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

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Leptospirosis has been reported in farmed deer in New Zealand since 1980 but knowledge was limited. Studies presented in this thesis investigated the epidemiology and distribution of infection associated with serovars Hardjobovis, Pomona, and Copenhageni in farmed deer, efficacy of a vaccine and the influence of maternally derived antibody, and aspects of exposure of humans to leptospirosis. Serology, bacteriology, and pathology were employed as determinants of infection and vaccine efficacy.

A serological survey of 2016 deer from 110 herds confirmed leptospirosis throughout New Zealand with 61.3% of herds infected with Hardjobovis and 3.6% with Pomona alone, 16.4% with dual infections with those serovars, and none with Copenhageni, giving an overall herd prevalence of 81.3%.

Epidemiological studies involved serological analysis of samples from a serum bank involving weaner, yearling and adult deer (n=10/group) from 15 farms sampled 3-monthly for 21 months, and intensive blood and urine sampling of young deer on three farms over 1-2 production cycles (n=15-65/group). Infection with Hardjobovis followed an age-related endemic cycle with some animals infected by 3-4 months of age and seroconversion peaking at up to 57% at12-15 months and mild kidney lesions typical of a host-adapted organism. Infection with Pomona followed an epidemic pattern with seroprevalence of up to 100%, more severe kidney lesions, clinical signs and evidence of reduced growth and reproduction, typical of an accidental host relationship. Leptospira were observed in 30.4% of urine samples and 37.0% of kidneys.

Vaccination with "Leptavoid 3" (Schering-Plough Animal Health Ltd) was studied on one non-infected, one Hardjobovis infected, and two dual Hardjobovis/Pomona infected herds. Vaccination produced sustained titres in uninfected young and adult deer, and no maternally derived antibody interference was observed in progeny vaccinated at approximately 3-4 months of age. In infected herds, vaccination enhanced seroprevalence and antibody titres, and reduced urine shedding by 44% and culture from urine and kidneys by 37% in the face of continued natural challenge.

Analysis of previous data combined with pooled data from the above studies, indicated that 73.0% of lines and 29.0% of individual deer at slaughterhouses had kidney lesions indicative of leptospirosis with a relative risk (RR) of 1.08 and 1.6 for the relationship between seropositivity to Hardjobovis and Pomona, respectively. The overall RR between positive serology, lesion and culture was 2.1. The kidney culture rate ranged from 2.5-33% between herds demonstrating significant risk of exposure to humans, particularly slaughterhouse workers.

This study has provided an understanding of the epidemiology in farmed deer and control options available to the industry

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Preface

Leptospirosis is a contagious and infectious spirochaete bacterial disease caused by various serovars of the genus *Leptospira* (OIE 2000). Leptospiral infections are an emerging health problem in human and veterinary medicine, having an important economic impact on production animal industries, and being one of the most frequent zoonoses in the human population (Plank and Dean 2000; Levet 2001).

There is limited information about leptospirosis in deer (Prescott 1993). Evidence from surveys of free-ranging and farmed deer from 1957 to 2005 indicates that *Leptospira* have been prevalent in many deer populations internationally (Ayanegui-Alcérreca et al 2003). Nineteen serovars have been reported in deer, but only serovars Hardjobovis, Pomona, Copenhageni, and Roumanica have been isolated (Ayanegui-Alcérreca et al 2004).

Leptospirosis in farmed deer in New Zealand has been reported intermittently for about 25 years (Ayanegui-Alcérreca et al 2003). Nevertheless, not much was known of its prevalence, epidemiology, subclinical effects, prevention, and human health significance (Wilson et al 1998). Bacterial cultures and serological results from New Zealand suggest that serovars Hardjobovis and Pomona are the most frequent pathogens in red deer (*Cervus elaphus*) and Copenhageni has been linked to occasional outbreaks (Flint et al 1986; Griffin 1987; Ayanegui-Alcérreca et al 2003).

The research presented in this thesis was in response to the Deer Industry's desire for robust data on the national distribution and on-farm epidemiology of leptospiral infections, as well as the effectiveness of vaccination in reducing risk to animals and humans. Data will provide guidance for the deer industry in determining the need for an industry-wide control strategy, and if so, the most appropriate course(s) of action.

Thesis format

- Chapter 1 contains an extensive literature review of the biology of leptospirosis and its role in farm animal disease in general, but with a particular reference to deer. This Chapter contains review papers entitled: "Leptospirosis in New Zealand farmed deer: A review" and "A review of international reports of leptospirosis in deer" which have been submitted to scientific journals for publication.
- Chapter 2 describes methodology common to the research presented in Chapters 3 to 8.
- Chapters 3 to 8 describe individual research projects written largely in the format for papers to be submitted to scientific journals. The methodology specific to each chapter is described in them.
- Chapter 9 is a general discussion, which brings the research into context, to allow a broad presentation of the relevant results of the different research projects.
- Appendices 1 to 4 contain the raw data for Chapters 3 to 8.

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List of Publications

- Ayanegui-Alcérreca, M.A., Wilson, P.R., Heuer, C., Collins-Emerson, J., 2003, Leptospirosis in farmed deer. Proceedings of the Deer Branch of the New Zealand Veterinary Association 20, 135-76.

- Ayanegui-Alcérreca MA, Wilson PR, Mackintosh CG, et al. Deer and leptospirosis: A review of the world situation. Proceedings of the 1st World Deer Veterinary Congress and the Deer Branch of the New Zealand Veterinary Association 2004;21:34-38.

- Ayanegui-Alcérreca MA, Wilson PR, Mackintosh CG, et al. Epidemiology and control of leptospirosis in New Zealand farmed deer: a summary of Massey University research. Proceedings of the Deer Branch of the New Zealand Veterinary Association 2005a;22:128-131.

- Ayanegui-Alcérreca MA, Wilson PR, Mackintosh CG, Collins-Emerson, J. M., Heuer, C. and Midwinter, A. Deer leptospirosis vaccination: a preliminary report. 2005b. Proceedings of the Deer Branch of the New Zealand Veterinary Association 2005a;22: 132-12.

- Dean G, Ayanegui-Alcerreca A, Wilson PR, et al. An outbreak of leptospirosis on a Southland deer farm. Proceedings of the Deer Branch of the New Zealand Veterinary Association 2005;22:88-123.

- Wilson PR, Ayanegui-Alcerreca MA, Castillo-Alcala F, et al. Leptospirosis in farmed deer in New Zealand: implications and industry options Proceedings of the Deer Branch of the New Zealand Veterinary Association 2005;22:124-127.

Presentations

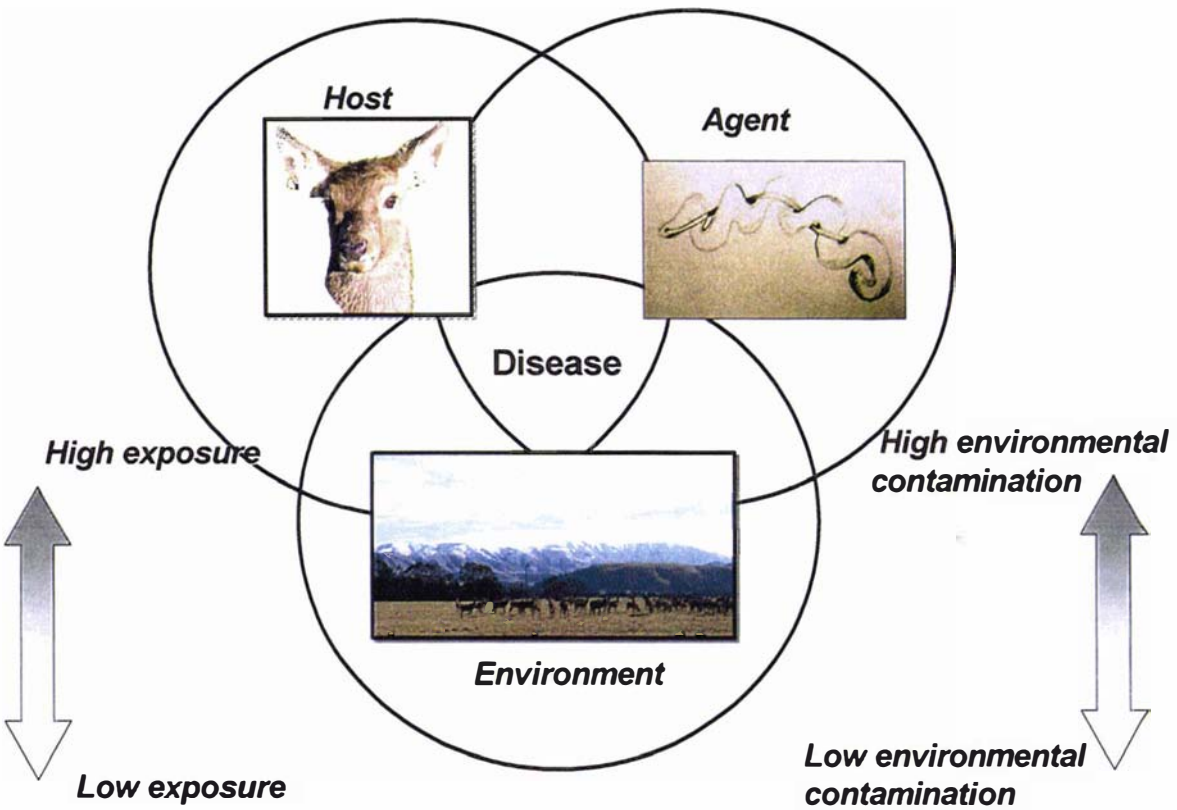
- **Ayanegui-Alcérreca MA***, Wilson PR, Dean G, Midwinter, A., Collins-Emerson, J., An Outbreak of Leptospirosis in New Zealand farmed deer caused by *Leptospira Icterohaemorrhagie* sv. Pomona. Microbes outside the square, New Zealand Microbiological Society, 17-19 November 2004, Palmerston.

- Heuer C. Dorjee S, Ridler, A.L. , Ayanegui-Alcérreca, M.A, Collins, J.M., Midwinter, A., West, D.M., Jackson, R., Wilson, P., MA, Collins, J.M., Midwinter, A., West, D.M., Jackson, R., **Wilson, P***., eds Human occupational leptospirosis atributable to livestock exposure In:. Leptospirosis:Disarming the threat. 4th Scientific Meeting of the international Leptospirosis society: International Leptospiral Society (ILS), 2005;156-123. 14-16 Novembre 2005, Chaing Mai, Thailand.

*= Speaker

Chapter 1

Literature review an overview of *Leptospira* in general, and leptospirosis in farmed deer with particular reference to New Zealand.



1.1 Introduction

A number of reports of leptospirosis in different deer species can be found since the late 1950s. However, little is known of this bacterial infection in deer species or the deer's role in the epizootiology of the disease (Ayanegui-Alcérreca et al 2004). Primary knowledge of leptospirosis comes from cattle and swine (Ellis 1994). Several features of the disease and its epidemiological implications in farm or wild animals are inadequately understood because of difficulties in diagnosis, the complexities of the host-leptospire relationship and the varying patterns of infection (Bolin and Prescott 1999).

An anonymous report in 1980 was the first describing leptospiral disease in farmed red and wapiti deer (*Cervus elaphus*) in New Zealand (Anon. 1980). Later, a number of diagnostic laboratory reports were summarised along with the occurrence of clinical disease and subclinical manifestations in farmed deer (Wilson and McGhie 1993; Wilson et al 1998). Severe clinical leptospirosis was considered uncommon in deer in New Zealand (Griffin, 1987), but that claim was on the basis of little evidence, so its incidence may be higher than believed. Leptospirosis in deer in New Zealand has been associated with serovars Hardjobovis, Pomona, and Copenhageni (Wilson et al 1998). Of more than the 200 serovars of these bacteria known, only seven are found in New Zealand, being Hardjobovis, Pomona, Copenhageni, Tarassovi, Ballum, Balcanica, and Australis. The first two are of primary importance due to their prevalence in the livestock population (cattle, sheep, deer, swine) and in humans (Dowd 2003).

The New Zealand environment favours survival of the microorganism and infection is recognized as endemic in animals and humans (Hathaway 1978; Thornley et al 2002). A large part of the New Zealand ecosystem is occupied by agricultural-based production, characterized by high-density livestock (ruminant) farming in a semi-intensive to intensive pastoral system. Deer in the wild or in a farming environment are not only as susceptible to the infection as any other mammal, but available information suggests that they are playing a part in maintaining the infectious cycle. (Ayanegui-Alcérreca et al 2003, 2004).

Leptospirosis should be regarded as a set of separate diseases caused by individual serovars in an ecosystem (New Zealand), within particular hosts (in this case farmed deer species), rather than being viewed as a single disease with an epidemiology and host response in common with other domestic or wild species (Bolin and Prescott 1999; Crump 2001). At the centre of the epidemiological cycle of leptospirosis is the carrier (excretor carrier) animal, whose renal tubules are lined with leptospires (Prescott 1992). Chronic persistence of the bacteria in animals is one of the key points of the infection cycle. Each serovar tends to be maintained by a host group that carries and spreads the disease (maintenance host) (Adler and de la Peña-Moctezuma 2004).

This literature review gives a brief summary of the deer industry in New Zealand and then concentrates largely on the biology and epidemiology of leptospirosis relevant to New Zealand farmed deer.

1.2 Background to New Zealand Deer Industry

Man's husbandry of deer goes back a long way before deer farming was conceived. Red deer (*Cervus elaphus*) have provided mankind with meat for most of European history (Wallis, 1993; Alexander and Buxton 1994). While domestication of deer had been practised in China

for centuries, usually in enclosures, and of reindeer in Scandinavia, modern pastoral deer farming began in 1969 in New Zealand and in 1970 in Britain as an extension of traditional forms of utilisation (Alexander and Buxton 1994; Audigé 1995). Deer farming is the production of deer in an enclosed and controlled environment (Audigé 1995). There are 22 major deer-producing countries world wide, with nearly half of the farmed deer population of the world belonging to New Zealand. This country has been a pioneer and leader in this highly significant industry (Wallis, 1993; Alexander and Buxton 1994; Audigé 1995, Fletcher 1998).

The first deer were imported to New Zealand in 1851 and there were some 200 liberations of until the early 1900s. While 11 species were brought to the country only seven became established: Red (*Cervus elaphus*), Fallow (*Dama dama*), Sika (*Cervus nippon*), Rusa (*Cervus timorensis*), Wapiti (*Cervus elaphus*), White-tail (*Odocoileus virginianus*), and Sambar (*Cervus unicolor*). These animals adapted to their new and rich environment and people began trophy and meat hunting. The deer population was well adapted and growing by the 1920s (Wallis 1993).

In the 1950s and early 1960s wild deer were well established, competing with domestic animals for grazing, destroying natural vegetation and were considered a pest (Wallis 1993; Wilson 2001). By the end of the 1960s, deer were hunted and the meat was exported to the European markets as game. The New Zealand Game Industry and Exporters Association was founded and in 1969, deer farming regulations were invoked and deer farming began (Wallis 1993; Wilson 2001). Farming was established first with captured wild deer but progressively from the mid 1970s to the early 1980s, farm-bred deer fuelled industry growth (Wilson 2001). Since then, the deer industry has become well established with constant development. The estimated farmed deer population is around 1.7 million, with more than 4,500 farms involved and an average herd size of 400 (Statistics New Zealand 2002). More than 80% are Red deer, and 10% Wapiti, or Wapiti-Red crossbreeds, with the remainder being Fallow deer (Wilson 2001).

Thus, the New Zealand deer industry has been developing for the last 35 years and has a projected continued growth (Mackintosh, 2002). Deer farming has become a diverse enterprise, where animals can be farmed in large numbers in both individual and mixed species farming systems.

1.3 Leptospiral nomenclature

Leptospires are gram-negative, spiral or helical shaped bacteria (Euzéby 2002) belonging to the order *Spirochaeteles* (Greek, *spiro* = coiled and *Chaete* = hair) (Margulis 2000), and Family *Leptospiraceae*, which includes the genus *Leptospira*, containing both bacteria pathogenic to animals and humans and non-pathogenic or free-living organisms (Johnson and Faine 1984) **(Figure 1.1).** The genus *Leptospira* (Noguchi 1917) was historically based on morphology and cultural characteristic. Members of the genus *Leptospira* (Greek adj. *leptos* = thin) are thin-coiled hair-like organisms (Faine et al 1999).

Leptospires are classified by two systems: s*ensu stricto* and *sensu lato*. The *sensu stricto* classification is based on DNA similarities (Euzéby 2002). *Leptospira* can be dividing into 17 species (genomospecies) based on at least 70% relatedness by DNA/DNA homology and a maximum of 5% unpaired bases (Faine et al 1999; Matsunaga et al 2002). Seven of this genomogroup contain pathogenic *Leptospira* (Faine et al 1999). This categorization coexists

with the original serological classification in which antisera are used to establish antigenic relatedness between isolates (Bharti et al 2003).

Serological based classification (*sensu lato*) groups leptospiral strains into serovars and serogroups (Dikken and Kmety 1978). Typically, leptospires were divided in two species *L. interrogans*, (pathogenic serovars) and *L. biflexa*, primarily saprophytic (Bharti et al 2003). Serovar (Sv.) is the basic antigenic unit reflecting antibody reaction (cross-agglutination-absorption) to the structural component of lipopolysaccharide (LPS) (WHO 2003; Nascimento et al 2004a). This classification is based mainly on serological properties using specific reference serum. It does not reflect phylogenetic relationships (WHO 2003). In the present document, the *sensu lato* classification is used and microorganisms are named based on serovars (Table 1).

Antigenically related serovars are arranged for convenience into serogroups. The *sensu lato L. biflexa* contains more than 60 serovars (Bharti et al 2003). The pathogenic serovars (>200) *sensu lato L. interrogans*, have 24 serogroups (Bharti et al 2003). Serogroups consist of serovars that cross-agglutinate at a moderate to high titre, and it is a practical system for grouping strains (Dikken and Kmety 1978).

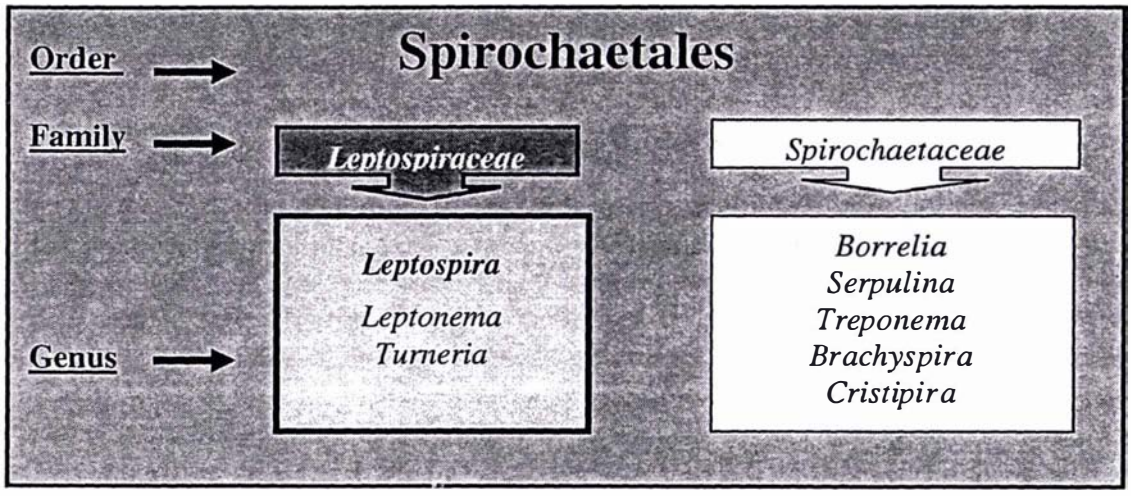


Figure 1.1Taxonomy of Spirochaetales and *Leptospira* (Adapted from Quin et al 2002).

Wolf and Broom (1954) defined the term serovar, which was adopted by the WHO (World Health Organization) in 1967. Although this system does not have a taxonomic purpose, it is a practical way of differentiating between leptospires on the basis of the host-infectious agent relationship (Sasaki et al 1999; WHO 2003). Most of the epidemiological and clinical research done with *Leptospirae* is based on the detection of antibodies against specific laboratory strains by the microscopic agglutination test (MAT). This test remains as a reference test for the disease, and it recognizes differences in the lipopolysaccharide (LPS) structure of the bacteria (OIE 2000, WHO 2003). Table 1 shows the accepted nomenclature for the genus *Leptospira* found in New Zealand.

When the serogroup and serovar are named together, serogroup should be written with upper case, and serovars in lower cases (Pasteur Institute 2002). Throughout the text of this thesis,

they will be referred to as serovars as this is the accepted convention for epidemiological research into this disease.

Note: Serovars Icterohaemorrhagiae and Copenhageni are extremely closely related at both the genotype and serologic profile level and it depends on the country or region of the world as to the way the serovar is reported. *Leptospira borgepetersenii* Hardjobovis and *Leptospira interrogans* Hardjoprajitno, were indistinguishable by the *sensu lato* classification, and for a long time were termed serovar Hardjo. However, they are genotypically distinct and belong to different species. Throughout the thesis, the name of the serovars will be quoted as in the original source of the information.

Table 1. - Genus and species classifications of the *Leptospira* found in New Zealand based on *sensu stricto* and *sensu lato* classifications of the seven strains known to exist in NZ. (Committee on the taxonomy of *Leptospira* 2002).

Sensu stricto		
Genus	**Species**	**Serovar (Sv.)**
Leptospira	***borgepetersenii***	Hardjobovis
Leptospira	***borgepetersenii***	Ballum
Leptospira	***borgepetersenii***	Balcanica
Leptospira	***borgepetersenii***	Tarassovi
Leptospira	***interrogans***	Pomona
Leptospira	***interrogans***	Copenhageni
Leptospira	***interrogans***	Australis*

Sensu lato			
Genus	**Species**	**Serogroup**	**Serovar (sv.)**
Leptospira	***interrogans***	Sejroe	Hardjobovis
Leptospira	***interrogans***	Ballum	Ballum
Leptospira	***interrogans***	Sejroe	Balcanica
Leptospira	***interrogans***	Tarassovi	Tarassovi
Leptospira	***interrogans***	Pomona	Pomona
Leptospira	***interrogans***	Icterohaemorrhagiae	Copenhageni
Leptospira	***interrogans***	Australis	Australis*

* sv. Australis was isolated only from a human, not from animals and its significance is unknown (Midwinter 1999).

1.4 History of Leptospirosis

1.4.1 Global Situation

Almost 120 years have past since the discovery of leptospiral infections (Gsell 1984). Descriptions of human disease consistent with leptospirosis have been recorded since before biblical times (Adler and de la Peña-Moctezuma 2004). The first records of leptospiral disease can be traced back to ancient Chinese and Japanese medicine where there were descriptions of "rice-harvest jaundice", "haunted fields" at harvest time in China, or the "7 day or autumn fever" in Japan (Faine et al 1999; Margulis 2000). These fevers were associated with season, occupation, and regions, closely corresponding to the general description of leptospirosis in humans long before their aetiologies were appreciated (Faine et al 1999).

Adolf Weil (1886) published in Deutsches Archiv für klinische Medizin the first description of "an acute infectious illness with spleen tumour, jaundice and nephritis" (Gsell 1984). Inada (1916) produced the first evidence of infection and transmition of the disease from infected humans to guinea pigs. Inada's work was translated and published by Flexner and Noguchi in 1916 and then recognized as the first experimental association of the spirochaete and the disease (Inada et al 1916; Faine et al 1999; Margulis 2000). By the 1920s and 1930s, a good part of the basic knowledge of leptospirosis was recorded. Noguchi published a description of the leptospire's structure, that was based on light microscopy (Gsell 1984; Faine et al 1999).A full review of the international history of leptospirosis is presented by Faine et al (1999).

1.4.2 Animal Medicine

Earlier, few reports could be found of leptospirosis in animals, but the relationship between animal infections as a source for the human disease was apparent (Adler and de la Peña-Moctezuma 2004). Leptospirosis was first identified as a disease in domestic animals in a case involving dogs (serovars Canicola and Icterohaemorrhagiae) in the 1930s and the potential as a source for infection of humans, was soon recognized (Gsell 1984; Faine et al 1999). In the late 1930s and 1940 in Russia, leptospirosis was accepted as a problem in food animal production (Gsell 1984; Faine et al 1999).
Leptospira have now been reported worldwide in diverse deer populations (farmed, wildlife, and zoo). There is serological evidence for 19 serovars but culture and pathology evidence for only four (Mackintosh 1984, Ayanegui-Alcérreca et al 2004). The international situation regarding leptospirosis in deer species has been reviewed by Ayanegui-Alcérreca et al (2006b) and is attached at the end of this chapter.

1.5 Biology of Leptospirosis

Infections by the pathogenic members of the spirochaete genus *Leptospira* cause leptospirosis. Leptospires are motile bacteria. The cells have a hook at each end making them S or C-shaped (Holt et al 1994). Although cytochemically Gram-negative, they do not stain well with conventional bacteriological dyes. They are usually visualized by dark-field microscopy, silver, or immunofluorescence staining techniques (Johnson and Faine 1984).

The cytoplasmatic cylinder consists of nuclear material, cytoplasm and cytoplasmic membrane (Holt et al 1994). The general layered structure of leptospires resembles that of typical gram-negative bacteria, with inner membrane, peptidoglycan wall, and external membrane with LPS (Figure 1.2) (Adler and de la Peña-Moctezuma 2004). Leptospires have two flagellae, one fixed to the inner membrane at each extreme of the bacterial body (Holt et al 1994; Biberstein 1990) giving leptospires their characteristic motion and shape (Adler and de la Peña-Moctezuma 2004).

The leptospiral genome consists of two (large and small) circular chromosomes with more than 4,700 predicted genes (Ren et al 2003; Bharti et al 2003). Most of the genes required for growth are located on the large chromosome, but some essential genes lie on the small one (Nascimento et al 2004a). The bacterial genome is large compared with other prokaryotes or spirochaetes, which is considered an indication of the ability of the *Leptospira* spp. to live and adapt to diverse environments, either in an animal host or free in the environment (Bharti et al 2003).

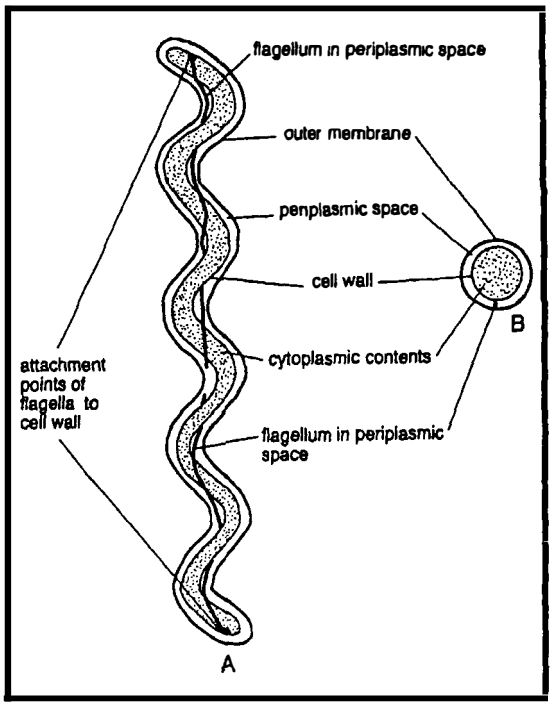


Figure 1.2. Diagrammatic illustration of a typical spirochaete, indicating important structural features (A) and their relationship in cross section (B). The flagellae are attached to the cell wall at each end of the organism. (Quin et al 2002).

These bacteria are classified as fastidious microorganisms and they do not grow in traditional culture media. *Leptospira* culture media contain 10% rabbit serum or 1% bovine serum albumin, have fatty acids as a carbon source, and are at a pH of 6.8-7.4 (Bharti et al 2003) (For media composition see chapter 2). Leptospires are oxidase and catalase positive, many have lipase activity and some produce urease (Bharti et al 2003). Leptospires are obligate aerobes, and bacterial generation time is 12 hrs (Johnson and Faine 1984). Saprophytic leptospires will grow at 11-13° C and the optimum growth occurs at 28-30° C. Pathogenic leptospires will grow at 28-30° C, but not at 13° C. *In vitro* growth occurs at a pH of 7.2-7.6 in aerobic conditions. These microorganisms can be converted to spherical forms by 1M NaCl solution/ 2h (Faine et al 1999). Leptospires are killed by drying, freezing, heat (50°C/10 min), soap, bile salts, detergents, acidic environment and putrefaction. They persist in moist, temperate environments at neutral or slightly alkaline pH (Biberstein, 1990).

1.6 Epidemiology of Leptospirosis

Leptospirosis has a worldwide distribution. Pathogenic leptospires show variations between regions and countries in different animal species in terms of prevalence, epidemiological and management factors, which all influence the manifestation of leptospirosis (Bharti et al 2003). Infections are more common in the tropics where more serovars and possible hosts are present and the environment is generally wet, than in temperate regions, but transmission of the bacteria occurs in both (Bharti et al 2003).

Infection results from exposure to infected urine, either directly or indirectly via contaminated soil or water. The prevalence of the different serovars depends on the reservoir animals present and the serovars that they carry, local environmental conditions, the industrial, agronomic, and agricultural practices, and the host-serovar associations between the animals in the population (Table 3) (Hathaway 1978; Bharti et al 2003). The extent to which leptospires are transmitted in an animal population or herd depends on factors such as climate, population density, and degree of contact between the host (maintenance or accidental), herd, and individual immune status (Boqvist 2002b).

There are differences in the global distribution of some *Leptospira* species (*sensu stricto*). *Leptospira kirschneri, L. interrogans,* and *L. borgpetersenii* have a worldwide distribution with *L. interrogans* and *L. borgpetersenii* being mainly clustered in Asia and Europe, respectively. *L. noguchii* and *L. santarosai* are mainly found in the American continent, and *L. weilii* is found mostly in Asia (Baranton and Old 1995). Regional differences have been reported in deer (Ayanegui-Alcérreca et al 2004). The regional divergence or the number of serovars reported could be also be influenced by the surveillance system in each region or country (see the "Review of international reports of leptospirosis in deer" at the end of this chapter).

Table 2- Criteria for definition of host-adapted and incidental host categories for leptospiral infections (Heath and Johnson 1994; Radostits et al 1999).

Maintenance (reservoir) host	Incidental (accidental) host
• High susceptibility to infection, but low pathogenicity to the host. • Endemic transmission within the species (high prevalence). • Tendency to cause more chronic than acute disease (low titres in culture positive animals, mild pathology). • Long kidney phase (long-term shedders).	• Relatively low susceptibility and high pathogenicity to the host. • Sporadic transmission within the species (low prevalence). • Tendency to cause acute and severe rather than chronic disease (high titres in culture positive animals, acute and dramatic pathology). • Short kidney phase (short-term shedders).

Leptospira hosts can be maintenance or accidental, as defined and described in Tables 2 and 3. Maintenance hosts are animals that carry leptospires in their renal tubules where the bacteria proliferate and are shed in the urine for periods varying from months to more than a year. Shedders are essential as sources of infection for other animals or humans (Hathaway 1985). An infected maintenance host can remain symptom-less and shed the bacteria in the urine for a lifetime (Thiermann 1984, Faine et al 1999).

Exposure of susceptible animals to non-host-adapted serovars results in accidental or incidental disease, sometimes with dramatic outbreaks but generally of low prevalence. Accidental hosts are less important in the transmission of the infection as the shedding period is short (Hathaway 1981b; Prescott 1992; Heath and Johnson1994; Radostits 2001). However, these associations are not absolute, and under certain circumstances, most serovars can infect many host species (Alder and de la Peña-Moctezuma 2004). Some animal species can act as the reservoir for more than one serovar, or more than one serovar can infect a specific animal host (Bharti et al 2003; Adler and de la Peña-Moctezuma 2004).

1.6.1 Environmental Factors

Transmission of leptospires in the environment is affected by geography, climate, hosts (accidental or maintenance) interaction and human activities (Bharti et al 2003). Conditions necessary for the survival of *Leptospira* are a wet environment, with neutral or slightly alkaline water; although, leptospires are known to survive acidity of pH 5 to 6.2 for limited periods (Hellstrom, 1978). They can survive in cold water if it does not freeze (Ellis 1984; Elder 1986; Thiermannn 1984; Baranton and Old 1995). Indirect exposure depends on wet environmental conditions and or a mild climate that favours the survival of leptospires. Streams and ponds can become a source of infection, as can aerosols of urine in cowsheds and milk from infected cows (Biberstein 1990; Quin 1994).

Leptospiral infections of accidental hosts are abundant in tropical and sub-tropical regions because of environmental conditions and occur throughout the year. In dry regions, accidental host infections are associated with water holes with high animal concentrations. In temperate regions, such infections are usually seasonal and the autumn may also coincide with greater number of shedders (Ellis 1984; Thiermannn 1984; Prescott 1992). Contaminated bodies of water are an important source of infection to livestock, other animals, and humans (Biberstein 1990; Faine et al 1999). Pathogenic *Leptospira* species are capable of surviving as free-living bacteria for extended periods after urinary shedding. (Barnett 1999; Haake 2000). *Leptospira* species can survive for days or even weeks in diverse physicochemical conditions, which is a reflection of the adaptability of the genome to different conditions (Ellis 1984).

1.6.2 Animal factors

Infectious agents that can infect more than one host species are ubiquitous; 62% of human pathogens are zoonoses, and 77% of livestock pathogens and 91% of domestic carnivore pathogens, respectively, infect multiple hosts (Haydon et al 2002). More than one host species may constitute infection reservoirs (Haydon et al 2002). The triangular relationship between serovar, host, and the environment will determine the manifestations of disease and host-species relationship with an ecosystem. (Hathaway et al 1981b). Man's changing husbandry and management practices influence the infectious cycle. Higher animal stocking densities, extensive versus intensive, pastoral versus housed, multi- versus single-species production systems, together with environmental conditions that allow the survival of the leptospires, such as high humidity and temperature, all influence the diversity of hosts (Hellstrom 1978; Crump et al 2001).

1.7 New Zealand Livestock

New Zealand has an abnormally high occurrence of leptospirosis compared with other countries. It is endemic in farmed animal species and in humans, it is referred to as "hyper-endemic" (Crump et al 2001; Thornley et al 2002). New Zealand native fauna did not include recognized host species for the *Leptospira* serovars now reported in this country. However, in the early days of colonisation, given that infections could only be introduced in animals that survived the long and hard conditions they had to endure during sea voyages, only those organisms that could persist as chronic infections, i.e. those with mild or subclinical pathology, namely host-adapted serovars could be introduced (Crump et al 2001). Subsequent importation of animals by air has potentially altered this dynamic, although quarantine protocols require treatment to eliminate the risk of introduction of other serovars.

Leptospira probably arrived to New Zealand when the different reservoir or maintenance host species were introduced to the country (Table 3). In the late eighteenth century, Captain Cook introduced the pig (*Suis scrofula*), and goat (*Capra hircus*) (Crump et al 2001). The brown rat (*Rattus norvegicus*), black rat (*Rattus rattus*), and house mouse (*Mus musculus*) were introduced between 150 to 200 years ago. Other species followed; hedgehogs (*Erinaceus europaeus*), other feral species such as rabbits (*Oryctolagus cuniculus*), the brush-tailed possum (*Trichosurus vulpecula*), and various domestic animals including, cattle, sheep, and horses (Hathaway 1978; Crump et al 2001).

Table 3. – Currently accepted host-serovar relationships for *Leptospira* serovars present in New Zealand. The serovars isolated from farmed deer in New Zealand are in bold (Brockie 1977a,b; Marshall et al 1976; Hathaway 1978, 1981a,b; Flint et al 1986; Flint et al 1988a; Mackintosh 1993; Day 1997; Wilson et al 1998; Midwinter 1999).

Serovar	Reservoir Host	Accidental host	Farmed Deer Culture/Serology
Hardjobovis	**Cattle**	H	Yes/Yes
Pomona	Swine	C, S, H	Yes/Yes
Copenhageni	Norway rat (*Rattus norvegicus*)	C, D	Yes/Yes
Ballum	Black rat (*Rattus rattus*) Mouse (*Mus musculus*) Hedgehog (*Erinaceus europaeus*)	C	No/Yes
Balcanica	Possum (*Trichosurus vulpecula*)	C	No/Yes
Tarassovi	Swine	D+	No/Yes
Australis**		H	No/Yes

C = cattle, D = dog, H = human, S = sheep, D+ = clinically healthy dogs.
**** Two isolated cases.**

1.7.1 Cattle

Cattle in New Zealand are the recognized reservoir for serovar Hardjobovis. The major impact is related to kidney invasion and its potential as a zoonosis rather than as a cause of reproductive failure or clinical disease (Mackintosh 1981). Hellstrom (1978) showed that 60% of New Zealand cattle and 80% of the herds had titres to Hardjo. It appears that most animals become infected between one to three years of age. Mackintosh (1981) reported a 38%

seroprevalence of Hardjobovis at the herd level in 136 dairy farms in the lower North Island and 10% seroprevalence of Pomona within those farms positive to Hardjo. Mackintosh (1981) also reported an association between Pomona positive herds and breeding sows and dairy beef calves. Sporadic infections in cattle can occur with Pomona because of direct or indirect contact with the swine. Leptospiruria has been detected in cattle for up to 3 months with Pomona and 19 months with Hardjo (Hellstrom 1978; Mackintosh 1981).

In New Zealand, serovar Pomona can cause abortion in adult cows or fatal haemolytic diseases in calves. Hellstrom (1978) reported 18% of the animals had titres to Pomona. It appears that most animals become infected between one to three years of age (Hellstrom 1978; Mackintosh 1981). Cattle are a well-adapted reservoir host for Hardjobovis, characterised by a weak agglutinin response to natural infection and the ability to remain as a urine shedder, transmitting infection, for months or years (Mackintosh 1981; Radostits et al 1999).

1.7.2 Swine

New Zealand studies of leptospirosis in pigs showed that infections with serovar Pomona occurred after 10 weeks of age. Young animals did not have serological titres, but by the time of slaughter, most showed serologic evidence of infection. This suggests that the infection is acquired in the flow to the finisher pen, and by replacement gilts when they are mixed with adult sows (Kemp 1980b; Bolt and Marshall 1995). Bolt (1990) reported from a slaughterhouse survey that the serological prevalence for Pomona serovar was 53% and some of those animals were culture positive. Serovar Tarassovi was not detected by Bolt (1990), but Ryan (1978) reported 7% prevalence and a positive isolate.

1.7.3 Sheep and Goats

Under some circumstances sheep may be maintenance hosts, independent of cattle, for serovar Hardjo, which can cause neonatal lamb losses due to starvation because of acute agalactia or congenital infections. In one survey of sheep in the Manawatu district in New Zealand, 3.8% carried agglutinins for serovar Pomona, although sheep are not considered to be active carriers (Bruére and West, 1990).

Vermunt et al (1994), reported clinical pathological and aetiological evidence of three outbreaks of leptospirosis due to serovar Pomona in New Zealand lambs, with characteristic haemoglobinuria, jaundice, liver and renal damage. Bahaman et al (1980), isolated leptospira Hardjo from sheep. Marshall et al (1979a) and Mackintosh et al (1981) were able to establish a controlled infection in sheep with Hardjobovis, whereas serovar Balcanica attempts were unsuccessful. West et al (2004) reported titres to Hardjobovis and Pomona in flocks, and Pomona was isolated from an aborted foetus. Human cases of leptospirosis caused by Hardjobovis and Pomona were reported in meat workers that were only exposed to sheep (Baker and Lopez 2004). In a serological survey in New Zealand Flint et al (1988b), reported titres to Ballum, Brastislava (possibly cross reaction with Ballum since early infections with Pomona can cause cross-reactivity with serovar Bratislava), Hardjobovis, Pomona, Australis, Copenhageni and Tarassovi in goats with highest titres clustered in the first four. In spite of serological evidence of widespread exposure to several leptospires species, no isolates were recovered from urine taken from goats.

1.7.4 Other farm animals

Serological reactions against *Leptospira interrogans* serovars in New Zealand (182 samples), suggested the occurrence of sporadic infections in horses Hilbink and Penrose (1990). The data in this work indicate the level of exposure to leptospire in New Zealand horse population is unlikely to have a role as a maintenance host for leptospires in this country.

In dogs, the clinical syndrome has been observed in the Auckland region (Hilbink et al 1992). Mackintosh et al (1980a) reported cases of clinical leptospirosis in hunt hounds caused by Tarassovi and Pomona. A regional survey in Auckland showed that more than 5% of dogs tested against serovar Copenhageni were positive (Hilbink et al 1992). In a nation-wide New Zealand serologic survey of dogs, positive titre rates for the following *Leptospira* serovars were: Copenhageni 0.9% (78/8730), Ballum 0.7% (41/5835) and Canicola (1/6375) (Hilbink et al 1992). O'Keefe et al (2002) reported 14.2% seroprevalence in a sample of 446 dogs in the Lower North Island with serovar copenhageni in 9.5% (41/433) of animals.

1.8 Farmed Deer in New Zealand

Leptospirosis is now a well-recognized cause of clinical disease in farmed deer in New Zealand. A full review of leptospirosis in deer in New Zealand has been summarised for publication in the New Zealand Veterinary Journal (Ayanegui-Alcérreca et al 2006a) (Attachment 1, Chapter1)

1.9 A review of international reports of leptospirosis in deer

This is the subject of a reviewintended for submission to an internationally peer reviewed veterinary journal. (Ayanegui-Alcérreca et al 2006b) (Attachment 2, Chapter1)

1.10 Leptospirosis in Humans

Humans have never been proven to be important in the transmission of the disease. Human infection results from exposure to infected animals or contaminated environments (Plank and Dean 2000). Traditionally, in humans leptospirosis was considered to be an occupational disease associated with activities such as mining, sewers, farming, the meat processing industry, veterinary practice and military work (Campagnolo et al 2000). In tropical areas, human infections are linked to crop farming (rice, bananas) or agricultural practices, although the whole population may be exposed to a contaminated environment particularly when there is high seasonal rainfall (Bharti et al 2003). In temperate countries, human cases occur mainly linked to occupational or recreational activities, particularly water related (Faine et al 1999; Crump et al 2001).

Livestock farming is a major occupational risk for leptospirosis throughout the world. The highest risk is associated with dairy farming and with serovar Hardjo, particularly with those performing the milking (Levett 2001). Leptospirosis is New Zealand's most common occupationally acquired infectious disease, and the incidence is high in comparison with other temperate developed countries, and it is considered hyper- endemic (OSH 2001).

By 1980, the reported annual incidence of leptospirosis in the human population in New Zealand was one of the highest of the world, with 90% of the reported cases related to animal occupational activities, mainly in the dairy industry. Serovar Hardjo was two times more frequent than Pomona, and in a sero-survey of dairy workers, titres to serovars Hardjobovis, Pomona, and Copenhageni were present (Mackintosh 1981). The three most frequently reported serovars in humans from 1990 to 1998 were Hardjobovis (46.1%), Pomona (24.4%) and Ballum (11.9%). A declining trend in dairy industry workers and a rise in the case rate in meat industry workers have occurred. Hardjobovis was found in people working with cattle, sheep and farmed deer, Pomona was in people associated with pigs, cattle and sheep, and Ballum with rodents and Cattle (Thorneley et al 2002; Baker and Lopez 2004; Bell 2005) (For more detail see section 1.16 on Zoonoses).

1.11 Bacterial factors and disease mechanisms of leptospirosis

Leptospirosis is a dynamic process that involves factors related to the serovars and their hosts (Faine et al 1999). The virulence of each serovar depends not only on the genotypic characteristic, but much more on the expressed phenotypic properties of the organism by which means pathological changes may be effected in a defined host (accidental or adapted) (Faine et al 1999; Bharti et al 2003). The pathogenesis for each serovar remains inadequately understood, and the basic evidence obtained from different serovars has to be interconnected to attempt to have a broader vision of the disease mechanism (Faine et al 1999; Adler and de la Peña-Moctezuma 2004).

1.11.1 Bacterial Pathogenic Factors

The leptospiral life cycle requires the ability to respond and adapt to a complex array of environmental conditions as free-living organisms or as host parasites (Nascimento et al 2004a). The first and most important bacterial factor is its capacity to survive. In bacterial terms, *Leptospira* have a large and complex genetic pool, which is a reflection of the diversity of the genus. (Ren et al 2003; Nascimento et al 2004b).

Different mechanisms by which *Leptospira* complete its pathogenic cycle causing tissue damage and diseases have been recognized. The functional pathogenic genes and their relationships have been based on the evidence obtained from other bacteria, (Ren et al 2003; Adler and de la Peña-Moctezuma 2004) and are mostly indirect due to the lack of genomic interchange or genomic vehicle systems in the genus (e.g. bacteriophages, replicons or transposons) (Ren et al 2003; Nascimento et al 2004 a,b). A number of putative virulence factors have been suggested (Levett 2001; Adler and de la Peña-Moctezuma 2004). The natural habitat of pathogenic *Leptospira* is in the tubules of the mammalian kidney from which they are excreted in urine, often for several months or even years. They are also found in the genital tract of a carrier animal. The first pathogenic mechanism is the presence in the carrier host and the environment (Biberstein 1990; Quin et al 2002).

1.11.1.1 Motility

Motility is based on rotational movement generated by the two flagella (Figure 2). Chemotaxis and motion of the leptospire are generally acknowledged as two of the most

relevant pathogenic factors. Around 50 well-conserved genes are related to motility and more than 12 to chemotaxis (Nascimento et al 2004 b). The combination of both permits the dissemination of the bacteria in the host tissues (Ren et al 2003; Bharti et al 2003). Pathogenic leptospires have been shown to have a positive chemotaxis toward haemoglobin, which can be associated with the haemolytic syndrome of the acute infection (Yuri et al 1993).

Motility enables rapid movements, especially in highly viscous environments, allowing them to escape host defences through invasion of tissue. Flagella movements permit the bacteria to breach connective tissue, mucosal and cutaneous barriers, and penetrate tissue and basal membranes. Leptospires are extracellular parasites, so they must penetrate the hosts epithelial and endothelial cells when spreading and establishing an infection (Adler and de la Peña-Moctezuma 2004). Transcytosis without disturbance of cytoeskeletal structures have been observed in *in vitro* models (Barocchi et al 2002). This pathogenic quality permits the colonization of locations within the host where the immune system is less efficient, such as the tubular space in the kidneys (Baranton and Old 1995; Moens and Vanderleyden 1996).

