | 0          | 0              | 0          | 48          | 0      | n/a            | n/a        |
| 110        | 9        | 0              | 0          | 0              | 0          | 48          | 0      | 0              | 0          |
| 111        | 9        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |

| Sample no. | Line no. | Kidney culture | Kidney PCR | Uterus culture | Uterus PCR | Hardjobovis | Pomona | Foetus culture | Foetus PCR |
|------------|----------|----------------|------------|----------------|------------|-------------|--------|----------------|------------|
| 112        | 9        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 113        | 9        | 0              | 0          | 0              | 0          | 48          | 0      | 0              | 0          |
| 114        | 9        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |
| 115        | 9        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 116        | 9        | 0              | 0          | 0              | 0          | 24          | 0      | n/a            | n/a        |
| 117        | 9        | 0              | 0          | 0              | 0          | 24          | 0      | 0              | 0          |
| 118        | 9        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 119        | 9        | 0              | 0          | 0              | 0          | 0           | 0      | 0              | 0          |
| 120        | 9        | 0              | 0          | 0              | 0          | 0           | 0      | n/a            | n/a        |