1.11.1.2 Attachment

After host tissue colonization, the outer membrane of pathogenic leptospires plays a central role due to direct contact with the host cell structures. The molecules associated with the outer membrane mediate this interaction (Cullen et al 2004). The membrane composes primarily phospholipids, outer membrane proteins (OMPs) and lipopolysaccharides (LPS). Virulent leptospires adhere to epithelial cells *in vitro* (Levett 2001). Pathogenic serovars attach to cultured cells within 3 hours of incubation, whereas non-pathogenic do not (Barocchi et al 2002). Lipopolysaccharides and OMPs are involved in cell attachment and phagocytosis in the presence of homologus antibodies. Virulent leptospires become associated with neutrophils, but are not killed by them (Merien et al 1997). Lipopolysaccharides stimulate adherence of neutrophils to endothelial cells and platelets causing aggregation, suggesting a role in the development of thrombocytopenia (Levett 2001).

The outer membrane proteins in spirochaetes shift with the environmental-host setting (Cullen et al 2004). This difference in expression of OMPs is thought to be linked with adaptation and survival. The system that allows the up and down regulation of these proteins in the mammalian host, contributing to the survival of the bacteria, and completion of pathogenesis (Cullen et al 2004). During infection, a small number of these proteins are expressed and considered to be linked to the interaction between the organism and the cell (Haake et al 1993; Barnett et al 1999). Some of these proteins expressed during infection are *immunoglobulin-like* proteins or Tool-like receptors (*ligA*, li*gB* and *ligC*), which may contribute to the early steps of the infection and colonization of tissues. The first two are expressed in low passage cultures and during mammalian infections (Nascimento et al 2004a,b). Pathogenic leptospires may adhere to renal epithelial cells when studied *in vitro*, whereas a-virulent strains like *L. biflexa* serovar Patoc adheres non-specifically to a glass surface (Yang et al 2000).

1.11.1.3 Endotoxin LPS (Lipopolysaccharide)

Lipopolysaccharide is the major surface component, and contributes to the pathology associated with the disease. Endotoxic activity has been reported in several serovars (Faine et al 1999; Kalambaheti et al 1999; Peña-Moctezuma et al 2001). Despite its similarities with the LPS of other gram-negative bacteria, it exhibits biological activity at much lower toxic potencies (ten-fold less, in an *in vitro* assay) than other gram-negative bacteria (Isogai et al 1986, 1989 and 1990 a, b; de la Peña-Moctezuma et al 1999). Differences in LPS biosynthesis are thought to account for serovar diversity among leptospires, and act as part of the host adaptation and account for some immunoevasion mechanisms (; Nascimento et al 2004b). Leptospiral LPS is mitogenic for B-lymphocytes and activates macrophages (Faine et al 1999). The LPS stimulates adherence of neutrophils to endothelial cells and platelets, allowing the bacteria to spread in different organs and systems, causing aggregation and a suggested role in the development of thrombocytopenia (Isogai et al 1989, 1990b). Modulates of the immune response, particularly of macrophages via the CD14 and Tool-like receptor2 (TLR2) receptor molecules has been reported (Werts et al 2001).

1.11.1.4 Cytotoxins and Haemolysins

Several haemolysins (haemolysin associated protein 1 Hap-1 or LipL32) and sphingomyelinase-like proteins have been reported *in vivo* and as part of the genomic sequence in different serovars. Haemolysins are considered important in leptospiral infections (Seger et al 1990, 1992;Bharti et al 2003). Haemolysis produced by leptospires had been associated with phospholipase activity, in the host cell membranes (Quin et al 2002) although a definitive role for these enzymes in the pathogenesis of infection has not been established. Nevertheless, the presence of these enzymes in the bacteria is considered important in the development of the cellular damage seen in this disease (Quin et al 2002). Both phospholipase A and sphingomyelinase C activities (selective for erythrocytes membranes) observed in some serovars (Autumnalis, Grippotyphosa, Icterohaemorrhagiae, Hardjo and Pomona) are linked to pathogenicity *in vivo* (Segers et al 1990; Segers et al 1992). These lipases are probably responsible for the haemoglobinuria in young mammals and non-host adapted species infected with those serovars (Segers et al 1990; Lee et al 2002).

Serovars Pomona and Copenhageni produce cytotoxin (glycolipoprotein), with a cytotoxic activity fraction building up in the kidney and affecting renal tubule function (Burth et al 1997; Merien et al 1997). The proposed mechanism is that of active palmoleic and oleic acid of leptospiral origin intercalating into the host membrane cells, blocking or using the normally occurring fatty acids of the cell, leading to leakage of the cell contents (Vinh et al 1986; Burth et al 1997; Pereira et al 1997). A similar fraction in serovar Canicola inhibits Na^+, K^+, and ATPase activity. (Burth et al 1997). Renal failure in experimental leptospirosis in the guinea pig showed pathophysiological changes in tubular function, characterized by hypokalemia with a corresponding elevated urinary fractional secretion of potassium (Abdulkader et al 1993).

Table 4. -A Summary of the pathogenesis and origin of the pathophysiology of the disease at the cellular level.

Pathogenesis	Mechanism (Bacterial pathogenic factors)	Biological action at the cellular level (Author/year)
Source	**Direct:** Contaminated by urine or infected tissue, (kidney, blood, liver, eye) and excretions (milk, reproductive mucosa). **Indirect: Urine-contaminated water, soil, mud**	**Transmission: Direct**: most probable for serovars maintained in endemic or zoonotic infections. **Indirect:** more important in accidental infections. (Baranton and Old 1995; Faine et al 1999; Barocchi et al 2002).
Entry	**Motility** (Flagella) and **chemotaxis** (Haemoglobin): Penetrate through mucosal surface or skin abrasions. Susceptible host * From 1 to 10 bacteria can cause an infection.	**Motility and chemotaxis** will allow the bacteria to move towards the tissues of the susceptible host. **Chemotaxis** to haemoglobin provides the ability to migrate through host tissue. (Faine et al 1999; Bharti et al 2003).
Survival	**Motility** (Flagella and chemotaxis) **and Attachment**: Epithelial surface of the host	**Fibronectines-binding proteins (OMPs, *LigA, LigB*)** bind to epithelial cells and attach to the extra-cellular matrix attachment to epithelial and endothelial cells, neutrophils, and gains access to bloodstream. **LPS:** stimulates adherence of neutrophils to endothelial and platelets (Isogai et al 1990a,b;Yang et al 2000; Levett 2001).
Spread Bacteraemic phase	**Motility, Chemotaxis and Attachment** The host becomes bacteraemic for hours or up to 7 days. Pyrexia and myalgia occur	**LPS:** Stimulates adherence to haematopoietic cells allowing the bacteria to spread and causing aggregation and thrombocytopenia. Leptospires reach and replicate in liver, kidneys, spleen, genital tract, and central nervous system. Proliferation occurs in parenchymatous organs (Merien et al 1997; Bolin and Prescott 1999; Barocchi et al 2002).
Cellular damage Acute phase	**Cellular damage** either bacterial mediated or immune response mediated. Small-vessel vasculitis, cell leakage and eventually haemorrhages. Microscopic lesions appear in 2 or more days.	**LPS** stimulates the specific antibody synthesis, actives neutrophils, macrophages, and complement fixation. Parenchymatous organs; membrane damage to the attached tissue by the action of cytotoxins, haemolysins. (Bharti et al 2003; Adler and de la Peña-Moctezuma 2004).
LPS	Stimulates **adherence** of neutrophils to endothelial cells, but without specific antibodies will not activate macrophages.	Release of lymphokines such as tumour necrosis factor (TNF-α) from monocytes through the endotoxic activity of the *Leptospira*. Stimulates specific humoral (IgM and IgG) and cellular immune responses. (Yang et al 2000).
Cytotoxins	**Phospholipase, Sphingomyelinases, Haemolysin-associated protein-1 (Hapl or Lip32):** damage mechanism is a cytotoxic pore-forming protein. Presence or activity may vary within the serovars. **Glycolipoprotein** is a potent inhibitor of the Na^+-K^+-ATPase in renal epithelial cells.	**Phospholipase, Sphingomyelinases, and Hapl**: The proposed mechanism is active palmoleic and oleic acid of leptospiral origin intercalates into the host membrane cells, blocking or occupying the normally occurring fatty acids of the cell, leading to leakage of the cell contents. (Vinh et al 1986; Burth et al 1997; Pereira et al 1997:Haake et al 2000). **Glycolipoprotein** is responsible for the major toxicity, and the inhibitor activity of the glycoprotein has been linked to the physiological changes in the glomerular filtrate in the lower nephron (Na^+-K^+-bomb) and a membrane perforation effect in some cellular models. (Burth et al 1997; Yang et al 2000).
OMPs	Interaction with cellular matrix and inmuno-evasion. Cytotoxic effects of different outer leptospiral membrane structures have been demonstrated.	The expression of TNF-α, a pro-inflammatory cytokine produced by monocytes/macrophages and by injured renal cells. Activation is mediated by lipopolysaccharide, OMPs and lipases (Plank and Dean 2000; Yang et al 2000).
Immune-Related	**Immunoglobulin-like proteins (*LigA, LigB*)**: Act as a fibronectin to cell receptors). **Monocytes chemo-attractant protein-1 (MCP-1)** and the iNOS (Nitric Oxide system), produced by mesangial cells in damaged kidney tissue. **LPS** Mediated damage.	The MCP-1 stimulated, activates the nitric oxide system (potent pro-inflammatory effect) when medullary hypoxic injury occurs from the *in vitro* models. Then it can be speculated that induced chemokine synthesis that may be involved in damage renal tissue, acing as a monocytes chemotactic (Yang et al 2000; Matsuga et al 2003).

1.11.2 Disease Mechanisms at the Cellular Level (Pathophysiology)

The pathogenicity of leptospires relates to the virulence of the infecting serovar and the receptivity of the host species. While disease may be harsh in the immature maintenance host, the most dramatic and acute disease will occur in accidental hosts (Table 2) (Quin et al 2002). The pathogenic mechanism by which the bacteria cause damage can be either direct damage by *Leptospira* or related to the host immune response to the infection (Bharti et al 2003)

1.11.3 Pathogenesis and Clinical Disease

Leptospires are extracellular parasites and the main distinctive lesion is vascular damage. The major capillary damage and therefore petechial haemorrhages in the mucous membranes is common to all pathogenic serovars and occurs during the septicemic phase. The clinical manifestation will therefore be related to the extent of the damage and organs affected (Plank and Dean 2000; Adler and de la Peña-Moctezuma 2004).

Leptospires enter the body through mucous membranes or small cuts and abrasions in the skin. Once the leptospires gain entry, leptospiremia lasts until the appearance of specific antibodies (Table 4, Figure 3) (Faine et al 1999). Agglutinin antibodies are the core of the defence against the infection and are specific to the antigenic profile to the serovar involved (Faine et al 1999). These antibodies attach to the bacterial surface and opsonise them for phagocytosis by reticuloendothelia cells, tissue cells, and immune complex formation (van der Ingh and Hartman 1986). If a sufficient number of leptospires accumulates in circulation, the bacteria will reach the different tissues and cell damage will occur as result of direct toxic effects of the bacteria or the immune response associated against it (Figure 3) (Faine et al 1999).

Leptospires that reach the proximal renal tubules, genital tract, eye and brain and mammary gland may evade circulating antibodies and the antibody-complement system. Leptospires sequestered in the renal tubules or in uterine lumen can persist and multiply in these sites, and may be excreted and transmitted to susceptible, in-contact animals, primarily by urine. (Heath and Johnson 1994; Faine et al 1999).

Leptospires enter the kidney via the haematogenous route, migrate through the vascular endothelium, and persist in the interstitial space, to finally migrate into tubular lumina from where they are shed (Marshall 1976). The infection is focused in the renal parenchyma, causing an interstitial nephritis and persistence of the leptospire in these lesions followed by a prolonged leptospiruria. Renal failure in experimental leptospirosis in the guinea pig showed pathophysiological changes in tubular function (Table 4, Cytotoxins) (Abdulkrader et al 1996).

In the acute presentation, leptospiral glycolipoprotein has been linked to the physiological changes in the glomerular filtrate in the lower nephron (Table 4) (Burth et al 1997; Yang et al 2000). Infected human patients and animals can develop hypokalemia and non-oliguria suggested to be from impaired absorption in the proximal tubule (Yang et al 2000). Renal lesions build up because the infection persists there long after it has been cleared from other tissue sites. Such lesions are frequently macroscopically observable as grey or white foci (white spots) in the renal cortex (Boqvist 2002b).

Localization and persistence of the organisms in the uterus of pregnant animals may result in foetal infection, with subsequent abortion, stillbirth, birth of weak neonates, or birth of healthy but infected offspring (Ellis 1994; Bolin and Prescott 1999). If the foetus becomes infected in the third trimester, it could produce specific antibodies and may overcome the infection (Quin et al 1994). The pregnant uterus, and especially the placenta, has well vascularised tissues, which facilitates bacterial location in the bacteraemic phase. The placenta it is also abundant in immune cells, particularly macrophages and T lymphocytes. However, these cells have a phagocytic function primarily, and secondly, they release immunosuppressor cytokines. Thus, they do not initiate an inflammatory response, and it becomes an ideal environment for the leptospires and other microorganisms involved in abortions to spread and establish (Tizard 1995). An animal usually aborts only once. In the maintenance host, infection often persists in both the male and female genital tract (Ellis 1994; Bolin and Prescott 1999).

1.11.4 Pathology and Clinical Presentation

Leptospirosis in veterinary medicine is recognized with a variety of signs and presentation depending on the affected organs in accidental or host adapted infections. Endothelial and small vessel damage causes ischemia and vascular damage to the involved organs (Boqvist 2002b).

1.11.4.1 Acute.

The acute clinical presentation has wide clinical manifestation that includes septicaemia kidney (acute interstitial nephritis, haemoglobinuria, kidney failure); liver (hepatitis, icterus, liver failure); blood (haemolytic anaemia); platelet deficiency; spleen (splenomegaly, and jaundice); lungs (rare in animals, haemorrhagic pneumonitis); uterus (infertility and abortion (second and third trimesters), placentitis, autolysis of the foetus, stillbirths or birth of infected animals with liver lesions); eye (autoimmune uveitis); central nervous system (haemorrhagic meningitis) among others (Radostits et al 1999).

1.11.4.2 Sub-acute

In the sub-acute form, the pathogenesis is similar to that of the acute septicaemic form, except that the reaction is less severe. It occurs in all species, and is more common in adult cattle and horses where occult cases with no clinical illness but with rising antibodies can be observed. These are difficult to explain but may be associated with strains and serovars of varying pathogenicity, immune status, density and frequency of the microbial challenge, environmental conditions, or number of carriers in relation to severity (Bolin and Prescott 1999; Radostits et al 1999).

1.11.4.3 Chronic

The chronic phase in most animals is characterized by chronic nephritis and uraemia. Not all shedding animals will develop kidney lesions. The less dramatic pathology and the persistence of the bacteria in inmuno-privileged sites are characteristic of host-maintenance or reservoir infections (Faine et al 1999; Radostits et al 1999).

Animals infected with host-adapted *Leptospira* serovars may persistently shed the organism for the duration of the animal's life (Heath and Johnson 1994; Faine et al 1999). For the most part, leptospiral infections are apparently subclinical, particularly in non-pregnant and non-lactating animals, distinguished only by the presence of antibody or of minor lesions of interstitial nephritis at slaughter (Prescott 1992; Faine et al 1999).

1.11.5 The Disease in Deer

The following section is complemented by a review of leptospirosis in deer in New Zealand at the end of this chapter.

In red deer, clinical signs may range from sudden death in per-acute cases to general malaise, sometimes accompanied by haemolytic disease, severe nephritis, haematuria, haemoglobinuria and jaundice in acute cases (Fairley et al 1984; Fairley et al 1986; Haigh and Hudson 1993; Dean et al 2005). Serovars Pomona, Copenhageni, Icterohaemorrhagiae, and Roumanica infections have been reported causing serious disease outbreaks (Gou 1981; Mackintosh 1984; Ayanegui-Alcérreca et al 2004). Red deer appear to be a maintenance host for serovar Hardjobovis, with subclinical disease, mainly presenting as kidney lesions (Ayanegui-Alcérreca et al 2004).

Trainer et al (1961), using an experimental model for leptospirosis in white-tailed deer (*Odocoileus virginianus*) with serovar Pomona, observed signs ranging from sub-clinical disease to abortion in four of five pregnant white-tailed does. In the same report, renal histopathological changes were observed and antibody titres of 1:100,000 were reported 10 to 15 days after challenge.

1.11.5.1 Macroscopic Pathology

The most severe pathology seen in deer in New Zealand has been associated with serovar Pomona. In acute cases with this serovar, reports include; jaundice, haemolysis, haemoglobinuria, enlarged kidneys (dark red or pale) and enlarged livers, with probable failure, and spleen damage (Anon 1980; Fairley et al 1984; Wilson and McGhie 1993; Dean et al 2005). In experimental infections with Pomona in the United States of America, the common findings were dark red urine, and kidneys that were dark red or pale kidneys, two to three times the normal size with subcapsular haemorrhages in the acute form or, fibrosis in the chronic form (Ferris et al 1960; Reilly et al 1962a).

Abortion due to leptospirosis in deer has only been reported in white tailed deer linked to Pomona following experimental challenge (Ferris et al 1960; Reilly et al 1962a). Reilly et al (1962a) also reported lung congestion after experimental infection with Pomona in white tailed deer.

Multi-focal white kidney spots, capsular adhesions and cortex scaring are common in subclinical cases of *Leptospira* Hardjobovis (Wilson and McGhie 1993; Wilson et al 1998). Wilson et al (1998) in a survey monitoring serology and kidney lesions at slaughter reported that a considerably greater proportion of kidney lesions were observed in lines with high serologic titres particularly to Hardjo and Pomona. These observations were made in animals without apparent health problems (Wilson et al 1998).

1.11.5.2 Microscopic Pathology of Leptospirosis with References to Deer

Vasculitis appears primarily to affect the capillaries, consistent with the observation that lesions are most severe in filter organs such as the liver, lungs, and kidney (Hadlow and Stoner 1955; Prescott 1992; Faine et al 1999; Plank and Dean 2000).

In deer, the histological lesions reported are similar to those seen in adult cattle and pigs with focal, interstitial, diffuse inflammatory cell infiltration in the renal cortex and leptospires within the lumen of cortical tubules (Wilson et al 1998). Affected cortical tubules are generally dilated and have absent or flattened epithelium; many contain casts of protein material, neutrophils, or cytoplasmatic and nuclear debris. In a more chronic stage, fibrosis is the main observation. Glomerular changes are involved interstitial reaction. The liver and spleen may also be damaged (Ferris et al 1960; Reilly et al 1962a; Fairley et al 1984).

1.11.6 Clinical Pathology

Diagnosis of leptospirosis must be confirmed by laboratory testing. The only confirmatory diagnostic measure at the early stage of disease is culture of blood. Leptospiruria is accompanied by albuminuria of varying degrees and persists for varying lengths of time in the different species. The presence of jaundice, icterus, and haemoglobinuria depend on the leptospiral clinical presentation (Radostitis et al 1999). Renal damage is reflected in abnormal urinalyses, characterized by proteinuria, sterile pyuriria, haematuria, and hyaline and granular casts (Prescott 1992; Faine et al 1999).

1.11.7 Differential diagnosis of Leptospirosis in Deer

Differential diagnosis in deer should include causes of sudden death such as, yersiniosis, clostridial diseases, malignant catarrhal fever (MCF) and copper toxicity and other causes of haemolysis such as nitrate poisoning, onion poisoning, brassicas (Haigh and Hudson 1993) cold-water overload haemoparasites Babesia, and Theileria, undiagnosed haemolytic anaemia in young red deer (Wilson et al 2004; Rawdon et al 2005). White spots in the kidney have been reported with MCF and other bacteraemic infections (Haigh and Hudson 1993). Red urine has been observed in the environment from deer on red clover, but the colour change occurred after urination (Niezen et al 1992).

1.12 Diagnosis

The criteria for diagnosing leptospirosis may be different for medical and epidemiological or epizootological investigations. The diagnosis of leptospirosis is less difficult on a herd basis than in a single animal (Radostits et al 1999). In clinical cases the serovars can be identified by serology and/or culture. For epidemiological studies serology can be the diagnostic tool, but ultimately the microorganism must be isolated and characterized to validate the serological data (Table 5) (Faine et al 1999).

1.12.1 Acute Case Diagnosis

The first step is an accurate clinical history followed by serological testing. In animals, the prime test is the MAT , with a full panel of the serovars present in the country (Tables 2 and 3) (OIE 2000, WHO 2003). An agglutinating titre $\geq$1/100 is considered to be a positive test is. (Note: an alternative titre is often appropriate for epidemiological studies). Paired samples are part of the protocol in clinical cases (5-7 or 15 days after the first sample), although titres may remain negative up to four weeks after exposure. Information on the vaccination history has to be considered when interpreting titres (Faine et al 1999; Radostitis 1999; OIE 2000). In humans the priority is in identifying the genus then serovar. Therefore, genus-specific ELISAs or the MAT (Patoc serovar) are used as screening tests, but even a low titre should be taken in consideration. (Faine et al 1999).

In the acute phase (3 to 7 days after infection) (Figure 3), darkfield microscopy can be performed on blood, peritoneal or pleural exudates, and urine. In this same period, culture from blood or encephalic liquid, can be attempted but can take from 10 days to 6 months to obtain a definitive result. At post-mortem, diagnostic cultures from kidney, liver, or spleen are useful, as is histopathogy with silver staining. Some rapid polymerase chain reaction (PCR) techniques are under development and in use for human diagnosis (Table 5) (Faine et al 1999; Quin et al 2002).

1.12.2 Chronic Cases

In chronic and carrier infections, the priority is for identification of the serovar involved. For this situation the MAT, with a full panel of the serovars present in the country, is the preferred screening test. If used at the individual level, a paired sample should be used (Faine et al 1999; OIE 2000).

In endemic areas with host-adapted serovars, animals will tend to have a low but sustained titre. If cross-reacting serovars are present, then the highest titre tends to be the more specific. In chronic cases where serology is not producing a clear result, even with repeated sampling, negative serology cannot be considered definitive and other means have to be used to demonstrate the presence of the *Leptospira*. When infected with a non-host adapted serovar, the antibodies will rise rapidly to high titres and will drop over a period of weeks or months. (Faine et al 1999). In chronic cases even though serology is the primary tool, the sensitivity of diagnosis is increased by the inclusion of other methods of detection in an effort to provide a clear result and the correct interpretation of the serological data. (Table 5) (Faine et al 1999; Quin et al 2002).

1.12.3 Epidemiological Research

Screening populations for epidemiological surveillance establishes which serovars are present and their seroprevalence. The MAT is used since the technique can identify the serovar. However, serology is indirect evidence of the presence of the bacteria and not a confirmation of infection (Table 5) (Faine et al 1999; Quin et al 2002).

The interpretation of the titre will vary with different serovars. For example, Hardjobovis or Bratislava will generally give a lower titre compared with serovars like Pomona or

Copenhageni. For epidemiological studies even titres of 1/24 or 1/48 can be considered significant. Serovar Pomona can cross-react with other serovars like Copenhageni. Therefore, if low titres are included for this serovar, the disease prevalence may be over-estimated or under-estimated. In the case of Hardjobovis in New Zealand where the serovar is considered endemic, Hellstrom (1978) and Blackmore et al (1979) reported that titres of 1/24 or greater could last for approximately 10 year in bovines and humans. Mackintosh (1981) suggested that an average titre of 1/192 for Hardjobovis was an indicator of a "high risk" herd in an endemic cycle and an average of 1/24 represented a "low risk" herd.

Table 5. - Summary of diagnostic tests and their use in the medical and epidemiological investigations.

Disease state	Investigation	Primary Test	Secondary test	Observation.
Acute	**Medical**	Animals (MAT)	GP, HP, SS, Dfm, BC, CPT	Serovar has to be identified, can be individual, or herd level.
		Humans (ELISA)	MAT, GP, HP, SS, PCR, BC, CPT, Dfm	Genus has to be identified and after that the serovar, in general individual level.
Chronic	**Medical**	Animals (MAT)	GP, HP, SS, Dfm, BC, DNA	Serovar most be identified (serology), and Isolated (genus) if possible. Herd study.
		Humans (MAT)	CPT, ELISAs, BC, DNA	Serovar must be identified (serology), Isolate when possible. Individual level.
Acute (Outbreak)	**Epidemiology**	Animal or Humans (MAT and BC)	GP, HP, Dfm, CPT, DNA	Serovar has to be identified, and culture at the population level.
Chronic	**Epidemiology**	Animal or Humans (MAT and BC)	GP, HP, Dfm, BC, DNA	Serovar must be identified (serology), and Isolated (genus). Population level.

Diagnostic test (taxonomic level of identification): MAT= Microagglutination (serovar), ELISAs (genus), Dfm = Darkfield microscopy (Genus), SS= Silver staining (Genus), HP=Histopathology (Suggests Genus), GP= Gross Pathology (Suggests Genus), CPT= Clinical Pathology Test (Suggest genus), PCR= Polymerase Chain Reaction (Genus), BC= Bacterial culture (Genus), DNA (Genus, species and serovar) MAT plus BC (Genus, Serovar).

Guidelines for the control of leptospirosis contain tables indicating the number of samples required within a group size to detect a given percentage of reactors in a population, with different statistical probabilities. For example in a herd of 300 animals, with a prevalence of 20%, and with 95% confidence limits, 14 animals would be required to determine infection status of the herd (Faine et al 1999). While serological tools can be used as way to monitor a population eventually, the only confirmatory evidence comes from culture. This is particularly important for the correct identification of the serovars present in a country due to cross-reactivity of antibodies between some serovars, and to the future development of a bacterin because immunity is serovar-specific (Faine et al 1999; Quin et al 2002). Culture of the serovar from urine and tissue from specimens collected at slaughter, postmortem and or clinical cases will be required. Culture and isolation can be achieved *in vitro* (culture media) or *in vivo* (animal models). Finally, the isolates have to be characterized by molecular (DNA) and serological tests (serovar specific MAT) (Table 5) (Faine et al 1999; Quin et al 2002).

1.12.4 Diagnostic techniques

A variety of assays have been developed in an attempt to provide accurate diagnosis of leptospirosis as recently reviewed by O'Keefe (2002). Direct methods for detection of bacteria, their antigens, or genomic material, include darkfield microscopy (DFM), special histopathology techniques such as silver staining, bacterial culture, animal inoculation, immunofluorescence, immunohistochemestry (peroxidase), polymerase chain reaction (PCR), DNA finger printing. Indirect techniques, detecting antibody, include microagglutination (MAT), macroagglutination, ELISAs, latex agglutination and complement fixation (O'Keefe 2002).

Ideally, diagnostic tests should be evaluated, and the sensitivity (Se) and specificity (Sp) determined for the environment, animal or human species, and type of investigation (medical or epidemiological) in which they are going to be used (Greiner and Gardener 2000). This is particularly important in the study of leptospirosis, due to the fastidious nature of the microorganism, with a well recognized serovar variation between regions and with different host-serovar relations (Vijayachari et al 2001). Nevertheless, only limited or inconsistent information is available for most of the reported diagnostic techniques for infection with this bacteria.

Smits et al (2001) reported a Sp of 93.9% and Se 89.8%for the complement fixation test. Levett (2001) reported a Se 92% and Sp of 95% for the indirect haemagglutination assay compared with the MAT, but low Se has been reported in endemic areas in the acute phase of the disease. Gusenhoven et al (2000) and Plank & Dean (2000) reported 60% to 87% Se for an IgM ELISA in humans. The same authors reported100% Se and 93% Sp for a different commercial IgM ELISA when compared with the MAT. Vijayachari et al (2001) reported a 40.2% Se and 61.5% Sp for DFM in plasma. Faine et al (1999) reported a 17% Sp for culture, whereas for the MAT a range of Sp and Se from above 98% and 93% to 67% and 41%have been reported in animals and humans with different serovars (Faine et al 1999; OIE 2000; Vijayachari et al 2001).

1.12.4.1 Dark-field Microscopy

Dark-field microscopy has been used as a rapid screening tool to identify leptospires in the urine of animals, but has the disadvantages of low specificity and sensitivity. Direct visualization of the organism is problematic, and artefacts present in body fluids are difficult to distinguish from leptospires (Bolin and Prescott 1999). This technique is useful in situations where laboratory resources are limited and can aid in establishing a rapid presumptive diagnosis. A concentration of 10^3 to 10^4 leptospires/ml is necessary for one cell per field to be visible and it requires a high degree of operator skill (Weyant et al 1999; OIE 2000). Dark-field microscopy should not be relied upon as the sole diagnostic criterion.

1.12.4.2 Silver Staining

The silver stain technique is not specific for *Leptospira* since it will also stain other bacteria, and cannot distinguish between *Leptospira* serovars. However, the bacterial morphology of the spirochaete in sections, particularly the size and nature of the helix, are particularly helpful in distinguishing *Leptospira* from other pathogenic spirochaetes such as *Borrelia* (Faine et al 1999). Numerous silver staining histopathological techniques, including the

Warthin-Starry and Van Orden methods, have been described for visualizing leptospires in tissues. As with other microscopic techniques, sensitivity is poor for samples in which the number of leptospires is small (Weyant et al 1999; Levett 2001).

1.12.4.3 Histopathology

A variety of histopathological stains have been applied to the detection of leptospires in tissues. Leptospires were first visualized by silver staining and the Warthin-Starry stain is widely used for histologic examination (Levett 2001). Silver stains or immunohistochemical stains on tissues will allow the detection of leptospires or leptospiral antigens in renal tubules, interstitium of the kidney, liver, lung, or placenta. Low sensitivity and difficult staining conditions are disadvantages of this diagnostic technique. Spirochaetes are often present in small numbers in affected tissues, particularly in chronic leptospirosis and histopathology cannot identify serovars (Bolin and Prescott 1999).

Histopathological examination of haematoxylin-eosin (H&E) stained sections of affected tissue may be useful, but will not a permit a visualization of leptospires. Histopathological lesions of leptospirosis are highly characteristic in renal tissues (Prescott 1992), with diffuse interstitial inflammatory infiltrate, which is more severe in the cortico-medullary junction. The infiltrate is primarily composed of plasma cells and secondly, lymphocytes and macrophages. In acute infections, vascular damage can be observed and some neutrophils and necrotic epithelial cells in the tubular lumina. Kidneys of chronically affected animals have a mild to diffuse lymphocyte infiltration, scattered macrophages and scarring tissue. Lung or liver lesions in acute cases will show fibrinoid necrosis of the blood vessels, haemorrhages, mononuclear infiltrates, thrombosis and necrotic changes in the functional cells (Prescott 1992).

1.12.4.4 Bacterial Culture

Culture isolation is the definitive, sensitive diagnostic test, but in practice, bacteriologic culture is too expensive and slow for many purposes. Culture may take 8 to 20 weeks and some researchers recommend culture for up to 6 months before a negative diagnosis can be made (Ho and Blackmore 1979; Wagenaar et al 2000; O'Keefe 2002). The choice of specimen (blood, urine, kidney, spleen and liver) to be collected for isolation from the infected host will depend on the phase of the disease (Johnson and Faine 1984).

The growth curves of leptospires follow the same lag, logarithmic and stationary phases seen for other bacteria culture, but with longer time scales. In optimum conditions, the growth of a pathogenic specimen is about 6-8 hrs, measured by viable count, where one log unit can be the expected growth in one day (Faine et al 1999). A similar time is reported for pathogens adapted to their hosts.

The media used for the culture of leptospires include Korthof and Stuart broths, Fletcher semisolid medium and EMJH medium, (Ellinghausen-McCullough-Johnson-Harris). Tween 80-albumin medium that are liquid, can be modified to semi-solid by the addition of agar. The cultures are then incubated at 30 °C for up to 8 weeks or longer. A drop of the culture is examined by dark-field microscopy once weekly for five weeks and then twice a month for up to 4 months before calling a culture negative (Johnson and Faine 1984; Weyant et al 1999; Faine et al 1999).

1.12.4.5 Animal Inoculation

Virulent leptospires can cause infection in laboratory animals, which can be used for primary isolation (Faine et al 1999). Weaning gerbils, hamsters (<55 g) and guinea-pigs (<200 g) can be inoculated intraperitoneally with 0.5-1.0 ml of neutralised urine, unclotted blood or a 10 % tissue suspension in EMJH or 1 % BSA. Cardiac blood is taken aseptically when a temperature rise is detected or at 5, 9, 10 and 14 days post inoculation. Animals surviving for 21-day post-inoculation can be euthanized and organs and blood samples taken for serology (Quin et al 1994; Faine et al 1999).

1.12.4.6 Immunofluorescence (Fluorescent Antibody; IFA)

This method is often preferred to silver staining, because it is a fast and accurate diagnostic method for detecting the leptospires and is easier to manage than other stains. While the characteristic morphology is often less evident in IFA than in dark-field microscopy, this technique is extensively used to demonstrate leptospires in veterinary specimens. The technique is not serovar-specific using commercial antibodies, but in Canada, a serovar-specific IFA test has been developed. (Quin et al 1994; Faine et al 1999; Bolin and Prescott, 1999; Levett 2001). This technique can be used for urine, other body fluids, and frozen tissues (Faine et al 1999). While it is considered less sensitive than molecular techniques, it is comparable or better than culture. (Bolin et al 1989b).

1.12.4.7 Immunohistochemistry

Immunohistochemistry has proven to be sensitive and specific for detection of leptospires in recent outbreaks. Although this technique has not been directly compared with direct immunofluorescence, it represents a promising non-fluorescence-based alternative (Weyant et al 1999; Levett 2001). Horseradish peroxidase (HRPO) or metallic gold labelling, when coupled to immunoglobulin G (IgG), has been demonstrated to be a practical marker for antigen detection in tissues.

This technique has the advantage that it can be used with both light and electron microscopy and does not require a fluorescence microscope. It can be performed with formalin-fixed tissues, even in those that have been stored for long periods, and avoids the disadvantages of artefacts arising from formalin fixation, impermanence and poor morphological definition associated with immunoflourescent staining (Ellis et al 1983; Faine et al 1999). Yener and Keles (2001) demonstrated that this technique could show the presence of *Leptospira* even in kidneys that showed no gross lesions in cattle.

1.12.4.8 Polymerase Chain Reaction (PCR)

In recent years, PCR-based methodologies have been developed for the rapid detection of *Leptospira* DNA. The PCR assays are more sensitive than conventional methods such as culture and dark-field microscopy. PCR analysis of blood samples has been reported useful within the first 10 to 14 days after the onset of disease, when leptospiral DNA may be detected prior to the development of the humoral immune response (Wagenaar et al 2000; O'Keefe, 2002).

PCR of urine samples can differentiate the carrier or shedding state while serological tests can only detect antibody regardless of infection status at the time. Furthermore, PCR can differentiate *Leptospira* spp. serovars. Suitable samples for this PCR include serum, urine, CSF, and aqueous humour (Weyant et al 1999; Heinemann et al 2000). The primers described by Merien et al (1992) amplify a 331-bp fragment of the 16S rRNA gene of both pathogenic and non-pathogenic leptospires, which in a contaminated sample, might produce a false positive result. Gravekamp et al (1993) described the detection of all pathogenic serovars by the use of two primer pairs. However, despite the theoretical and practical advantages of PCR, it has not been widely used for research or diagnostic purposes (Levett 2001).

1.12.4.9 DNA fingerprints, Ribosomal RNA

The genetic diversity of the members of the family Leptospiraceae has led to the investigation of molecular approaches for identification. Whole-chromosome restriction endonuclease patterns and ribotyping are used for serovar identification and PCR restriction fragment length polymorphism methods can differentiate some *Leptospira* species. Randomly amplified polymorphic DNA fingerprinting and arbitrarily primed PCR represent two promising new approaches (Weyant et al 1999).

1.12.4.10 Serological Techniques

Most cases of leptospirosis are diagnosed by serology. Antibodies are detected in the blood approximately 5 to 7 days after the onset of clinical signs (Levett 2001). Serologic tests are widely available and inexpensive (Wagenaar et al 2000) and can be divided into two groups;

a) Genus-specific tests such as complement fixation, sensitised erythrocyte lysis, macroscopic slide agglutination, immunofluorescence, serovar Patoc slide agglutination test, indirect haemagglutination, and counter-immuno-electrophoresis.
b) "Serovar-specific" tests such as ELISA, Dot-ELISA, microcapsule agglutination, IgM dipstick and latex agglutination and the microscopic agglutination test assay (MAT). Although there are recognized cross-reactivity between serovars of the same serogroup that can preclude exact identification of the species (Smit et al 1999: Plank and Dean 2000; Levett 2001).

1.12.4.11 Microscopic agglutination test (MAT)

The MAT is the reference method for leptospiral diagnosis. It is a screening test that detects agglutinating antibodies in serum of the host by mixing it in various dilutions with live or killed leptospires. Antileptospiral antibodies present in the serum cause bacteria to agglutinate and form clumps, and the degree of agglutination is visually assessed using dark-field microscopy. The unit that the test uses to report this result is the titre, which is the final dilution giving 50% a detectable reaction. The titre is also called positive titre (Plank and Dean 2000; WHO 2003).

The MAT is highly sensitive and serovar-specific. It is a complex test to control, difficult to standardize and interpret and is usually performed only in reference laboratories or regional

laboratories. A stock of serovar-specific live organisms must be maintained for antigens (Carter and Ryan 1975; Plank and Dean 2000; Levett 2001; O'Keefe 2002). Nevertheless, the MAT remains the only serological assay widely accepted and capable of producing serovar-specific results, so it remains the "gold standard" test, along with culturing, for the diagnosis of leptospirosis in both humans and animals (Weyant et al 1999; O'Keefe 2002). The MAT detects both immunoglobulin-M (IgM) and Immunoglobulin-G (IgG) classes of antibodies. The assay requires a positive control to maintain the standard identification on the reference cultures. Positive control antisera for most serovars are available commercially. Alternatively, standard antisera may be prepared in rabbits (Alexander 1986).

Formalized antigens have been used in the MAT to overcome some of the difficulties of keeping live specimens. Titres obtained with these antigens are somewhat lower than those seen with live antigens and more cross-reactions are detected. The technique is qualitatively different from that seen with live antigens (Alexander 1986). Antibody titres against local isolates are often higher than titres to the laboratory stock strain of serovars of the same group (Chapter 2) (Faine et al 1999; Weyant et al 1999).

The MAT is a specific test, and for optimum sensitivity, it should employ serovars existing in the country in which the animals are found (OIE 2000; WHO 2003). Sensitivity can be improved by using local isolates rather than reference strains, but reference strains assist in the interpretation of results between laboratories. The specificity of the MAT between serovars of different serogroups is reliable (Hardjobovis and Pomona do not cross-react) (Faine et al 1999; OIE 2000). However, there is significant cross-reaction with other serovars within the same serogroup or genomo-group, particularly when the antibody titre of the infecting serovar is high. Usually the closely related serovar will react at a lower level than the infecting serovar (eg. Pomona and Copenhageni) (OIE 2000; Ayanegui-Alcérreca et al 2003).

As an individual animal test, the MAT is very useful. Acute infection can be demonstrated by a four-fold change in antibody titre in a paired sample from acute to convalescent serum since the titres rises fast and can be related to a recent infection (Radostitis et al 1999; OIE 200). The World Health Organization and the Office of Epizooties accepts titre of 1/100as a cut-off for a positive sample although others accept 1/200, 1/400 or 1/800 depending on the animal species, the serovar involved and whether it is an individual sample or a herd sample (WHO 2003). In an area of low prevalence, even a low titre can be considered diagnostic if it is accompanied by the concurring clinical and epidemiological factors. In highly endemic areas, many individuals are likely to have persistent antibodies due to past infections and a relatively low titre in a single sample may be difficult to interpret (WHO 2003). To increase the sensitivity in a highly endemic situation, at the serological level, paired sampling can be used. Group or herd level sampling is another way to overcome the sensitivity problem in the MAT (Faine et al 1999; WHO 2003)

In an accidental host infection a single sample with a high titre, together with the appropriate the clinical history is considered positive (e.g. Pomona outbreaks in calves or deer weaners). With host-adapted serovars like Hardjobovis in cattle, the test is less sensitive so a titre of 1/100 to 1//400 is considered the cut-off point to relate the titre with a recent infection. More than one cow with a titre 1/300 is considered evidence of infection in the herd (Radostitis et al 1999; Bolin and Prescott 1999)

At the herd level the test has a better sensitivity. Samples from 10 animals or 10% of the herd is, in general, considered adequate to detect the infection at the herd level, although the more animals sampled the higher the sensitivity (Hathaway et al 1986). With an accidental host, the test will be easier to interpret. With a host-adapted infection, the herd diagnostic test increases the probability of finding either several animals with low titre or at least one with the cut off point of 1/100 (OIE 2000).

The MAT has limitations in the diagnosis of chronic infection in individual animals. Both in abortion, chronic renal or genital carriers because of the low titres reactions associated with the previous clinical situations and can persist for a long time, complicating the interpretation of the results. A paired sample increases the sensitivity in abortion situations (Radostitis et al 1999; OIE 2000). Vaccination history should also be taken into account since the MAT does not distinguish between antibodies induced by vaccines from natural infection (OIE 2000). In vaccinated cattle herds, animals will show a rise in titre from 1/100 to 1/400 and titres will drop after 3 months. Some animals, particularly those that received several vaccinations, will sustain a higher titre for a longer time. If the herd is vaccinated, paired samples plus some direct leptospiral method should be used in conjunction with a good clinical history (Bolin 2003).

1.12.4.12 Macroscopic Agglutination Test

The macroscopic agglutination test is a screening test that uses dead antigens but suffers from lack of specificity (Quin et al 1994). The assay uses the thermo-resistant L. biflexa Patoc agglutination antigen. It consists of a dense suspension of formalin-treated heated cells of L. biflexa serovar Patoc. The antigen reacts broadly with antibodies to diverse pathogenic serovars. The test is conducted on a glass slide by mixing one drop of each serum at a 1:10 dilution with an equal volume of antigen. The slide is rotated for some minutes and agglutination is assessed against a black background (Alexander 1986).

1.12.4.13 ELISA (Enzyme Linked Immunosorbent Assay)

The ELISA is a relatively new technique compared with others discussed. It can detect specific IgM and IgG antibodies with high sensitivity (Gussenhoven et al 1997). While some of these assays have been more sensitive than the MAT, they have a number of disadvantages including a lack of specificity. Antibody levels do not necessarily reflect the immune status of an animal, since both protective and non-protective antibodies are measured and seropositives animals may harbour infection in their kidneys (Faine et al 1999; Yan et al 1999).

A variety of different antigens have been tested in ELISA tests including carbohydrate antigens (Matsunaga et al 2003), LPS (Yan, et al 1999), sugar chains, outer membrane proteins (Flannery et al 2001), whole lysed bacteria, and formalin- fixed whole culture extract (Weyant et al 1999; O'Keefe 2002). One commercial IgM ELISA was found to be 100% sensitive (93% specific) when compared with the MAT and in 29% of cases detected antibodies at an earlier stage of the infection (Gussenhoven et al 1997; Smits et al 2000). In some ELISA assays cross-reactivity has been reported between serovars, possibly due to the inner envelope antigen of *Leptospira* sharing some common cross-reacting antigens. Even though cross-reactivity is low, it should be taken into account when choosing an ELISA (Cho et al 1989).

The main advantage of the ELISA test is that it can provide information about the isotype (IgG or IgM) of the type of reacting antibody. This is particularly useful in monitoring in endemic areas of disease in animals or humans at the individual level because the presence of IgM can be regarded as the result of new and recent infection. Antibody detectable by IgG-based indirect ELISA persists for months or even years, whereas in humans, IgM ELISA titres began to decline after 2 months and reduced to 67% by one year (Brandao et al 1998). A dipstick ELISA has been designed to detect IgM. It is based on the heat-stable antigen of *Leptospira biflexa* adhered to a nitrocellulose strip. The strips are tested with human serum, and a secondary antihuman-IgM antibody labelled with horseradish peroxidase is used to indicate a positive or negative reaction. (Gussenhoven et al 1997; Smits et al 2000; Plank and Dean 2000).

1.12.4.14 Latex Agglutination Test

Latex agglutination is performed by mixing antigen-coated dyed latex beads with the serum on a slide, and observing the reaction within seconds, or after 2 to 10 minutes, at room temperature. The sensitivity of the assay varies with the stage of the disease from 72% in the first 10 days of the illness, to 88.2% in later stages. The specificity was 93.9% and 89.8%, respectively. The latex agglutination test provides a quick clinical diagnosis. The serovar and serogroup identification has a greater relevance in the epidemiological field (Smits et al 2001).

1.12.4.15 Sensitised Red Blood Cell Methods

A number of methods using sensitised red blood cells have been described including a haemolytic assay requiring complement, and a haemagglutination assay. These assays detect both IgM and IgG antibodies using heat stable peptidoglycan, or polysaccharides derived from LPS (Faine et al 1999). The indirect haemagglutination assay developed at the Center for Disease Control, USA was shown to have a sensitivity 92% and specificity of 95% compared with the MAT, but low sensitivity has been reported when used in endemic areas in the acute phase of the disease (Levett 2001).

Complement fixation (CF) was widely used, but the method was not standardized. Complement Fixation was applied to veterinary diagnosis, but species differences were noted (Faine et al 1999). The CF test, like the haemolytic test, utilizes a *L. biflexa* antigen and has only genus-specific activity. The assay uses lamb, or O type human red blood cells and guinea-pig complement. A titre of 1:100 is considered significant, and in the convalescent-phase the serum titre can range from 1:1,000 to 1:100,000. The test is used in endemic areas (Alexander 1986; Faine et al 1999).

1.13 Immunological responses to leptospirosis

Research in ruminants has shown some differences in the spectrum of the responses at the cellular and humoral levels between species (Estes and Brown 2002). The overall structure and function of the immune system and lymphohaematopoietic tissues follow a similar pattern in most ungulates (Howard 1992; Tizard 1995; Lastras et al 2000).

For the present review, there is little information specific to deer. The research on the immune responses to infections in farmed deer to Mycobacterial diseases and in New Zealand offer the basis for the assumption that the deer immune responses are similar to those observed in the bovine species (Howard 1992; Mackintosh and Griffin 2004; O'Brien et al 2004). Thus, most of this discussion will be extrapolated from what is already known of the disease in cattle.

1.13.1 Humoral Immunity

Most of the substantial evidence and research on immunity to *Leptospira* has been centred on the humoral response, opsonizing and bactericidal antibodies directed to outer membrane structures, particularly to the lipopolysaccharide antigens and some outer membrane proteins which mediate the bacterial recognition (Faine et al 1999; Adler and de la Peña-Moctezuma 2003). Following infection, specific antibodies are induced in the early phases of the disease; these antibodies are highly serovar-specific. Antibody production in infected animals begins a few days after the onset of leptospiraemia (Quin et al 1994). Maximum titres are achieved within 2-3 weeks of the initial infection (Brown et al 2003).

The first serological response is that of IgM antibodies. They will retard bacterial growth, but will not kill or clear all the organisms. IgM immunoglobulins rise rapidly but commonly decline to undetectable concentrations by four weeks of infection (Radostitis et al 1999; Adler and de la Peña-Moctezuma 2004). Within two weeks of infection, IgG antibodies appear, and at three months after bacterial challenge, they represent 80% of the immunoglobulins detected in the MAT (Radostitis et al 1999). The antibodies cause lysis of circulating leptospires by complement activation, or by mediated phagocytosis in the macrophages and granulocytes. Immunoglobulins IgG_1 and IgG_2 are both able to fix complement, which is an important mechanism to control leptospirosis in its own right. Bovine IgG_2 antibodies also act as opsonins decreasing the number of leptospires (Naiman et al 2002). An anamnestic response develops after re-exposure to organisms of the same leptospiral genotype. Resistance to reinfection appears to depend on antibodies directed against serovar or serogroup-reactive antigens (Adler and de la Peña-Moctezuma 2003).

Deer have been farmed for a relatively short period of time, some information on humoral responses specific for the deer as a production animal were reviewed. Wilson and Schollum (1984) and Wilson et al (2001) in small trials, reported low titres against *Leptospira* (Hardjobovis and Pomona) and a classical humoral response curve as those of other mammals in vaccinated deer with commercial vaccines. Wilson (1984) monitored antibody responses to *Clostridium* in rising yearling deer, where no antibodies were detected after the first vaccine, but a low peak response was observed 2 weeks after the booster vaccine.

1.13.2 Cellular Immunity

Cell-mediated responses have received less attention in the case of leptospira research than the humoral immunity. It was previously thought that protective immunity against leptospirosis was largely humoral. Anti-leptospiral lipopolysaccharide immunoglobulins in some animal models provide good passive and active protection against some serovars such as Pomona, Icterohaemorrhagiae, or Copenhageni (Faine et al 1999; Naiman et al 2002).

However, some research was undertaken with serovar Hardjo in cattle in an attempt to understand why antibodies to this particular serovar are difficult to induce, and why they did not correlate with protection. This challenged the concept that protective immunity to this infection is primarily humoral and indicate the importance of exploring further the cellular immune response in these group of infections (Adler and de la Peña-Moctezuma 2004). Most serovars are capable of inducing a good antibody response to the LPS structure, but some like Hardjobovis and Hardjoprajitno in cattle, or Bratislava in pigs, show immunoglobulin titres in the MAT or ELISA assays. One line of research focused on the cell-mediated immune response to Hardjo and cattle, which has proven to be important. The other was the study of the antigenic structures such as the LPS, which has been shown to be different with other Gram negative bacteria, and possibly has a modulatory effects on the coordination of the immune response by the its interaction with the innate immune system (de la Peña-Moctezuma 1999; Werts 2001).

Brown et al (2003) showed that immune response induced by Hardjobovis vaccines (i.e. "Leptoavoid" or "Spirovac") correlated with the induction of substantial cellular immune response. For other serovars, vaccines are designed to induce the production of antibodies against LPS and prevent the urinary shedding of the bacteria. However, this was not true for bacterins containing different serovars, and hence redirected research to explore other aspects as of the immune response such as T cell responses, innate immunity, LPS, and outer membrane proteins regulation (Naiman et al 2001; Brown et al 2003). Based on recent studies it appears that these Hardjobovis vaccines induced a strong and long lasting cell-mediated immune response, which was characterized by antigen-specific IFN-γ -producing T cell, IgG_1 and IgG_2 antibodies The data suggested that a good cellular response enhanced vaccine efficacy (Naiman et al 2001; Naiman et al 2002). There is no vaccine cross-protection between Hardjo and Pomona, so in areas with both serovars present, multivalent bacterins are recommended (Broughton et al 1984; Campagnolo et al 2000).

1.13.3 Innate Immunity

Resistance to infection by the innate immune system has a greater degree of specificity than previously thought in its ability to discriminate between self and foreign pathogens, and is the key to the acquired specific response (Zinkernagel 2003; Akira and Takeda 2004). A family of evolutionary conserved receptors, known as Toll-like receptors (TLRs), have a role in the primary modulation of immune response. The receptors are type I integral membrane glycoproteins, similar to the receptor of interleukins. They are present in monocytes, macrophages, endothelium, dendritic cells, T cells and epithelial cells. Each receptor has been linked to a ligand that will trigger a particular cytokine route (Akira and Takeda 2004; Jenner and Young 2005).

1.13.4 Maternal immunity in ruminants

Many mammalian species including ungulates have syndesmocorial or epitheliochorial placentation virtually impermeable to immunoglobulin and newborns are hypogammaglobulinaemic (Salmon 1999; Kehrli and Harp 2001). Because the neonatal immune system is immature, transfer of colostral immunoglobulins during the first few hours of life is essential for the survival of the new born (Kehrli and Harp 2001; Van de Perre 2003). In addition, the newborn will receive a continuing supply of immunoglobulins from the mother's milk to provide passive mucosal protection until weaning (Salmon 1999; Kehrli and Harp 2001).

Although ungulates can be immuno-competent at birth, antigenic stimulation is required to establish a primary immune response. However, this response requires a lag period (Morein et al 2002).

The immunological status of the newborn contributes to endemic infections in animal populations, sustaining a continuous prevalence of infectious diseases such as leptospirosis in herds (Morien 2002; Zinkernagel 2003). The fundamental role of maternal antibodies is to attenuate infections to permit a "physiological vaccination" of the offspring by allowing a gradual exposure to pathogens while the immune system matures, thereby providing active immunization (Zinkernagel 2003).

Vaccination programs that target the mother are aimed at ensuring protection in the first period of life and to reduce the risk of infection (Morein et al 2002). While the newborn is protected, it is recognized that passive immunity can compromise later immuno-responsiveness. An important practical consequence of the immunomodulatory effects of the passive maternal immunity is the effects on immune response of the offspring to the vaccine (Barrington and Parish 2001). The more largely acknowledged effect is the direct interaction of the maternal neutralizing antibodies with the antigens on a vaccine. IgG_1 accounts for 80% of the immunoglobulin in the colostrum, with an estimated 16-day half-life in calves, with more than 97% catabolized after 80 days. Most vaccine programs will began between 2 to 4 months of age as colostral antibody declines (Tizard 1995; Barrington and Parish 2001).

Palit et al (1991) reported an effective vaccine response to challenge with serovar Hardjobovis in calves between 4 and 18 weeks of age, regardless of their age or maternally derived antibody titres. McDonald and Rudge (1957) challenged young calves at ten days of age showing titres from 1:300 to 1:10 000 and had 100% survival, whereas the twenty-five days of age calves showed titres from 1:100 to 1:3,000 where only one animal died. Gillespie and Kenzy (1958) and Kenzy et al (1961) vaccinated one group of calves at one or two months of age with Leptospira Pomona and a second group at six to eight months. The animals were challenged after 6 months when 76% of those vaccinated in the first 2 months became infected whereas only 8% of animals vaccinated at an older age became infected.

Bolt (1990) reported in pigs in New Zealand, that the optimum leptospiral vaccine response was after 10 weeks of age and was negatively correlated with the dam's serological titre. Schollum and Marshall (1985) vaccinated calves with a commercial New Zealand leptospiral vaccine containing serovars Hardjobovis and Pomona detecting a 30% to 40 % seroconversion in calves immunized at 3 months of age, compared with an 80 to 100% seroconversion in those vaccinated at six months of age.

In deer, maternal antibodies may be important in a leptospiral vaccination program, because the highest risk of clinical disease has been reported in the autumn period, when deer are only 3-5 months of age (Gou, 1981, Mackintosh 1984). Wilson et al (2001) in a study on immunological responses to multiple vaccines (*Yersinia, Clostridium* and *Leptospira* (Hardjobovis and Pomona)) reported low antibodies titres against these serovar in weaners vaccinated at 3 months of age, but without apparent interference between vaccines. Mackintosh et al (2001) observed good immune responses to vaccination with a *Yersinia pseudotuberculosis* vaccine in fawns over 12 weeks of age, but the results suggested that maternal antibodies may have interfered with the immune response to vaccination in animals less than 12 weeks of age.

1.14 Treatment

The primary intention of treatment is to control the infection prior to irreparable damage to the liver and kidney occurring (Radostitis et al 1999) but treatment is often disappointing because animals usually are presented only when septicaemia has occurred. The second aim is to control leptospiruria of carrier animals (Radostitis et al 1999). Animals with acute leptospirosis can be treated with streptomycin (12.5 mg/kg twice a day for 3 days) singly, or combined with ampicillin, or large doses of penicillin-G. Leptospires are susceptible to erythromycin, tiamulin, and tylosin although these antibiotics cannot remove the renal carrier state. Chronic states may be treated with a single dose of streptomycin (25 mg/kg) or in countries such as the USA where it is no longer available; a 20mg/kg dose of long-acting oxytetracycline appears effective (Presscott and Nicholson 1988; Presscot 1991;Bolin and Prescott 1999). Smith et al (1997a), report two injections of amoxycillin at a dose of 15mg/kg IM administrated 6.5 weeks after a controlled infection in heifers with Hardjo as an acceptable alternative to dihydrostreptomycin. In deer, Mackintosh (1993) and Haigh and Hudson (1993) propose streptomycin as the drug of choice. Oxytetracycline is useful for the treatment of the clinical cases, but is not recommended to stop leptospiruria.

Standardized procedures have not been developed for antimicrobial susceptibility testing of leptospires. Clinical experience, studies with experimental animals and *in vitro* susceptibility testing have generally correlated. There is strain variability in susceptibility to penicillin and oxytetracycline, penicillin-G, ampicillin, amoxycillin, bacampicillin, cyclacillin, piperacillin, mezlocillin, doxycycline, chlortetracycline, erythromycin, cefotaxime, and moxalactam were effective in preventing the death of infected animals (Kemp 1980a;Smith et al 1997a: Weyant et al 1999; Alt et al 2001; Plank and Dean 2000). The effect of sulphamethazine was ambiguous *in vitro* and drug resistance was reported by Prescott and Nicholson (1988).

1.15 Prophylaxis, Control and Management

Control of leptospirosis is based on management, vaccination, and treatment. In all cases, the aim should be the limitation of direct and indirect transmission of the bacteria by the renal carrier animals (Radostitis et al 1999).

1.15.1 Leptospiral Vaccines

Vaccines as a general concept do not prevent infection but reduce it or prevent the infectious cycle of disease (Figure 3) (Babiuk 2002; Zinkernagel 2003). Specificity is a key issue, and for a vaccine agent to be efficient it must mimic as closely as possible the antigenic challenge of a natural infection (Babuik et al 2002; Zinkernagel 2003). Immunological protection in leptospirosis is serovar specific, so vaccines should contain the serovars enzootic to a given country (Bolin et al 1989 a, c).

Most vaccines protect hosts via neutralizing antibodies or opsonizing immunoglobulins. Successful vaccines will provide protection for the individual as well the population, particularly those stimulating the Th_2 cells (humoral response) (Zinkernagel 2003). Leptospiral vaccines are formalin-inactivated bacterins containing one or more serovars of a 10-day-old culture with an approximate concentration of 0.7 to 1.0 x 10^9 bacteria/ml. Phenol

and merthiolate are commonly used as preservatives and alum is a common adjuvant in leptospiral vaccines.

Vaccine seeds are cultivated in low protein growth medium. Most vaccines are prepared with low passage virulent field strains (monotype pure cultures) to avoid saprophytic Leptospira. These are better than avirulent laboratory strains and require less antigenic mass. It is important to conserve the integrity of the outer membrane of the organism in the vaccine since that is where most of the important immunogens are located (Bey 1992; OIE 2000).

There are many vaccines available worldwide with different composition for cattle (Morter 1980; Ellis 1984; Bolin et al 1989 a,c). These products should not be used without an understanding of local serovars (Theirman 1984). Leptospiral vaccines should be formulated for use in a particular animal species in a specific geographical region and should contain only those serovars (and genotypes) specific to a particular class of animal (OIE 2000).

In New Zealand, the commercial products licensed for use in deer include "Leptavoid-3" (Hardjobovis, Pomona and Copenhageni), "Leptavoid-2" (Hardjobovis and Pomona) (Schering-Plough Animal Health Ltd), which was the vaccine used in the research presented in this thesis, "Leptoshield" Pfizer Animal Health trivalent and bivalent vaccine (which is the CLS technology of "Spirovac" from Pfizer UK), and the 7-in-1 multivalent combination "Ultravac 7-in-1") (Hardjobovis, Pomona and *Cl. perfringens* type D, *Cl. tetani*, *Cl. septicum*, *C. chauvei* and *Cl. novyi* type B) Pfizer Animal Health NZ (Index of Veterinary Specialties, New Zealand 2005).

1.15.2 Vaccination

Vaccination attempts to mimic exposure to an infectious microorganism, but as a sterile antigenic challenge, where animals will develop immune protection against a disease, but without the exposure to an infectious microorganism (Zinkarangel 2003). It is suggested by Thrusfield (1995), that an 80% sustained herd immunity should be sufficient to limit infectious cycles. Commercial leptospiral vaccines aim at 80% protection of the challenged animals in controlled challenge trials. In animals vaccinated before the first exposure to the infection, the stock should be protected against infection, particularly establishment of bacteria in the kidney, but it should not be considered a 100% effective. In shedding animals, the vaccination attempts to reinforce the immunity against re-infection, but may not eliminate the shedder status (Figure 3). The objective of a vaccination programme in different generations is to limit the number of infected animals (Dowd 2003). Once the vaccine program has begun it has to continue, firstly to keep the pressure on the source of infection, and secondly, to avoid reintroduction of the infection from other animals or the environment.

Immunity to vaccines or natural challenge has been measured by the titre of agglutinating antibodies in the MAT even though they only represent a part of the immune response (Bolin 2003). Bacterins induce lower titres to the MAT than natural infection. The duration of immunity measured by the agglutinating antibodies following vaccination usually is no more than 6 to 12 months (Faine et al 1999). However, protection and immunity to the disease and to reinfection can persist for a least a year in cattle (Figure 3) (Radostitis et al 1999; Bolin and Prescott 1999).

In New Zealand, vaccination has been used successfully to prevent leptospirosis in young calves (cattle) and has apparently worked well on deer farms in the face of outbreaks (Marshall and Manktelow 2002; Dean et al 2005; Wilson, personal comm.). The combined Hardjobovis-Pomona bacterin has been used in cattle in New Zealand since the late 1970s protecting against infection and leptospiruria for Hardjobovis and outbreaks in calves caused by Pomona (Marshall et al 1979b, 1982; Broughton et al 1984; Mackintosh 1981) In cattle vaccination involves two doses spaced 4 to 6 weeks apart, with annual or bi-annual reinforcement required due to the short term of the immune memory response to these bacterins (Faine et al 1999; Radostitis et al 1999). Mackintosh (1981) reported 80% antibody prevalence for Hardjobovis, and above 90% for Pomona in vaccinated cattle in New Zealand. Titres to both serovars remained elevated in most cattle for 5 to 6 months, but titres to Hardjobovis in some animals lasted almost a year.

In deer, only one small vaccine study is published (Wilson and Schollum 1984). Antibody responses were of lower magnitude and shorter duration than in cattle, thus it is recommended that weaners in herds at risk be vaccinated in autumn followed by annual booster vaccination (Mackintosh 1993). However, because there is no information to confirm the validity of this recommendation, this issue was addressed as part of the research objectives of his thesis.

Vaccination strategies in the dairy and pig industries have in New Zealand proven successful to control animal infection and human exposure (Mackintosh 1980b; Bolt 1990; OSH 2001; Thorneley et al 2002, Marshall and Manktelow 2002, Baker and Lopez 2004).

Annual incidence of human cases in the dairy industry where vaccination has been used for more than two decades declined from 5.7/ 100, 000 in 1990-1992 to 2.9/100 to in 1996-1998. However, the incidence rose again to 3.8/100,000 in 2002, attributed to meat workers. While the pig industry has a regular practice of vaccination, the sheep and deer industries are now of concern in relation to human exposure (Thorneley et al 2002; Baker and Lopez 2004; Bell 2005).

1.15.3 Control and management

Control of leptospirosis in livestock has been centred on prevention to exposure, vaccination, and selective treatment of animals (Bolin 2003). Vaccination programs aim to provide herd immunity to break the infectious cycle, reducing clinical disease and the urinary shedding. However, in practice, vaccination may prevent leptospiruria particularly in non-infected animals at the time of vaccination or shorten the shedding period in animals that become infected after vaccination (Figure 3) (Mackintosh et al 1980d; Heath and Johnson 1994; Smith 1997b).

Infections with host-adapted and non-host-adapted Leptospira serovars required different control strategies. An optimal program to control leptospirosis should prevent clinical disease and urinary shedding (Bolin 2003). Control strategies should limit direct and indirect contact between susceptible animals, the carrier and the contaminated environment, and other sources of infection (Bolin 2003). However, in a pastoral farming situation, many of these proposals are not practical.

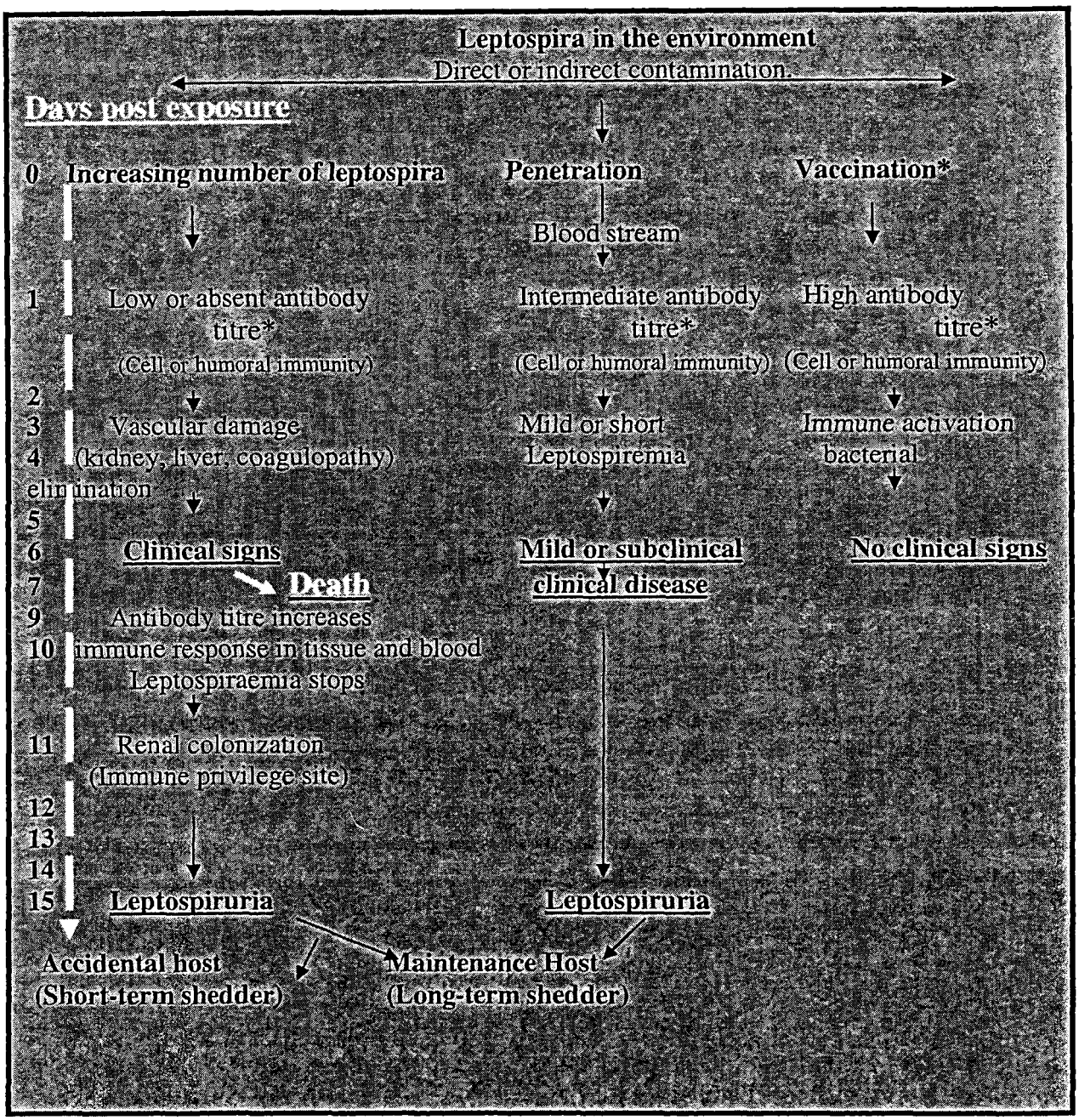


Figure 1.3 - A schematic diagram of the progression of leptospiral disease and immunity, and the role of vaccines. Adapted from (Greene et al 1998). * Antibodies can be either the result of vaccination, passive immunity (colostral) or the result of previous infection.

Research into the epidemiology, management, and control of leptospirosis in production animals has been conducted since the early 1950s (Marshall and Manktelow 2002; Bruére 2003) providing a baseline of knowledge for extensive farming pastoral systems in New

Zealand. However, more information was needed before inferences or practices from other production systems could be validated for control of the disease in deer.

In an extensive pastoral system like New Zealand the strategy has to be centred on control rather than eradication (Mackintosh 1981; Bolin 2003; Dowd 2003). Realistically, vaccination control programs should aim for containment rather than eradication (Radostitis et al 1999; Alonso-Andicoberry et al 2001).

Vaccine programs should aim to prime the immune system of animals in each new generation before they are exposed to infection, as well as newly introduced individuals to a herd, and then stimulate memory of the immune response by periodic revaccination (Babiuk et al 2002; Zinkarangel 2003). The New Zealand dairy industry experience demonstrates that persistent vaccination can control the infection in a herd over 1 to 2 generations at the population level. Similar results are reported from the pig industry (Bolt 1990; Dowd 2003). Approximately 20% of beef cattle are vaccinated while data for sheep and deer are unknown. Control must include all susceptible or possible hosts in mixed species grazing system (Dowd 2003).

Mackintosh (1981), suggested that the persistent vaccination of a previously infected herd could achieve freedom from the vaccine serovars. For the sheep industry, Dowd (2003) calculates that 7 to 10 years will be required to have 80-90% stock vaccinated. In deer, because a hind can have a productive life up to 15 or more years, a longer period should be considered.

Three decades of a vaccination program in the New Zealand dairy industry has reduced the disease impact in animals and humans. Similar results are likely in the other industries. However, the real impact in control will not have the same effect if it is constrained either to the herd level or to only one species. The figures presented by Dowd (2003) and the evidence from sheep (Heuer and Davies 2004) and deer (Wilson et al 1998, Wilson et al 2005) suggests the problem has to be addressed in these the deer and sheep industries.

Management of the disease at the herd level, including vaccination, should also consider the following:

- Herd infection status using serology MAT and other diagnostic criteria. as above;
- Identification of susceptible and/or infected alternative or source hosts, particularly relevant in the multi-species grazing farm (Mackintosh and Wilson 2005).
- Particular behaviours that could be establish a higher risk of infection such wallows on deer farms.
- Open or closed status of the herd since many clinical disease outbreaks have been observed following mixing of deer from multiple origins in their first year of life particularly in the during autumn Dean et al (2005).
- Other sources of contamination such as environmental sources common water bodies (ponds, dams, streams) in the property or sheared with other properties. The presence of wildlife that could a source of infection.

1.16 Leptospirosis as a zoonoses in New Zealand

In developed countries, leptospirosis continues to be a disease of considerable economic importance in animal husbandry. However, the main occurrence of human disease remains in tropical and subtropical developing countries (Plank and Dean 2000; Levett 2001). Human infections may be acquired by occupational, recreational, or vocational exposure through direct contact with infected animals or indirect with a contaminated source. Livestock farming is the major recognized occupational hazard factor throughout the world and accounts for most of the infections in farmers, veterinarians, abattoir workers, or meat inspectors and other farm animal-related occupations in New Zealand (Wilks 1997).

Leptospirosis has been called "Dairy farmer fever" mainly (Hardjobovis and Pomona) because traditionally this group of workers have been the biggest single group affected by the disease. The risk of exposure was predominantly livestock farming workers (No. of workers exposed to different animal species: dairy 212, pig 19, beef 6, deer 5), forestry (17), and the rest of the cases had no defined occupation (Thorneley et al 2002). Recent statistics from New Zealand (1990-1998 show that, although farm workers still make up the majority of leptospirosis, meat processing workers now have a proportionally higher rate, 80% more than livestock farm workers (OSH 2001; Thorneley et al 2002). Data from the recent reports on human leptospirosis cases associate with deer are summarized the review on Leptospirosis in New Zealand farmed deer attach to this chapter.

The clinical appearance of the disease in humans varies in accordance with the severity of the infection and may range from a flu-like illness to meningitis, acute respiratory distress syndrome, hepatic, or renal failure (Yang et al 1997). Leptospirosis should be taken into account in the differential diagnosis of any patient with acute febrile illness or non-specific symptoms who has a fitting epidemiologic history. Laboratory diagnosis is by bacterial culture or PCR, but serology remains the primary diagnostic tool using paired samples in convalescent individuals with a fourfold increase in the serovar specific titre as diagnostic. However, the detection of leptospiral IgM-class antibodies by an ELISA represents a sensitive and specific serologic test also used in diagnosis (Campagnolo et al 2000).

Effective and comprehensive animal vaccination programmes such as "Leptosure" (Dairy Cattle Veterinarians, New Zealand Veterinary Association) in the dairy industry, or the control imposed in the pork industry, where the trend is for only vaccinated and certified pigs being accepted for slaughter have prove efficient, and similar strategies should be considered by other high risk industries such as the deer and sheep (Wilson et al 2005).

1.17 Research objectives

The research presented in this thesis deals with the epidemiology and control of leptospiral infection in the farmed deer of New Zealand. It was prompted by concerns within the deer industry of the growing awareness of the prevalence of leptospirosis in the national farmed deer herd as an animal and human health issue.

1.17.1 General Objective

The general objective was to provide the New Zealand Deer Industry with an enhanced understanding of the epidemiology and distribution of leptospirosis in farmed deer and the role of a vaccine as a possible control measure to reduce animal and human risk of disease.

1.17.2 Specific Objectives

- Establish an understanding of the epidemiology of leptospirosis infection in commercial deer herds.
- To investigate the effectiveness of vaccines and vaccination strategies to control and/or prevent leptospirosis in farmed deer.
- To define the regional prevalence of various serovars of leptospirosis in farmed deer.

1.17.3 Research Hypotheses

- The epidemiology of leptospirosis in a deer herds is similar to that in other farmed ungulate species in New Zealand
- Hardjobovis and Pomona are the most significant serovars in farmed deer.
- Leptospirosis is widespread throughout the New Zealand farmed deer population.
- An appropriate vaccination strategy, based on epidemiological and immunological data, will reduce animal and human risk of leptospirosis infection in the New Zealand Deer Industry

Attachment 1

Leptospirosis in New Zealand farmed deer: A review.

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Leptospirosis in New Zealand farmed deer: A review.

Abstract

This paper reviews current knowledge of leptospirosis in farmed deer in New Zealand. Leptospirosis is an emerging zoonosis and animal health issue internationally and there have been several reports in farmed deer in New Zealand over the past 25 years, and more recently, human cases linked to farmed deer. Most knowledge evolves from serological studies with culture evidence from some, suggesting infection is widespread, and there are several reports of clinical disease, both individual cases and outbreaks. Mixing of young stock from several sources appears to be a significant risk factor for outbreaks. Serological evidence of serovars Hardjobovis, Pomona and Copenhageni has been reported with serovar Hardjobovis having the highest prevalence either individually or mixed with serovar Pomona. Infection and disease caused by serovar Copenhageni and others appear uncommon. Deer appear to be maintenance hosts for serovar Hardjobovis, incidental or accidental hosts but a maintenance population for serovar Pomona with some infections persisting for several months, and accidental hosts for serovar Copenhageni.

Serovar Pomona appears to produce more severe clinical disease than serovar Hardjobovis although the relative risk has not been determined. Disease is usually manifest by haemolysis, jaundice, renal lesions, haemoglobinuria and often sudden death. Kidney lesions are commonly observed at slaughter and evidence suggests many are associated with leptospiral infections. Slaughterhouse workers appear to be at greatest risk of contracting the disease from deer. Vaccination produces serological responses, but its effectiveness in protecting from disease, and prevention or reduction of shedding in urine, has not yet been reported in deer. More robust knowledge of the epidemiology of leptospiral infections in deer, and the effectiveness of vaccines and vaccination regimes is needed to assist industry to develop a strategy to manage this disease.

Key words: Deer, farmed deer, New Zealand, leptospirosis, Leptospira, Pomona, Hardjobovis, Copenhageni, zoonosis, review.

Introduction

Leptospirosis is a bacterial disease with worldwide distribution caused by pathogenic *Spirochaetes* belonging to the genus *Leptospira* (Sonrier 2001). It is a significant animal and human disease. *Leptospira* spp. are classified into 17 genomospecies (23 serogroups) with seven being pathogenic and endemic to feral and domestic mammals (Faine et al 1999). All mammals can serve as maintenance hosts for one or more of the >250 known serovars of *Leptospira* (Plank and Dean 2000). There is an increasing appreciation of this disease and its zoonotic potential internationally, (Ellis 1994; Crump et al 2001).

Serological evidence of a low prevalence in feral deer had been published prior to establishment of the deer farming industry (Daniel 1966, 1967), although Anon (1977) and Hathaway et al (1981c) later failed to detect serological evidence of infection in wild deer. The first published report describing leptospiral disease in farmed red deer (*Cervus elaphus*) and wapiti (*Cervus elaphus*) in New Zealand was in 1979 (Anon 1980). Subsequently, there have been many leptospirosis outbreaks and individual cases on deer farms, and infection is

now recognised as being widespread throughout the deer industry (Ayanegui- Alcerreca et al 2004). Human cases associated with deer have increased (Bell 2005). Thus, leptospirosis is now a well recognised clinical disease in farmed deer in New Zealand but there is limited knowledge of its prevalence, epidemiology, clinical disease incidence risk, subclinical effects, prevention and human health significance (Wilson et al 1998). This paper reviews the literature on leptospirosis in deer in New Zealand.

Leptospiral serovars present in New Zealand

Classification of leptospires has been described by Faine et al (1999). The serovars isolated from animals in New Zealand are from the *L borgpetersenii* and *L interrogans* genomospecies. Serovars isolated from animals other than deer in New Zealand include Hardjobovis from cattle and sheep (Hathaway 1979; Bahaman et al 1980; Mackintosh et al 1980c; Marshall et al 1982), Ballum from rats, mice and hedgehogs (Brockie 1977a,b; Marshall and Manktelow 2002), Tarassovi from pigs and dogs (Ryan and Marshall 1976; Mackintosh et al 1980a), Balcanica from possums, cattle, sheep (Hathaway et al 1978; Mackintosh et al 1980c, Mackintosh et 1981), Pomona from pigs, cattle, sheep and dogs (Te Punga and Bishop 1953; Vermunt et al 1994; Bolt and Marshall 1995; Mackintosh et al 1980a), and Copenhageni from rats (Brockie 1977a,b). Serovars Australis and Canicola have been reported only from humans in New Zealand (Midwinter 1999). Serological evidence of serovars Hardjobovis, Pomona Copenhageni, Tarassovi, Ballum, Balcanica and Australis, and culture evidence for serovars Hardjobovis, Pomona, and Copenhageni have been reported from deer in New Zealand, as summarised in Tables 1 and 2.

Occurrence in deer

Clinical and research reports of leptospirosis in New Zealand deer in the technical literature are presented in Table 1 while a summary of quarterly Animal Health Laboratory reports is presented in Table 2. The first report of disease caused by leptospirosis in farmed deer was in 1980 (Anon 1980). It was subsequently proposed that severe leptospiral disease was uncommon (Griffin 1987) despite renal and liver lesions, suspected abortions, red water, and positive serology reported in deer from which *Leptospira* spp. had been isolated (Fairley et al 1984; Flint et al 1986). More recent reports suggest that infection is widespread amongst farmed deer (Flint et al 1988b; Wilson et al 1998; Ayanegui-Alcérreca et al 2004) and clinical disease and mortalities are common, based on laboratory reports summarised in Table 2. Observation of widespread infection is supported by anecdotal information from veterinary practitioners, and the observation of large numbers of kidneys at deer slaughter premises with lesions resembling those caused by leptospirosis, sometimes containing leptospires in histological sections (Wilson et al 1998).

Serological surveys show variation in prevalence of various serovars. Flint et al (1988a) sampled two North Island and three South Island deer herds, observing an overall titre prevalence of serovars Australis 13%, Ballum 47%, Bratislava 53%, Copenhageni 43%, Hardjobovis 39% Pomona 14% and Tarassovi 26%. However, all titres above a dilution of 10 were included, in contrast to titres of 96 and 100 being used as the cut point in other reports. Titres above a dilution of 80 were observed only for Copenhageni (n=1), Bratislava (n=1), Pomona (n=2) and Hardjobovis (n=38). Wilson et al (1998) sampled 601 red and red x wapiti stags, mainly 1- and 2-years of age, and 21 adult hinds from 53 farms (10 or 12 deer

per farm) in the lower North Island. Using a positive titre interpretation at a dilution of ≥ 96, serovar Hardjobovis was present on 73.6%, Pomona on 41.5%, Copenhageni on 11.3% and Tarassovi on 15.1% of farms although it is likely those Tarassovi titres were cross-reactivity. That serovar has also been reported by Flint et al (1988a). The within-slaughter line prevalence (12 samples per line) ranged from 8-100% between farms for serovars Pomona and Hardjobovis, and from 8-92% for serovar Copenhageni. Reichel (1999) suggested that the prevalence of leptospirosis in the national herd may not be as high as suggested by Flint et al (1988a) and Wilson et al (1998), based on a 9.8% and 1.4% prevalence of titres to Hardjobovis and Pomona, respectively. However, that study tested a randomly selected sub-sample of 417 sera from 1150 samples comprising two samples each from 575 farms reducing the chance of detecting infection at the herd level. A preliminary analysis for serological prevalence from samples in a serum bank from 14 herds in the lower North Island sampled in 1992–3 (n=730 samples) showed 51.8 % (49.6-53.9 CI) prevalence for serovar Hardjobovis (Ayanegui-Alcérreca et al 2003).

Geographical, sex, age and possibly serological interpretation may contribute to variation in prevalence estimates between studies. It is likely that leptospirosis is under-diagnosed or mis-diagnosed on deer farms because many diseases and mortalities are not subject to veterinary investigation (Wilson 2004).

Host: serovar relationships for deer

The definitions of host:serovar relationships applicable to leptospirosis have been discussed by Hathaway (1981b) and summarised in Table 3. It would appear that lack of culture evidence and infrequent serological evidence for serovars Balcanica, Ballum, Tarassovi and Australis (Flint et al 1988a; Wilson et al 1998) would suggest that deer would only be accidental hosts to these serovars.

Serovar Copenhageni has been cultured from the urine of deer from one Nelson property (Flint et al 1986), and there is serological evidence of causation of acute disease in weaners on one Hawkes Bay farm (Walker pers. com.). These limited reports, coupled with its low prevalence in serological surveys (Flint et al 1988a; Wilson et al 1998) suggest that deer are an accidental host to serovar Copenhageni.
Serology and culture indicate that deer are common hosts to serovar Pomona (Table 1). The only report of persistent shedding of leptospires in deer (Fairley et al 1984) shows this serovar was shed for at least eight months in one deer, and at least 2.5 – 5 months in other deer in the same herd. Those authors stated however, that "As might have been expected vaccination did not prevent leptospiuria in animals which were already infected", inferring but not confirming that the deer from which urine was collected had been vaccinated. The period of shedding may have been longer in non-vaccinated deer. Thus, the proposition by Fairley et al (1984) that deer may not be maintenance hosts for serovar Pomona lacks definitive evidence. Those authors presented evidence of transmission of Pomona from cattle to deer, whereas Dean et al (2005) demonstrated transmission by newly introduced young deer. Serological evidence of serovar Pomona infection in a herd in a longitudinal study of almost 2-years duration (Ayanegui-Alcérreca et al 2004) suggests that farmed deer can at least be a maintenance population, if not an individual reservoir host, for serovar Pomona.

The substantial evidence of widespread infection with serovar Hardjobovis (Tables 1 and 2) coupled with evidence of persistent infection within herds over 21 months (Ayanegui-

Alcérreca et al 2004) is strong support for the hypothesis that farmed deer are maintenance (reservoir) hosts and population for this serovar.

Relationship between serovar and disease

Table 4 presents the relationships between host status and expression of disease. Most cases of clinical leptospirosis in deer have been associated with serological and/or culture evidence of serovar Pomona (Tables 1 and 2). However, while it appears that serovar Hardjobovis is not commonly associated with clinical disease, it is not possible to rule out that this serovar can, on occasion, cause clinical disease. Wilson and McGhie (1993) reported that serovar Pomona was associated by serology with 90% of diagnostic laboratory submissions for leptospirosis in deer, while serovar Hardjobovis was associated with 10% of cases submitted. Only two cultures were reported from 70 submissions although few were requested or attempted, and both yielded serovar Pomona. It is difficult to make conclusions about disease causation based on laboratory reports of serology alone as in some of the cases reported, because many apparently healthy deer present as sero-positive to a number of serovars (Wilson et al 1998). A recent survey of veterinary practitioners indicated that a small number had observed clinical signs and outbreaks associated with serovar Hardjobovis (Ayanegui-Alcérreca unpublished). One outbreak of redwater and jaundice in a group of weaner deer was associated with serological evidence of serovar Copenhageni in the absence or titres to serovars Pomona or Hardjobovis (Walker, pers com.). Of 18 submissions for investigation of sudden death, 16 yielded Pomona titres, whereas only two deaths yielded Hardjobovis titres (Howell 1991; Wilson and McGhie 1993).

In contrast to clinical disease, the appearance of kidney lesions resembling those caused by leptospirosis are frequently identified at deer. Wilson et al (1998) reported higher prevalence of gross kidney lesions from herds with serological evidence of infection, with both serovars Pomona and Hardjobovis, than from sero-negative herds, and produced histological evidence that suggested that leptospiral infection was likely to be the main cause of lesions. However, only three of ten randomly sampled kidneys that were culture positive had histological lesions suggesting that infection may not produce gross or histological lesions in all cases.

Epidemiology of infection in deer

Little is known about the epidemiology of leptospiral infections in farmed deer, but this is the subject of current research by the authors to be published in due course. It would appear that serovar Hardjobovis is endemic in the majority of herds (Flint et al 1988a; Wilson et al 1998; Ayanegui- Alcerreca et al 2003). The majority of reports of clinical disease associated with leptospirosis in deer are from deer in their first year of life (Table 1). One instance of death of a four-day-old calf with lesions typical of leptospirosis and spirochaetes in renal tubules was reported by Fairley et al (1986). In a review of laboratory submissions for leptospirosis in deer from 1987 to 1992, Wilson and McGhie (1993) reported that 77% of reported mortalities occurred in deer less than 12 months of age with the majority of deaths occurring during autumn, corresponding to a period when animals are commonly moved from farm to farm and mixed with other animals. Fairley et al (1986) provided evidence of infection with serovar Pomona of young deer transmitted from cattle recently introduced to the property, and Dean et al (2005) demonstrated introduction of infection by serovar Pomona to a property by newly introduced weaner deer. It is likely that infection with serovar Copenhageni was by association with rats (Flint et al 1986).

Preliminary data of samples from a serum bank of 10 weaners, yearlings and adult deer from 14 farms observed over a two year period, suggested an age difference in prevalence of serovar Hardjobovis being 7-31% in weaners at 3-4 months of age, increasing to 50% at 15 months of age and 71% in adults. This demonstrates that where infection is endemic, it occurs primarily in the first year of life (Ayanegui-Alcérreca et al 2003).

Clinical signs and gross pathology

With the exception of two cases reported by Wilson and McGhie (1993) in which serovar Hardjobovis was implicated, and one case of sudden death with jaundice and redwater associated with serovar Copenhageni (Walker, pers com), all clinical cases confirmed with leptospirosis have been associated with serovar Pomona. A recent survey of veterinary practitioners (Ayanegui-Alcerreca, unpublished) confirms that few cases can be attributed to serovar Hardjobovis. Dean et al (2005) provide the only report of confirmed leptospirosis in live deer, describing lethargy and red urine observed on the deer yard floor when treating the mob in the face of an outbreak. This phenomenon has been observed earlier by one of the authors (PRW). Photosensitisation, corneal opacity and jaundice were reported by Bertram (1986) in a young red deer with a titre to Pomona of 1:200, and histological lesions suggestive of leptospirosis, but in the absence of leptospires. The principal presenting sign of leptospirosis in case reports (Fairley et al 1984, 1986; Dean et al 2005) and in submissions to veterinary diagnostic laboratories was sudden death with or without gross pathology (Wilson and McGhie 1993, 40% of investigations), and redwater (Table 2). Poor reproductive performance was investigated in 4% and abortion in 2% of laboratory submissions but leptospirosis could not be confirmed as the cause. Other signs prompting investigation for leptospirosis included jaundice, low weight gain, anaemia and fading.

The only detailed descriptions of necropsy findings typical of leptospirosis in deer are by Fairley et al (1984, 1986), and Dean et al (2005). Jaundice, enlarged kidneys that were sometimes pale with radial bands of tissue, swollen liver with rounded borders, red urine and anaemia have been described, but are not observed in all cases. Dean et al (2005) described the absence of gross lesions in two deer dying suddenly from leptospirosis. Of 33 laboratory reports reviewed by Wilson and McGhie (1993), jaundice was described in 73% of cases, with redwater in 33%, kidney lesions in 33%, liver lesions in 6% and decreased fat reserves in 6%.

Gross kidney lesions, ranging from small numbers to multiple red and/or white spots, red and/or white mottling, and fibrotic scarring are frequently observed at slaughter in apparently clinically healthy animals (Wilson et al 1998). Some kidneys were observed with severe lesions suggesting active pathology, and leptospires were isolated from some of those kidneys. A higher frequency of kidneys with lesions was observed in deer from seropositive lines. There have been no studies reported of sub-clinical effects of leptospirosis in deer, but the extent of lesions in kidneys from some herds and individuals suggest reduction of growth rate may occur in young animals.

Histopathology

The main histological lesions observed specific to leptospirosis are reported from the kidney (Table 2), with lesions in some other organs, particularly the liver (Wilson and McGhie 1993), usually typical of acute septicaemic disease. Those authors described the frequency of lesions in 34 cases submitted to diagnostic laboratories for histopathology being haemoglobinuric nephrosis (26%), nephrosis (18%), visible leptospires (41%), haemoglobin casts (15%), interstitial nephritis (24%) nephritis (6%) and other (21%). Fairley et al (1984, 1986) provide the most comprehensive histological description of leptospirosis from clinical cases. Liver lesions included centrilobular necrosis, hepatocyte vacuolation and sometimes bile retention, erythrophagocytosis and heamosiderin pigmentation. Renal lesions include cortical segmentation with radial bands of inflammatory tissue, infiltration of mononuclear cells, interstitial inflammation and oedema, nodules of lymphocytes and fibrosis. Tubules are often dilated and contain casts and hyaline droplet degeneration. Glomerular lesions including peri-glomerular fibrosis, proliferation of Bowman's capsule and glomerular atrophy were seen in areas involving interstitial pathology. Leptospires are often seen in tubules when stained with the Warthin-Starry stain.

Wilson et al (1998) and Wang et al (1992) described histological lesions related to various categories of gross lesions observed in kidneys at a deer slaughterhouse as summarised above. Lesions in kidneys with white spots consisted of extensive focal interstitial infiltration of lymphocytes and plasma cells in the cortex and accumulation of leucocytes in tubules, typical of leptospirosis in other species. Leptospires were observed in the tubules of kidneys from three of 10 culture positive kidneys. Lesions were more severe and common in kidneys from sero-positive farms.

Treatment and vaccination

Treatment of clinical cases of leptospirosis in deer in New Zealand has been reported by Howell (1991) and Dean et al (2005). Anecdotal reports from veterinarians suggest use of streptomycin or oxytetracycline, as recommended by Mackintosh (1993) is common. These antibiotics are commonly used for prophylactic treatment of in-contact deer when outbreaks are experienced or exposure is imminent such as described by Dean et al (2005). Vaccination has been reported, either in the face of an outbreak (Fairley et al 1984; Dean et al 2005), often with concurrent prophylactic antibiotic treatment, or where management of risk either to humans or animals is desired (Howell 1991).

In the only published study of serological responses to vaccination, Wilson and Schollum (1984) reported few serological responses in six deer to a sensitiser dose, while all but one produced a serological response following a booster four weeks later. Initial responses were similar to those observed in cattle, but titres fell to undetectable levels after three to five months whereas titres reported in cattle generally persisted for 12 months. Serological responses were significantly higher in deer that were sero-positive before vaccination. While annual vaccination of hinds to provide passive immunity to the weaners, followed by vaccination of the weaners in autumn has been recommended in herds at risk (Mackintosh 1993), no studies of protection against either natural or artificial challenge, or the effectiveness of various vaccination regimes have been reported in deer. This is despite there being three leptospiral vaccines registered for this species in New Zealand (Index of Veterinary Specialties, New Zealand). Fairley et al (1984) demonstrated that vaccination did not stop shedding of leptospires from animals already infected with serovar Pomona. Recent research (unpublished) prompted Wilson et al (2005) to suggest that vaccination will likely

play an important role in any industry strategy for managing the risk of leptospirosis to animals and humans within the New Zealand deer industry.

Human leptospirosis associated with deer

Leptospirosis is hyper-endemic in New Zealand and it is a frequent cause of disease in farmers and meat workers (Crump et al 2001), and the zoonotic potential of deer leptospirosis has been reviewed by Wilson and Davies (2003). Observations of urine shedding in clinical cases and in-contact deer (Fairley et al 1984, 1986), and from an infected herd without clinical disease (Flint et al 1986), along with isolates from kidneys of apparently clinical normal deer at a Deer Slaughter Premise (Wilson et al 1998) suggest a risk to humans associated with both live and dead deer. There are unpublished reports of farmers, veterinarians and slaughterhouse workers contracting leptospirosis. Bell (2005) described acute febrile disease with non-specific clinical signs including fever, malaise, myalgia, meningitis, conjunctivitis as well as anorexia, abdominal pain, nausea and vomiting in humans caused by leptospirosis. Other clinical forms include "flu", jaundice, haemorrhage and enlargement of the spleen and liver, with the most severe form being hepatic and renal failure or massive pulmonary haemorrhage (Weil Syndrome) which is sometimes fatal. Bell (2005) reported eight cases in deer slaughterhouses in Southland in 2002-3 with a risk of 5/38 workers in the second year. Brown (2005) reported two severe cases requiring hospitalisation in workers at an Otago Deer Slaughter Premise. That author reported a cost of approximately $5000/case to the Deer Slaughter Premise. To date no fatalities have been reported.

Conclusions

This review of deer leptospirosis in New Zealand deer has shown serological, bacteriological and pathological evidence of infections with serovars Hardjobovis, Pomona, and Copenhageni. Most serological surveys suggest infection is widespread on deer farms in this country, but the incidence rate of clinical disease is unknown. It seems that in New Zealand deer behave as a maintenance host species for serovar Hardjobovis with widespread infection but little if any disease. At the individual level, deer appear to act as an incidental/accidental host to serovar Pomona with severe disease but at the population level could act as an alternative maintenance population. Clinical disease, both as individual cases and outbreaks has been reported frequently in laboratory reports relative to other deer diseases, mainly associated with serovar Pomona. Clinical presentation and epidemiology appear similar to that in cattle in New Zealand. The role in human cases is becoming more apparent. More research is needed to define control strategies for the infection in farmed deer in New Zealand.

Table 1. Summary of clinical and research reports of leptospirosis in deer in New Zealand. MAT = Microscopic Agglutination Test, ND = Not done or not described, Weaner = 3-13 months of age, Neg = Negative; Pos = Positive.

Author/ Year Source/ report type	Deer Species+ Age	Sample Size	No. of serovars tested	Serovar MAT Seroprevalence	Culture	Lesions Gross/ Histopathology	Region
Daniel (1966) Wild deer, serological survey	Red deer + Mixed age	109	8	Pomona, 0.9%	ND	ND	North Island (Kaingaroa)
Daniel (1967) Wild deer, serological survey	Red, fallow, virginian, sika + Mixed age	279	8	0	ND	ND	North and South Island
Fairley et al. (1984) Farmed deer, clinical report	Red deer + 3-4 months	12/70	5	Pomona 1:100-1:12800, 66.6% Pomona + Hardjo, 8.3% Hardjo 1:100, 8.3%	Pomona, Hardjo	Yes	North Island (Rotorua)
Fairley (1984) Farmed deer, clinical report	Red deer + Weaners	16/>100	ND	Pomona 1:24-1:768 62.5%	Pomona Hardjo	Yes	South Island (Southland)
Inglis (1984) Wild deer, serological survey	ND	24	ND	Pomona, 16.6% Pomona+ Icterohaemorrhagiae* 16.6% Icterohaemorrhagiae*, 8.3% (*copenhageni)	ND	ND	South Island (Nelson)
Wilson & Schollum (1984) Farmed deer, vaccine trial	Red deer + Adult hinds	6	2	Hardjo, 1:24-1:384, 33%	ND	ND	North Island (Manawatu)
Flint (1985) Farmed deer, serological survey	ND	6/26	ND	Hardjo, 23%	Hardjo	ND	South Island (Nelson)
Williams (1985) Farmed deer, serological survey	Not stated Yearlings	120 (10 properties)	ND	Hardjo, Copenhageni	ND	ND	South Island (Nelson)
Fairley et al (1986) Farmed deer, clinical report	Red deer + Fawns and weaners	4 clinical 16 in-contact	7	ND (clinical) In-contacts: Pomona, 62% Hardjo, 12%	Pomona (5/6) Hardjo (1/6)	Yes	South Island (Southland)
Flint et al (1986) Farmed deer, serological survey + culture	Red deer + Adult hinds	27	5	Hardjobovis 1:32-1:128 21.4%	Hardjobovis (8/27) Copenhageni (3/27)	ND	South Island (Nelson)
Flint (1988a) Farmed deer, serological survey (5 farms)	Red, fallow deer + Mixed age	360	7	Hardjo 1:10-1:10240, 39% Pomona 1:10-1:320, 14% Copenhageni 1:10-1:320, 43% Australis 1:10-1:20, 13% Ballum 1:10-1:80, 47% Bratislava1:10-1:160 53% Tarassovi 1:10-1:80, 26%	ND	ND	North and South Island
Wilson and McGlue (1993) Farmed deer, retrospective study	Red deer , Wapiti cross, Fallow deer +Mixed age	309 tested 345 death	ND	Hardjo Pomona Copenhageni	Pomona	Yes	North and South Island
Wilson et al (1998) Farmed deer, serological survey + culture (53 farms)	Red deer, Wapiti cross + Mixed age	675	4-7	Hardjo 1:24-1:1536, 73.6% Pomona 1:24-1:1536, 41.5% Copenhageni 1:241:1536, 11.3% Tarassovi ,15.1	Pomona (1/10) Hardjobovis (3/10) Not typed (6/10)	Yes	North Island (Lower)
Reichel (1999) Farmed deer, serum bank survey	Red deer + Adult hinds	417	3	Hardjo >1:100, 9.8 % Pomona >1:100, 1.4% Copenhageni >1:100, 0.2%	ND	ND	North and South Island
Dean et al (2005) Farmed deer, clinical report	Red deer + Adult hinds and weaners	75	3	Pomona 1:24-1:3072, 26% Hardjo 1:24- 1:48, 1.3%	ND	Yes	South Island (Southland)

Table 2. Data summarised from quarterly veterinary diagnostic laboratory reports in various issues of Surveillance (Ministry of Agriculture and Forestry and its predecessors, Wellington, New Zealand) describing leptospirosis cases in deer since first reported in 1980.

Year (Vol. No.)	No. cases**	Age/sex	Location/ source	Signs/Lesions	Serology*	Histology	Mortality rate±
1980 (3)	1	Adult hind	NR Farm	Haemolysis, redwater	Pomona (Pomona, Hardjo, Ballum and Tarassovi in 5/12 deer tested in same farm	Nephrosis	1/42
1989 (3)	6	NR	NI(5), SI(1) Farm	Redwater	Pomona	NR	NR
1990 (2)	Several	NR	North and Central NI Farm	Jaundice, anaemia	NR	Nephrosis	10 %
1990 (3)	1	Yearlings	NR	Focal renal scarring	Pomona	NR	55/1000
1991 (1)	2	Yearlings Weaners	NI Farm NI Farm	Sudden death NR	Pomona 1:200-1:12800 Pomona	Chronic interstitial nephritis	55/3000
1991 (4)	1	Weaners	NR Farm	Jaundice, redwater, sudden death	Pomona >1:800	Nephrosis, hepatic degeneration	10/60
1993 (1)	1	Yearlings	NI Slhouse	White spotted kidneys(27/30)	NR	Chronic interstitial nephritis	
1993 (2)	1	Yearlings	Slhouse	White spotted kidneys	Hardjo 1:25600 Pomona 1:800	NR	
1993 (3)	Several	Yearlings Weaners	SI Slhouse Farm	White spotted kidneys Jaundice, redwater	Hardjo Pomona NR	Leptospires in tubules NR	4/350
1996 (2)	1	Yearling hinds	NR Farm	Diagnosed pregnant, did not calve	Hardjo 1:50-1:400	NR	
1997 (3)	1	Fawns	NR Farm	Watery blood, sudden death	Pomona	Nephrosis, + leptospires in tubules	5/1174
1998 (1)	2	NR	NR Slhouse	White spotted kidneys	Not done	Leptospires in tubules	
1999 (4)	1	NR	NR Slhouse	NR	NR	Nephritis, + leptospires in tubules	
2000 (2)	2	Weaners	NR Farm	Suuden death, redwater.	NR	Nephrosis	7/500 NR

2000 (3)	Several	Weaners	NI Farm	Sudden death, redwater	Pomona	Leptospires in tubules	25/176 NR
2003 (4)	1	Yearling hinds	SI Slhouse	Enlarged spleen and dark kidneys	Ballum	Intravascular haemolysis	
2004 (3)	3	Weaners	NR SI Farm	Diffusely jaundiced Depression, red water Sudden death	Pomona >1:1600 Pomona >1:800 NR	NR Chronic interstitial nephritis Leptospires in tubules	3 20/450 16/110
2005 (1)	1	Yearlings	SI Slhouse	White spotted kidneys	Pomona	Chronic interstitial nephritis	

* Highest titre is given where available, ** Number of reports where available, NI = North Island, SI = South Island, NR = Not reported, Slhouse = slaughterhouse, ± Mortality rate (No. of deaths per 100 head of population)

Table3. - Epidemiological definitions of leptospiral host status (Hathaway 1981b).

Host status	Epidemiological definition
Maintenance host or reservoir.	An animal that is capable of acting as a natural source of infection for its own species.
Incidental or accidental host.	An animal that gets infected with a serovar hosted by another animal species, but in which the serovar does not persist.
Maintenance population.	Population of a species of animals that acts as a continuous reservoir of infection in a specific ecosystem.
Alternative maintenance population.	In general defined by the reservoir animal, but if not present other animal species can serve as a maintenance population.

Table 4. – Relationship between leptospiral host category and clinical and/or sub-clinical expression of disease (Heath and Johnson 1994)

Maintenance (reservoir) host	Incidental (accidental) host
• High susceptibility to infection, but low pathogenicity to the host • Endemic transmission within species (high titre prevalence) • Tendency to cause more chronic than acute disease (low titre in positive culture animals, mild pathology) • Long kidney phase (long term shedders).	• Relatively low susceptibility, and highly pathogenicity for the host • Sporadic transmission within the host species (low titre prevalence) • Tendency to cause acute and severe rather than chronic disease (high titre in positive culture animals, acute and dramatic pathology) • Short kidney phase (short term shedders).

Attachment 2

A review of international reports of Leptospirosis in deer.

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Note: To be submitted as a review paper.

A review of international reports of Leptospirosis in deer.

Abstract

Leptospira infection in feral and farmed deer occurs worldwide and can be a significant medical and economic problem. This paper reviews the literature on leptospirosis in deer first available from 1957 to 2005 including reports from 16 countries. Forty five (68.2%)were from wild deer including four investigations where evidence for leptospirosis was absent, 16 (24.3%) were from farmed deer with nine of those from New Zealand, three were experimental infection of captive deer, and two were from a zoo. The majority of reports are from red (29.5%), white-tailed (16.5%), fallow deer (*Dama dama*) (17.7%), and roe (9.5%). Most reports (72.7%) are based on serology only with evidence for 19 serovars, with the most common serovars reported being Pomona (26.2 %), Hardjobovis (14.9 %), Copenhageni (14.7%), Grippotyphosa (10.5%) and Ballum (6.3 %). However, only serovars Hardjobovis, Pomona, Copenhageni and Roumanica, have been cultured from deer.

Disease, culture and gross and/or histopathological lesions were described in 18 (27.2%) reports involving red, white-tailed, wapiti and Sika deer, following either natural or artificial challenge, and associated with serovars Pomona, Hardjobovis, Copenhageni, Roumanica and Ballum However, proof of causation of clinical disease, requiring culture, has been described only for Pomona, Copenhageni and Roumanica, although subclinical kidney lesions are caused by Hardjobovis. Evidence suggests that various deer species can act as a reservoir (maintenance) host for Hardjobovis, an accidental individual and alternative population maintenance host for Pomona and possibly accidental hosts for serovars Roumanica and Copenhageni. Insufficient evidence exists for establishment of the host:organism relationship for other serovars, or whether there is a deer species difference in susceptibility to infection by the various leptospiral serovars.

Key Words: Deer, *Leptospira*, leptospirosis, international, review.

Introduction

Leptospirosis is a contagious, emerging disease of animals and humans caused by infection with the spirochaete *Leptospira* (OIE 2000). Leptospirosis can be acquired through direct contact with animal reservoirs and/or from the environment that has been contaminated by urine (Kawabata et al 2001). The disease has a wide range of clinical manifestations including weight loss, fertility problems, abortions, sudden death, septicaemia with liver and kidney damage, and haemolysis and haemoglobinurea among other problems (Sasaki et al 1999) causing significant economic loss in a range of domesticated livestock species in many countries. The ability of pathogenic leptospires to infect a wide range of hosts is a risk factor for disease in both humans and domestic animals (Haydon et al 2002) and contributes to leptospirosis being one of the most frequent and widespread zoonoses globally (Levet 2001).

Reports link leptospiras to a wide range of deer species in wild, farm and zoo environments. The organism has caused significant disease and economic loss in farmed deer in New Zealand (NZ) (Ayanegui-Alcérreca et al 2006a). Relatively little is known of leptospirosis

and the role of deer in the epizootiology of leptopiral infections in most countries (Haigh and Hudson 1993; Heath and Johnson 1994). Evidence from free-ranging and farmed deer indicates that *Leptospira* are prevalent in many deer populations globally, but information in the species is scarce compared with that available for other animals or humans (Ellis 1994; Ayanegui-Alcérreca et al 2004). An understanding of the occurrence, epidemiology and host:organism relationships is essential to underpin the rationale for and development of control strategies for this disease, particularly in farmed deer populations as exist in NZ and elsewhere. A detailed review of leptospirosis in deer in NZ with particular reference to the farming environment is published elsewhere (Ayanegui-Alcérreca et al 2006). This paper reviews the international literature of leptospirosis in deer.

Scope of this review

This review summarises information from 66 accessible publications in the technical literature since the first available report in 1957 as summarised in Table 1. It reports observations from 18 countries, with the majority being from the United States of America (USA) (27.2%), NZ (24.2%) and Australia (10.5%). Evidence exists for 19 serovars based on serological evidence alone but four serovars only based on culture evidence (Table 3). Forty five (68.1%) reports were from wild or feral deer while 16(24.2%) were from farmed deer, 3 (4.5%) were from captive deer and 2 (3.0%) were from zoo deer. In this review, discussion of data relating to the distribution and epidemiology of leptospirosis in deer applies only to natural infection, excluding data from the three experimental investigations involving artificial challenge from those considerations. Further, data from four investigations which failed to detect evidence of leptospirosis are not included in summaries of distribution or epidemiological evaluations.

Of 45 reports in wild deer, 33.3% were from the USA, and of reports in farmed deer, 81.2% (13/16) were from NZ with two from Australia and one from China. The deer species in which evidence of leptospirosis was most commonly reported were red deer, wapiti and their hybrids (29.5% of reports), fallow deer (*Dama dama*) (17.7%), white-tailed deer (*Odocoileus virginianus*) (16.5%),and from roe deer (*Capreolus capreolus*) (9.5%) (Table5.2). Some reports did not state taxonomical classification.

Taxonomy

For taxonomic purposes and as an aid to epidemiological studies, more than 250 serovars have been classified into 23 serogroups based on antigenic relationships (OIE 2000). Leptospiras can be calssified as *Sensu lato* where Leptospiral strains have been grouped in serovars and serogroups based on heterogeneity of the carbohydrate component of lipopolysaccharide (Nascimento et al 2004a) and *Sensu stricto,* containing 17 genomospecies based on DNA relatedness (Faine et al 1999). Serological based classification has been the convention for most epidemiological descriptions and research (Sasaki et al 1999), and is the convention adopted in this paper. It should be noted that while Copenhageni and Icterohaemorrhagiae are extremely close in DNA profile and are antigenically indistinguishable, they are reported here using the terminology used in the original publications. The originally recognized serovar Hardjo currently recognized two distinctive serovar which, are Hardjobovis and Hardjopajitno, though serologically virtually

indistinguishable are genetically distinct species. The terminology for these serovars used here was those reported in the original publications.

Serological and bacteriological data.

Most reports refer to serological data alone while few have included bacterial isolates, pathology evidence, or clinical disease. Culture of the microorganism rather than serology is the highest diagnostic standard for study of leptospirosis (Faine et al 1999). Only four serovars (Pomona, Hardjobovis, Copenhageni, and Roumanica) have been cultured from deer out of the nineteen serovars in the reports (Gou, 1981; Mackintosh 1984; Flint et al 1988a; Wilson et al 1998).

Table 1 summarises serological, bacteriological and pathological (gross and histopathology) evidence for the presence of nineteen serovars in deer. Serological testing, employing the microscopic agglutination test (MAT), was used in every report, while culture evidence was presented in 10 reports. The most frequent serovars reported were Pomona (26.2%), Hardjo, Hardjobovis (14.9%) and Copenhageni and/or Icterohaemorrhagiae) (14.8%) Grippotyphosa (10.6%), and Ballum (6.3%) in addition to 13 other serovars.

Leptospirosis has a worldwide distribution. Pathogenic leptospires show variations between the serovars present in each region and countries in terms of prevalence, epidemiological and environmental factors (Bharti et al 2003) (Tables 1 and 3). The most frequently reported from America and Australasia was Hardjo or Hardjobovis, Pomona, and Copenhageni and or Icterohaemorrhagiae, whilst for Europe and USA it was Grippotyphosa and Ballum (Tables 1 and 3).

The gold standard for this seroepidemiological studies has been the microagglutination test which is the reference method for leptospiral diagnosis. It, since the technique can identify the serovars (Plank and Dean 2000). While serological tools can be used as way to monitor a population eventually, the ultimate confirmatory evidence comes from a characterized culture of these fastidious microorganisms by serological tests (serovar specific MAT) and/ or molecular (DNA) (Faine et al 1999). Pomona, Hardjobovis, Copenhageni (Red and Wapiti and its hybrids) and Roumanica (Sika) had been isolated from framed deer, where as and the first serovar has also been cultured from wild deer (Red and White tailed) and experimental challenges in captive white tailed deer (Tables 1 and 5).

Serological titres are an indirect evidence of contact between the animal and the bacteria (OIE 2000) and do not necessarily confirm current infection. However, there is significant cross-reaction with other serovars within the same serogroup or genomo-group, particularly when the antibody titre of the infecting serovar is high. Usually the closely related serovar will react at a lower level than the infecting serovar (E.g. Pomona and Copenhageni, or Hardjobovis and Tarassovi) (Hellstrom 1978; Faine et al 1999; OIE 2000). In an area of low prevalence, even a low titre can be considered diagnostic, whereas in highly endemic areas, many individuals are likely to have persistent antibodies due to past infections and a relatively low titre in a single sample may be difficult to interpret (WHO 2001). Suggesting that serological evidence alone is not necessarily diagnostic of exposure to or infection by a given serovar, this is particularly important for the correct identification of the serovars present in a country and the possible cross-reactivity of antibodies between some serovars (Faine et al 1999). Although the titre

dilution is an important consideration in determination of the possibility of cross-reactivity, a titre of ≥1:100 is considered as the standard cut-off point to define infection (OIE 2000).

From all reports presented in this review, only serovars Mini, Andaman and Batavie had shown titres lower than 1:100. The other 16 serovars had been reported at least once above the reference titre, with serovars Pomona, Hardjobovis, Copenhageni and/or Icterohaemorrhagiae, Grippotyphosa and Ballum as the most prevalent ones it is notable that the strong serological evidence for infection with previous serovars (Tables 1 and 3). .Hardjobovis, Pomona, Copenhageni and Roumanica has been confirmed by culture for each serovar, with Pomona being the most commonly isolated (Table 5). However ultimately, proof of infection for those serovars for which strong inference of infection cannot be derived from serology alone can only be achieved by culture such as Grippotyphosa (serological evidence) and Ballum (pathological evidence) or the other 13 reported serovars (Table 1).

Clinical disease and mortalities

There are 6 reports of clinical disease caused by leptospirosis in wild deer. Abdulla et al (1962) in Canada reported positive serology to Grippotyphosa, Pomona and Icterohaemorrhagiae in white tailed deer, the reaction to the later serovar were considered a cross reaction with Pomona, while the titres from the other two were concluded as positive titres. From the seropositive animals to Pomona the most relevant observations were a titre range 1:60 to 1: 1,14,580, the only lesion observed from this animals were interstitial nephritis in the cortical area of the organ, with a focal distribution. The severity of the lesion was described as parallel to the elevation in titre. Four isolates were reported in this same paper. It was noticeable that the highest serological titres, histological lesions and cultures came from animals that were found near a cattle herd that had recently experience a Pomona outbreak. Kingscote et al (1987) also in the same country reported positive serology in 6 of 24 Wapiti deer to serovars Autumnalis, Icterohaemorrhagiae and Bratislava. Interstitial nephritis was described in 8 or 20 collected kidneys, three of those were from seropositive animals, and one kidney was positive to the immunofluorescence antibody test (IFA) to serovars Autumnalis and Bratislava.

Two reports were from the USA. Reilly et al (1962b) isolated Pomona from the urine of a white tailed adult male with a antibody titre of 1:100. The isolate was used later to in a control challenge of a seronegative 7 month-old female, that developed fever, a titre of 1:800, but the strain could not be re-isolated. Roth et al (1964) cultured Pomona from the renal tissue of one seropositive white tailed deer from a group of five animals, three were seropositive to the cultured serovar, four presented focal interstitial nephritis at the histopathological examination.

Corrigall (1978) in the UK described a disease course of 6 days, in a Ballum seropositive 2-years-old red deer male (with a titre rise in 10 days from 1:100 to 1:1600). The signs were congested oral and mucosal membranes, incoordination, dullness with a high leucocytes count and death. The postmortem results included congested and oedematous lungs, jaundice, yellow pericardial fluid, haemorrhages in the liver border, gut mucosal surface, pale pulpy kidney. Dufree and Presidente (1979) from Australia reported interstitial nephritis from the kidneys of 8 deer, 3 were from a group of 35 seronegative fallow deer, and 5 from a group of 19 Rusa deer where 68.4% were seropositive to Hardjo.

Clinical disease caused by leptospirosis in farmed deer in China has been reported in a summary 1953-1963 by Gou (1981) associated with, Roumanica characterized by acute clinical course of 10 days, with haemoglobinuria "port wine" coloured urine, pyrexia, haemolytic anaemia, jaundice with a mortality >90%. Postmortem results were haemorrhagic lungs, necrotic lesions in the liver and kidneys, bacterial isolation from postmortem specimens, and positive serology to this serovar 1:400 from more than 200 affected animals. The reported incidence rate was 75%, outbreaks occurred mainly in autumn and winter, with no sex or age predilection, the source of infection was considered to be mice acting as a carrier host, outbreaks were controlled with vaccination (Gou 1981).

In NZ, Leptospirosis in farmed deer can range from subclinical to acute outbreaks with acute septicaemia, haemolysis, anaemia and haematuria, where morbidity can reach 50% and mortality 10% (Wilson and McGhie 1993). In farmed deer in NZ most clinical leptospirosis cases have been associated with serovar Pomona in young animals (3 to 12 months) during autumn and early winter when deer are often moved between farms and mixed with other herds or mobs (Wilson and McGhie 1993; Dean et al 2005). In that country, despite infection with Hardjobovis being commonly recognised in farmed deer with positive serology, mild kidney lesions and positive isolates from urine and kidneys, its role as a causative agent for clinical disease remains equivocal (Fairley et al 1984 and 1986; Flint 1986; Wilson et al 1998; Ayanegui-Alcérreca et al 2005). It has been suggested that males are more susceptible than females (Reilly et al 1962b; Harrington 1975).

Three authors in the early 1960s in USA reported on the experimental controlled challenges with Pomona in the white tailed deer. Ferris et al (1960) challenged 4 adult females with an swine Pomona isolate, reported moderate pyrexia, a subclinical presentation on three of the animals and clinical disease only in one. The study relevant outcomes were re-isolation of the challenge strain in the bacteraemic phase, rise in the leukocyte counts, titre against Pomona up to 10,000,000, kidney tubular and glomerular degeneration, liver and spleen lesions with hemosiderin deposition and foci of necrosis. Trainer et al (1961) a similar strain observed sub-clinical disease and abortions in four of 5 pregnant does, renal histological changes were observed and antibody titres of 1:100,000 were reported 10 to 15 days after challenge. Reilly et al (1962a) reported pyrexia, anorexia, diarrhoea, weight loss, with re-isolation of the challenge strain in the bacteraemia phase, accompanied with a sudden rise of agglutinating titres 1:1000 to 1: 256,000 at the necropsy lung congestion was observed, renal haemorrhages and focal areas of necrosis.

Serovars Pomona, Copenhageni, (Icterohaemorrhagiae) and Roumanica infections in deer have been reported as more severe than those with Hardjobovis, with the latter usually being sub-clinical (Mackintosh 1984; Ayanegui-Alcérreca et al 2004). Copenhageni and Roumanica both appear to cause clinical disease in farmed deer and are probably associated with incidental contact with rodent reservoir hosts (Haigh and Hudson 1993).

Culture and serological titres

Data from the 12 reports of culture and related titres are presented in Table 5. Serovar Pomona was isolated from four natural infections, in both white-tailed in North America and red deer in NZ and titres range from low, to 1:4860. Recovery of Pomona was reported after artificial infection of white-tailed deer and serological titres were consistently high (Table 5 (Ferris et al 1960; Trainer et al 1961; Reilly et al 1962a). Culture following natural infection with serovars Hardjobovis (titres up to 1:1536) and Copenhageni (titres up to 1:64) have been

cultured from red deer in NZ. The report of culture of Roumanica was not accompanied by serological data (Gou, 1981).

Habitat of deer with evidence of leptospirosis

Screening populations for epidemiological surveillance establishes which serovars could be present and their seroprevalence. The prevalence of the different serovars depends on the reservoir animals present and the serovars that they carry, local environmental conditions, the industrial, agronomic, and agricultural practices, and the host-serovar associations between the animals in the population (wild or domestic) (Hathaway 1981b; Bharti et al 2003). From the naturally occurring leptospirosis in deer, Hardjobovis, Pomona, Copenhageni and Roumanica were presented with strong serological and clinical, bacteriological or pathological evidence from farmed deer. Pomona has been most frequently serovar documented from wild deer, followed by Ballum with pathological and serological evidence, and Grippotyphosa only with serological data. There have been only two reports on leptospirosis from zoo deer, so few conclusions can be drawn about the presence of leptospirosis in zoo deer.

The serovars found with evidence of leptospiral infection in deer of various origins, wild, zoo or farmed, are presented in Table 4. The majority of serovars have been reported from wild deer. It is notable that Hardjobovis has been reported from a greater number of farmed deer than wild deer. This may reflect that many of these reports are from New Zealand where this serovar is common in cattle, and grazing proximity between cattle and deer is often close. More than 70% of farmed deer herds surveyed in New Zealand present evidence of infection with this serovar. The apparently higher proportion of reports from wild deer demonstrating infection with Pomona may suggest closer association with the maintenance host(s) for this serovar, (commonly swine) than in a domestic farming environment. This would be consistent with evidence that deer are an accidental individual host, but possibly an alternative maintenance population host for this serovar (Ayanegui-Alcérreca et al 2005).

Relationship between serovar and deer species host

Serovar Pomona has been cultured from White-tailed and red deer, while serovars Hardjobovis and Copenhageni have been cultured from red deer only (Table 5). Roumanica has been cultured only from Sika deer in China. Serological evidence exists for nine serovars in white-tailed and red deer, with Pomona being the most common in both deer species, and Hardjobovis being common in red deer in NZ. Five and four serovars have been reported in wapiti and Fallow deer, respectively, with Pomona and Hardjobovis being the most common (Table 1). There is a small number of reports of various serovars in other deer species. It is not possible to draw conclusions about susceptibility of various deer species to the range of serovars from the information available.

Regional distribution of serovars

There are differences in the global distribution of some *Leptospira* species in various animal species. While *L. kirschneri, L. interrogans* and *L. borgpetersenii* have a worldwide distribution, *L. interrogans* and *L. borgpetersenii* are clustered mainly in Asia and Europe, respectively, *L. noguchii* and *santarosai* are found mainly in the American continent, and *L. weilii* is found mainly in Asia (Baranton and Old 1995). The number of serological and culture reports for each serovar reported from deer in the Americas, Asia, Australasia and Europe are presented in (Table 3). Regional differences have been observed in deer the 19 reported serovars belong to the first three species. However, apparent regional differences could be linked to the surveillance system in each region or country. Furthermore, there have been limited numbers of reports either published or accessible from many countries and regions. Thus, few conclusions can be drawn about the regional distribution of leptospiral serovars in deer around the globe.

Leptospiral host definitions for deer

The relationship between leptospira and deer hosts (accidental or maintenance) is complex. Hosts can be principally defined at the individual level as maintenance (reservoir), in which pathogens can be permanently maintained, usually resulting in a high prevalence of asymptomatic infection, or accidental (incidental or spill-over) involving transmission from the normal maintenance host usually resulting in clinical disease (Haydon 2002) The alternative definition, population maintenance host, relates to maintenance of infection within the population due to transmission between non-maintenance hosts. Serological data can be useful in helping to define the host status of an animal species for leptospiral serovars (Radamas et al 1992; OIE 2000). However, high seroprevalence at a single point in time may indicate an outbreak in the host population or accidental host, rather than pathogen persistence in it (Haydon 2002).

Few reports exist that allow reasonably robust determination of host status for serovars in deer other than Hardjobovis, Pomona and Copenhageni, and those reports relate only to farmed red deer in NZ (Ayanegui-Alcérreca et al 2006a). It is proposed that red deer in the NZ agricultural environment are a maintenance host for Hardjobovis, an accidental individual and possibly a maintenance population host for Pomona, and an accidental host for Copenhageni. Evidence summarised in Table 1, particularly relating to the effect of artificial challenge (Ferris et al 1960; Trainer et al 1961; Reilly et al 1962a) suggests that white-tailed deer may be accidental hosts for Pomona. It is possible that Sika deer in China (Gou 1981) may act as an accidental host to Roumanica, since the mouse is considered the maintenance host for this serovar. However, more evidence is needed. No conclusions can be drawn about the host relationships between other deer species and serovars reported in Table 1.

It is possible that the host:serovar relationship in a feral environment may be different to that in a farmed environment because domestication of a wild animal species such as deer may alter the "natural" ecology under which this co-evolution occurred (Gay 2001). The low prevalence (0.3 – 0.9% for Pomona, and no serological evidence of Hardjobovis) reported from wild deer in NZ (Daniel 1966, 1967) contrasts significantly with the high prevalence of infection by both those serovars in farmed deer in the same country reviewed by Ayanegui-Alcérreca et al (2006a).

In accordance with the previously described concepts and the data obtained in deer, a difference in regional distributions had been observed. The environment in which animals

exist also seems to play a big part (Nidality). The documentation on deer leptospirosis published in NZ can be a good example of how once the animals' ecosystems are change and their niche relations also changes, diseases can occurred. In the 1960's, the deer population was basically a feral, by the early 1980's (500,000 farmed animals) at the beginning of the deer farming industry to today (more than 2,000,000 farmed animals) (Statistics NZ 2002). Population demographics, the environmental relations from feral to an intensive pastoral system, interaction with other animal species can be reflected in the changes of the epidemiology of the disease clinical cases and changes in prevalence at the individual and herd level from zero to>70% depending on the herd and serovar (Ayanegui-Alcérreca et al 2003).

Deer leptospirosis as a zoonosis

Wilson and Davies (2003) included leptospriosis as a potential zoonosis associated with deer, citing Edelstein and Rowden (1991) who reported leptospirosis in humans following exposure to elk in the USA, and based on evidence of gross and histological kidney lesions consistent with leptospirosis and culture from kidneys of apparently normal deer presented for slaughter reported by Wilson et al (1998). More recent evidence shows a high incidence of leptospirosis in deer slaughterhouse workers in NZ as reviewed by Bell 2005 and Brown 2005.and there are anecdotal reports of deer farmers and veterinarians contracting leptospirosis from farmed deer in that country.

Conclusion

Given the worldwide distribution of a large number of serovars of leptospira, and the importance of leptospirosis as an animal disease and zoonosis, coupled with the global distribution of 17 genera and 42 species of deer, there are remarkably few detailed studies of this genus of organisms in deer. This limits the conclusions that can be made about leptospirosis in deer, and many of the relationships described proposed in this review are tentative and must be interpreted with caution because of the paucity of substantial information. Serological evidence of 19 serovars of leptospira infections has been reported variably in eight species of deer of wild, farmed or captive origin in many countries, but there are reports of culture of only serovars Pomona, Hardjobovis, Copenhageni and Roumanica. Clinical cases appear uncommon in wild deer where infection is likely to be incidental or accidental, but are more commonly reported from farmed deer, possibly reflecting the economic significance of deer farming, and the ability for veterinary diagnosis and intervention. Subclinical infections of some serovars appear common, particularly for Hardjobovis in farmed deer in NZ. The host status appears to vary depending on the region, environment or country with deer appearing to be a maintenance host for Hardjobovis in NZ and an accidental host for other serovars. More research is needed to fully understand leptospirosis in deer worldwide.

Table 1. – Publication, country, deer species, sample size, diagnostic method and lesion status in global reports of leptospirosis in deer (1957-2005) by region. (Expt = experimental challenge, ND = not done or not reported, NS = not stated). (Deer species taxonomic names are presented in Table 5.2)

Author/ Year	Origin	Country	Deer Species	Sample Size Seroprevalence (%)	Leptospira, Serovar, (Titre range, or positive cut point)	Culture	Kidney Lesion	
							HP	GP
Americas								
Uhart et al 2003	Wild	Argentina	Pampas	14 (21.4)	Overall *Leptospira* Pomona (1:100-1:400) Wolfii (1:25-1:100)	ND	ND	ND
Mathias et al 1999.	Wild	Brazil	Pampas	41 (9.7) (4.8) (2.4) (2.4)	Overall *Leptospira* Hardjo, Wolfii, Mini. (≥ 1:100)	ND	ND	ND
Deem et al 2004	Wild	Bolivia	Gray Brocket	11 (45.5)	Overall *Leptospira* Autumnalis, Ballum, Icterohaemorrhagiae, Sejroe. (≥ 1:100)	ND	ND	ND
Abdulla et al 1962.	Wild	Canada	White-tailed	75 (28.0) (28.0) (4.0) (6.6)	Overall *Leptospira* Pomona (1:60- 1:1,620) Icterohaemorrhagiae(1:60-1:180) Grippotyphosa (1:540-1:1,620)	Pomona	ND	ND
Bourque and Higgins 1984	Wild	Canada	Moose	84 (1.1)	Grippotyphosa (1:400)	ND	ND	ND
Kingscote et al 1987.	Wild	Canada	Wapiti	24 (25.0) (25.0) (8.3) (4.1)	Overall *Leptospira* Autumnalis Icterohaemorrhagiae Bratislava (1:50- 1:200)	ND	Yes	Yes
Parras et al 1992.	Zoo	México	White-tailed	27 (85.1)	Overall *Leptospira* Autumnalis, Icterohaemorrhagiae Pomona, Others (≥1:100)	ND	ND	ND
Luna-Alvarez et al 1996	Zoo	México	White-tailed	10(10.0)	Autumnalis, Icterohaemorrhagiae Pomona, Others (1:100-1:800)	ND	ND	ND
Wedman and Driver, 1957.	Wild	USA	Wapiti	187(16.0)	Pomona (1:10- ≥1:100)	ND	ND	ND
Reynolds and Smith, 1958.	Wild	USA	Wild deer	628	(0)	ND	ND	ND
Shotts et al 1958.	Wild	USA	White-tailed	438 (1.5)	Pomona (1:100)	ND	ND	ND
Ferris et al 1960.	Captive Expt	USA	White-tailed	4 (100.0)	Pomona (1:100-1:10,000,000)	Yes	Yes	Yes
Trainer et al 1961.	Captive Expt	USA	White-tailed	5 (100.0)	Pomona (1:10-1:100,000)	Yes	Yes	Yes
Reilly et al.	Captive	USA	White-	7(85.0)	Pomona	Yes	Yes	Yes

1962a.	Expt		tailed		(1:640-1:25,600)			
Reilly et al 1962b.	Wild	USA.	White-tailed	101(22.7) (7.9) (3.9) (1.9) (10.8) (4.9) (4.9) (0.9)	Overall *Leptospira* Pomona (1:50-1:600) Icterohaemorrhagiae (1:50:1:100) Canicola (1:50) Hebdomanis(Hardjo) (1:50-1:100) Autumnalis (1:50-1:100) Grippotyphosa (1:50-1.100) Ballum (1:50-1:100)	Pomona	ND	ND
Trainer et al 1963.	Wild	USA	Wild deer	1,256 (26.0) 478 (29.4) (16.7) (11.7) (2.0) (0.2)	Overall *Leptospira* Sub sampled Leptospira Pomona Autumnalis Grippotyphosa Icterohaemorrhagiae (≥1:100)	ND	ND	ND
Roth et al 1964.	Wild	USA	White-tailed	5 (20.0)	Pomona (1:400) Canicola/Ballum (1:100)	Pomona	Yes	Yes
Shotts and Hayes 1970	Wild	USA	White-tailed	1,544 (18.9) (13.6) (5.2) (1.6) (0.9) (0.6) (1.6)	Overall *Leptospira* Grippotyphosa Pomona, Canicola Australis Icterohaemorrhagiae, Others (≥1:100)	ND	ND	ND
Harrington et al 1975.	Wild	USA	Wild deer	578 (8.3) (4.3) (2.2) (1.5) (1.3) (1.0) (1.0)	Overall *Leptospira* Pomona Autumnalis Australis Ballum Hebdomanis (Hardjo) Grippotyphosa (≥1:100)	ND	ND	ND
Cirone et al 1978.	Wild	USA	Black tailed	12 (100.0) (83.3) (58.3) (25.0) (25.0) (16.6) (8.3) (8.3)	Overall *Leptospira* Canicola Pomona Autumnalis Wolfii Icterohaemorrhagiae Australis Tarassovi (1:40-1:2,560)	ND	ND	ND
Fournier et al 1986.	Wild	USA	White-tailed	248 (7.2) (4.0) (3.2) (0.8) (0.8)	Overall *Leptospira* Grippotyphosa (≥1:100) Pomona, (1:100-1:6400) Hardjo (≥1:400) Canicola (≥1:400)	ND	ND	ND
Ingebrigtsen et al 1986.	Wild	USA	White-tailed	115 (2.6)	Leptospira spp (≥1:100)	ND	ND	ND
Goyal et al 1992.	Wild	USA	White-tailed	204 (43.0)	Pomona and / or Bratislava (≥1:100)	ND	ND	ND
New et al 1993.	Wild	USA	White-tailed	518 (2.0) (11) (6) (3) (0.2)	Overall *Leptospira* Hardjo (1:250-1:1000) Pomona (1:250-1:500) Icterohaemorrhagiae (≥1:250)	ND	ND	ND

					Canicola (≥1:250)			
Alonso-Aguirre et al 1995.	Wild	USA	Wapiti	456 (2.8) (2.8) (1.7)	Overall *Leptospira* Pomona Hardjo (≥1:100)	ND	ND	ND
Bender and Hall, 1996.	Wild	USA	Elk (Wapiti)	11(81.) (81.8) (36.6) (18.1)	Overall *Leptospira* Bratislava (1:100-1:1,400) Pomona (1:200-1:800) Grippotyphosa(1:100- 1:400)	ND	ND	ND
Asia								
Gou, 1981	Farmed	China	Sika	220 (95.0)	Roumanica Mortality (90%)	Yes	Yes	Yes
Australasia								
Munday, 1972.	Wild	Australia	Fallow	127 (0.8)	Grippotyphosa (1:30)	ND	ND	ND
Dufree and Presidente 1979	Wild	Australia	Fallow Rusa	58 (22.4)	Hardjo (≥1:32)	ND	ND	ND
Milner et al 1981.	Farmed	Australia	Rusa	12 (25.0)	Hardjo (1:28: 1:1024)	ND	ND	ND
English, 1982.	Wild	Australia	Fallow	86 (1.7)	Pomona Titre cut point NS	ND	ND	ND
Presidente and Westbury 1984.	Farmed	Australia	Fallow Rusa	116 (28.4)	Hardjo Titre cut point NS	ND	ND	ND
McKenzie et al 1985.	Wild	Australia	Fallow Red Rusa	677 (15.6) (14.5) (0.6) (0.3)	Overall *Leptospira* Hardjo Pomona Tarassovi Titre cut point NS	ND	ND	ND
English, 1985.	Wild	Australia	Fallow	76 (2.6)	Pomona (1:100)	ND	ND	ND
Daniel 1966.	Wild	NZ	Red Fallow	109 (0.9)	Pomona (1:200) Andaman (<1:200)	ND	ND	ND
Daniel 1967.	Wild	NZ	Sika	279 (0.3)	Pomona (1:10)	ND	ND	ND
Anon 1980.	Farmed	NZ	Red	42 (2.4)	Pomona Titre cut point NS	ND	Yes	Yes
Fairley et al 1984	Farmed	NZ	Red	12 (83.3) (75) (16.6)	Overall *Leptospira* Pomona (1:400-1: 3200) Hardjobovis (1:100)	Pomona and Hardjobovis	Yes	Yes
Fairley 1984	Farmed	NZ	Red	16 (62.5) (62.5) (6.2)	Overall *Leptospira* Pomona (1:24-1:768) Hardjobovis (1:24)	Pomona and Hardjobovis	Yes	Yes
Inglis 1984.	Wild	NZ	Wild deer	21(71.4)	Pomona and Copenhageni (1:50-1:100)	ND	ND	ND
Wilson & Schollum 1984.	Farmed	NZ	Red	6 (16.6)	Hardjobovis (1:384)	ND	ND	ND
Flint 1985	ND	NZ	Not	26 (23)	Hardjo	ND	ND	ND

			stated					
Williams 1985.	Farmed	NZ	Not stated	120 (Seroprevalence NS)	Hardjobovis Copenhageni	ND	ND	ND
Flint et al 1986.	Farmed	NZ	Red	27*(33.0) (33.0) (14)	Overall *Leptospira* Hardjobovis (1:32- 1:128) Copenhageni (1:0)	Copenhageni and Hardjobovis	ND	ND
Flint et al 1988a.	Farmed	NZ	Red	360 (42.5) (39.0) (14.0) (43.0)	Overall *Leptospira* (≥1:10) Hardjobovis (1:10-1:1024) Pomona (1:10-1:320) Copenhageni (1:10-1:320) other serovars	ND	Yes	Yes
Howell 1991.	Farmed	NZ	Red	55 (40%)	Pomona (1:200-1:6,400)	ND	Yes	Yes
Wilson and McGhie 1993.	Farmed	NZ	Fallow Red x Wapiti	345	Hardjobovis Pomona Copenhageni other serovar	Yes	Yes	Yes
Wilson et al 1998.	Farmed	NZ	Red x Wapiti	675 (47.7) (45.0) (12.0) (3.2)	Overall *Leptospira* (≥1:96) Hardjobovis (1:24- 1:1,536) Pomona (1:24-1:1,536) Copenhageni (1:24- 1:1,536) Other serovars	Hardjobovis and Pomona	Yes	Yes
Reichel 1999.	Farmed	NZ	Red	417 (19.8) (19.8) (1.4) (0.2)	Overall Leptospira Hardjobovis (1:25-1:1,600) Pomona (1:100) Copenhageni (1:50-1:100)	ND	ND	ND
Dean et al 2005	Farmed	NZ	Red	75(98.6) (90.6) (58.6)	Overall *Leptospira* Pomona (1:24- 13072) Hardjobovis (1:24-1:96)	ND	Yes	Yes
Europe								
Sebek et al 1976	Wild	Austria	Red Roe	623 (4.5)	Leptospira spp (≥1:100)	ND	ND	ND
Treml and Nesnalova, 1993	Wild	Czech Republic	Red Roe	534 (4.3)	Grippotyphosa Titre cut point NS	ND	ND	ND
Zitek and Babicka, 2000	Wild	Czech Republic	Fallow Red Roe Sika	745 (11.2)	Overall *Leptospira* Pomona Icterohaemorrhagiae Grippotyphosa Sejroe Canicola / Sejroe (1:100-1:3,200)	ND	ND	ND
Blancou, 1983.	Wild	France	Roe	189	(0)	ND	ND	ND
Hubner and Horsch, 1977.	Wild	Germany	Red Roe	144 (5.5)	Grippotyphosa Titre cut point NS	ND	ND	ND
Weber and Christoph, 1981.	Wild	Germany	Fallow Red Roe	399 Red (2.1) Roe (1.8) Fallow (5.7)	Icterohaemorrhagiae, Ballum, Grippotyphosa. (1:200)	ND	ND	ND
Witt et al 1998.	Wild	Germany	Fallow Roe	1,821(2)	Overall *Leptospira* (≥1.100)	ND	ND	ND
Cuteri et al 1999	Wild	Italy	Fallow	224	(0)	ND	ND	ND

Cerri et al 2003	Wild	Italy	Wild deer	143 (1.39)	Not specified	ND	ND	ND
Travnicek, 1999.	Wild	Slovakia	Fallow	24	(0)	ND	ND	ND
Twigg et al 1973.	Wild	United Kingdom	Fallow Red	210 (0.65)	Icterohaemorrhagiae, Batavie (1:30)	ND	ND	ND
McDiarmid, 1975.	Wild	United Kingdom	Chinese-water Fallow Red Roe Sika	1	Bratislava	ND	ND	ND
Corrigall, 1978.	Wild	United Kingdom	Red	3 (100.0)	Ballum (1:100- 1:1,600)	ND	Yes	Yes
Mefodév and Valvova, 1972.	Wild	Former USSR	Wild deer	n NS (7.0)	Overall *Leptospira*	ND	ND	ND
Kovacic et al 1983.	Wild	Yugo-slavia	Wild deer	270 (7.0) (3.7) (2.9) (0.4) (0.4)	Overall *Leptospira* Ballum Pomona Grippotyphosa Sejroe Titre cut point NS	ND	ND	ND
Kovacic et al 1986.	Wild	Yugo-slavia	Wild deer	128 (7.0) (3.9) (3.1) (2.3) (1.5) (0.7) (0.7)	Overall *Leptospira* Ballum Grippotyphosa Saxkoebing Sejroe Pomona Australis (1:100-1:1000)	ND	ND	ND

Table 2. Deer species and number of reports of leptospirosis in the 66 used publications.

Deer species	Taxonomic name	Number of reports	(%)
Red	*Cervus elaphus spp*	19	29.5
Wapiti	*Cervus elaphus canadensis spp*	4	
Red x Wapiti hybrid	*Cervus elaphus*	2	
Fallow	*Dama dama*	15	17.7
White-tailed	*Odocoileus virginianus*	14	16.5
Roe	*Capreolus capreolus*	8	9.5
Rusa	*Cervus timorensis*	4	4.7
Sika	*Cervus nippon*	4	4.7
Pampas	*Ozotoceros bezoarticus celer*	2	2.4
Black tailed deer	*Odocoileus hemionus columbianu*	1	1.1
Gray Brocket deer	*Mazama gouazoubira*	1	1.1
Chinese water deer	*Hydropotes inermis*	1	1.1
Moose	*Alces alces*	1	1.1
Wild deer		8	9.5
Not stated		1	1.1
Total		85	100

Note: some reports included more than one deer species

Table 3. – Number of reports of each serovar reported in deer in four regions from 1957 to 2005. Excluding experimental challenges.

Region Number of reports (%)	Serovar (number of reports)			Culture and pathology (positive)
	Leptospira. interrogans	*Leptospira. borgepetersenii*	*Leptospira. kirschneri*	
Americas 23 (36.5)	Pomona (19) Icterohaemorrhagiae (10) Autumnalis (8) Bratislava (5) Canicola (5) Wolfii (3)	Hardjo (6) Ballum (4) Mini (1)	Grippotyphosa (6)	Pomona
Asia 1 (1.5)	Roumanica (1)			Roumanica
Australasia 23 (36.5)	Pomona (15) Copenhageni (7) Andaman (1) Australis (2) Balcanica (1) Bratislava (1)	Hardjobovis (15) Tarassovi (2) Ballum (2)	Grippotyphosa (1)	Hardjobovis Pomona Copenhageni
Europe 16 (25.3%)	Icterohaemorrhagiae (3) Pomona (3) Australis (2) Bataviae (1) Canicola (1) Saxkoebing (1)	Ballum (4) Sejroe (3)	Grippotyphosa (6)	Ballum Pathology

Table 4. Number of reports (and percentage of publications in which each serovar has been reported within each habitat) in naturally infected deer.

Serovar	Habitat (%)			Total (%)
	Farmed	Wild	Zoo	
Pomona	12 (75.0)	20(44.4)	2(100)	34 (53.9)
Hardjo or Hardjobovis	12 (75.0)	9(20.0)	0	21 (33.3)
Copenhageni or Icterohaemorrhagiae	6 (37.5)	12(26.6)	2(100)	20(31.7)
Grippotyphosa	0	13(28.8)	0	13 (20.6)
Ballum	2 (12.5)	8(17.7)	0	10 (15.8)
Other (14 serovars).	9 (56.2)	28(62.2)	2(100)	39 (61.9)
Number of publications	16	45	2	63

Table 5. Leptospiral serovars isolated from deer, type of infection, number of reports, country, proportion of sample culture positive, and serological titres reported.* number of cultures was not stated.

Serovar	Infection	No. Studies	Country	Deer Species	% Culture	Titre range.
Pomona	Experimental	3	USA	White-tailed	16-80	$1:10^4$-1: 10^6
	Natural	4	NZ	Red	0.5-80.0	1:24-1:3200
	Natural	3	Canada/USA	White-tailed	3.3-33.3	1:100-1:540
Hardjobovis	Natural	4	NZ	Red	1.48-61.5	1:10-1:1536
Copenhageni	Natural	1	NZ	Red	23.0	1:32
Roumanica	Natural	1	China	Sika	Positive *	1:400

Chapter 2

General Material and Methods



2.1 Introduction

This Chapter describes the materials and methods common to many chapters throughout this thesis for the identification of *Leptospira* infected and non-infected deer herds, sample collection, and handling of the animals.

2.2 Animal ethics

All procedures involving the uses of animals in that were undertaken during the course of this had animal ethics approval from the Massey University Animal Ethics Committee, Palmerston North, and New Zealand.

2.3 Animals and farms

All the deer used in these studies were Red or Red x Wapiti cross bred deer (*Cervus elaphus*) from farms or locations in New Zealand. Management practices were standard for commercial pastoral deer farming. Deer used at the Massey University Deer Research Unit were similarly managed.

2.4 Herd screening and survey

The owners and managers of the commercial deer farms and research deer herds that were included in the study took part voluntarily on the basis of their interest in the research. The study aimed to identify infected and non-infected deer herds in the different parts of the country and the type of serovars that were present based on serology, and in some cases infections were confirmed by culture. Blood samples from 12 to 20 two-year-old or young adults were collected as described in section 2.8.1. In addition, they were used to identify suitable herds for the vaccine efficiency trials. In total, thirty-four herds were screened, twenty-eight from different farms randomly sampled in the North and South Islands at Deer Slaughter Premises (DSP) at Feilding, and Hokitika, and the rest of the samples were collected from six farms in the lower North Island. As a result of this screening process, four herds went on to participate in the vaccine efficacy trials.

A regional survey was performed to determine the distribution of infection among the different regions of the country. Veterinary practices were utilised to identify herds that had no vaccination history. The co-operation of farmers resulted in a total of 79 herds being included in the regional survey. The veterinarians collected twenty blood samples per farm, in similar manner to that described above, mainly from rising two-year-old hinds (Chapter 7). After collection, samples were shipped to Massey University, tested for agglutinating antibodies against *Leptospira* (Sv. Hardjobovis, Sv. Pomona and Sv. Copenhageni). The survey also included a questionnaire that collected data for a risk factor analysis (Chapter 7) that was sent back with the blood samples (Appendix 3).

2.5 On-farm vaccine trials

Four deer herds from the lower North Island (Manawatu and Hawkes Bay) were identified through the results of the herd screening processes and went on to participate in the research. The data obtained from these farms were used in Chapters 4, 5, 6, 7 and 8 and the identification numbers of the farms will remain constant. The general description of the farms is presented in Table 2.1 and the raw data is presented in Appendix 2.

Farms 1, 2 and 3 had no previous history of vaccination against *Leptospira*. Farm 4 had been vaccinated in the past and vaccination was stopped two years before the trials began. (Appendix 2).

Year 1= 2003, Year 2=2004, Year 3=2005.

Table 2.1 Description of trial farms. Hb. & Pom. = Hardjobovis and Pomona, Hb. & Pom. R = Hardjobovis and recent introduction Pomona.

	Farm Number						
	1	2		3	4		
Location	**Manawatu**	**Hawkes Bay**		**Wairarapa**	**Manawatu**		
Farm Topography	Flat	Hill and Flat		Hill and Flat	Hill and Flat		
Farm	Commercial	Commercial		Commercial	Research		
Production type	Venison & Breeding	Venison, Breeding & Velvet		Venison & Breeding	Research, Breeding &Venison,		
Deer Species	Red-Wapiti cross	Red		Red-Wapiti cross	Red & Red-Wapiti cross		
Herd Management	Closed	Closed		Open	Closed		
Serovar #	Hardjobovis	Hb. & Pom. R	Hb & Pom	Hb & Pom.	Negative.		
No./Yr trial	Year 1	Year 1	Year 2	Year 2	Year 1	Year 2	Year 3
Deer farm/herd demographics.							
Deer fenced Ha.	40	396	396	190	22	22	22
No. of Deer	398	1357	1395	1500	162	185	191
Weaner males	71	316	350	370	29	38	35
Weaner females	73	261	350	370	36	30	38
Yearling males	27	42	15	0	0	0	0
Yearling females	62	100	120	0	7	16	0
Adult males	10	8	10	10	11	11	18
Adult females	170	630	550	450	79	90	100
Weaning month	May	Jun	Feb	Jun	Jun	Feb	Feb-Mar
Other animals							
Herd Management°	Open°	Open°	Open°	Open°			
Cattle dairy	0	0	0	200°	0	0	0
Cattle beef	0	150°	150°	80°	0	0	0
Sheep	80°	200°	300°	200°	0	0	0
Pigs	0	3	0	0	0	0	0
Horses	0	20	20	0	0	0	0
Dogs	7	6	6	6	0	0	0

Serovar involvement based on serology confirmed by culture; ° indicates which species were farmed with the "open" classification.

2.5.1 Leptospiral diagnosis by herd and brief clinical history of the herd

Farm 1

The herd was Hardjobovis positive in samples collected from the DSP but had no recorded clinical cases of leptospirosis. The herd was one of the farms in the serum bank from a PhD study by Audigé LJM, 1995, "Deer Herd Health, and Production Profiling" and it was positive to the same serovar. (The samples from this serum bank were used to generate the data in Chapter 3). The farm used for the 1995 research work, was sold. However, the animals on the new farm were part of the original herd or its progeny and it remained mostly as a closed herd since then.

Farm 2, Year 1

The farm had had a history of low weaning rates in first calving hinds for the past 5 five years. In the same period two horses had presented with recurrent uveitis and one sudden death that was considered similar to those of leptospirosis acute cases by the veterinary practitioner. In the first year (2003), when the 20 rising 2-year-old hinds from herd screening were sampled, the herd was seropositive only to Hardjobovis. By the second and third sampling of the weaners in the trial groups, the introduction of Pomona was detected. The original hinds in the screening sample were re-sampled and titres to both serovars were detected. The detection of Pomona titres coincided with the introduction of beef bulls from diverse origins and was considered the source of serovar Pomona. In this first year, three pigs were also present at the farm, but were removed as soon as the titres for this second serovar were detected, but one of the pigs had been on the farm for years and the other two for several months. There was ample opportunity for environmental spread from sheep and cattle.

Farm 2, Year2

The herd had a dual Hardjobovis and Pomona infection status throughout.

Farm 3

Pomona and Hardjobovis seropositives were found in yearlings at slaughter in December 2003,and acute and chronic kidney lesions were observed. Clinical cases and live shedding of leptospiral organisms with positive serology results predominantly to serovar Pomona had been observed in June 2004. Eight post mortems were performed on deer from this farm during the study with only one diagnosed as having leptospirosis alone. The herd had concurrent problems with copper deficiency, infection with septic leg lesions linked to *Fusobacterium*, and was positive for Johne's disease. There was ample opportunity for environmental spread from sheep and beef cattle.

Farm 4

This research farm (Massey University Deer Research Unit) was seronegative and was used to establish the base pattern of humoral response to the vaccine, without the natural challenge of

infection. This herd had been sporadically vaccinated in the past with a bivalent commercial bacterin ("Ultravac-7" (CSL NZ Ltd) containing serovars Hardjobovis and Pomona). Vaccination was stopped 2 years before the present study began so antibodies were detected in adults and maternal antibodies were present in the offspring. No kidney lesions were observed in three years of observations.

2.5.2 Weaners used in farm trials.

Farm 1

Eighty weaners were randomly selected for each treatment group (vaccinated and control) (Table 2.2). During the trial, one had died with a broken neck in the paddock

Farm 2

In year one, all 80 animals survived. The only animals vaccinated for leptospirosis on the farm were those in the trial. In the second year, 80 animals were selected one month before weaning. At the end of year 1, some hinds were vaccinated. Half of the progeny used in year 2 were the offspring of vaccinated hinds while the other half were from control hinds The weaners were allocated randomly to either the control or vaccinated weaner groups for this year's trial. (Table 2.2). Five animals were lost through the year, two were never found while the other three died during yarding. Post mortem revealed that the animals had cataracts. Because of the rise in the titre prevalence to serovar Pomona, it was decided to vaccinate all cattle and remaining deer on this property, with the exception of the control animals in the trials.

Farm 3

This was considered to be a highly infectious herd, on the basis of kidney lesions, the high prevalence of Pomona shedding and acute death in a weaner. Only the control weaner deer were left unvaccinated. For the remainder of the ruminants (cattle, sheep and adult deer) on the property, vaccination was recommended in consultation with his veterinarian.

Farm 4

In year 1, 20 weaners with negative serology to serovars Hardjobovis, Pomona and Copenhageni were used to establish a basic humoral response to the vaccine. At the end of the first year, half of the breeding hinds were vaccinated. Half of the progeny from each group of hinds were vaccinated in year 2.

Table 2.2 Weaner trial groups by farm.

Farm/ Serovar.	Year	Duration	Vaccine		Sex	No.	No. weaners	
			Hind	Weaner			Start	Finish
1 Hardjobovis.	1	*May 03 -May 04*	No	*No*	♂	20	80	79
					♀	20		
				Yes	♂	20		
					♀	20		
2 Hardjobovis, recent Pomona	1	Jun. 03 -Feb. 04	No	No	♂	20	80	80
					♀	20		
				Yes	♂	20		
					♀	20		
2 Hardjobovis, and Pomona	2	Feb. 04 -Dec. 04	No	No	♂	10	80	75
					♀	10		
				Yes	♂	10		
					♀	10		
			Yes	No	♂	10		
					♀	10		
				Yes	♂	10		
					♀	10		
3 Hardjobovis, and Pomona	2	Jul. 04 - Dec. 04	No	No	♂	16	105	105
					♀	24		
				Yes	♂	32		
					♀	33		
4 Negative	1	Jul. 04 - Dec. 04	No	No	♂	3	20	20
					♀	2		
				Yes	♂	7		
					♀	8		
	2	*Feb.04 - Dec.04*	No	No	♂	11	71	71
					♀	5		
				Yes	♂	8		
					♀	9		
			Yes	No	♂	12		
					♀	8		
				Yes	♂	9		
					♀	9		
	3	Feb-05 - Mar-05	No	No	♂	31	76	76
			Yes	No	♀	35		

2.5.3 Hinds used in vaccine trials

Adult hinds were mixed-age pregnant (confirmed by ultrasound) animals. In year 2 the hinds on farm 2 were grouped into calving mobs according to vaccination treatment until weaning to guarantee colostral antibody status of progeny. On Farm 4 hinds in calving mobs were involved in other research trials, so progeny were identified to dam by repeated behavioural observation.

Table 2.3 Summary of pregnant hind vaccine trails

Farm No.	Duration	Trial		No. Hinds	Serology	Observations
		Year 2	Year 3	Total		
2	Oct.03 - Feb. 04	Control		100	30	Sixty hinds were chosen to monitor the serology, but pregnancy and weaning rates were recorded in the 200 animals.
		Vaccine		100	30	
4	Sep. 04 - Feb.05	Control (n=40)	Control	20	20	Half of the vaccinated and half of the control hinds received one bacterin booster dose, while the rest remain as controls.
			Vaccine	20	20	
		Vaccine (n=40)	Control	20	20	
			Vaccine	20	20	

2.6 Vaccination technique

A skin area of 4 to 5cm square on the anterior neck was clipped then swabbed with a 70% ethanol solution prior to vaccination. A skin fold was held and the needle carefully inserted to ensure subcutaneous placement. Two ml of the Trivalent Leptospiral vaccine ("Leptavoid–3"Schering-Plough Animal Health Limited, New Zealand) was administered aseptically using a new sterile syringe and 20g 1 inch needle for each animal. Vaccines used in the efficacy studies were from batch numbers 7762D1, 7762A1 described in chapters 5 and 6.

2.7 Deer restraint

For blood sampling and/or vaccination of the adult deer the animals were generally restrained in small pens or race with three to five animals. If needed, physical restraint in a commercial pneumatic deer-restraining device was use. Blood collection and vaccination of weaners was done in small pens with 4 to 5 deer at a time. The young deer were gently restrained by the person collecting the sample and, if required, by an additional operator.

2.8 Sample collection and handling

2.8.1 Blood (serum) sampling

Blood samples were collected from the jugular vein with a new 20 gauge, 1 inch needle for each animal (Precision Glide Becton Dickinson Vacutainer Systems Plymouth UK) into 10ml blood,

red top tubes without anti-coagulant (Vacutainer ® No Additives, Becton Dickinson, Franklin Lakes, NJ. USA).

After collection either on farms or at slaughterhouses, samples were left to clot at room temperature followed by refrigeration for 1-12 hrs at 4°C for clot retraction. Clotted samples were centrifuged at 1008 g units for 5-10 minutes and, the serum was aliquoted into two 1.5ml plastic storage tubes which were labelled and stored frozen at –20 °C until the masterplates for serology were set up for the MAT. Additional aliquots were frozen for future purposes from all samples collected from farm trials (Chapters 4 to 6), from samples collected for the regional survey (Chapter 7) and at slaughter (Chapter 8).

2.8.2 Serum Bank

Initial serological investigation was carried out on the sera of a deer serum bank collected during the PhD study of Audigé LJM, 1995, "Deer Herd Health and Production Profiling". Sera was collected and identified in 1992 and 1993 and remained frozen at -20°C until tested. See Chapter 3 for details.

2.8.3 Urine

For urine collection, hinds were selected for sampling because of anatomical and behavioural features. Urine was collected from sequential groups of 4-8 deer 15 to 30 minutes after administration of a diuretic, "Salix" (Intervet, New Zealand: furosemide 50mg/ml) at 1-1.5 mg/kg (50 to 100 mg per animal) (adapted from Fairley et al 1986). The dose was delivered intramuscularly in the lateral neck using 3ml, sterile syringes and 20 g x 1 inch needles. A new 70ml plastic clinical specimen collector was held in a gloved hand a few centimetres beneath the vulva after urination began. Five to 50 ml middle-stream urine was collected. As many urine samples as possible were taken at each sampling period.

2.8.4 Urine handling

As soon as possible after the samples were obtained, each was identified and sub-cultured on the farm and later at the laboratory. The specimen collectors containing the individual urine samples were later used to check for the presence of leptospires using dark-field microscopy.

For sub-culture, one ml of the urine was drawn off with a 3ml sterile syringe. Once in the syringe, a 20g, 1.5 inch, sterile needle was attached. The top of a transport tube containing 5 ml sterile culture media (EMJH (Ellinghausen-McCullough-Johnson-Harris) medium with 200 µg/ml of 5'-fluorouracil) was swabbed and cleaned with a 70% ethanol solution and two drops (200 µl) of urine were injected through the top. The tubes were conserved and transported in plastic containers at room temperature back to the laboratory for further sub-culturing within 2 to 8 hrs after collection.

2.8.5 Urine Dark-field examination

The method was adapted from Hathaway (1978). Urine samples were centrifuged at 500 *g* units for 5 minutes, to remove cellular bodies and crystalline debris. A loop of supernatant was examined by dark-field microscopy for leptospires. A sample with two or more motile leptospiral organisms was considered positive. According to Turner (1970), more than 10,000 leptospires per ml must be present in the sample to detect one leptospire per high-power field. Therefore, failure to detect one leptospire by this technique does not rule out the presence of the organism. This technique was used in the study as an adjunct to serology, culture and pathology findings.

2.9. Slaughter

Animals from each farm were slaughtered at the end of vaccine trials. In addition, lines from different farms in the North and South Islands were randomly sampled for herd screening to identify candidate farms 1-3, regional survey data and to study kidney lesions. Deer were processed at Deer Slaughter Premises (DSP), Feilding, Hastings, and Hokitika, using normal commercial slaughtering practises.

2.9.1 Blood collection at slaughter

Disposable rubber gloves were worn for handling and collection of all organs and tissues. After deer were electrically stunned and hung inverted, the slaughterer incised the neck in the normal manner, severing the carotid artery and sometimes the brachial vessels, and usually the jugular vein, avoiding risk of oesophageal contamination. Ten ml of blood was collected by free-catch directly in into an open 10ml glass tube without anti-coagulant (Vacutainer ®). Carcass identity was recorded for correlation with kidney collection. Blood samples were then handled as described in point 2.7.1. Blood samples that were collected in the DSP were identified and matched to the kidneys from each deer carcass. The samples were collected in two periods.

Period 1, 1992-1993

Blood samples were collected at the Feilding DSP located in the southern North Island of New Zealand. The data were described by Wilson et al (1998), and supplied by the senior author to complete the observations between serology and kidney lesions. (Data are presented in Chapter 3 and 8).

Period 2, 2003-2004

Samples reported here were collected either for herd screening to identify suitable herds for studies presented in Chapters 4, 5 and 6 or, for the regional seroprevalence survey reported in Chapter 7, and slaughterhouse leptospirosis kidney lesion study in Chapter 8. Serum from the samples collected at the Hokitika DSP were separated, identified and frozen at the slaughter plant facilities and later transported to the Massey University Leptospirosis Laboratory to be tested.

Samples collected at Feilding or Hastings DSPs were transported to Massey University to be processed as described above.

2.9.2 Kidney collection and gross pathology examination

Both kidneys from each deer were collected from the inspection tray following evisceration and MAF inspection, placed into clean and dry plastic bags, identified, and matched to the carcass and animal identification numbers. Each kidney was examined and a description of gross pathology recorded. Macroscopic lesions were categorised. Kidney samples were drawn from three collection periods.

Period 1, 1992-1993

This was retrospective data used for analyses in Chapter 8. Kidney samples collected in this period were from the southern North Island of New Zealand, slaughtered at a Feilding DSP. The records for the lesions from the study in period one had been scored numerically 1-6, as described by Wilson et al (1998).

Period 2, 2003-2004

Samples were collected for studies presented in Chapters 4 – 8. Kidney samples were collected in Westland (Hokitika DSP) where they were predominantly one-year-old stags and from the southern North Island (Feilding DSP). The kidneys from the Feilding or Hastings DSPs were taken back to Massey University postmortem room, for gross pathology description and photographs. From animals in the three herds involved in epidemiological and vaccination studies, one kidney was used for histopathology whilst the second kidney was transferred to a clean, dry, labelled plastic bag and stored at 10°C refrigeration until used for bacterial culture.

2.10 Histopathology

One kidney was used to take a sample for histopathology. A 5mm wedge section through the middle portion of the kidney was fixed in 10% formalin/saline for histopathology studies. After the kidney sections were fixed, a 4-5 mm section was trimmed and processed routinely in a tissue processor (Leica Jung, 10509). The tissues were then embedded into paraffin (Paraplast, Oxford Labware, St. Louis, MO, USA) and cut in three micron sections on a microtome (Microtech Rotary, 4055). Sections were stained with standard haematoxyl and eosin (H&E), mounted in Entella medium (Merck, Darmstadt, Germany) and examined using a light microscope at 40x, 200x, and 400x magnification.

Lesions were described and a score of 0 (no), or 1 (yes) given for the following observations: focal/diffuse cellular infiltration (lymphocytes, plasma cells); glomerular adhesion; scarring; and tubule necrosis consistent with the description of multi-focal interstitial nephritis of leptospirosis (Prescott 1992).

2.10.1 Formalin/saline solution 10%

(Saif and Ward 2000).

• 37-40% Formaldehyde	100.0 ml.
• Distilled water	900.0 ml.
• Monobasic sodium phosphate.	4.0 g.
• Dibasic sodium phosphate	6.5 g.

2.9 Serology

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

2.9.1 Serum master plates

Serum was set in standard 96 well, flat-bottomed serology plates (master plate), with saline solution to a first dilution of 1:6. One hundred and fifty µl of sterile saline solution was dispensed with a multi-channel pipette into all the plate wells. A serum aliquot was thawed at room temperature. The tube with the serum sample was vortexed, for 3-5 seconds. Using a pipette (sterile yellow tip) 30 µl of the serum was dispensed into a well. The plate was sealed with Parafilm® or sealing tape and carefully sealed so that the serum would not leak between wells. The lid of the plate was replaced and the master plate then stored in the -20°C freezer.

The number of the master plate was written on the side of the plate bearing the letter A-H and the IDs of sera, which were recorded, on a master plate sheet was stored on the master plates and standard file. The location and ID of any given serum was registered and located by its column (Numbers1-12) and row (letters A-H).

2.9.2 Dark-field microscopy

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

Dark-field microscopy was the routine optical microscopic technique used to examine specimens such as reference cultures, urine and kidney cultures or urine samples. The spirochaete's shape and movement, if present, becomes evident by diffusion and condensation of light through the microscope.

A drop of the culture or fluid to be examined was placed onto a microscope slide with either a flamed platinum loop or a "dropper". Serology, cultures and fresh urine samples were all read without a cover slip. The slide was then secured and examined with the 4x, 10x, and 20x

objectives. The microscope light was set at its lowest, with good contrast, and switched from the lowest objective to exam the slide for leptospires for agglutination during MAT.

2.9.3 Microscopic agglutination test (MAT)

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

The MAT was performed to test the animal's serum reactivity to laboratory standardised and recognised *Leptospira* serovars Hardjobovis, Pomona and Copenhageni to determine a positive or negative status of the animals or the herds. MAT is considered the "gold standard" because of its high specificity in the serodiagnosis of serogroups or serovars.

Serum master plates were taken out of the freezer and allowed to thaw. Sterile standard saline was poured into a clean petri dish and wells of the serology plate were prepared by filling with 25 µl of the sterile standard saline using the multi-channel pipette. A row of sera from the master plate was serially diluted by placing the combs of the multidiluter machine in the wells, mixing, and then taking up a 25 µl sample of serum. The mixing combs are designed to hold 25µl and hence transfer from one dilution row to the next was achieved by raising the combs and then lowering into the next dilution row. Repeating this process through successive rows in the serology plate made serial dilutions. The process resulted in eight; two-fold dilutions covering the range 1:24 – 1:3072.

The *Leptospira* reference culture of live bacteria 4-14 days old was checked for density by comparing with the McFarland nepholometer scale (tube No.3) (Mac Fadden 2000) which equates to approximately 1-2 $x10^8$ organisms /ml. The culture was then examined under the dark-field microscope to see whether any auto-agglutination had occurred. If more than a few clumps were observed then the culture was not suitable for use. If deemed suitable for use, 25 µl of antigen (leptospiral culture) was added to each well using the multi-channel pipette. The plates were placed in re-sealable plastic bags and kept at room temperature (20-30°C) for 1.5 to 4 hours.

The plate containing the standard antiserum (sera) was prepared in the same manner. In addition to the standard, a well containing 25 µl standard saline and 25 µl of antigen was prepared to act as a negative control. The dropper was then used to place a sample from each of the eight dilutions from each well onto a microscope slide.

The end-point of agglutination reaction was deemed the dilution at which approximately 50% of the organisms had been agglutinated or lysed. The Standard plate 1 was read first to ensure that the antigen had reacted appropriately with its antiserum standard. The serology plates were then placed in a bucket of Virkon® solutions for a minimum of half an hour after use to decontaminate them.

2.9.3 Serotyping and identification of leptospire isolates

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

Leptospiral isolates were classified as belonging to a serovar by the use of a simple agglutination procedure. Antisera of known specificity to serovars Hardjobovis, Pomona, Copenhageni, Tarassovi and Balcanica were used. An aliquot of 30 µl of a 1:6 pre-diluted reference serovar serum was placed in sterile Eppendorf tubes and conserved at –20°C until used. Serum samples were thawed and 25µl of this serum in was placed in the first row of the wells of the serology plates; a dilution as the one used for the MAT serology was done. The appropriate isolate cultures were added and the reaction was observed after 1.5 - 4 hours incubation at room temperature. The end agglutination to the specific reference sera was used to identify the serovars involved in the different cultures.

2.10 Bacteriology

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

2.10.1 Maintenance of leptospiral cell cultures for serology

A stock of standard and previously identified leptospiral cultures (laboratory adapted strains) of serovars Hardjobovis, Pomona, and Copenhageni was kept for use with the MAT. The laboratory strains were cultured in different culture bottles depending on the volumes required to test the sera samples. EMJH (Ellinghausen-McCullough-Johnson-Harris) medium with 200 µg/ml of 5'-fluorouracil was used as contamination inhibitor. Leptospiral cultures were incubated and maintained at 28-30°C; a backup culture was keep at room temperature at all times. The bacterial stock was subcultured when needed into fresh 5 ml culture bottles as needed.
Optimum culture for MAT was when the *Leptospira* reference culture of live bacteria 4-14 days old was checked for density by comparing with McFarland nepholometer scale (tube No.3), with approx equivalent to 1-2 $x10^8$ organisms /mL.

- **EMJH media (Ellinghausen-McCullough-Johnson-Harris).**

(Premix powder medium available from Difco).
Composition per litre:

Na_2HPO_4	1.0g
NaCl	1.0g
KH_2PO_4	0.3g
NH_4Cl	0.25g
Thiamine	5.0mg
Rabbit serum	100.0mL

Ph 7.5 +/- 0.2 at 25°C.

Preparation: Add components, except rabbit serum, to distilled/deionised water and was brought up to volume to 900.0 ml mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 min

at 15 psi pressure/ 121°C. Cool to 25°C. Aseptically add sterile rabbit serum. Aseptically distribute into sterile tubes or flasks.

- 5'-fluorouracil for use as an antibiotic in EMJH medium.

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

5'-fluorouracil	1.0 g
2M NaOH	1-2 ml.
Distilled H_2O	50 ml

pH adjusted to 7.6, and the volume is taken up to 100ml with distilled H_2O then filter-sterilised.

2.10.2 Urine Culture

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

From the subculture at the collection site (See 2.7.4) three more subcultures were done. A 100 µl sample was taken and cultured into another labelled culture Bijou bottle of EMJH media with 200 µg/mL of 5-Fluorouracil as a selective and inhibitory agent. The process was repeated yet again to obtain three serial dilutions from the subculture tube from the farm. Culture bottles were labelled and held at 28-30 °C, examined at 2 weekly intervals by dark-field microscopy for 3 to 6 months.

The procedure was carried out aseptically in a biohazard cabinet and utilising a Bunsen burner. Positive cultures were sub-cultured and the serovar typed using a specific MAT with reference antisera.

2.10.3 Kidney Cultures

Adapted from standard technique reported by Hathaway et al 1978, Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

Kidneys were collected and handled as described above. A flamed to red, No. 20 surgical blade, was used to seal and sterilise a $4cm^2$ area on the kidney surface. Once the surface was sealed, a portion of approximately $3cm^3$ was aseptically removed using sterile, scissors, forceps, and scalpel. The dissection instruments were kept in a 70 % ethanol solution and flamed to red before each of the incisions were done to obtain the piece of kidney tissue to be used. The tissue sample was transferred into the lid of a plastic sterile disposable Petri dish and chopped into $1\text{-}mm^3$ pieces with clean and flamed surgical scissors. The chopped pieces were transferred into a 10 ml sterile syringe and triturated by passing the tissues through the syringe, into a 5ml culture Bijou bottle with of EMJH media with 200 µg/mL of 5-Fluorouracil.

A 100µl aliquot of the supernatant was transferred aseptically into a 5 ml culture bottle; the procedure was repeated until there was 5 dilutions or subcultures from each kidney.

Cultured bottles were identified, incubated and monitored as described in the above section. The procedure was carried out in a biohazard cabinet with a Bunsen burner. Positive cultures were sub-cultured and the serovar typed using a specific MAT with reference antisera.

2.10.4 Long term storage of leptospiral cultures

Massey University, IVABS Leptospirosis Laboratory SOP, based on Guidelines for the control of leptospirosis, Faine (1982).

Positive cultures or isolates from the urine and kidney samples were sub-cultured in liquid EMJH medium with 5-Fluorouracil until the culture was dense, actively growing and free as possible of agglutination and obvious contamination. The procedure was done under aseptic conditions in the biohazard cabinet with the Bunsen burner. Seventy µl of sterile glycerol and 930 µl of culture were placed in a sterile Eppendorf tube. The cap was closed, the sample mixed, marked with the number of the isolate and the serovars identified. Then the tube was placed at –20°C in a freezer.

2.11 Serological interpretation

Interpretation is based on those of Mackintosh (1981), Bolt (1990) and Thrusfield (1995).

The microagglutination test was used to detect the concentration of agglutinating anti-leptospiral immunoglobulins in serum expressed as a titre. In the MAT, this term is used to express the highest serum dilution at which 50% or more of leptospires agglutinated a specific serovar. A serum sample in which the level of agglutination was less than 50% in all the test wells was designated a titre of zero (seronegative). Seroprevalence was used to describe the percentage of serum samples, which were seropositive at a designated minimum dilution.

2.11.1 Manipulation and transformation of titre data for statistical purposes.

The geometric mean titre is the usual form of average used for serological studies (Paul and White 1973). Geometric dilutions are equally spaced on a logarithmic scale corresponding to a log transformation to base 2. Serum was diluted in geometric series, with a constant ratio of two between successive dilutions. The dilutions were coded as the value of these logarithms: 0= >1:24, 1:24=1, 1:48=2, 1:96=3, 1:192=4, 1:384=5, 1:768=6, 1:1536=7, 1:3072=8. The logarithm of zero is "minus infinity", therefore when calculating means of coded titres, seronegative samples have to be excluded. Hence, the mean titres were calculated only for seropositive animals. Geometric mean of the coded titres were calculated for descriptive analysis, and back transformed to the reciprocal titre of the titre to be expressed in the text as presented in the following formulae with a worked example in Table 2.4.

- **Mean coded titre** =Σ coded titre/ n
- **Geometric Mean Titre (GMT)** = Coded Titre/12 x antilog (Coded mean x $\log_2$).
- **Standard error (SE)** and 95% confidence interval of the mean coded titre.
- **SE** = ± Standard deviation of the coded titres$\sqrt{}$ n (where n= number of titres)
- **95% Confidence interval (95%CI)=** Arithmetic Mean of the coded titre ± 1.96 x SE
- **Reciprocal Titre Transformation=** $2^{(GMT)} * (12)$

Table 2.4 Example of manipulation, with sample number, titre and coded titre, (above) and description of back transformation of the Geometric mean of those titres (below).

Sample	Titre	Code
1	3072	8
2	0	
3	0	
4	192	4
5	768	6
6	48	2
7	96	3

Statistic	Result
Total samples	5
Number of titre +ve (n)	5
GMT	4.09
SD of the coded titre	2.40
SE	0.91
(95% CI)	1.78
GMT (95% CI)	4.09 (±1.78)
Reciprocal titre (GMT)	205.117

2.11.2 Titre dilution range and cut-off points for the MAT

The MAT in this research was used based on the OIE and WHO recommendations as the "gold standard" in diagnosis of leptospirosis (Smith et al 1994; Faine et al 1999). The MAT was used as a test for screening and monitoring study populations and not for clinical diagnosis of individual animals. A MAT titre ≥ 1:100 is the convention to declare a sample positive for clinical disease. However, for epidemiological (population) studies there is justification for including lower titres in some circumstances. The decision for titre cut points from 1:24 in some circumstances in this thesis was based on the following considerations:

- Blackmore et al (1976), recommended inclusion of these low titres, because the use of a higher titre cut-off for an epidemiological investigation could underestimate the true prevalence of the disease or exposure in a herd.

- Blackmore et al (1979), in humans found a significant correlation between the history of clinical leptospirosis and these titres. Hathaway (1978) with wildlife, Hellstrom (1978), and Mackintosh (1981) with cattle and humans regarded this titre as sensitive and specific.
- Interpretation of the titre varies with different serovars for example Hardjobovis (*L. borgpetersenii*) will generally give a lower titre than serovars Pomona or Copenhageni (*L. interrogans*) in response to infection (Bolin and Prescott 1999; Faine et al 1999). Hardjobovis LPS has been suggested to be poor inducers of agglutinating antibodies (Bolin et al 1989a,c).
- From the review of leptospirosis in deer it was decided to concentrate the research on serovars Pomona, Copenhageni and Hardjobovis. The first two serovars appeared to behave as non-host adapted serovars with high titres and lower prevalence, while Hardjobovis was regarded as an adapted serovar with high prevalence and lower titres. The nature of the host-organism relationship justifies different cut points for each serovar.
- For Pomona the titre reaction is strong, but can cross-react with Copenhageni. Where both serovars reacted the higher titre and prevalence was considered to be the reacting serovar (Faine et al 1999).

The cut point for serovar Hardjobovis in this thesis is generally 1:24, although some analyses examined alternative cut points, as stated in the sections concerned, while the cut point for serovars Pomona and Copenhageni was generally 1:48.

The doubling dilution range from 1:24 to 1:3072 in the MAT was chosen because it was the most frequently used in the New Zealand research in domestic animals (Hathaway 1978; Hellstrom 1978; Mackintosh 1981) and it was consistent with the existing information in farmed deer (Failey et al 1986; Wilson et al 1998), thus providing consistency in interpretation for comparison with existing information.

Chapter 3

A longitudinal retrospective serological investigation of leptospirosis on deer farms



3.1 Introduction

Leptospirosis was first reported in farmed deer in New Zealand in 1979 (Anon 1980). Subsequently there have been a number of reports of clinical disease and serological evidence of infection in New Zealand as reviewed by Ayanegui-Alcerreca et al 2006 a,b (paper submitted for publication in the New Zealand Veterinary Journal, accompanying Chapter 1 of this thesis). A number of studies, largely serological, have been conducted on a range of deer species globally, as reviewed by Ayanegui-Alcerreca et al (2004), and updated and presented in a paper submitted for publication in the New Zealand Veterinary Journal, accompanying Chapter 1 of this thesis.

The epidemiology of leptospirosis in various domestic animal species and countries has been reviewed elsewhere (Michna 1970; Ellis 1984; Prescott 1992; Health and Johnson 1994; Faine et al 1999; Radostitis et al 1999). While the epidemiology and control of leptospirosis in dairy cattle and pigs in New Zealand has been well researched, as reviewed by Marshall and Manktelow (2002), little is known of the epidemiology of leptospiral infections in farmed deer or sheep in New Zealand. New Zealand has a unique temperate pastoral farming system for deer, often carried out in association with sheep and cattle farming, suggesting that the epidemiology of leptospirosis in deer may have unique characteristics in this country. One of the key objectives of research presented in this thesis is the development of an understanding of the epidemiology of this infection in deer to underpin control and management strategies should they be desired or desirable at the individual farm, regional or national levels.

This chapter presents a summary of serological analyses for leptospirosis in samples stored in a serum bank from a previous two-year longitudinal observational study involving 16 deer farms in the southern North Island (Audigé 1995). The aim of this retrospective analysis was to evaluate the pattern of serological evidence for leptospiral infection by farm, age, sex, year and season. The three serovars investigated in this study (Hardjobovis, Pomona and Copenhageni) are those previously isolated by culture from farmed deer in New Zealand confirming their presence, since serology offers only indirect evidence of infection (Farley et al 1984 and 1986; Flint 1986; Wilson et al 1988).

3.2 Material and Methods

A deer serum bank was established with samples collected in 1992 and 1993 by Audigé (1995). The samples were part of a 2-year longitudinal observational study involving 16 commercial deer farms from the lower New Zealand North Island and remained stored frozen at the Institute of Veterinary Animal and Biomedical Sciences, Massey University. Full details of that study are presented in Audigé (1995) and Audigé et al (1994 a, b). A total of 1494 serum samples were tested from 754 deer comprising 419 from weaners, 471 from yearlings and from 604 adults.

3.2.1 Farms, Animals and sampling regime

Sixteen farms were selected within 2h driving distance from Massey University. Farms chosen were typical of commercial deer farms located in the southern North Island regions of Wanganui, Taihape, Hawke's Bay, Wairarapa, Manawatu and Horowhenua to fit farm size, management and other criteria described by Audigé 1995). All deer on the study farms were

red deer (*Cervus elaphus*). No samples were analysed from farm number 12 which withdrew from the study early. Samples were analysed from farm number 14 which withdrew after year 1. Sampling of animals commenced in March 1992 and finished in September 1993.

Numbers sampled at each period, and summary serological data for each serovar are detailed in Tables 3.3, 3.4 and 3.5. Raw data are in Appendix 1. Sampling commenced in March 1992. Age classes were 3-4 to 12-month-old (weaner), 15 to 24-month-old (yearling), and deer 2 years of age and older (adult), including approximately equal numbers of both males and females were selected and, identified with unique duplicated tags, for repeated sampling over the following 21 months to September 1993. Weaners from the first year moved to the yearling group in the second year, yearling animals from the first year became part of an enlarged adult group in the second year and adults from the first year remained in this classification stratum for the whole length of the study. A new group of weaners was introduced in March of the second year to maintain the balance of each age stratum for the full longitudinal study.

Blood sampling was undertaken in March, June, September and November/December year 1 (1992) and March, June and September/October year 2 (1993). Thus, weaners from year 1 were sampled at 3, 6, 9, 12, 15, 18 and 21 months of age, weaners from year 2 were sampled at 3, 6, and 9 months of age and yearlings from year 1 were sampled at 15, 18, 21, 24, 27, 30, and 33 months of age.

In addition to blood sampling, a full dataset of production parameters, animal characteristics including a biochemical profile, and daily climatic data was established over the duration of the study (Audigé (1995). Estimated foetal loss rates were uniformly low, averaging 0.6%, for yearling and 0.9% for adult hinds (Audigé et al 1999) indicating that no abortion outbreaks occurred. Further, no mortalities attributable to leptospirosis were observed (Audigé et al 2001).

Note: The numbering of farms (Appendix 1) described in this chapter is that used for the original study (Audigé 1995) and is relevant only to this chapter. For other chapters, four different farms were given numbers 1-4, and that numbering system is used throughout the remainder of this thesis.

3.2.2 Re-sampling of farms

Seven of the farms mentioned in section 3.2.1 that were originally sampled in 1992-3, were re-sampled in 2004. Twenty blood samples were collected from yearling hinds, and serological analyses performed as described below. Farm 13 that had altered its policy from breeding to finishing bought-in weaners, had experienced an outbreak of leptospirosis and was now vaccinating. Data from the six non-vaccinated farms in this section were also used for the regional seroprevalence study described in Chapter 7. Relevant data of farming practices and stocking policies were collected.

Since the sampling regime in 1992-3 was different to that in 2005, it was necessary to establish criteria for defining herd status, since the intent of the re-sampling was solely to establish whether or not the herds were infected. The seroprevalence calculated for the 1992-1993 data at the herd level included all animals and all samples. In 2004-2005, farm leptospiral status was established for Hardjobovis on the criteria of at least 3 seropositives

>1:24, or one ≥ 1:96, and for serovars Pomona and Copenhageni of at least 1 seropositive ≥ 1:96 (See Chapter 7). In 1992-1993 the same titre cut points were used.

3.2.3 Blood Collection, Identification and Storage

Blood samples were collected as described in Chapter 2. Serum was removed from clotted blood after centrifugation and transferred into 1.5 plastic Eppendorf tubes. In addition to samples aliquoted for immediate purposes of the original study, five aliquots of each sample were identified with farm and animal number, date of sampling, sex, and age. Samples were packed and recorded and remained stored at –20°C for undetermined future use.

For the purposes of this study, one aliquot of serum was used from each animal. Samples were thawed at room temperature and vortexed prior to serology.

3.2.4 Serology

Samples were tested for leptospiral antibodies to serovars Hardjobovis, Pomona and Copenhageni according to the method described in Chapter 2. A titre of ≥1:24 was used as the cut-point for serovar Hardjobovis and ≥1:48 was used as the cut-point for serovars Pomona and Copenhageni as described in Chapter 2 section 2.11.2 unless otherwise stated. Animals in this study had no history of vaccination against Leptospira (except farm 13 when re-sampled in 2004-5), therefore with the exception of farm 13, the detected titre was considered the result of the natural exposure to the bacteria.

3.2.5 Statistical analysis

Data are presented by season corresponding to the month of collection (March = Autumn, June = Winter, September = Spring and November/December = Summer). Descriptive analysis was expressed as seroprevalence (proportions) with a 95% confidence interval (95%CI), and geometric mean of the coded titres with (95%CI) as described in Chapter 2. Because numbers varied between sampling points, data were assessed by power analysis calculator with NCSS 2000 and PASS 2000 (Jerry Hintze ©2001) to confirm suitability of data for statistical analyses.

Statistical analyses were performed for s Hardjobovis, Pomona and Copenhageni. Associations between sero-conversion and year, age, farm, sex and season were investigated using a logistic regression model, employing SAS for Windows (SAS Institute Inc., Cary, Nc, USA). Logistic regression analysis was chosen to explore possible associations with and between serology and the different demographic and production data available from the original study. The original study (Audigé 1995) was set out to establish a profile of deer farming practices and outcomes in general and was not designed for the particular outcome of leptospirosis infection. However, it was considered appropriate for that purpose. Seroprevalence accounting for different variables was explored with a model estimating the effect of year, season, farm, sex and age on the seroprevalence of the three serovars at different titre cut-points, but ≥1:48 provided the best fit for statistical analysis. The model adjusted for repeated measurements on individual animals. Odd Ratios and were obtained from the model, and relative risk

3.3 Results

Summaries of raw data including animal numbers are presented in Tables 3.3, 3.4 and 3.5, and complete raw data are presented in Appendix 1. Regression analyses showed no differences attributable to sex for s Hardjobovis and Copenhageni. A sex effect was found for Pomona related only to farms 9 and 15. Thus, data for both sexes within age-groups were pooled for further statistical analyses. The overall seroprevalences within each farm are presented in figure 3.1.

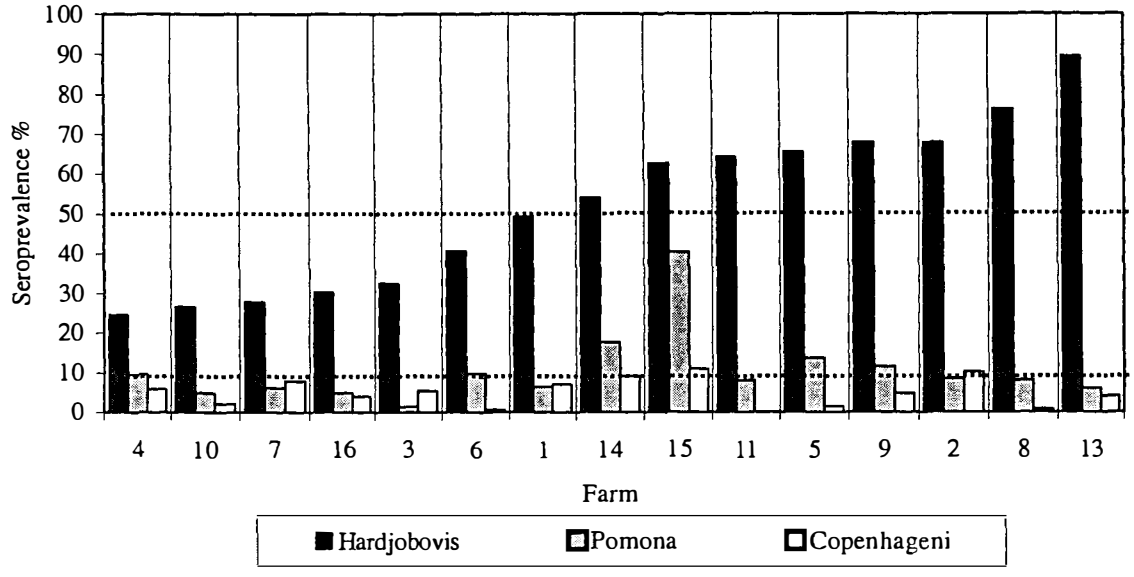


Figure 3.1 Overall seroprevalence s Hardjobovis (≥1:24), Pomona and Copenhageni (≥ 1:48), (all ages, all samplings and both sexes and years combined within each farm) with farms ranked by the seroprevalence of Hardjobovis. (Data for serovar Hardjobovis for each year separately is presented in Figure 3.3)

3.3.1. Serovar Hardjobovis

Samples positive for serovar Hardjobovis were present in all age groups on all farms (Table 3.3, Figure 3.2), with a total seroprevalence of 51.8% (49.6%-53.95%, CI) and a titre range zero to 1:3072. Seroprevalence for Hardjobovis ranged from 24.6% to 89.40% between farms, with eight having a seroprevalence of ≥50% (Figure 3.1). Overall seroprevalence in weaners was 40.69% (males 43.3% and females 38%) with a reciprocal GMT of 67.4. Yearlings had a seroprevalence of 51.86% (males 51.8% and females 51.4%) with a reciprocal GMT of 79.5 and adults had a seroprevalence of 63.81% (males 63% and females 60%) with a reciprocal GMT of 65.3. Between farms, seroprevalence in weaners ranged from 16.6% to 85.3% with a mean reciprocal GMT between 33 and 93, seroprevalence in yearlings ranged from 25% to 94.87% with a mean reciprocal GMT between 33 to 130, while seroprovalence in adults ranged from 26.6% to 92.89 % with a mean reciprocal GMT between 24 to122. The overall seroprevalence of Hardjobovis in both years was around 50% (Figure 3.3). Most animals were seropositive on more than one occasion.

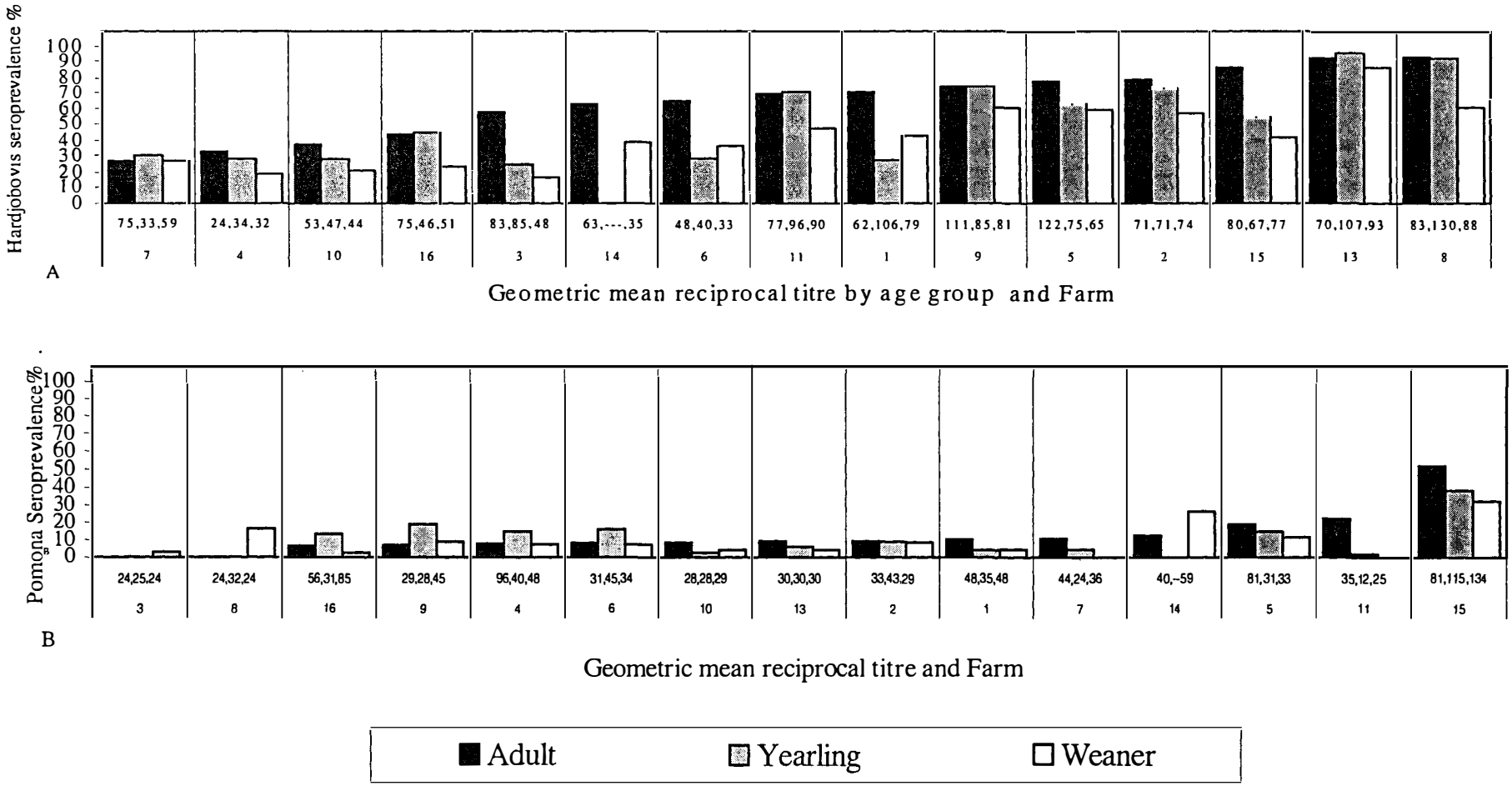


Figure 3.2. Overall seroprevalence for serovar Hardjobovis (cut point of ≥1:24) (Top), and Pomona (cut point 1: 48) (below), from each farm, all samples combined, at each sampling, ranked by the seroprevalence in the adult group

3.3.1.1 Farm, age, season

Seroprevalence and confidence intervals for Hardjobovis on each farm for both years, in order of seroprevalence for year 1, are presented in Figure 3.3. The regression model estimated the effect of year, month, farm, and age on the seroprevalence of Hardjobovis at a titre cut point of ≥1:48, adjusting for repeated measurements, with the model being Hardjobovis seroprevalence = Farm _ year * month _ age _ year * age, for each fixed effects. (* = interaction).

Six of the seven farms with seroprevalence <50% in the first year remained below 50% in the second year. Likewise, farms with a seroprevalence >50% in year 1 (n=8) (Figure 3.3), maintained high seroprevalence in the following year. The statistical analysis showed that the variable "Farm" was significant (F- value =18.54; p <0.001). Odds ratios (OR) in the regression model for the farm effect ranged from 0.64 to 1.97 in the farms below 50% seroprevalence, and for those farms above 50% seroprevalence, the OR ranged from 4.43 to 15.

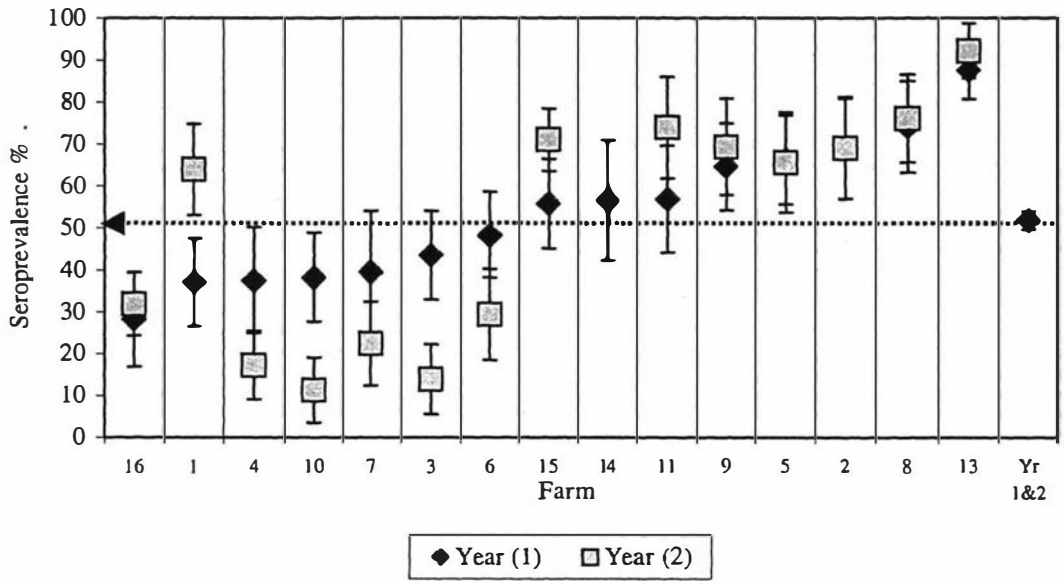


Figure 3.3 Seroprevalence of Hardjobovis (±95% CI), all ages, all samplings, and both sexes combined, within each farm in years one and two with farm ranked by the seroprevalence in the first year. The final column presents the average seroprevalence, all farms in both years.

3.3.1.2 Age and season cohorts by seroprevalence

The overall seroprevalences for all farms combined at each sampling period for each age group, are presented in Figure 3.4. Regression analysis confirmed an age difference (F- value = 29.11, p <0.001), with weaners having a lower seroprevalence than yearlings and adults, but seroprevalence in adults and yearlings was not significantly different (Figure 3.2 and 3.4). Seroprevalence in weaners was 84% in March year 1, fell to 18% in June, rose to 76% in September, and was 53% in December. The following year, the seroprevalence in those animals was constant around 80 %. Weaners in year 2 began at 33.3% seroprevalence, peaked at 51.9% in June and decreased to 27.4% by September. Seroprevalences in yearlings in year

1 were 97.4%, 62.4%, 96.3%, and 72.2% at respective samplings, and remained stable at around 80% in those animals as adults in year 2. Seroprevalence in adult animals was 60% to 70% with a transient drop in seroprevalence in March year 2.

The interaction Year*month and Year *Age _ month were significant (F- value (9.19), $p<0.001$, and F- value (3.29), $p<0.0426$, respectively). Seroprevalence variations can be observed in Figure 3.4, and were significant in the regression model in December (Summer) of the first production cycle ($p=0.05$), March (Autumn) ($p=0.0059$) and June (Winter) ($p<0.001$) of the second year.

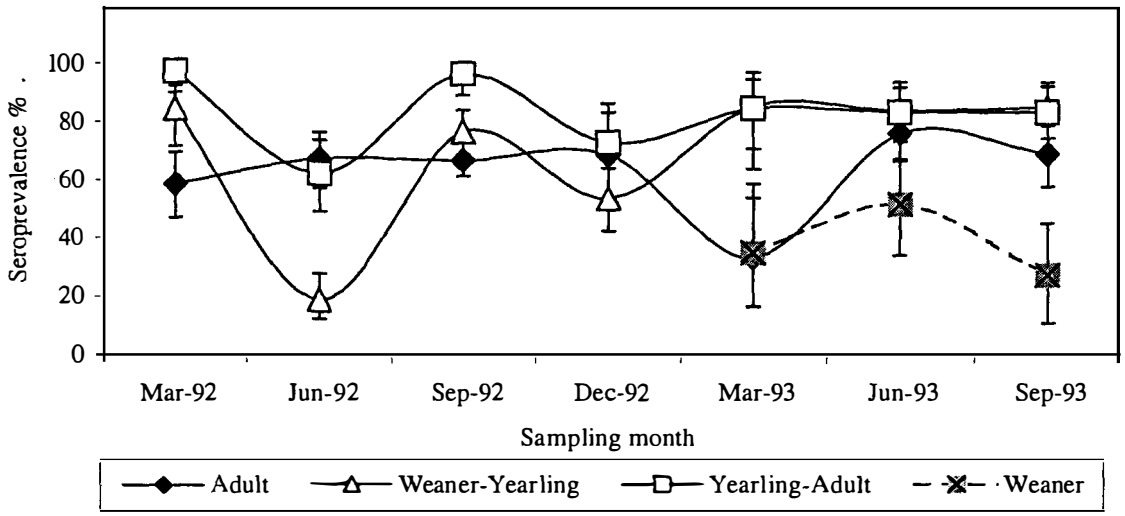


Figure 3.4. Seroprevalence for serovar Hardjobovis, all farms combined, for each age group at each sampling. Note, weaners in year 1 become yearlings in year 2, and yearlings in year 1 become adults in year 2. Weaners in year 2 were sampled only in that years.

3.3.1.3 Age and seasonal cohorts by titre.

The seroprevalences and geometric mean of titres for each age group by farm are presented in Figure 3.2. The overall GMTs and 95% CI of each age group, all farms by sampling month are presented in Figure 3.5, demonstrating a similar pattern between age groups. The overall reciprocal GMT for Hardjobovis was 70.3 (mode =1:48). In adults the reciprocal GMT was 65.3 (mode =1:48), in yearlings it was 79.5 (mode =1:96) and in weaners it was 67.4 (mode = 1:48). The seroprevalence and GMT tend to remain high in adults, but are more variable in weaners.

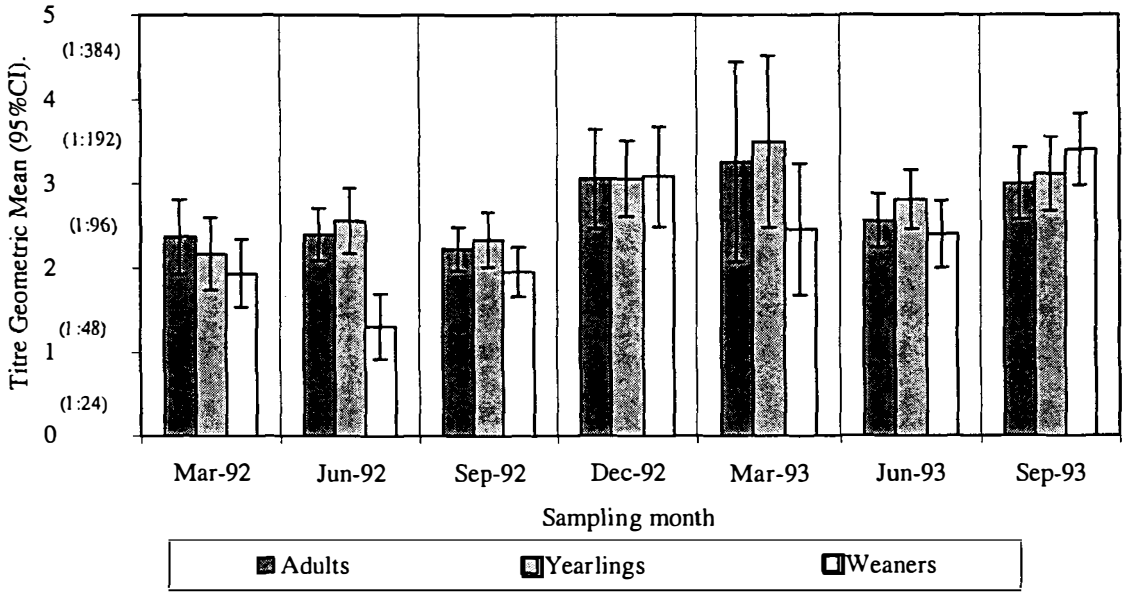


Figure 3.5 Geometric mean of coded titres (the reciprocal titres are presented in brackets) for serovar Hardjobovis (and 95%CI) for each age group, all farms combined, at each sampling period in years 1 and 2.

3.3.2 Serovar Pomona

The seroprevalences and mean reciprocal GMTs of each age group within farms, both years is presented in Figure 3.2. Mean seroprevalence for Pomona was 10.5 % (9.2% -11.9% CI), ranging from 1.34% to 40.36% between farms with five farms having a seroprevalence ≥10%. Titres ranged from zero to 1:3072. Serorevalence in weaners was 10% (males 23.7% and females 7.6%) with a reciprocal GMT of 32.3. Seroprevalence in yearlings was 9% (12.5% males and 5.6% females) and a reciprocal GMT of 48 and in adults was 14%, (17.4% males and 8.9% females) with a reciprocal GMT of 42.6. The seroprevalence in the weaner groups ranged between farms from zero to 32.0% with a reciprocal GMT range from 24 to 134. The within-farm seroprevalence for yearlings ranged from zero to 38.2% with the reciprocal GMT ranging from 12 to 115. The seroprevalence in adults ranged from zero to 51.7% with a reciprocal GMT range between farms of 24 to 81. The reciprocal GMT of all data combined was 39.9.

3.3.2.1 Farm, age, year, season and sex

The highest seroprevalence was in farms 14 (17.6%) and 15 (40.36%) (Figure 3.1). The regression analysis estimating the effect of farm, age-year, month, and sex on the seroprevalence of Pomona was significant at a cut point of ≤1:24 (χ^2 = 47.2, p<0.001) and at a cut point of ≥1:48 (χ^2 = 54.17, p<0.001) but not when other cut points were used in the analysis. The statistically significant effects were Farm (χ^2 15.96, p<0.001), sampling year (χ^2 9.49, p=0.002) where the OR of having a positive seroprevalence in year two if positive in year one was 3.047 (1.49-6.19 CI), sampling month (χ^2 9.41, p=0.002) and sex (χ^2 10.67, p<0.001) with the OR of 1.63 (1.21- 2.19 CI) of been positive in the male group.

The effect of sex on titre seroprevalence for Pomona was statistically significant at titre cut points ≤1:24, ≤1:48, ≤1:96. Pomona seroprevalence in adults, both years all sampling points combined, was 9.8% in females and 17.2% in the males with a χ^2 of 7.18 ($p<0.001$) for the difference in the proportion. Similarly, the seroprevalence in yearling hinds was 7.0%, and in stags 14.64% with the χ^2 being 8.43 ($p=0.003$) for the difference in the proportion. The seroprevalence in weaner females was 6.9% and in weaner males, 9.8%. The difference was not significant. The sex effect was not significant when Farms 9 and 15 were removed from the analysis. Farm 9 had a seroprevalence of 5.7% in females and 16.6% in males (χ^2 of 4.32 ($p=0.03$) and Farm15 had a seroprevalence in females of 27.9% and in males 48.9% (χ^2 of 7.34 ($p=0.006$)). Farm, year, and sampling month remained significant for all age groups ($p<0.05$).

3.3.2.2 Pomona titre distribution and geometric means.

Four of the 15 farms showed a seroprevalence higher than 10% with an overall reciprocal GMT of 42.7. Four farms had a seroprevalence >10%, ranging from 11.5 to 40.7%, with GMT ranging from 30.7 to 102. Other farms where titres were detected had a low seroprevalence, low titres and few animals showed a repetitive positive titre through the cohort series. Animals with at least one titre ≥1:96, which is regarded as indicative for active infection, particularly in the non-host adapted serovars, where some reactors were found on 11 farms. The highest seroprevalence for Pomona was Farm 15.

3.3.2.3 Pomona "case" farm

Farm 15 was the only farm that showed a pattern of seroconversion suggestive of an active infection, whereas the titre pattern on other farms was suggestive of sporadic infection or non-specificity. Summary data for this farm are presented in Figure 3.6. The total seroprevalence including all samples both years was 40.36% with seroprevalence and reciprocal GMT (in brackets) in weaners, yearlings and adults of 32.7% (134), 38.29% (115) and 51.78% (81), respectively (Figure 3.2). A higher seroprevalence was observed in males as above. Titre seroprevalence in many individual animals increased in sequential samples, typical of active infection during the onset and convalescent phases with a non-host adapted serovar. Seroprevalence was highest in males in March both years, but overall seroprevalence was highest in September year 2. Active infection was identified by consistently high titres through more than one sampling point, whereas sporadic infections were defined by low infection prevalence.

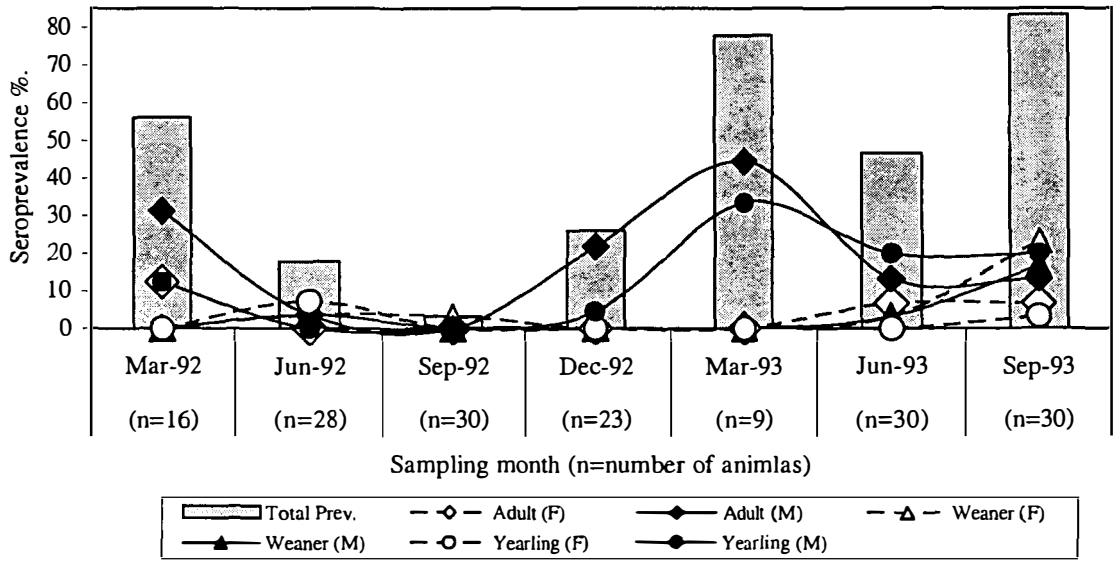


Figure 3.6 Total and individual seroprevalences by age and sex for Pomona on Farm 15.

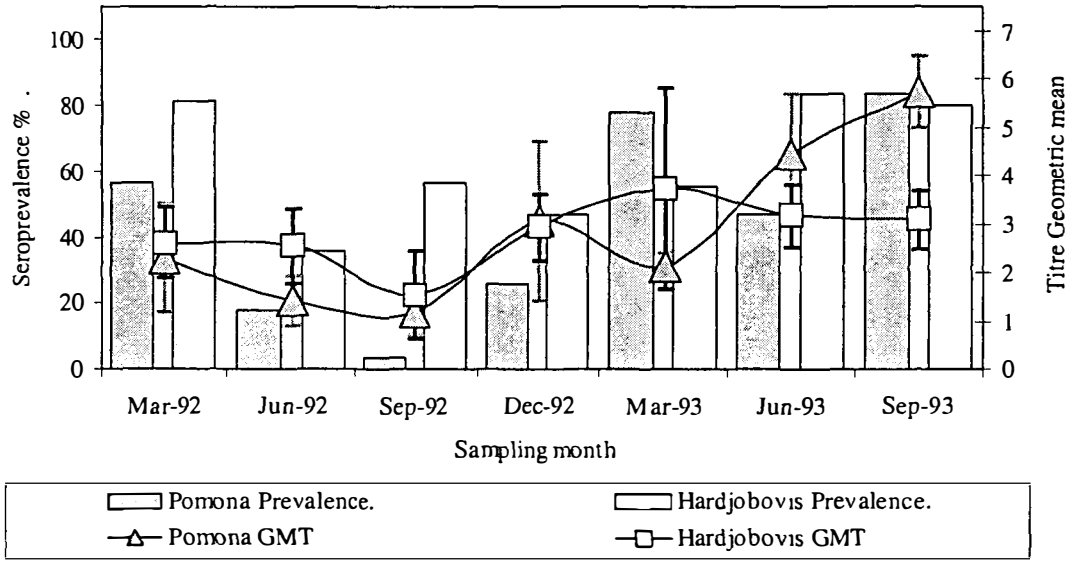


Figure 3.7 Comparative seroprevalence and GMT for serovars Pomona and Hardjobovis on Farm 15.

Seroprevalence and GMT data for both Hardjobovis and Pomona from deer on Farm 15 are presented in Figure 3.7, to demonstrate patterns for each serovar. For serovar Pomona, the GMT increased as seroprevalence increased in the second year while the GMT for serovar Hardjobovis remained relatively stable in year 2 despite there being a higher Seroprevalence.

3.3.3 Serovar Copenhageni

Summary data for serovar Copenhageni are presented in Table 3.5. Nine farms had at least one titre ≥1:48, with 27 animals having a titre of ≥96. Mean seroprevalence was 4.76% (3.9%-5.8% CI), ranging from zero to 10.17% with two farms having a seroprevalence of ≥10%, and the titres ranging from 0 to 1:384. The reciprocal GMT of seropositive samples was 33.1 (mode 1:24). Seroprevalence, all animals and both years combined, was 6% in weaners (6.4% females and 2.5% males), 6% in yearlings (8.1% females and 5.1% males) and 4% in adults (4.9% females and 4.4% males). Most farms had only one sample seropositive to Copenhageni. Few animals were seropositive on more than one occasion, and in those instances, titres were within one dilution. Of the animals with titres ≥1:96, eight had no titres to other serovars. Most seropositive samples were from Farm 15 which had the highest seroprevalence to Pomona.

In the regression analysis for serovar Copenhageni, seroprevalence, farm, year, age season, or sex was not significant. Regression analysis and Relative risks for the relationship between serovars Copenhageni as a response variable and Pomona and Hardjobovis as effect variables are presented in Table 3.1. There was a significant positive relationship between seroprevalence for serovars Copenhageni and Pomona at all dilutions tested, whereas there was a positive relationship with Hardjobovis only at ≥1:24 when the titre to Copenhageni was ≥1:96.

Table 3.1 Summary of a regression analysis and Relative Risk for the relationship between Copenhageni and Hardjobovis and Pomona at different cut points. (Only statistically significant comparisons are included).

Response variable Copenhageni titre	Effect variables		χ^2 (p)	Relative Risk (95%CI)
	Serovar	Titre		
≥ 1:24	Pomona	≥ 1:24	19.8 (<0.001)	1.6 (1.3-2.1)
		≥ 1:48	33.8 (<0.001)	2.4 (1.8-3.1)
		≥ 1:96	12.9 (=0.006)	2.5 (1.4-4.1)
≥ 1:48	Pomona	≥ 1:24	14.7 (<0.001)	1.8 (1.3-2.4)
		≥ 1:48	32.6 (<0.001)	3.1 (2.1 –4.2)
		≥ 1:96	22.1 (=0.003)	4.1 (2.2-6.9)
≥ 1:96	Pomona	≥ 1:24	13.2 (<0.001)	2.5 (1.6-3.5)
		≥ 1:48	18.7 (<0.001)	3.9 (2.2 -5.9)
		≥ 1:96	12.7 (<0.001)	5.2 (2.1- 10.8)
	Hardjobovis	≥ 1:24	4.96 (=0.02)	0.57 (0.31-0.9)

3.3.5 Re-sampling of farms

A summary of the seroprevalence and herd status for the re-sampled farms is presented in Table 3.2. Excluding the vaccinated herd (no. 13), all but one herd remained seropositive to serovar Hardjobovis. No farm was seropositive for Pomona on both occasions, but two were seronegative on both occasions. Five farms remained negative to serovar Copenhageni. The mean seroprevalence for Hardjobovis was similar both years. The seroprevalence of Pomona was higher on the two farms infected in 2004-5 than in 1992-3.

Table 3.2. Seroprevalence of each serovar, and infection status of re-sampled herds sampled in both 1992-3 (92-3), and 2004-5 (04-5).

Farm	Seroprevalence %						Herd status					
	Serovar						Serovar					
	Hardjobovis		Pomona		Copenhageni		Hardjobovis		Pomona		Copenhageni	
	92-3	04-5	92-3	04-5	92-3	04-5	92-3	04-5	92-3	04-5	92-3	04-5
3	32.6	85	1.3	0	5.3	0	(+)	(+)	(-)	(-)	(-)	(-)
7	27.8	45	6.0	65	7.7	0	(+)	(+)	(-)	(+)	(-)	(-)
9	67.7	28	11.4	0	4.7	0	(+)	(+)	(+)	(-)	(-)	(-)
10	26.5	0	4.9	0	2.0	0	(+)	(-)	(-)	(-)	(-)	(-)
11	64.2	15	8.0	56	0	0	(+)	(+)	(-)	(+)	(-)	(-)
15	62.5	100	40.3	0	10.8	0	(+)	(+)	(+)	(-)	(-)	(-)
13	89.4	65*	5.9	45*	3.9	20*	(+)	(+)*	(-)	(+)*	(+)	(+)*
Mean[+]	49.9	54.5	12.8	60.5	5.7							

Notes		
	1992-1993	2004-2005
3	No Pomona > 1:48, 3 Copenhageni = 1:96	New farm location, same herd. Deer/sheep, Closed
7	No Pomona > 1:48, 2 Copenhageni = 1:96	No changes in location or stock, deer only
9	3 Pomona > 1:48, 1 Copenhageni = 1:96	No changes in location or stock, deer only
10	No Pomona > 1:48 no positive paired sample	Velvet and breeding, closed herd, deer only
11	No Pomona > 1:48 no positive paired sample	No changes in location or stock, mixed species
15	Several animals with positive > 1:96, 8 samples Copenhageni ≥1:96	No changes in location or stock, mixed species.
13	1 Pomona > 1:48, 2 Copenhageni = 1:96	Vaccinated, had an outbreak 2 year previously

[+] Mean of positive farms, *= Vaccinated herd.

3.4 Discussion

This study utilised a serum bank from a 2-year longitudinal observational study to investigate some aspects of the epidemiology of leptospirosis in farmed deer based on serological evidence. Single point serological surveys and reports such as those described by Fairley et al (1984, 1986), Flint et al (1986), Wilson and McGhie (1993) and Dean et al (2005) are useful as an initial assessment of the disease within populations, but they do not contribute greatly to understanding of its epidemiology (Faine et al 1999). Two earlier serological surveys provide only farm and individual animal prevalence data of leptospirosis in farmed deer in New Zealand (Flint et al 1988a; Wilson et al 1998). The present research is the most comprehensive longitudinal study of this disease in farmed deer that fulfils the criteria for such studies (Hathaway et al 1986; Faine et al 1999). The existence of a serum bank, supported by productivity and health data, allowed a cost- and time-effective investigation that logistics and financial resources would usually prohibit.

While it is known that serovar Pomona (Fairley et al 1984, 1986; Wilson and McGhie 1993; Dean et a 2005) and possibly Copenhageni (Walker pers. comm.) can cause clinical disease in deer, and that abortion occurred following artificial infection of white-tailed deer with serovar Pomona (Ferris et al 1960; Trainer et al 1961; Reilly et la 1962a), there is no convincing evidence that serovar Hardjobovis is pathogenic in deer (Ayanegui-Alcerreca et al (2006a) this thesis). The database on production and health upon the study farms described in this chapter (Audigé 1995) showed no evidence of subclinical or clinical losses or abortion or other reproductive losses associated with leptospiral infections on any of the 15 study farms.

3.4.1 Serovar Hardjobovis

This study demonstrated a 100% farm and 51.8% individual animal seroprevalence for Hardjobovis. Flint et al (1988a) reported individual animal seroprevalence of 29% using a titre cut point ≥1:20. That study gave only a partial description of the origin of data, preventing calculation of farm prevalence. Wilson et al (1998) reported a farm seroprevalence of 73.6% (n=622 deer/53 farms) with a cut point of ≥1:96, but when all titres ≥24 were included, as used in that study, the farm seroprevalence was 81.1%. The individual animal seroprevalence was 63.7%. The regional survey of 107 farms in 2004-5 (n=2044 sampled deer) presented in Chapter 7 showed 65% farm seroprevalence and 63% individual animal seroprevalence for Hardjobovis titres using a cut point of ≥1:24. Thus, it would appear that serovar Hardjobovis has been, and continues to be, widespread on deer farms in New Zealand and there is no clear evidence of different seroprevalence over that time.

The higher individual animal seroprevalence seen both in the study of Wilson et al (1998), and in the more recent seroprevalence data presented in Chapter 7, was largely attributed to the presence of weaners in the present study. Weaners had the lowest over all seroprevalence (40.6%).

The predominance of serovar Hardjobovis in this study parallels the findings from surveys in dairy cattle in New Zealand in which Hellstrom (1978) showed that 80% of herds and 60% of individuals were seropositive and Mackintosh (1981) showed that 38% of herds and 66.3% of individuals were seropositive. Cordes et al (1982), studied 250 dairy herds where 7,500 cows were tested, and reported 12.5% herd seroprevalence at a titre cut point of ≥1:200, and 88% if all titres ≥1:20 were included. Bahaman et al (1984b) showed a 62% seroprevalence of Hardjobovis in >800 cows tested in a cross sectional study in the Taranaki district and Lake (1973) reported 18% seroprevalence of Hardjobovis in 890 serum samples from cattle herds linked to clinical leptospirosis in humans where Hardjobovis was the predominant serovar. Similar seroprevalences in cattle, in other countries with extensive farming in temperate climates, have been reported. In Australia, Elder et al (1986) reported Hardjobovis as the predominant serovar with seroprevalence ranging from >35% to 65% between years and Black et al (2001) reported 15% seroprevalence of Hardjobovis from unvaccinated beef cattle at slaughter. Similar seroprevalences are reported from the United Kingdom (Hathaway et al 1986; Little et al 1992 a,b).

Lack of evidence in the original study (Audigé 1995) linking serovar Hardjobovis with reproductive losses or disease is consistent with observations from cattle in New Zealand (Hathaway 1981c) where kidney invasion and potential as a zoonosis rather than clinical disease is the primary concern (Mackintosh 1981). The link between serovar Hardjobovis and kidney lesions in deer is discussed in Chapters 4 and 8.

Farm and year effects were observed in seroprevalence of Hardjobovis. While the overall Hardjobovis seroprevalence was around 50% each year, the variable "year" was significant in the regression analysis. These variations are likely to be attributable to individual farm differences in environment, management practices, and climate within years, and climate within farms between years, as observed in cattle in Australia (Elder et al 1986). The variations were largely attributable to the difference in weaners where the seroprevalence was higher in year 1 than in year 2. The high titres in the first year appear to be maternal antibody since the seroprevalence decreased from March to June and there was a slight reduction in

GMT for those that remained positive. The high titres that year may have been due to high maternal antibody concentrations as a result of assumed high challenge in the year before the study. The maternal antibody effect appeared less in the second year. Further evidence for colostral antibody is presented in Chapters 4 and 5. In cattle, Hellstrom (1978) reported an average duration of maternal antibodies of 120 days. However, a month by herd interaction was not significant therefore validating the aggregation of herds.

Climatic data from Audigé (1995) showed an overall mean rainfall of 130.66 and 88.7 mm in years 1 and 2, respectively. However, it was not appropriate to analyse climatic variables in this study because of the complexity of the epidemiology of leptospirosis and the many confounding influences between farms. However, numerous reports of human cases of leptospirosis are associated with wet environment (Levett 2001). Yearly fluctuations on the seroprevalence of Hardjobovis and Pomona in cattle were correlated with humidity but not with the total rainfall or rain days (Elder et al 1986). Hellstrom and Marshall (1978) reported a correlation between seroconversion of Hardjobovis among cattle and the wettest period of the year.

This study analysed seroprevalence in deer from 3-4 months of age, while studies of Flint et al (1988a) and Wilson et al (1998) sampled deer 12 months of age and older. This information is essential for determination of control strategies. Deer of 3-4 months of age were seropositive on most farms, suggesting that exposure to infection occurs early in life and/or that maternal antibody persists for at least that period. It was not possible to distinguish between infectious and maternal antibody titres since post-vaccine maternal antibody titres observed from an uninfected herd post-vaccination (Chapter 6) were of a similar magnitude. However, the seroprevalence had dropped to its lowest level by June, suggesting strongly that the March titres were colostral in origin. Seroprevalence and GMT in September suggest that infection had occurred by 6-9 months of age on most of the properties. The continued increase in seroprevalence to yearling and adult ages, being statistically higher than in weaners, supports the proposal that exposure to infection occurs in the first year of life. In dairy cattle, Hellstrom (1978) reported that maternal antibodies declined by 3 months of age, that infection and urine shedding was uncommon before 7-8 months of age, and was most common in first calving heifers at two years of age. Bahaman et al (1984b) and Little et al (1992 a, b) reported the highest seroconversion in 1- and 2-year-old dairy cattle. A similar pattern related to age and physiological status has been reported in other species including Balcanica in possums (Hathaway 1978), Ballum in mice (Hathaway et al 1983) and Pomona (Bolt 1990; Ryan 1978) and Tarassovi (Ryan 1976a,b) in domestic pigs.

No seasonal effect on seroprevalence overall was identified in this study, although there was a month effect in weaners, which is explained as an age rather than season effect. Seasonal variations in seroprevalence of Hardjobovis in cattle have been reported by Elder et al (1986) and Little et al (1992b).

3.4.2 Serovar Pomona

This study showed an overall 26.6% farm and 24.6% individual animal seroprevalence for Pomona using the definitions that two animals with titres ≥1:48 were required to represent herd infection, and using a cut point of 1:48 for an individual animal, though the prevalence estimate decreased when a higher titre cut of was used. Other authors use various interpretations, making direct comparisons difficult. The mean seroprevalence within farms

was 10.5% with 5 farms >10%. No age difference was observed, probably due to low power, because of the small numbers. Flint (1988a) reported 2.79% individual animal seroprevalence for Pomona, while Wilson et al (1998) reported 41.5% of farms and 12.0% of individual animals were seropositive with some being dual infections with serovar Hardjobovis. Survey data in Chapter 7 showed 4% of the herds had Pomona alone and 14% had dual infections with Hardjobovis. Pomona seroprevalence in all three studies was lower than Hardjobovis seroprevalence.

Only a small proportion of deer showed titres ≥1:96 and fewer showed positive paired samples with titres ≥ 1:96. Few farms had deer with serial samples positive for serovar Pomona. This pattern is suggestive of either sporadic infection or non-specificity rather than widespread endemic infection in the study herds. Farm 15 with a 40.6% overall seroprevalence was the only one that presented what was considered an epidemic pattern typical of a non-host adapted serovar (Hathaway 1981b,c). The pattern of seroconversion observed in this farm was similar to those observed in longitudinal studies of two farms infected with serovar Pomona presented in Chapter 4. It is notable that this farm was not seropositive for Pomona when re-sampled in 2004-5.

The pattern of serological results for Pomona is similar to that recognised for this serovar in cattle in New Zealand in which the infectious cycle can be sustained for only a limited time and the serovar does not become truly endemic in herds (Hellstrom 1978). In New Zealand dairy cattle, Hellstrom (1978) reported 36% of herds and 18% of individuals were seropositive, Bahaman et al (1984b) reported 80.0% of herds of ten herds and 4% of individuals were seropositive and Cordes et al (1982) reported 6.0% of herds and 3.5% of individuals were seropositive to Pomona. In Australia Elder et al (1986) reported seroprevalence between 20% to 45%.

In farmed deer the majority of clinical leptospirosis cases involving serovar Pomona have been during the autumn and early winter in their first year of life (Wilson and McGhie, 1993, Dean et al 2005).

The significant sex effect on seroprevalence of Pomona was attributable to two farms. It is likely that management practices differ between animal classes within herds. However, it is possible that on farms with high prevalence of infection with serovar Pomona the higher frequency of wallowing in stags and their direct physical contact through fighting during the rut, including spraying of urine may predispose stags to infection.

3.4.3 Serovar Copenhageni

This study showed 13.3% of farms and 10.5% of individual animals were seropositive for Copenhageni. The mean herd seroprevalence for serovar Copenhageni was 4.76%, and four farms showed a seroprevalence slightly higher than >10%. Flint (1988a) reported 16.7% individual animal seroprevalence with a titre cut point of ≥1:40 and Wilson et al (1998) reported 11.3% seroprevalence at the herd level, where five herds showed titres ≥1:96 in more than one animal, in the absence of titres to Pomona. The regional survey presented in Chapter 7 showed a seroprevalence for Copenhageni of 0.44% at the individual level. Few animals showed an increased titre in paired samples without a concurrent high titre for serovar Pomona. There was a significant correlation between seropositivity for Pomona and Copenhageni, but not with Hardjobovis. Therefore, with the possible exception of eight animals with titres ≥96, which could indicate infection (Appendix 1), it is concluded that

other titres were likely to be the result of cross-reaction. In pigs, Ryan (1978) observed a high cross-reactivity with Pomona and in cattle Hellstrom (1978) observed the same.

Other authors present more convincing evidence that deer can be infected with serovar Copenhageni. Inglis (1984) reported six of 10 deer with titres ≥1:100 and Flint et al (1986) subsequently isolated Copenhageni from the urine of four of 27 deer on the same property. Infection was suggested as accidental through contact with the brown rat, which is the recognised maintenance host for this serovar (Flint 1986).

3.4.4 Re-sampling of Farms

Establishment of herd status in 1992-3 was on the basis of two or more sequential titres in any animal of 1:48 or more since up to seven samples were collected over the sampling period. Only one sample was collected per animal in 2004-5. Thus, infected farm status may have been underestimated in 2004-5, or overestimated in 1992-3. However, the seroprevalence and herd status for serovar hardjobovis were consistent between years in five of the six non-vaccinated herds. There were two herds positive for serovar Pomona in both periods, but the infection was in different herds in 2004-5. In four of the six non-vaccinated herds, titres to Pomona at ≥1:48 were present in some animals in 1992-3, giving the seroprevalences presented in Table 3.2. It is possible that had similar serial samples been collected in 2004-5, more titres may have been detected. This suggests that the MAT may have varied in specificity, and that titres in that range may not be due to infection. These observations of herd status and seroprevalence support the hypothesis that farmed deer are maintenance hosts for serovar Hardjobovis and accidental/incidental hosts for serovar Pomona, similar to the cattle in New Zealand (Hathaway 1981b,c), and Copenhageni.

No titres were detected to serovar Copenhageni that suggest a herd level infection. The titres observed in 1992-3 were likely to be cross-reactivity to Pomona as discussed previously. Samples from farm 13 were from 12-month-old deer that had been vaccinated as weaners eight months earlier in March/April. This pattern of antibody persistence is similar to that observed in vaccine trials presented in Chapter 5.

3.5 Conclusions

- This study has helped define the epidemiological pattern of infection with leptospiral serovars Hardjobovis, Pomona and Copenhageni in farmed deer.
- The high seroprevalence of Hardjobovis demonstrates widespread infection with Hardjobovis within and between farms and this is, along with other evidence, shows Hardjobovis is the predominant serovar in farmed deer in New Zealand with an endemic pattern of infection.
- The pattern of infection with Hardjobovis was similar between years with highest prevalence in older deer, and lowest prevalence in weaners. Data suggest that infection occurs through the first year of life and is persistent.
- Evidence demonstrates that infections with serovar Pomona were present but at a low herd and individual animal prevalence. The pattern within and between farms resembles a sporadic or epidemic rather than endemic infection.
- Most serovar Copenhageni titres were considered cross-reactivity to serovar Pomona with only a few titres possibly the result of the natural infection.

- Data support the proposal that deer are maintenance hosts for serovar Hardjobovis, and accidental hosts for serovar Pomona and Copenhageni.

Table 3.3. Summary of number (n) and % of deer sampled that were Hardjobovis seropositive at a cut point of ≥1:24, and reciprocal titre of the geometric mean titre (GMT) at each sampling period, by age category and sampling month.

Hardjobovis		March 92		June 92		September 92		December 92		March 93		June 93		September 93	
Farm	Age	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT
1	Weaner			0.0 (10)		30.0 (10)	36			16.7 (6)	96	46.2 (26)	79	76.5 (17)	101
	Yearling	25.0 (4)	24	11.1 (9)	96	11.1 (9)	192					57.1 (7)	0	100 (1)	120
	Adult	11.1 (9)	4	70.0 (10)	57	63.6 (11)	74					94.1 (17)	67	90.0 (10)	50
2	Weaner	100 (5)	32			70.0 (10)	112			54.5(11)	45	60.0 (10)	71	20.0 (10)	605
	Yearling	100 (5)	159			88.9 (9)	83			22.2 (9)	48	100 (8)	66	66.7 (9)	83
	Adult					100 (9)	47			14.3 (7)	96	100 (8)	86	87.5 (8)	120
3	Weaner	87.5 (8)	47	0.0 (9)	0	20.0 (10)	40	0.0 (9)	0	0.0 (5)	0	20.0 (15)	59	0.0 (16)	0
	Yearling	20.0 (5)	48	40.0 (5)	85	100 (1)	192	100 (1)	96		0	16.7 (6)	48	0.0 (6)	0
	Adult	57.1 (7)	68	75.0 (12)	129	66.7 (15)	93	0.0 (2)	0		0	33.3 (9)	43	55.6 (9)	62
4	Weaner	25.0 (5)	48	0.0 (10)	0	63.6 (11)	28	0.0 (5)	0	8.3 (12)	96	30.8 (13)	24	0.0 (13)	0
	Yearling	20.0 (4)	24	20.0 (5)	24	80.0 (5)	32			14.3 (7)	85	40.0 (10)	38	9.1 (11)	48
	Adult	50.0 (4)	24	25.0 (4)	96	50.0 (4)	40			0.0 (2)	0	50.0 (6)	24	14.3 (7)	48
5	Weaner	71.4 (7)	45	11.1 (9)	24	100 (9)	81	40.0 (5)	96	0.0 (5)	0	85.7 (14)	51	58.3 (12)	211
	Yearling	85.7 (7)	80	28.6 (7)	24	100 (8)	54	25.0 (4)	24	0.0 (3)	0	80.0 (5)	90	80.0 (5)	268
	Adult	50.0 (2)	48	66.7 (6)	207	80.0 (10)	136	16.0 (7)	160	66.7 (3)	0	87.5 (8)	80	66.7 (3)	96
6	Weaner	90.0 (10)	61	38.5 (13)	24	22.2 (9)	24	44.4 (9)	24	0.0 (3)	0	27.3 (11)	24	9.1 (11)	48
	Yearling	33.3 (6)	96	45.5 (11)	30	0.0 (9)	0	20.0 (5)	48	0.0 (1)	0	45.5 (11)	37	18.2 (11)	66
	Adult	50.0 (2)	24	75.0 (12)	57	80.0 (10)	48	100 (1)	48	0.0 (1)	0	62.5 (8)	34	37.5 (8)	74
7	Weaner											50.0 (12)	49	0.0 (9)	0
	Yearling	25.0 (4)	24	12.5 (8)	24	60.0 (10)	34		0	0.0 (4)	0	54.5 (11)	39	0.0 (11)	0
	Adult	20.0 (10)	31	20.0 (5)	96	60.0 (10)	34		0	0.0 (5)	0	37.5 (8)	42	0.0 (7)	0
8	Weaner		0	0.0 (10)	0	80.0 (10)	43	77.8 (9)	312	100 (2)	192	80.0 (15)	81	50.0 (16)	79
	Yearling	100 (4)	384	100 (5)	62	80.0 (5)	75	100 (1)	384	100 (3)	439	100 (10)	143	75.0 (8)	87
	Adult	87.5 (8)	114	100 (5)	41	100 (6)	71					100 (4)	175	80.0 (5)	101
9	Weaner	16.7 (7)	51	22.2 (9)	24	50.0 (10)	38	100 (8)	198	80.0 (5)	95	66.7 (15)	97	50.0 (14)	171
	Yearling	71.4 (6)	48	100 (10)	192	60.0 (10)	39			100 (1)	96	100 (6)	219	83.3 (6)	202
	Adult	50.0 (2)	48	90.0 (0)	68	50.0 (10)	37	50.0 (2)	192	50.0 (2)	96	88.9 (9)	126	85.7 (7)	503
10	Weaner	28.6 (7)	24	0.0 (8)	0	90.0 (10)	53	11.1 (9)	48	0.0 (3)	0	11.1 (18)	24	5.6 (18)	384
	Yearling	50.0 (2)	48	0.0 (8)	0	77.8 (9)	53	0.0 (4)	0		0	16.7 (6)	48	16.7 (6)	24
	Adult	75.0 (4)	28	22.2 (9)	85	62.5 (8)	37	66.7 (3)	36		0	20.0 (5)	48	0.0 (6)	0
11	Weaner		0	100 (1)	24	33.3 (9)	48	40.0 (5)	107		0	100 (2)	107	45.5 (11)	210
	Yearling	80.0 (10)	27	50.0 (2)	768	40.0 (10)	66	50.0 (6)	238	100 (6)	211	100 (4)	167	80.0 (10)	267
	Adult	100 (5)	130	71.4 (7)	27	30.0 (10)	68	66.7 (3)	227	100 (3)	89	100 (1)	96	85.7 (7)	102
13	Weaner	100 (1)	96	40.0 (10)	46	90.0 (10)	82	100 (10)	102	100 (5)	54	94.7 (19)	106	85.0 (20)	111
	Yearling	83.3 (6)	77	100(10)	212	90.0 (10)	223	100(7)	83			100 (6)	139	100 (6)	78
	Adult	100 (5)	103	88.9 (9)	40	88.9 (9)	109	100 (3)	42			100 (4)	119	66.7 (3)	96
14	Weaner	0.0 (2)	0			63.6 (11)	36	0.0 (5)	73						
	Yearling														
	Adult	66.7 (9)	54			68.4 (19)	45	25.0 (4)	48						
15	Weaner	50.0 (2)	48	10.0 (10)	24	10.0 (24)	24	40.0 (5)	152			69.2 (13)	86	61.5 (13)	129
	Yearling	83.3 (6)	52	0.0 (8)	0	60.0 (10)	44	40.0 (10)	384	60.0 (5)	103	88.9 (9)	98	88.9 (9)	80
	Adult	87.5 (8)	104	90.0 (10)	85	70.0 (10)	31	87.5 (8)	85	50.0 (4)	358	100 (8)	160	100 (8)	106
16	Weaner	0.0 (2)	0	10.0 (10)	192	40.0 (10)	30	0.0 (10)	0	0.0 (4)	0	63.2 (19)	49	0.0 (19)	0
	Yearling	66.7 (6)	43	20.0 (10)	192	20.0 (10)	48		0	0.0 (1)	0				
	Adult	40.0 (5)	31	37.5 (8)	53	33.3 (9)	42	50.0 (2)	0			90.0 (10)	58	10.0 (10)	384

Table 3.4. Summary of number (n) and % of deer sampled that were Pomona seropositive at a cut point of ≥1:48, and reciprocal titre of the geometric mean titre (GMT) at each sampling period, by age category and sampling month.

Pomona		March 92		June 92		September 92		December 92		March 93		June 93		September 93	
Farm	Age	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT
1	Weaner			20 (10)	107	0 (10)	0			0 (6)	0	0 (26)	24	5.8 (17)	32
	Yearling	0 (4)	0	11.1 (9)	48	0 (9)	0					0 (7)	0	0 (1)	0
	Adult	11.1 (9)	48	30 (10)	128	0 (11)	24					5.8 (17)	32	10 (10)	40
2	Weaner	0 (5)	0			10 (10)	32			9.0 (11)	48	10 (10)	384	10 (10)	32
	Yearling	0 (5)	24			11.1(9)	26			11.1(9)	48	12.5 (8)	48	0 (9)	0
	Adult					0 (9)	24			0 (7)	0	25 (8)	66	12.5 (8)	27
3	Weaner	0 (8)	24	22.2 (9)	36	0 (10)	24	0 (8)	24	0 (5)	0	0 (15)	0	0 (16)	24
	Yearling	0 (5)	24	0 (5)	0	0 (1)	26	0 (1)	24			0 (6)	0	0 (6)	24
	Adult	0 (7)	0	0 (12)	24	0 (15)	24	0 (2)	0			0 (9)	0	0 (9)	0
4	Weaner	0 (5)	24	20 (10)	48	0 (11)	0	0 (5)	0	0 (12)	0	15.3 (13)	48	7.6 (13)	96
	Yearling	0 (4)	0	20 (5)	48	40 (5)	24			0 (7)	0	20 (10)	48	9.0 (11)	48
	Adult	25 (4)	96	25 (4)	96	0 (4)	0			0 (2)	0	0 (6)	0	0 (6)	0
5	Weaner	14.2 (7)	32	22.2(9)	36	11.1(9)	0	0 (5)	0	40 (5)	36	7.1 (14)	27	0 (12)	24
	Yearling	0 (7)	24	42.8 (7)	38	12.5 (8)	48	0 (4)	0	66.6 (3)	66	0 (5)	24	0 (5)	24
	Adult	50 (2)	48	0 (6)		30 (10)	0	0 (7)	0	33.3 (3)	96	12.5 (8)	48	0 (3)	0
6	Weaner	0(10)	24	0 (13)	0	11.1 (9)	29	0 (9)	0	66.6 (3)	66	9.0 (11)	192	9.0 (11)	96
	Yearling	16.6 (6)	48	0 (11)	24	22.2 (9)	48	0 (5)	24	100 (1)	48	9.0 (11)	27	18.1 (11)	48
	Adult	0 (2)	0	8.3(12)	29	20 (10)	128	0 (1)	0	0 (1)	24	0 (8)	24	12.5 (8)	48
7	Weaner			0 (1)	0							0 (12)	0	0 (9)	24
	Yearling	25 (4)	48	0 (8)	0	0 (10)	36			25(4)	48	0 (11)	0	0 (11)	24
	Adult	30 (10)	44	0 (5)	0	0 (10)	32			40 (5)	36	0 (8)	0	0 (7)	24
8	Weaner			0 (10)	0	20 (10)	48	66.6 (9)	45	50 (2)	96	0 (15)	0	6.25 (16)	32
	Yearling	0 (4)	24	0 (5)	0	0 (5)	0	0 (1)	0	0 (3)	24	0 (10)	0	0 (8)	24
	Adult	0 (8)	24	0 (5)	0	0 (6)	0					0 (14)	24	0 (5)	0
9	Weaner	14.2 (7)	32	11.1 (9)	29	10 (10)	29	0 (8)	24	60 (5)	44	0(15)	24	0 (13)	24
	Yearling	16.6 (6)	0	20 (10)	48	10(10)	192			0 (1)	0	16.6 (6)	48	50 (6)	48
	Adult	0 (2)	29	20 (10)	32	0 (10)	24	0 (2)	0	50 (2)	48	0 (9)	24	0 (7)	0
10	Weaner	0 (7)	24	12.5 (8)	32	10 (10)	48	11.1 (9)	27	0 (3)	24	0 (18)		0 (18(	24
	Yearling	0 (2)	0	0 (8)	24	11.1 (9)	48	0 (4)				0 (6)		0 (6)	0
	Adult	25 (4)	32	22.2 (9)	48	0 (8)	24	0 (3)				0 (5)	24	0 (6)	24
11	Weaner			0 (1)	0	0 (9)	0	0 (5)	0			0 (2)	0	0 (11)	24
	Yearling	10 (10)	27	0 (2)	0	0 (10)	0	0 (6)	0	0 (6)	24	0 (4)	0	0 (10)	0
	Adult	60 (5)	38	28.5 (7)	48	0 (10)	0	33.3 (3)	48	33.3 (3)	48	100 (1)	48	0 (7)	0
13	Weaner	0 (1)	0	0 (10)	24	10 (10)	32	0 (10)	24	20 (5)	27	5.2 (19)	48	0 (20)	24
	Yearling	33.3 (6)	36	10 (10)	32	0 (10)	24	0 (7)	0			0 (6)	0	0 (6)	0
	Adult	0(5)	0	0 (9)	24	22.2 (9)	36	33.3 (3)	48			0 (4)	0	0 (3)	0
14	Weaner	0 (2)	0	100 (1)	96	36.3 (11)	68	0 (5)	24						
	Yearling														
	Adult	0 (9)	24			15.7 (19)	85	25 (4)	32						
15	Weaner	0 (2)	24	20 (10)	34	10 (10)	29	0 (5)				15.3 (13)	59	92.3 (13)	1568
	Yearling	33.3 (6)	36	25 (8)	36	0 10)	0	10 (10)	32	60 (5)	48	66.6 (9)	1045	77.7 (9)	666
	Adult	87.5 (8)	90	10 (10)	27	0 (10)	24	62.5 (8)	219	100 (4)	56	75(8)	223	75 (8)	164
16	Weaner	0 (2)	24	0 (10)	0	10 (10)	33	0 (10)	0	0 (4)	0	0 (19)	0	5.2 (19)	96
	Yearling	0 (6)	0	0 (10)	0	20 (10)	86			0 (1)	0				
	Adult	20 (5)	32	0 (8)	0	11.1 (9)	96	0 (2)	0			10 (10)	0	0 (10)	0

Table 3.5. Summary of number (n) and % of deer sampled that were Copenhageni seropositive at a cut point of ≥1:48, and reciprocal titre of the geometric mean titre (GMT) at each sampling period, by age category and sampling month.

Copenhageni		March 92		June 92		September 92		December 92		March 93		June 93		September 93	
Farm	Age	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT	% (n)	GMT
1	Weaner			0 (10)	24	0 (10)	0			83.3 (6)	67	0 (26)	0	0 (18)	24
	Yearling	0 (4)	0	22.2 (9)	132	11.1 (9)	48					0 (7)	0	0 (1)	0
	Adult	0 (9)	0	0 (10)	0	9.0 (11)	29					0 (17)	0	20 (10)	36
2	Weaner	0 (5)	0			10 (10)	32			18.1(11)	34	0 (10)	0	20 (10)	42
	Yearling	0 (5)	0			0 (9)	24			33.3 (9)	48	0 (8)	24	11.1 (9)	32
	Adult					11.1 (9)	48			14.2 (7)	27	0 (8)	0	12.5 (8)	48
3	Weaner	0 (8)	0	11.1(9)	96	0 (10)		0 (8)	0	0 (5)	24	6.6 (15)	32	6.2 (16)	48
	Yearling	0 (5)	0	20 (5)	96	0 (1)		0 (1)	0			33.3 (6)	66	16.6 (6)	32
	Adult	0 (7)	0	0 (12)	24	0 (15)	24	0 (2)	0			0 (10)	0	11.1(9)	48
4	Weaner	0 (5)	0	20 (10)	36	0 (11)	0	0 (5)	0	16.6 (12)	29	0 (13)	24	7.6 (13)	32
	Yearling	0 (4)	0	0 (5)	24	0 (5)	0			0 (7)	0	10 (10)	48	18.1 (11)	36
	Adult	0 (4)	0	0 (4)	0	0 (4)	0			0 (2)	0	0 (6)	24	0 (6)	0
5	Weaner	0 (7)	0	0 (9)	24	0 (9)	24	0 (5)	0	0 (5)	24	0 (14)	0	0 (12)	0
	Yearling	0 (7)	0	0 (7)	24	12.5 (8)	32	0 (4)	0	33.3(3)	48	0 (5)	0	0 (5)	0
	Adult	0 (2)	24	0 (6)	0	0 (10)	0	0 (7)	0	0 (3)	0	0 (8)	0	0 (3)	0
6	Weaner	0 (10)	0	0 (13)	24	0 (9)	0	0 (9)	0	0 (3)	24	0 (11)	0	0 (11)	0
	Yearling	0 (6)	0	0 (11)	0	0 (9)	0	0 (5)	0	0 (1)	0	9.0 (11)	96	0 (12)	24
	Adult	0 (2)	0	0 (12)	0	0 (10)	0	0 (1)	0	0 (1)	0	0 (8)	0	0 (8)	0
7	Weaner			0 (1)	0							0 (12)	0	0 (9)	0
	Yearling	0 (4)	0	0 (8)	0	40 (10)	40			0 (4)	24	0 (11)	0	0 (12)	0
	Adult	0 (10)	0	0 (5)	0	50 (10)	59			0 (5)	0	0 (8)	24	0 (7)	24
8	Weaner			0 (11)	0	0 (10)	24	66.6 (9)	24	0 (2)	0	0 (15)	0	0 (16)	0
	Yearling	0 (4)	24	0 (5)	0	0 (5)	0	0 (1)	0	33.3 (3)	48	0 (10)	0	0 (8)	0
	Adult	0 (8)	0	0 (5)	0	0 (6)	24					0 (4)	0	0 (5)	0
9	Weaner	14.2 (7)	48	0(9)	24	10 (10)	48	0 (8)	0	0 (5)	0	13.3 (15)	39	0 (13)	24
	Yearling	0 (7)	0	0 (10)	24	10 (10)	32			0 (1)	0	0 (6)	24	16.6 (6)	48
	Adult	0 (2)	0	10 (10)	48	0 (10)	0	0 (2)	0	0 (2)	0	0 (9)	24	0 (7)	24
10	Weaner	0 (7)	0	0 (8)	24	0 (10)	0	11.1 (9)	48	0 (3)	0	0 (18)	0	0 (18)	0
	Yearling	0 (2)	0	0 (8)	24	0 (9)	0	0 (4)	0			0 (6)	0	0 (7)	0
	Adult	0 (4)	0	11.1(9)	48	0 (8)	24	0 (3)	0			0 (5)	0	0 (6)	0
11	Weaner			0 (1)	0	0 (9)	0	0 (5)	0			0 (2)	0	0 (11)	24
	Yearling	0 (10)	0	0 (2)	24	0 (10)	0	0 (6)	0	0 (6)	24	0 (4)	24	0 (10)	24
	Adult	0 (5)	0	0 (8)	24	0 (10)	24	33.3 (3)	0	0 (3)	0	0 (1)	0	0 (7)	0
13	Weaner	0 (1)	0	0 (10)	24	30 (11)	44	0 (10)	44	0 (5)	44	5.2 (19)	44	5 (20)	44
	Yearling	0 (6)	0	0 (10)	24	0 (10)	0	0 (7)	0			0 (6)	0	0 (6)	0
	Adult	0 (5)	0	0 (9)	0	11.1(9)	0	33.3 (3)	0			0 (4)	0	0 (3)	0
14	Weaner	0 (2)	0	0 (3)	24	18.1 (11)	32	0 (5)	24						
	Yearling														
	Adult	0 (9)	0	100 (2)	48	0 (19)	24	25 (4)	24						
15	Weaner	50 (2)	96	10 (10)	40	0 (10)	0	0 (5)	0			0 (13)	24	61.5 (13)	57
	Yearling	0 (6)	0	12.5 (8)	0	0 (10)	0	10 (10)	24	40 (6)	133	11.1 (9)	133	33.3 (9)	133
	Adult	0 (8)	0	0 (10)	0	0 (10)	24	62.5 (8)	192	0 (4)	24	0 (8)	24	12.5 (8)	32
16	Weaner	0 (2)	0	10 (10)	48	0 (10)	24	0 (10)	24	0 (5)	0	5.2 (19)	48	5.2 (19)	32
	Yearling	0 (6)	0	0 (10)	0	10 (10)	29			0 (1)	0				
	Adult	0 (5)	24	0 (8)	24	11.1(9)	48	0 (2)	0			0 (11)	24	0 (10)	0

Chapter 4

Longitudinal observations of *Leptospira* in naturally infected commercial farmed red deer herds



4.1 Introduction

There is extensive information available on the epidemiology and pathology of leptospiral infections in cattle and swine, but there have been few published studies of natural or experimental infection in deer (Ayanegui-Alcérreca et al 2006b, this thesis). Most evidence of leptospiral infections in deer worldwide is serological. Serovars Hardjobovis, Pomona and Copenhageni have been isolated from deer in New Zealand.

Ferris et al (1960), Trainer et al (1961) and Reilly et al (1962 a, b) described experimental infections with serovar Pomona in captive white tailed deer (*Odocoileus virginianus*) causing haemolytic disease, abortion, and sub-clinical renal disease. Clinical and diagnostic laboratory reports provide evidence of clinical and sub-clinical manifestations of infection with this serovar in farmed deer in New Zealand (Fairley et al 1984 and 1986; Wilson and McGhie 1993; Wilson et al 1998; Dean et al 2005). Evidence of Hardjobovis in farmed deer herds is associated with kidney lesions and human infections, rather than clinical disease in deer (Flint et al 1986; Wilson and McGhie 1993; Wilson et al 1998; Bell 2005; Brown 2005). However, no systematic study of the epidemiology of leptospirosis in farmed deer has been done. An understanding of the epidemiology of a disease is essential for underpining control strategies at industry and individual farm levels.

Initial evidence of the epidemiological pattern of infection with serovars Hardjobovis and Pomona in farmed deer was provided by serological analysis from a serum bank containing samples from a 2-year longitudinal study presented in Chapter 3. That study confirmed that deer generally became infected during the first year of life and provided direction for this more detailed study into the epidemiology of leptospiral infections. If those previously described patterns were consistent in this collective of farms, they may reflect what is happening in the other commercial herds. The main study objective was to monitor the natural course of infection with serovars Hardjobovis, Pomona and if detected, Copenhageni, in commercial deer herds based on serological, bacteriological and pathology evidence.

4.2 Materials and Methods

4.2.1 Farm and animal selection and management

Three commercial deer farms from the lower North Island of New Zealand were selected based on their willingness to participate and serological evidence of infection with serovars *Leptospira borgepetersenii* sv Hardjobovis and/or *Leptospira interrogans* sv Pomona. Farm 1 (Hardjobovis positive) and Farm 2 (Hardjobovis and Pomona positive) were identified from seven farms screened on-farm early in 2003 by collection of 20 blood samples from rising 2-year-old hinds. Data are included in the regional survey presented in Chapter 7. Farm 3 (Hardjobovis and Pomona positive) was selected from nine herds screened by serology and the presence of kidney lesions in 12 – 15 deer/herd at the Feilding Deer Slaughter Premises (DSP) in December 2003- January 2004. Data are included in the study of relationships between serology, kidney lesions and culture presented in Chapter 8. This herd was re-confirmed in June 2004 with positive serum and urine samples by Dark Field Microscopy (DFM) from 7-month-old deer at weaning prior to the start of observations.

A general description of the farms and trial design is presented in Table 4.1. A more detailed summary of each farm is presented in Table 2.2, Chapter 2. The farms had no recorded history of vaccination against *Leptospira*. The animals used in the study were the non-vaccinated control animals from vaccination studies presented in Chapter 5. Observations in the present study spanned from June 2003 to December 2004. The year 2003 was unusually dry whereas 2004 was abnormally wet with severe flooding in the late February and constant rain throughout the year. Animals were dual identified with the farmer's identification tag and a distinctive colour ear tag. Animals were run under normal pastoral farming practices.

In addition to the young animals sampled as above, 58 adult hinds from Farm 2 were slaughtered in May Year 1 (2003). Blood and kidney samples were collected.

The number of subjects was established based on the number of animals needed to detect at least one seropositive animal in a population with a minimum prevalence of infection of 10% (Guidelines for the control of leptospirosis. Geneva, World Health Organisation (WHO), 1982; Faine et al 1999). The number ranged from 36-37 in a herd of 100 animals, to 42-43 in a herd of 500, at 99% and 95% confidence limits, respectively, when infection prevalence was 5%. That prevalence was chosen to ensure that sample size would be sufficient to detect infection even in herds with a low infection rate. Forty rising 1-year-old deer, of both sexes were randomly selected from one available mob per farm. Samples were collected approximately six weekly (Table 4.1).

Table 4.1 Summary of study farms and policies, leptospiral status, trial design, duration, sampling schedule and animal fate. (Full detail of farms is presented in Chapter 2).

Farm No. and Location	Deer herd & type of farm	Other Species	Serovar	Year	Trial design									
					Animals		Sampling							Fate (No.)
					Sex	No.								
1 Manawatu	Red and Wapiti cross. Commercial Venison, & Breeding. Closed herd	Sheep Open flock	Hardjobovis	2003			May	Jun	Jul	Sep	Nov	Feb-04	May-04	
					Male	20	B	B	B	B	B	B	B, K	S (20)
					Female	20	B	B	B, U	B, U	B, U	B, U	B	R (20)
2 Central Hawkes Bay	Red deer. Commercial. Venison, Breeding & Velvet Closed herd	Beef, Sheep Open herd/ flock Horses Closed herd	Hardjobovis *	2003				Jun	Jul	Sep	Oct	Dec	Feb-04	
					Male	20		B	B	B	B	B	B	R (20)
					Female	20		B	B, U	B, U	B, U	B, U	B, U	R (20)
			Hardjobovis & Pomona	2004			Feb	Mar	Apr	May	Jun	Sep	Nov-Dec	
					Male	20	B	B	B	B	B	B	B, K	S (16)
					Female	20	B	B, U	B, U	B, U	B, U	B, U	B, U	R (19)
3 Wairarapa	Cross Wapiti deer. Commercial. Open herd	Diary Beef Sheep Open herds/ flocks	Hardjobovis & Pomona	2004							Jul	Aug	Dec	
					Male	16					B	B	B, K	S (16)
					Female	24					B, U	B, U	B, K	S (21)
Total						**160**								**152**

* Initially Hardjobovis but with a subsequent introduction of Pomona B=Blood, U=Urine, K=Kidney, R=retained, S= slaughter

4.2.2 Leptospiral history of farms

Farm 1 was seropositive only to serovar Hardjobovis. It was one of the farms from the 1992-1993 retrospective serological study presented in Chapter 3 (Farm 3 in that study) when it was also infected with Hardjobovis, but had been relocated. The farm had no recorded history of clinical leptospirosis.

Farm 2 was seropositive originally only to serovar Hardjobovis. This farm had experienced low weaning rates from primiparous pregnant hinds, apparently losing offspring in the first month of life, but no clinical leptospirosis had been observed. Introduction of Pomona was detected shortly after the study began coinciding with the introduction of 150 beef calves to the farm and the herd remained positive to both serovars thereafter.

At initial screening (December 2003), Farm 3 had 72.2% of kidneys with severe interstitial nephritis and scarring, 100% seroprevalence of Pomona with titres ranging from 1:96 to 1536 and 63% seroprevalence of Hardjobovis with titres ranging from 1:24 to 1:192. In June 2004, 43 weaners sampled on-farm had 100% seroprevalence to Pomona with titres ≥1:96, 95% seroprevalence to Hardjobovis with titers ≥1:24, and the urine of 16/20 weaner hinds was DFM positive. In addition, coinciding with the beginning of observations, eight post-mortem examinations in weaners with sudden death yielded Pomona isolates from kidneys of three. However, only one death was attributed exclusively to leptospiral infection with the main lesion being severe interstitial nephritis in the absence of haemoglobinuria.

On Farms 1 and 2 in Year 1, 40 weaners involved in a vaccine trial (Chapter 5) were vaccinated against *Leptospira*. In the second year on Farm 2 and in Farm 3 all deer other than those in the study groups were vaccinated part way through the study along with all cattle. Half of the animals in Farm 2 in the second year came from vaccinated hinds.

4.2.3 Sample collection, storage and processing

Techniques for collection, processing, and storage are described in Chapter 2. Blood was collected from all animals at every sampling while urine was collected after the second visit. The final blood sample and kidneys were collected at the DSP from animals not retained on-farm. Serum was stored for serology for Hardjobovis, Pomona and Copenhageni, urine samples were examined by dark field microscopy and cultured, and kidneys were subject to gross and histopathological examination and culture.

4.2.4 Statistical analysis

Descriptive data were expressed as prevalence (±95%CI) including titres from 1:24 and the reciprocal geometric mean of the coded titres with a 95% confidence interval as described in Chapter 2. For presentation and analyses the three farms were categorised as four study groups (referred to as groups) defined by leptospiral status and year.

In addition to considering DFM, urine culture and kidney culture alone, an index of infection referred to as "live *Leptospira* index" was obtained by pooling data from DFM and urine and kidney culture within an individual such that any animal demonstrating a positive result at least once was considered to have been infected. This, in effect, is a cumulative measure of

infection status of individuals. The response variable "live *Leptospira* index" was negative = 0 or positive = 1 for statistical analysis. Associations between urine shedding, kidney lesions, and serology were investigated using the Chi-Square (χ^2) test in SAS for Windows (SAS Institute Inc., Cary, NC, USA) with $p \leq 0.05$ considered statistically significant. Relative Prevalence ratios (RP)($p \leq 0.05$) were estimated in Episheet 2002 (Rothman © 2002). Generalised mixed logistic regression models were used to estimate the prevalence of these responses within farm and sampling month including an adjustment for repeated measurements within the same animal. Effects were evaluated by RP for the associations between sampling months within farm, adjusting for sex and age, and Relative risk (RR) for the association Copenhageni titre results between Hardjobovis and Pomona, using SAS for Windows (SAS Institute Inc., Cary, NC, USA).

4.3 Results.

4.3.1 Serology

Seroprevalence and geometric mean data, all farms combined, are presented in Figure 4.1. Raw and summary data are presented in Appendix 2a and b, respectively. Farm 1 was positive for serovar Hardjobovis alone while Farms 2 and 3 were positive for both serovars Hardjobovis and Pomona.

4.3.1.1 Hardjobovis

The overall crude seroprevalence estimate, ignoring farm effect , from the three farms combined, was 43.48% (41.2-47.7% CI) with a titre range 0-3072, but seroprevalence varied between herds, between sampling months, and for Farm 2, between years. Farm 1 had an overall seroprevalence, all animals from all sampling points combined, of 31.70% (26.2-37.1% CI) (mode= 48) varying from 7.5% to 57.5% between sampling dates (Figure 4.1). The overall reciprocal GMT was 50.8. Farm 2 in Year 1 had an overall seroprevalence of 22.08% (16.8-27.3%CI) varying from 10% to 47.5% between sampling dates, and a reciprocal GMT of 44.5. In Year 2 that farm had an overall seroprevalence for Hardjobovis of 75.19% (69.9-80.4 %CI) varying from 52.9% to100% between sampling dates, and a reciprocal GMT of 64.4. Farm 3 had an overall seroprevalence for Hardjobovis of 63.7% (55.0%-72.8%) varying from 27.9% to 96.5% between sampling dates, and a reciprocal GMT of 54.6.

On Farm 1 the initial prevalence at weaning in May (5-6 months of age) was 57.5%. One month later the seroprevalence was 7.5%, ranging from 12.5-22.5% until November, and thereafter increased to 57.5% by May, at 17-18 months of age. The reciprocal GMT was 27.5 in May (Year 1), increasing to 103.5 in September, and ranging from 37.7-52.4 thereafter. With the exception of a lower seroprevalence at commencement of sampling in June, the seroprevalence and GMTs on Farm 2 follow a similar pattern. In Year 2 on that farm, seroprevalence was high from February to June, and fell thereafter. The GMT was again highest in September. On Farm 3, seroprevalence was 23% in June, 97.2% in August and 72.9% in Nov/Dec, and the GMT increased from 27.9 to 96.5 over that time.Thus, while the seroprevalence differed between farms the lowest seroprevalence was generally observed during winter months, but was above 50% in all herds by 12-19 months of age. Although titres ranged from 0-3072, the mean reciprocal titres remained between 27.5 and 115 (mode 1:48) for positive titres but most titres were between 1:48 and 1:96 unrelated to seroprevalence.

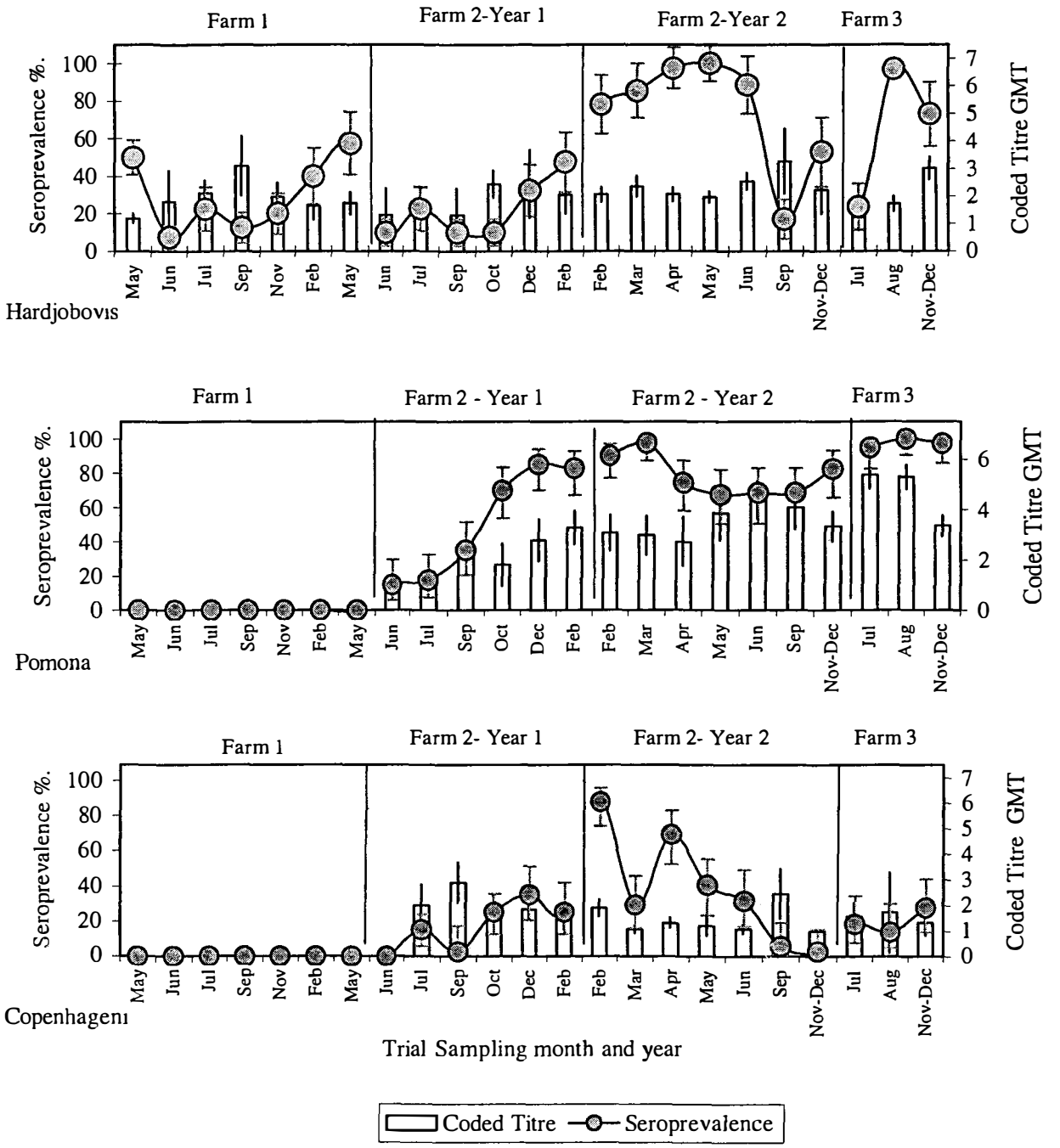


Figure 4.1 Seroprevalence (95%CI) with a cut point of ≥1:24 and coded geometric mean titre (95%CI) for serovars Hardjobovis, Pomona and Copenhageni from each farm all samples combined by month.

4.3.1.1 Group, year, sampling month and age effects

Regression analysis and χ^2 for the difference in seroprevalence of Hardjobovis, at titre cut point of ≥1:24, between groups (see section 4.2.4), years and sampling periods were significant (χ^2 197.3, p<0.001). There were significant differences in seroprevalence between sampling periods within each farm and year (Farm 1 χ^2 48.0, p<0.0001, Farm 2 Year 1 χ^2 28.0, p<0.0001, Farm 2 Year 2 χ^2 100.8, p<0.0001, and Farm 3 χ^2 45.3, p<0.0001).

Regression analysis estimating differences in seroprevalence of Hardjobovis at cut points ≥1:24, ≥1:48, and ≥1:96 for the fixed effects of study group, year, sampling period (month), sex, seropositivity to serovars Pomona and Copenhageni, and age in months showed the model Hardjobovis seroprevalence = group * Year * sampling month * age was significant (Table 4.2). Thus, there were differences in seroprevalence for Hardjobovis between group, month, year, and age.

Table 4.2 Summary of regression analysis for the relationship between the response variable seroprevalence to Hardjobovis at different titre cut points, and the effect variables group, Year, sampling month, and age in months.

Response variable Hardjobovis seroprevalence	Regression χ^2 (p)	Effect variable	Estimated χ^2, (p) in the regression
Titre ≥1:24	239.0 (p< 0.001)	Study Group	58.4 (p< 0.001)
		Year	105.3 (p< 0.001)
		Month	43.6 (p< 0.001)
		*Age	3.14 (p= 0.07)
Titre ≥1:48	199.5 (p< 0.001)	Study Group	44.96 (p< 0.001)
		Year	85.5 (p< 0.001)
		Month	23.1 (p< 0.001)
		Age	6.2 (p= 0.01)
Titre ≥1:96	73.6 (p< 0.001)	Study Group	13.7 (p< 0.001)
		Year	31.7 (p< 0.001)
		Month	8.47 (p= 0.003)
		Age	12.81 (p< 0.001)

*Not significant.

4.3.1.2 Pomona

Serovar Pomona was present on Farms 2 and 3 with an overall seroprevalence of 48.9% (45.6-52.2% CI) and titre range 0-12,228.

The original screening for Farm 2 demonstrated only serovar Hardjobovis. However, when the on-farm observations commenced in June Year 1, there was a 15% seroprevalence for Pomona increasing steadily to 82.5% by February the following year, with an overall seroprevalence of 50% (43.6-56.3%, CI) (Figure 4.1). Concurrently, the GMT rose from 26.1 to 117.1, averaging 61.9 (mode=96). In February Year 2, with a new group of weaners, seroprevalence was 90.2% and ranged from 68.5% to 97.5% thereafter, averaging 79% (74-83.9% CI). The reciprocal GMT ranged from 79.3 to 251.7, averaging 147. The seroprevalence on Farm 3 ranged from 94.7% to 100%, averaging 97% (93.8-100% CI) while the reciprocal GMT ranged from 123.2 to 501.2, averaging 343.8.

4.3.1.2.1 Group, Year, sampling month and age effects

Regression analysis and χ^2 for the difference in seroprevalence of Pomona, at titre cut point of ≥1:24, between groups 2-4 were significant (χ^2 464.0, p<0.001). The seroprevalence between sampling periods within each group was significant in Farm 2 Year 1 (χ^2 67.70, p<0.0001), Farm 2 Year 2 (χ^2 19.53, p=0.003), but not in Farm 3 (χ^2 3.77, p=0.15).

Regression analysis estimating differences in seroprevalence of Pomona at cut points ≥1:24, ≥1:48, ≥1:96 for fixed effects of group, year, sampling period (month), age in months, sex and seropositivity to serovar Copenhageni and Hardjobovis at different titre cut points showed the model Pomona seroprevalence = study group * Year * sampling month * age * Copenhageni titre was significant (Table 4.3).

Table 4.3 Summary of regression analysis for the relationship between the response variable seroprevalence to Pomona at different titre cut points, and the effect variables group, Year, sampling month and age in months, and seropositive titres to serovar Copenhageni.

Response variable Pomona seroprevalence	Regression χ^2 (p)	Effect variable	Estimated χ^2, (p) in the regression
Titre ≥1:24	641.0 (p< 0.001)	Study Group	24.3 (p< 0.001)
		Year	50.5 (p< 0.001)
		Month	4.09 (p= 0.04)
		Age	20.1 (p< 0.001)
		*Copenhageni ≥1:48	17.8 (p< 0.001)
Titre ≥1:48	539.0 (p< 0.001)	Study Group	11.0 (p< 0.001)
		Year	60.9 (p< 0.001)
		Month	7.7 (p= 0.005)
		Age	27.7 (p= 0.01)
		*Copenhageni ≥1:48	24.7 (p< 0.001)
Titre ≥1:96	492.0 (p< 0.001)	Study Group	4.5 (p= 0.03)
		Year	5.9 (p= 0.01)
		Month	60 (p= 0.003)
		Age	30.2 (p< 0.001)
		*Copenhageni ≥1:48	32.30 (p< 0.001)

* This variable was also significant at titre cut points of ≥1:24 and ≥1:96

4.3.1.3 Copenhageni

Titres against serovar Copenhageni were recorded only on the two farms positive to serovar Pomona with an overall seroprevalence of 8.1% (6.7-10.3% CI). Overall seroprevalence was 14.2% (10.0-19.2% CI) and 16.0% (11.5- 20.4% CI) on Farm 2 Years 1 and 2, respectively, and 8.0% (5.05-13.0% CI) on Farm 3. Reciprocal GMTs were 43.2, 34.5 and 51.4, respectively, with an overall GMT of 27.5 (mode=24). The highest titre was 1:384. Seroreactions to this serovar were always concurrent with a higher titre against serovar Pomona.

High seroprevalence on Farm 2 early in Year 2 was collectively attributed to cross-reactivity with serovar Pomona, and colostral antibody since half of the weaners monitored that Year were progeny of hinds vaccinated pre-calving with a vaccine containing this serovar. Regression analysis using seropositivity to Copenhageni as the response variable and

Hardjobovis and Pomona seropositivity as effect variables showed a significant positive relationship with Hardjobovis (1:24 only) and Pomona titres (≥1:24) ($p \leq 0.02$). Relative Risk are presented in Table 4.4.

Table 4.4. Relative risk (RR) (95%CI) for the regression analysis response variable Copenhageni titre against the effect variables Hardjobovis or Pomona titre at titre cut points ≥1:24, ≥1:48 and ≥1:96.

Response variable	Titre cut point	Relative risk and (95% CI).	
Copenhageni		Hardjobovis	Pomona
Titre ≥1:24	≥1:24	1.6 (1.4-1.8)*	1.9 (1.7-2.0)*
	≥1:48	1.9 (1.5-2.3)*	1.9 (1.6-2.1)*
	≥1:96	2.1 (1.4-2.9)*	2.0 (1.7-2.3)*
Titre ≥1:48	≥1:24	1.1 (0.8-1.4)	2.0 (1.8-2.1)*
	≥1:48	1.2 (0.85-1.6)	2.2 (1.9-2.4)*
	≥1:96	1.4 (0.8-2.3)	2.4 (2.0-2.7)*
Titre ≥1:96	≥1:24	1.1 (0.7-1.2)	1.9 (1.5-2.0)*
	≥1:48	1.4 (0.5-1.7)	2.1 (1.6-2.4)*
	≥1:96	1.7 (0.7-3.1)	2.2 (1.6-2.6)*

* Significant

4.3.1.4 Dual Hardjobovis and Pomona

A summary of the proportion of animals within overall seroprevalence at each sampling that were seropositive to Hardjobovis and Pomona alone or together (and 95%CI) on dually infected farms (2 and 3) is presented in Figure 4.2.

On Farm 2 in Year 1, overall leptospiral seroprevalence steadily increased from 22.5% to 90%. The proportion of seroreactors to Hardjobovis alone ranged from 2.5% to 12.5% and to Pomona from 7.5% to 65%. Dual seropositivity ranged from 0-10% in the first 4 sampling periods and increased to 30 and 40% in the last two, largely concurrent with the increase in Pomona seroprevalence alone. In Year 2 on Farm 2, a high proportion showed dual seropositivity, particularly earlier in the year. On Farm 3, most animals showed either Pomona or dual seropositivity.

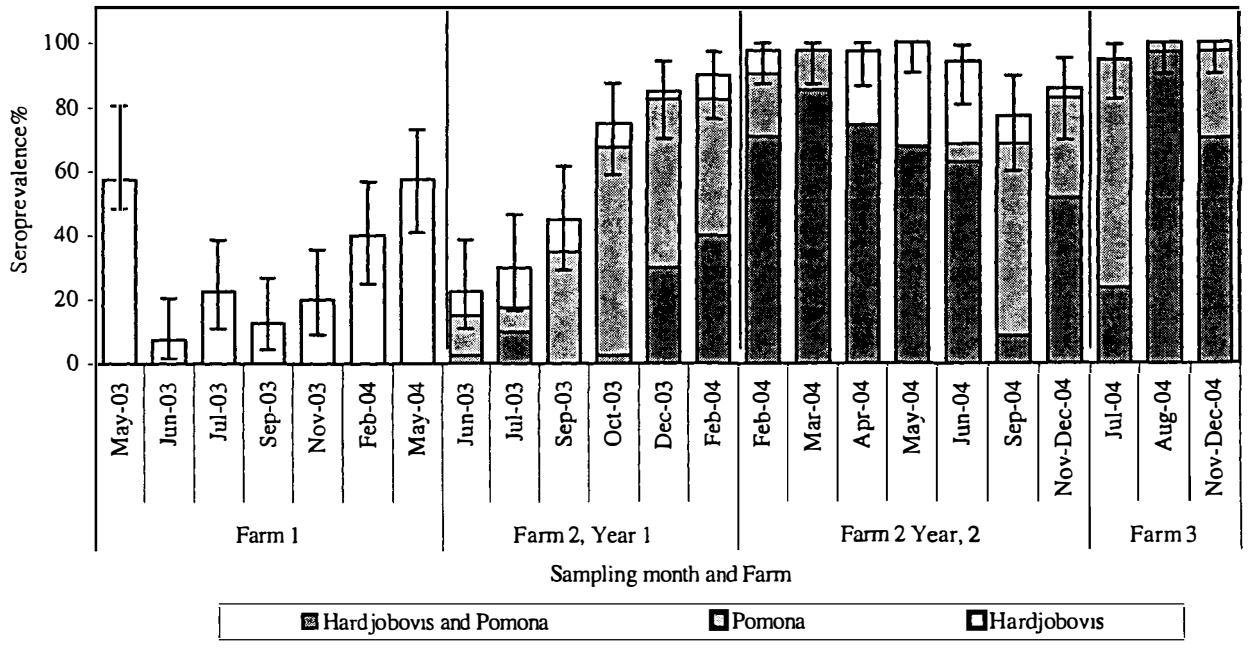


Figure 4.2 Proportion of animals within the overall seroprevalence (and 95% CI) at each sampling on dual Hardjobovis and Pomona infected farms that were individually or dually seropositive.

4.3.2 Urine dark field microscopy

The number of urine samples collected and the proportion DFM and culture positive by serovar, are presented in Table 4.5. Figure 4.3 presents the prevalence of positive urine samples and the observed seroprevalence of Hardjobovis and Pomona within study group by sampling period.

A total of 252 urine samples were collected with 38.4% DFM positive. The proportion of urine samples DFM positive ranged from 20-47.3% on Farm 1, 0-46.6% on Farm 2 Year 1, 14.2-60% on Farm 2 Year 2, and 57.8-100% Farm 3 (Figure 4.3). The proportion of urine samples DFM positive overall within each farm is presented in Table 4.5. The pattern of the proportion DFM positive generally mimicked seroprevalence on Farms 1 and 2.

Data at the individual animal level for urine collection and DFM positive and negative status are presented in Figure 4.4 along with the serovar determined by culture. A urine sample could be collected at least once in 97.5% of deer. Some hinds remained DFM positive for 5 months on Farm 1 and 8-9 months on Farm 2. Some hinds were never DFM positive, others were positive on only one occasion while others were DFM positive intermittently.

Overall, the proportion DFM positive between groups was significantly different (χ^2 36.7, $p<0.0001$) with 26.0, 23.6, 38.6 and 80.0% of samples positive on Farm 1, Farm 2 Years 1 and 2, and Farm 3, respectively. Differences in positive DFM between sampling periods were observed in Farm 1, Farm 2 Year 1 and Farm 3 (χ^2 8.7, $p= 0.03$, χ^2 12.5, $p= 0.01$ and χ^2 11.0, $p<0.001$, respectively).

In the regression analysis the model Urine shedding = group * sampling month * Hardjobovis ≥1:48 titre * Pomona ≥1:384 was significant (χ^2 was 35.8, p< 0.001). Individual effects were group (χ^2 of 11.7, p< 0.001), sampling period (χ^2 5.34, p< 0.02), seropositivity to Hardjobovis ≥1: 48 (χ^2 3.8, p< 0.05) and Pomona ≥1: 384 (χ^2 3.59, p< 0.05). Year and Copenhageni titres as effect variables were not significant.

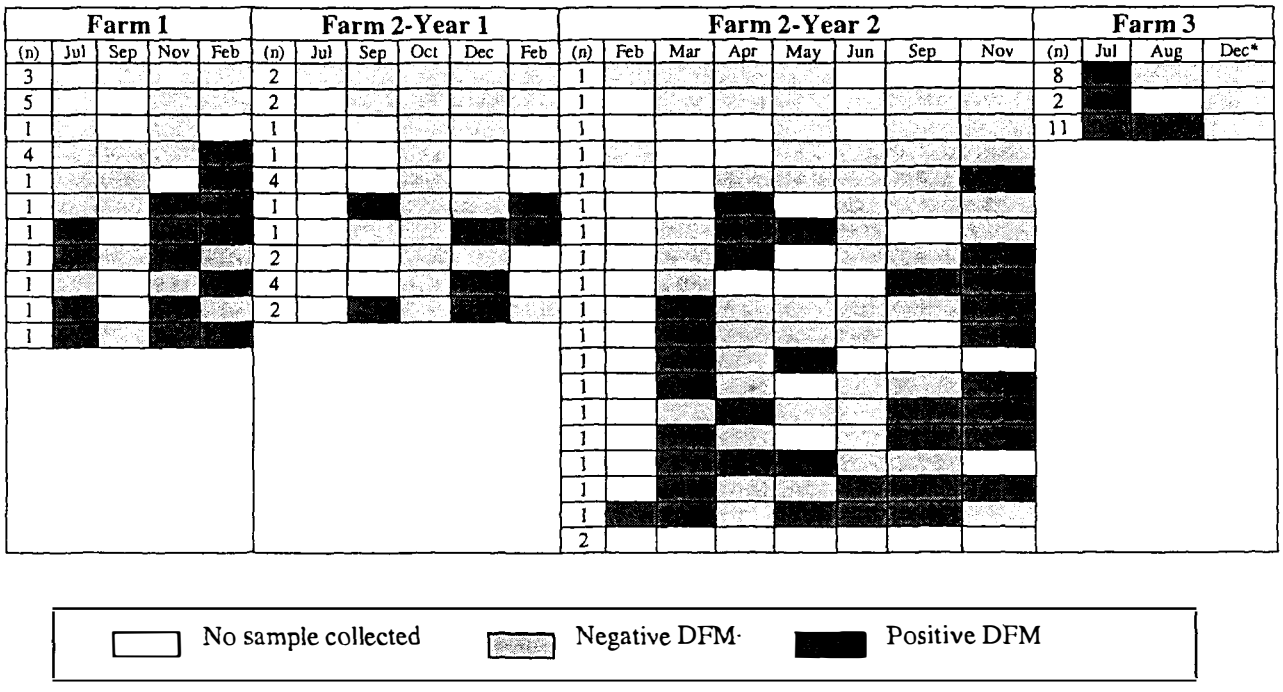


Figure 4.3 Number of deer (n) from which urine was collected and was DFM positive or negative at various combinations of sampling times on each farm.

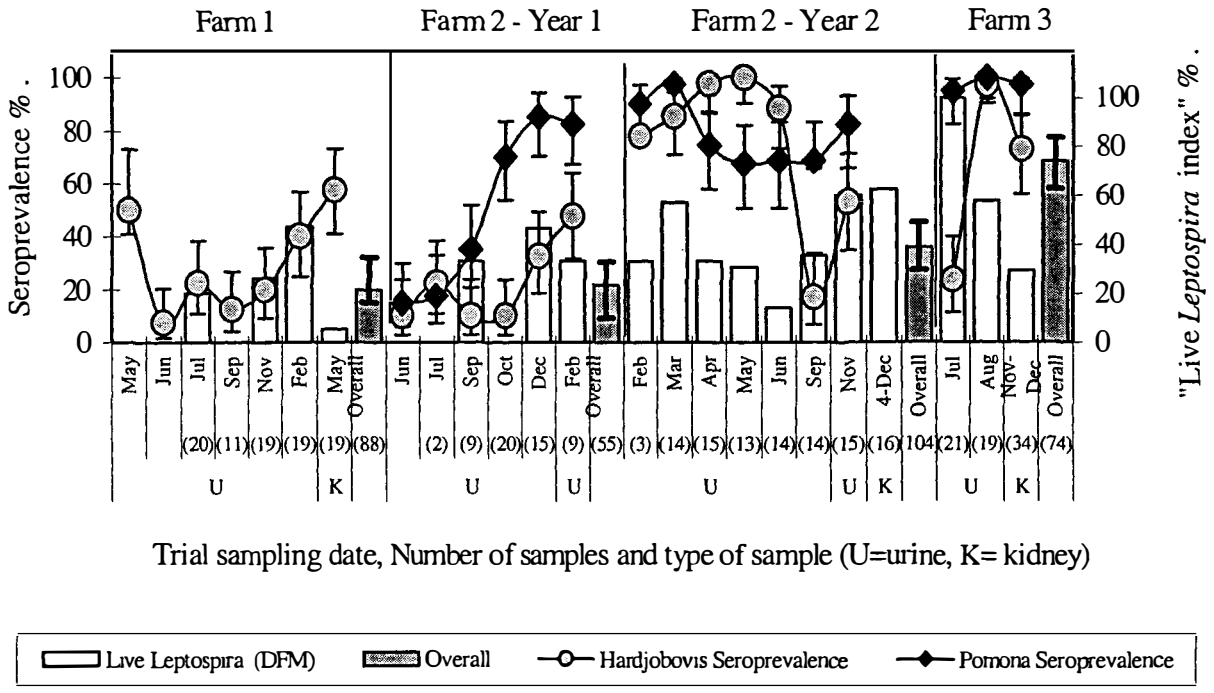


Figure 4.4 Seroprevalence (95% CI) for serovars Hardjobovis and Pomona, and the proportion (%) "Live *Leptospira* index" positive (95% CI) at each sampling period, and overall.

4.3.4 *Live* Leptospira *index*

A total of 39.9% of 323 individual samples were live leptospiral index (LLI) positive. Data are presented in Figure 4.5. The proportion that were LLI positive by farm were: Farm 1, 21.3% Farm 2 Year 1, 23.6% Farm 2 Year 2, 39.4% and Farm 3, 74.3% The difference in proportion between groups was significant (χ^2 54.6, p< 0.001). The proportion LLI positive in Year 1 (Farm 1 and Farm 2 Year 1) was 22.7% (33/144) and in Year 2 (Farm 2 Year 2 and farm3) was 53.9% (96/178), (χ^2 13.7, p< 0.001). All LLI positive animals were seropositive on at least one occasion.

The regression analysis for the response variable Proportion LLI positive showed the model DFM = farm * Year * age in months was significant (χ^2, 55.7, p<0.0001). Significant variables were farm (χ^2 37.2, p<0.0001), Year (χ^2 8.8, p=0.002) and age (χ^2 11.3, p<0.0001). The predictive Odds Ratio was 3.25 (1.49-7.0 CI) that LLI would be positive in Year 2. Seropositivity to Hardjobovis and Pomona at different cut points, sampling month and group as effect variables were not significant.

The relationship between LLI and seropositivity (Figure 4.5) was significant for Hardjobovis at titre cut point of ≥1:96 only (χ^2 6.2. p<0.01), and for Pomona at ≥1:24 (χ^2 21. p<0.001), ≥1:48 (χ^2 21.0, p<0.001), ≥1:96 (χ^2 17.7, p<0.001), ≥1:384 (χ^2 4.5, p<0.03). Odds ratios for the relationship between LLI and seropositivity at those cut points ranged between 2.99 (1.8-4.87CI) to 1.7 (1.0-2.8 CI). Thus it was more likely that a positive live leptospiral sample was from a seropositive rather than a seronegative animal.

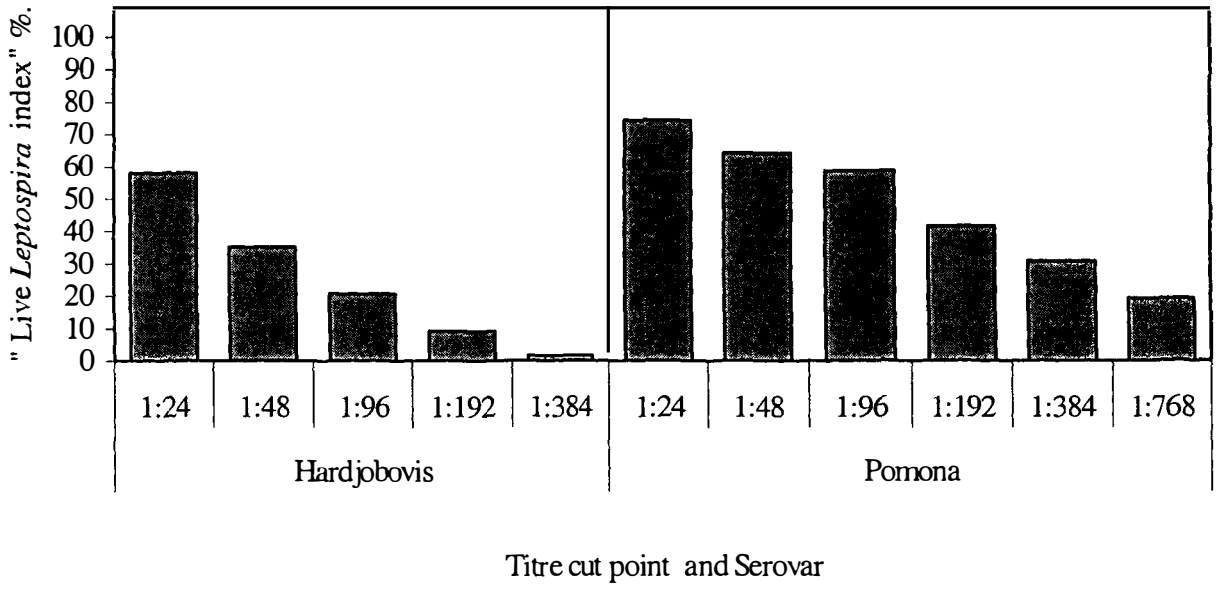


Figure 4.5 Proportion of animals LLI positive that were seropositive at ≥ various titre cut points for Hardjobovis and Pomona.

4.3.5 Leptospiral culture

Data for kidney and urine cultures are presented in Table 4.5. A total of 252 urine and 70 kidney samples were cultured from which 18 (7.1%) and 21 (30%) were positive, respectively. Forty-three were discarded due to contamination. Of the 281 cultures maintained, 38 (13.5%) were positive. The number of cultures by serovar from urine and kidney are presented in Table 4.5.

The proportion of positive cultures between study groups was different (χ^2 90.0, $p<0.001$) with 1.1%, 7.27%, 22.4% and 30.3% for Farm 1, Farm 2 Years 1 and 2, and Farm 3, respectively. There was a higher proportion culture positive in Year 2 (farms 2 and 3) than in Year 1 (Farms 1 and 2) (4.1% vs. 17.9% (χ^2 24.8, $p<0.001$). A positive Hardjobovis culture was obtained from the kidney from a post mortem specimen from a hind from Farm 2 Year 1 approximately 12 months after it was urine DFM and culture positive.

Table 4.5 Number of samples, proportion (%) positive and 95% CI for kidney pathology and culture, urine dark field microscopy and culture, and "live *Leptospira* index" positive, and serovar identification of isolates.

Farm/ Year	Kidney Pathology		Urine	Culture		"Live *Leptospira* index"	Typed Serovar		
	Gross	Histology	DFM	Kidney	Urine		Hb.	Pom.	Hb. & Pom.
1	20 (50%) (27.2-72.8)	20 (35%) (15.4-59.2)	69 (26.0%) (16.3-38.1)	20 (5%) (0.1-24.9)	69 (0%) (0-5.2)	89 (21.3%) (13.4-31.3)	1 (K)	0	0
2 Year 1	ND	ND	55 (23.6%) (13.2-37.0)	ND	55 (9.0%) (3.0-20.0)	55 23.6%) (13.2-37.0)	5 (U)	0	0
2 Year 2	16 (37.5%) (15.2-64.8)	16 (75%) (47.6-92.6)	88 (38.6%) (28.4-49.6)	16 (75%) (47.6-92.7)	88 (11.3%) (5.6-19.9)	104 (39.4%) (29.7-49.1)	5 (K) 5 (U)	3 (K) 3 (U)	4 (K) 2 (U)
3	34 (79.4%) (62.1-91.3)	34 (88.2%) (72.5-95.7)	40 (80.0%) (64.4-90.9)	34 (17.6%) (6.8-34.5)	41 (9.7%) (2.7-23.1)	74 (74.3.0%) (62.8-83.8)	2 (K)	4 (K) 4 (U)	0
Total	**70 (62.8%) (50.5-74.5)**	**70 (71.4%) (59-2-81.6)**	**252 (38.4%) (31.6-43.8)**	**70 (27.1%) (17.2-39.1)**	**252 (9.0%) (5.9-13.3)**	**322 (40.0%) (34.7-45.6)**	**8 (K) 10 (U)**	**7 (K) 7 (U)**	**4 (K) 2 (U)**

K= Kidney, U=Urine, ND = Not Done, Hb.= Hardjobovis, Pom.= Pomona.

4.3.6 Kidney gross and histopathology

Data from the 70 kidneys collected are presented in Table 4.5. Forty-four kidneys (62.8%) showed moderate to severe gross lesions (See Figures 8.6 – 8.8), 13.5% were from deer seropositive to Hardjobovis alone, 11.5% were from deer seropositive to Pomona alone, and 75.0% were from deer seropositive to both serovars. Lesions varied from a few foci (2-4 mm in diameter) to diffuse involvement of more than 80 % of the renal parenchyma. Farm 1 had the least severe lesions with less than 10% of the kidney surface involved. Farm 2 had kidneys with 20% to >60% of surface affected, and Farm 3 had more than half with more than 50% of the kidney the surface affected. The proportions with white spots were 50% (10/20), 37.5% (6/16) and 79.4% (28/34) from farms 1, 2 (Year 2) and 3, respectively. The difference in proportion between farms was significant (χ^2 11.3, p= 0.03). No direct relationship was detected between serological titre in the sample collected at the same time as the kidney, and kidney lesions.

Fifty kidneys (71.4%) had histopathological lesions. Prevalence of lesions were 35% (7/20), 75% (12/16) (31/34) for Farms 1, 2 (Year 2) and 3, respectively (χ^2 8.7, p= 0.01)) (Table 4.5).

The histopathological lesions (Figures 8.9 and 8.10) ranged from small intra-cytoplasmatic cortex infiltration characterised by mononuclear infiltrate (lymphocytes and plasma cells), to extensive lesions with tubular degeneration, interstitial fibrosis, extensive peritubular, and periglomelular lymphocytic infiltrate. The most frequent lesions were lymphocytic infiltrations and periglomelular damage. The mildest lesions were in kidneys from Farm 1 with the most severe lesions from Farm 3. The proportion of kidneys with various histological

lesions was different between farms. Overall, 70% had lymphocytic infiltration with 30% (6/20), 68.7% (11/16) and 94.1% (32/34) on farms 1-3, respectively, (χ^2 24.6, p< 0.001). Overall, 62.8% had peri-glomerular damage with 35% (7/20), 56.2% (9/16) and 82.3% (28/34) on farms 1-3 respectively, (χ^2 12.3, p= 0.002).

Several regression models were tested. The only effect variable associated with kidney gross and/or histopathogy was Pomona titre ≥1:96 (χ^2 7.76, p<0.001). The OR was 7.5 (1.4-38.2CI).

4.3.7 Adult deer, Farm 2

The seroprevalence of the 58 hinds sampled was 87.9% for Hardjobovis, 63.0% for Pomona and 27.5% for Copenhageni, with reciprocal GMTs 57.0, 38.9, 29.5, respectively. Five kidneys were culture positive to Hardjobovis.

4.4 Discussion.

This is the first detailed investigation of the epidemiology of naturally occurring leptospirosis infections in farmed deer providing information essential to underpin development of control strategies such as vaccination presented elsewhere in this thesis (Chapter 5 and 6), and the potential risk to humans (Chapter 8). This study focused largely on rising 1-Year-old deer because evidence from the 2-Year longitudinal study presented in Chapter 3 demonstrated that infection can occurred primarily during this stage of the deer's life. This discussion will be confined to serovars Hardjobovis and Pomona because titres to Copenhageni were regarded as cross-reactivity, particularly to Pomona, and maternal antibody from vaccinated dams in some younger animals, rather than evidence of infection.

This study set out to evaluate infection in two herds with each of serovars Hardjobovis and Pomona. Studies of infectious diseases on commercial farms are frequently subject to constraints, including identification and recruitment of potential candidate farms. Initially, investigations were to have commenced in the early autumn. However, a severe drought affecting weaning management decisions occurred in Year 1 and the few candidate farmers agreeing to participate were reluctant to accommodate early weaning and sampling. In Year two, one additional farm infected with Pomona was required. Severe flooding delayed weaning and transport to the study farm from a breeding herd. It was fortuitous that a Pomona infection was introduced to one of the study herds shortly after commencement, allowing real-time observations of establishing infection, concurrent with endemic Hardjobovis infection. However, despite the limitation that these infected herds where selected because they they were infected rather than being randomly selected, inferences are justified because the patterns of infection resemble those observed in the seroepidemiological study (Chapter 3) and were consistent between herds in the present study. Substantial data were obtained, and when combined with data from Chapter 3, provide a sound understanding of the epidemiology of leptospirosis on deer farms.

This study showed that infection with serovar Hardjobovis followed an annual cycle with an increasing proportion of animals becoming infected during the first 12 months, a pattern typical of endemic infections as seen in cattle (Hellstrom 1978; Ellis et al 1981; Bahaman et al 1984b; Hathaway 1983). Infection with serovar Pomona showed a pattern more typical of epidemic infections (Hathaway 1978; Hellstrom 1978; Mackintosh 1981). This observation

was similar to that observed in the serological epidemiological investigation presented in Chapter 3. Infection with Hardjobovis appeared to be sub-clinical. While Pomona was not associated with clinical disease on Farm 2, there was evidence of clinical disease caused by this serovar on Farm 3 on which a high proportion of deer were shedding leptospires of both serovars in urine on at least one occasion. Only a small proportion of DFM positive urine samples were culture positive, but a high proportion of kidneys were culture positive, particularly where Pomona was present.

4.4.1 Serology

Seroconversion is a commonly used tool in longitudinal observational studies of infectious diseases and has been used for investigations into Hardjobovis in cattle (Hellstrom et al 1978; Little et al 1992b), Pomona and Tarassovi in Pigs (Ryan 1976 a,b; Bolt 1990) and Balcanica in possums (Hathaway (1978). In this study, titres to Copenhageni are considered to be cross-reactions to Pomona, because there was no culture evidence, cross-reactions between these serovars are well recognised, and there was a correlation between them in this study. The correlation with Hardjobovis was only at Copenhageni titre1:24. This is probably due to confounding because of the high seroprevalence to Hardjobovis, because cross-reactivity between these serovars is not recognised elsewhere.

Single or multiple infections with serovars Hardjobovis and/or Pomona and/or Copenhageni have been demonstrated serologically in farmed deer (Fairley et al 1984 and 1986; Flint et al 1986; Wilson et al 1998). However, evidence reviewed by Ayanegui-Alcérreca et al (2006a,b) suggests that infection of deer with Copenhageni is uncommon. Studies from New Zealand and elsewhere also report single or dual-serovar infections in cattle (Little et al 1976; Hellstrom 1978; Ryan 1978; Cordes et al 1982; Kingscote 1985a,b; Hathaway et al 1984 and 1986; Little et al 1986; Grégoire et al 1987; Espi et al 2000;Alonso-Andicoberry et al 2001; Guitián et al 2001), sheep (Dorjee et al 2005) and pigs (Michna and Campbell 1969; Ryan 1978; Boqvist et al 2003).

While there were some overall similarities in the pattern of serology, particularly for Hardjobovis for which seroprevalence increased with time similar to that reported in Chapter 3, each farm showed a particular expression of infection. In cattle infected with serovar Hardjobovis and pigs with Pomona, it has been suggested that exposure to infection is usually in the first 6 to 12 month of life (Ryan 1978; Bahaman et al 1984a,b; Bolt 1990; Little et al 1992a,b). The increase of infection rate and rapid spread of infection seen in deer with serovar Hardjobovis has also been reported in cattle (Hellstrom 1978; Hathaway et al 1983). The pattern of serology for Pomona on Farm 2 differed significantly between years, as expected of a recently introduced infection in Year 1, presumed to be from newly purchased young cattle. That farm was positive only to Hardjobovis when screened before observations began whereas Farm 3 was seropositive to both Hardjobovis and Pomona when screened approximately 6 months before observations began. Seroprevalence to Pomona on Farm 2 increased during Year 1, and remained high during the second year, at a similar seroprevalence to Farm 3. Clinical outbreaks in deer have been reported associated with mixing of deer mobs and/or introduction of deer and cattle into the properties (Fairley et al 1984 and 1986; Wilson and McGhie 1993; Dean et al 2005) and in one case of Pomona infection described in Chapter 3. Clinical outbreaks associated with introduction of Pomona to cattle herds (Hellstrom 1978; Mackintosh 1981) and pigs (Mackintosh 1981) have been reported.

Variations between farms and years are also likely to be due to individual differences in climate, environment and management practices and policies. Year 1 was characterised by a severe drought and Year two by a severe flood and frequent rainfall. The seroprevalence to Hardjobovis observed in Farm 2 in Year 1 was lower than that observed in Year 2. Urine shedding was also higher in Year 2. Farm 3 had poor drainage resulting in animals being exposed to a very wet environment for months. Leptospiral outbreaks and clinical cases in humans and other animals have been associated with the rainy season (Faine et al 1999, Levett 2001). Variations in the prevalence of different serovars within and between years have reported in pigs (Boqvist el al 2005). Hellstrom (1978) and Elder et al (1986) reported in cattle a positive correlation between seroconversion and incidence rates of infection with serovars Pomona and Hardjobovis with persistently wet environments.

It is known that risk factors that need to be addressed when control measures are to be applied vary between farms. Between-farm and between-year differences were observed in the longitudinal seroepidemiological study of deer presented in Chapter 3. The importance of niche or nidus in the interaction between leptospiral serovar, host or hosts, and the environment, in the dynamics of infection were described by Hathaway (1978). Management policies were an important determinant of differences in leptospiral infection between and within piggeries in New Zealand (Bolt and Marshall 1995). Studies in pigs and cattle describe management, housing, mixing of species and geographical location as factors facilitating contact with and maintenance of different leptospiral serovars (Hellstrom 1978; Ryan 1978, Bolt and Marshall 1995; Boqvist et al 2003; Alonso-Andicoberry et al 2001).

The apparent introduction of Pomona from bulls on Farm 2 early in the study and the transmission to deer was permitted by cross-grazing and sharing of a common open water source with deer. However, within-deer herd transmission was also likely since several deer were shedding leptospires in their urine. Further evidence for within-species transmission was from a group of adult hinds sampled on this farm in April Year 1 that were positive only to Hardjobovis at that time, but positive to Pomona in October that year (Chapters 6 and 7) after being grazed below the watershed of the infected weaner group, and with no direct or indirect contact with the cattle.

Clinical outbreaks with Pomona have been reported in deer associated with cattle (Fairley et al 1984), specifically not associated with pigs or cattle (Fairley et al 1986) and where brought-in deer were the probable source of infection (Dean et al 2005). In cattle, serovar Pomona infection has been mainly associated with pigs (Mackintosh 1981), but also with sustained transmission within cattle herds in the absence of pigs (Doherty 1967a, b; Hellstrom 1978). In Year 2 of this study, a sustained seroprevalence of Pomona >80% was observed on Farms 2 and 3 suggesting that an outbreak could have been possible had other factors been conducive. In cattle up to 100% prevalence has been observed, not necessarily with expression of clinical disease (Doherty 1967a, b; Little 196; Mackintosh 1981).

The high seroprevalence of low titres to Hardjobovis in May in Farm 1 Year 1was considered the result of maternal antibody. It has been shown that maternal antibody levels in weaners, following vaccination of hinds in a non-infected herd, persisted for at least 6 months (Chapter 7). Further evidence is that seroprevalence and GMT decreased rapidly thereafter. Similar maternal antibody effects are reported in cattle and pigs (McDonald and Rudge 1957; Schollum and Marshall 1985; Palit et al 1991). On Farm 2 Year 2, the high seroprevalence in

February was likely to have been a combination of maternal antibody and natural challenge, given the number of deer shedding leptospires in urine at that sampling.

4.4.2 Urine shedding and culture

Despite that not all animals could be urine sampled every time, due to the logistics of obtaining the samples, and the possibly low sensitivity of DFM, particularly in animals shedding low numbers of organisms, analyses provide a crude estimate of shedding prevalence and persistence. It appears that rising 1-year-old deer can shed Hardjobovis and Pomona for at least 8 and 3 months, respectively. However, these data were collected over only those periods, so shedding is likely to persist beyond those times. One weaner hind on Farm 2 that was urine culture positive for Hardjobovis was also kidney culture positive 12 months later. Thus, farmed red deer have been shown to be persistent shedders of Hardjobovis and possibly Pomona. The only other report of shedding persistence in red deer (Fairley et al 1984) provided evidence of Pomona shedding for at least 9 months and Hardjobovis for at least 4 months. Reilly et al (1962a) reported shedding of Pomona for at least 35 days after experimental inoculation in white-tailed deer (*Odoicoileus virginianus*). In cattle, urine shedding of serovar Pomona from 28 to 118 days has been described (Reviewed by Hellstrom 1978). Shedding persistence of serovar Hardjobovis in cattle from various reports (Hellstrom 1978; Ris and Hammel 1978; Flint and Liardet 1980; Leonard et al 1992, 1993: Radostitis et al 1999) were 35, 10-118, 84, 17-174 and 207- 410 days. The duration of detectable leptospirurea in naturally infected heifers ranged from 1 – 82 weeks (average 53.1 weeks) (Mackintosh 1981), 28-84 weeks (Leonard et al 1992, 1993). The incidence of leptospiral shedding in deer generally increased with time during the first year of life in parallel with seroprevalence.

Evidence presented here and elsewhere in this thesis suggests that deer are maintenance hosts for serovar Hardjobovis. Persistent leptospiruria for more than 9-12 months should be sufficient to ensure an endemic cycle of infection in farmed deer, with each new generation of weaners becoming exposed to infection from persistently infected older generations. The persistence observed here supports that classification, although observations beyond the time periods studied here would be needed to determine the full extent of urine shedding persistency.

The observation of lower numbers being urine culture positive than DFM positive, and no cultures from DFM negative urine samples shows that DFM is a more sensitive test of shedding than culture. However, it could be that the time taken between sample collection and handling on-farm, and return to the laboratory for subculture resulted in loss of viability. Furthermore, the chemical conditions of deer urine may not be conducive to prolonged survival *in vitro,* or leptospiral strains in deer may not be adapted to the medium, since the protocol for culture was that described for cattle, in which culture from urine was more common (Mackintosh 1981, Hellstrom 1978).

4.4.3 Kidney pathology and bacteriology

Kidney gross and histopathology were used as an indicator of infection since renal lesions are highly characteristic (Prescott 1992; Radostitis 1999) although bacterial culture is the ultimate

evidence of infection (Faine et al 1999). The majority of macroscopic kidney lesions were chronic "white spots", indicative of interstitial nephritis typical of leptospiral infections in cattle, pigs, dogs and other animal species (Prescott 1992; Radostits et al 1999). Red spotting was also recorded but no clear association could be made with leptospiral infection.

Microscopic lesions were characterised by accumulation of mononuclear cells in the interstitial tissue, lymphocytic infiltration and periglomelular damage with different degrees of severity. Most lesions were chronic with few showing active haematic change. These observations were comparable to those reported for cattle and pigs infected with serovars Hardjobovis and Pomona (Prescott 1992; Radostits et al 1999). Renal tubules are considered to be the natural habitat of pathogenic leptospires but the degree of damage depends on adapted or non-adapted host-serovar status (Quin et al 2002), consistent with the more severe lesions associated with serovar Pomona in this study. Histological lesions observed here are comparable to those previously described in wild deer with Pomona (Abdulla et al 1962), and farmed red deer (Fairley et al 1984; Wilson et al 1998). Ferris et al (1960), Trainer et al (1961) and Reilly et al (1962a) reported similar but more severe changes in experimentally infected white-tailed deer with serovar Pomona.

The lower isolation rate, lesion prevalence and mildest lesions were observed associated with serovar Hardjobovis suggesting a low-grade virulence of this serovar. No clinical signs of disease associated with leptospirosis were observed in animals with this serovar in the study, and there is little data supporting that this serovar is pathogenic in deer (Wilson and McGhie, 1993, Ayanegui-Alcérreca et al 2006 a, b, and Appendix 3). The kidney lesions and isolation rate from deer kidneys where Hardjobovis was the predominant serovar were similar to those reported in cattle with this serovar (Mohanty and Singh 1970; Amatredjo et al 1976; Mackintosh 1981; Skilbeck et al 1988), in pigs with serovars Pomona, Bratislava or Tarassovi (Michna and Campbell 1969; Ryan 1978 and Boqvist et al 2003), and were typical of maintenance hosts. In deer isolates and kidney lesions with mild "white spotting" have been reported in deer infected with Hardjobovis by Wilson et al (1998). Those authors identified three Hardjobovis cultures from kidneys of 202 randomly selected deer, with six further cultures not typed. That culture rate was lower than the 11.4% isolation rate for this serovar from the three known infected herds in this study.

In the second year of this study when there was a high prevalence of dual infection with Hardjobovis and Pomona, the prevalence and severity of kidney lesion and isolation rate were greater. In deer, acute and chronic interstitial nephritis with white and red spotting has been associated with outbreaks of clinical disease and culture (Fairley et al 1984; Wilson and McGhie 1993; Wilson et al 1998; Dean et al 2005). Kidney lesions were reported by Abdulla et al (1962a) in Pomona infected wild deer. Severe kidney lesions were reported following experimental challenge with serovar Pomona by Ferris et al (1960), Trainer et al (1961) and Reilly et al (1962) in captive white-tailed deer. Similar findings were reported in cattle, with severe renal lesions caused by serovar Pomona (Te Punga and Bishop 1953). In this study, deer confirmed as infected with serovar Pomona had more severe lesions than those infected with serovar Hardjobovis. Kidney lesions and Pomona isolates have been reported from deer at slaughter (Wilson et al 1998) and following experimental infections in captive white tailed deer without clinical evidence of disease (Trainer et al 1961). Similarly in cattle, Hellstrom (1978) suggested that the infection with serovar Pomona can be sub-clinical.

Combined pathology and culture are useful to help define the host-serovar relationship (Michna and Campbell 1969; Hathaway 1978; Hellstrom 1978; Ryan 1978; Mackintosh 1981;

Ellis et al 1985; Ellis et al 1986; Bolt 1990) but a full understanding of infection should also combine urine shedding and serology as described for other production species including cattle and pigs (Michna and Campbell 1969; Amatredjo et al 1976; Cargill and Davos 1981; Ellis et al 1981; Gréigore et al 1987).

4.4.4 Use of multiple diagnostic tests

The sensitivity and specificity of the MAT varies, with higher titres to non-host adapted serovars. High titres denote recent, active infection with a sustained bacterial challenge. A low titre may denote an early, chronic or convalescent phase, or non-specificity. Infection by non-host adapted serovars usually results in high titres of shorter duration compared with sustained low titres of long duration following infection by host-adapted serovars. Thus, serology alone may over- or under-estimate infection (Michna 1970; Bolin and Prescott 1999; Faine et al 1999). In the present study, several direct and indirect diagnostic techniques were combined to monitor the development of the infections in the herds as suggested by Michna (1970), who emphasised that the reliability of a test depends upon its application at the appropriate time in the course of the infection, because each test measures specific events.

The better agreement between kidney lesions and culture, than between urine and culture and lesions and serology, reported in Chapter 8 for deer has also been reported in pigs and cattle (White et al 1982; Thiermann 1983; Kingscote 1985 a; Baker et al 1988, 1989, Scanziani 1991). The relationship between serology and kidney lesions is considered poor because they can be quite separate events on the time line of the infection, particularly in chronic cases such as those recorded in this study. Most immunological stimulation will occur during the bacteraemic phase, which lasts between 4 to 12 days, and the first invasion of the kidney. Once the leptospires reach the renal tubules they are maintained in an immuno-privileged space. Therefore, the titre is closely related to the primary infection of the individual and can be sustained for different periods, while the renal carrier estate is related to the presence of the bacteria in the kidney (Michna and Campbell 1969; Michna 1970; Greene et al 1998). Pathology in combination with urine shedding and culture was used to diagnose renal leptospirosis, firstly in the absence of other clinical manifestations and as direct evidence of impact on the animal health for both serovars and secondly, to establish the relevance of the renal carrier since control strategies in general are centred on the renal carrier (Bolin and Prescott 1999).

The combined diagnostic techniques enhanced the chances of a correct diagnosis. At the herd level the detection of infection by a *Leptospira* was achieved by all techniques, but the agreement between the test at the individual animal level was less accurate. The intent when combining the different techniques was to enhance the accuracy of identification of the infectious state confirmed by a positive culture of DFM at the individual and herd level. Serology and urine shedding by DFM were the best indicators of infection. Serology and culture combined was best at identifying serovar/s in a herd, and supporting herd level diagnosis based on MAT results. Culture and DFM are two tests with low sensitivity but combination of both in parallel increased the sensitivity for detection of active infection. The relationship of the titre cut point with sensitivity and specificity depend upon the endemicity of the disease, the serovar and the serovar-host status. Different titre cut points were included to demonstrate that the test results and variables associations were strong, as proposed by Vijayachari et al (2001).

4.5 Conclusions.

The results from this study allowed a better assessment and understanding of the infectious cycle in farmed deer with serovars Hardjobovis and Pomona, during the first year of life. These results together with the results form Chapter 3, where different age groups (rising-1-year, rising -2 -year and mixed-age adults) were studied, provided a good basis for understanding these infections in farmed deer. They also highlight the potential risk that deer may play in the epidemiology of leptospirosis in other animals, as well as a zoonosis.

- The seroconversion pattern for Hardjobovis suggested that infection is initiated in some animals before 3-4 months of age, with most deer becoming infected in their first year of life and the prevalence of leptospiral shedding in urine increasing during that period. There was no age-related pattern for Pomona.

- Data shows that shedding in urine can persist for more than 9 months.

- The presence of kidney lesions, positive bacterial isolates and positive serology are proof of active infections with Hardjobovis and Pomona in farmed deer and their capacity to support the infectious cycle.

- Hardjobovis was the most prevalent serovar, appearing to cause minimal disease, which was sub-clinical, characterised by mild kidney lesions, active bacterial shedding with a higher rate of seroconversion at 6-12 months of age. All these features indicate a host-adapted relationship with this serovar in farmed deer.

- Pomona infection resulted in high titres, was associated with severe and more frequent kidney lesions, and showed an endemic cycle that was age independent in contrast to Hardjobovis. This suggests a non-host adapted relationship at the animal level. That outbreak of clinical disease were not observed despite apparently high bacterial challenge, severe renal lesions and high within-herd seroprevalence, suggests that farmed deer can act as a maintenance population for pomona as previously proposed for cattle in New Zealand.

- Serology is a reliable technique to identify infection at the herd level, since the identity of the isolates matched those in the serology results.

- Kidney lesions are a good indicator of infection at the herd level.

- Combining diagnostic techniques enhanced the chance of a correct diagnosis.

- Urine shedding is confirmatory evidence of the role of deer in the infectious cycle of leptospirosis, establishing deer as an important contributor to the epidemiology of infection in other animals and humans at micro-ecosystem (farm) and macro-ecosystems (region or country) levels.

Chapter 5

Studies of a Trivalent Leptospiral Vaccine in Deer



5.1 Introduction

Vaccination is the most widely-adopted means for control of leptospiral infection in cattle and pigs in New Zealand (Dowd 2003). Vaccination is used globally in intensive and extensive farming situations, using bacterins specific to the serotypes present (Tripathy et al 1976; Hathaway et al 1983). In New Zealand vaccination has been shown to reduce clinical and subclinical disease and bacterial shedding in dairy cattle (Mackintosh 1981) and pigs (Bolt 1990), resulting in a significant reduction of animal and human cases (Marshall and Manktelow 2002). Serological surveys by Flint et al (1988a) and Wilson et al (1998) indicate that leptospiral infection of farmed deer in New Zealand is widespread and while the incidence rate of clinical disease has not been documented, there are many reports over the past 25 years (reviewed by Ayanegui-Alcerreca et al. 2006, this thesis), with some involving high infection and mortality rates (Wilson and McGhie 1993; Dean et al. 2005).

Leptospirosis in humans in New Zealand was reviewed by Thornley et al (2002), linking five cases to deer. More recently there has been an increase of human cases of leptospirosis notified in New Zealand (Baker and Lopez 2004) predominantly associated with serovar Hardjobovis and mainly attributable to cases in farm workers other than dairy, and meat workers. The risk of infection from beef cattle, sheep and deer in human disease has been identified but not been quantified (Heuer and Davies 2004). Bell (2005) and Brown (2005) reported 10 cases of leptospirosis in workers at two deer slaughter premises. Wilson et al (1998) reported 4.9% of randomly selected deer kidneys were culture positive. The risk of exposure to humans has been discussed in Chapters 8 and 9.

While there are two bivalent and one trivalent leptospiral vaccines licensed for deer in New Zealand, there is little published data on their efficacy in stimulating antibody production (Wilson and Schollum 1984, Wilson et al 2001), and there is no published data of their effect on urine shedding or kidney infection. Increasing concern in the deer industry about leptospirosis as an occupational disease, along with animal health concerns (Mackintosh and Wilson 2003), prompted this investigation of the efficacy of a candidate trivalent vaccine in a natural field challenge situation.

5.2 Materials and methods

5.2.1 Trial Design

Six trials were conducted over two years on four pastoral deer farms from the lower North Island of New Zealand. Farms were selected based on on-farm serological screening for serovars *Leptospira borgepetersenii* Hardjobovis and/or *Leptospira interrogans* Pomona and Copenhageni using the MAT and clinical history (Farms 1, 2 and 4) or slaughterhouse screening using serology and kidney lesions (Farm 3). Farm 4 was selected as a non-infected control, based on absence of gross and histopathological kidney lesions, lack of leptospires in urine by DFM, and serological evidence gathered during the previous 18 months. Farm 1 was confirmed positive for serovar Hardjobovis, and Farms 2 and 3 had dual infections with Hardjobovis and Pomona. No farm had evidence of infection with serovar Copenhageni.

The number of subjects in the vaccine and control groups was established based on the number of animals needed to detect at least one seropositive animal in a population with a minimum prevalence of infection of 10% (Guide lines for the control of leptospirosis. Geneva, World Health Organisation (WHO), Faine 1982), (Faine et al 1999). The number ranged from 36-37 in a herd size of 100 animals, to 42-43 in a herd size of 500, at 99% and 95% confidence limits, respectively, when infection prevalence was 10%. Fisher's Exact Test for two independent binomial samples was used to provide hypothesis-testing results (One tail χ^2). The alternative hypothesis tested that the infection prevalence in control animals was higher than the prevalence in vaccinated animals, with significance level of (α of 0.05 and a Beta (1-power) of 0.20 using NCSS 2000 and PASS 2000 (Jerry Hintze ©2001). The sample size by sex was determined by power analysis (PASS) using 95% confidence limits and hypothesis tests for the difference between two proportions with numbers chosen arbitrarily in the absence of information. A sample size of 15 achieved 86% power to detect a 70% difference in the seroprevalence with a 95% confidence limit. A minimum of 15 weaners, and 20 where available, were selected, since some losses can occur in the course of natural farming systems.

5.2.2 Farms, animals and sampling

Detail of farms and management, animals, and vaccination and sampling schedules are presented in Table 5.1. This study focussed on rising 1-year-old deer to the end of the production cycle defined as the slaughter date (Farm 3) or when a significant number of deer from the trial groups were slaughtered (1, 2 and 4). Animals on Farm 2 were red and on those on Farms 1, 3 and 4 were red and red x 25% wapiti cross-bred. They were identified in duplicate. Fifty to 75% of the deer in each group were vaccinated and were balanced between sexes where possible. Animals were randomly allocated within sex to vaccine and control groups that were managed together on pasture under normal farming practices. Blood was collected from all animals on every occasion as detailed in Table 5.1 and as many urine samples as possible were collected from hinds. Blood and kidneys were collected from deer available for slaughter, predominantly from males. Some study animals were retained on farms as herd replacements.

Concurrently, the control animals were used to investigate the effects of the natural infection with serovars Pomona and Hardjobovis (Chapter 4). Data from weaners on Farms 2 and 4 in Year 2 were the progeny of hinds that were part of maternal immunity trials (Chapter 6). Data relevant to maternal antibody is presented in that chapter.

Table 5.1. Farms, location, general description, leptospiral status and experimental procedures.

Farm No. and Location	Deer herd Type of farm	Other Species	Serovar	Yr	General trials design										
					Animals at the beginning of the trials			Sampling month/ Biological samples (Treatment).							Fate (No.)
					Group	Sex	No.								
No.1 Manawatu	Red and Wapiti cross. Commercial Venison, & Breeding. Closed herd	Sheep Open herd	Hardjo-bovis.	03	Sampling month			May	Jun	Jul	Sep	Nov	Feb-04	May-04	
					Control	♂	20	B	B	B	B	B	B	B K	S (19)
						♀	20	B	B	B U	B U	B U	B U	B U	R (20)
					Vaccine	♂	20	B (V.)	B (Bs.)	B	B	B	B	B K	S (20)
						♀	20	B (V.)	B (Bs.)	B U	B U	B U	B U	B U	R (20)
No. 2 Hawkes Bay	Red deer. Commercial. Venison, Breeding & Velvet Closed herd	Beef, Sheep Open herds Horses Closed herd	Hardjo-bovis.*	03	Sampling month				Jun	Jul	Sep	Oct	Dec	Feb-04	
					Control	♂	20		B	B	B	B	B	B	R (20)
						♀	20		B	B	BU	BU	BU	Bu	R (20)
					Vaccine	♂	20		B (V.)	B (Bs.)	B	B	B	B	R (20)
						♀	20		B (V.)	B (Bs.)	BU	BU	BU	Bu	R (20)
			Hardjo-bovis & Pomona	04	Sampling month			Mar	Apr	May	Jun	Aug	Sep	Nov	
					Control	♂	20	B	B	B	B	B	B	B K	S (15), R (4)
						♀	20	B	BU	BU	BU	Bu	BU	B U	R(18)
					Vaccine	♂	20	B (V)	B (Bs.)	B	B	B	B	B K	S(15), R (4)
						♀	20	B (V)	BU(Bs)	BU	BU	Bu	BU	Bu	R (19)
No.3 Pahiatua	Cross Wapiti deer. Commercial. Open herd	Diary Beef Sheep Open herds	Hardjo-bovis & Pomona	04	Sampling month						Jul		Sep	Dec	
					Control	♂	16				B		B	B K	S(16)
						♀	24				BU		BU	B K	S(24)
					Vaccine	♂	32				B (V.)		B (Bs.)	B K	S (32)
						♀	33				BU (V.)		BU (Bs.)	B K	S (33)
No.4 Manawatu	Red and Wapiti cross. Research Venison, & Breeding. Closed herd		Nega-tive.	03	Sampling month				Aug	Aug	Sep	Oct	Nov	Dec	
					Control	♂	3		B	B	B	B	B	B	S*(3)
						♀	2		B	B	B	B	B	B	R (2)
					Vaccine	♂	7		B (V.)	B	B (Bs.)	B	B	B	S*(7)
						♀	8		B (V.)	B	B (Bs.)	B	B	B	R (8)
				04	Sampling month			Mar	Apr	Jun	Aug	Sep	Nov		
					Control	♂	13	B	B	B	B	B	B		+
						♀	14	B	B	B	B	B	B		+
					Vaccine	♂	17	B (V)	B (Bs.)	B	B	B	B		+
						♀	18	B (V)	B (Bs.)	B	B	B	B		-

V = sensitiser vaccination; Bs = booster vaccination; B = Blood; U = Urine; K = Kidney; R = replacement; S = Slaughter; + = Other.

Farm 4 was a non-infected research herd monitored for two production cycles included to establish the basic pattern of humoral response to the vaccine without the confounding effect of natural challenge and providing a true negative control for pathology, immunology and bacteriology observations. Initial screening of this herd indicated some residual titres to vaccination of hinds two or more years previously. In Year 1, only deer that were seronegative were selected for vaccination. In Year 2, vaccination commenced at 3-4 months of age and included some animals that had low titres that were believed to be from colostrum as a result of residual vaccination titres in the dams.

Weaning rate data, combining number of progeny and number of lactating hinds determined by udder palpation in February, were collected from 97 hinds vaccinated in October, pre-calving, and 93 non-vaccinated hinds managed together on Farm 2, Year 1. Half of each group were first calvers (2-year-old). All were confirmed pregnant by rectal ultrasound early in gestation. Live weight was recorded on this farm from March to September and data from both years pooled for analysis. Weight was also recorded on Farm 4 Year 2. Farms 1, 2 and 3 had no previous history of vaccination against *Leptospira*.

5.2.3 Vaccination

The candidate vaccine investigated was the trivalent commercial vaccine "Leptavoid-3" (Schering-Plough Animal Health Ltd, New Zealand) as described in Chapter 2, containing serovars Hardjobovis, Pomona and Copenhageni. Batch 7762D1A was used on Farms 1, 2, and 4, while batch 7762A was used on Farm 3. Vaccination procedures are described in Chapter 2. The initial vaccination was followed by a booster 28-30 days later. Vaccination sites were inspected for tissue reactions at booster vaccination and at the subsequent sampling period.

5.2.4 Sample collection and storage

Procedures for blood and urine samples on-farm and blood and kidneys at deer slaughter premises are described in Chapter 2.

5.2.5 Microagglutination test (MAT)

Serum samples were tested for antibodies to serovars Hardjobovis, Pomona and Copenhageni using the MAT described in Chapter 2 (2.7.1 and 2.7.4). Titres on Farms 1, 2 and 3 were considered the result of the interaction between bacteria and/or vaccine antigens on the natural challenged farms while those on Farm 4 were considered due to earlier vaccination.

5.2.6 Bacteriology, gross pathology and histopathology

Techniques are described in Chapter 2.

5.2.7 Data analysis

Descriptive data were expressed as prevalence (proportions) and geometric means of the coded titres with a 95% confidence interval (95%CI). Each animal was sampled 6 times so paired samples that allowed establishment of a true cohort for each individual was obtained . All titres $\geq$1:24 for all serovars were included for the statistical analysis.

For analysis, the four farms in the two years were categorised as six populations coded as follows:

- Farm 1, Hardjobovis positive;
- Farm 2, Year 1, Hardjobovis positive, with a recent introduction of Pomona;
- Farm 2, Year 2, Hardjobovis and Pomona positive;
- Farm 3, Pomona positive with clinical disease and serological evidence for Hardjobovis;

- Farm 4, Negative herd Year 1;
- Farm 4, Negative herd Year 2.

To ascertain the overall pattern of GMT after vaccination in infected herds, data were pooled from each farm for each serovar for samples collected up to approximately 120 days.

In addition to considering DFM, urine culture and kidney culture alone an index of infection termed "live *Leptospira* index" was obtained by pooling data from DFM and urine and kidney culture within an individual such that any animal demonstrating a positive result at least once was considered to be or have been infected. This, in effect, is a cumulative measure of infection status of individuals after vaccination. The response variable "live *Leptospira* index" was negative or positive for statistical analysis.

The average time of shedding was estimated using the positive results from the LLI. Estimated shedding time intervals were calculated based on the assumption that shedding started and ceased half way between previous or subsequent sampling days, respectively, when samples were positive.

Associations between response variables urine shedding, renal findings, culture, serology, "live Leptospira index", and weaning percentage were investigated and the effect variables Farm, Year, sex and serology were investigated with logistic regression analysis and the corresponding Relative Risk. The model was Leptospira = Vaccine (No=0, Yes=1), Farm (1,2,3) * Year (1,2), Sex (Female=0, Male=1), serology at day zero, adjusting for the repetitive samples (in the females) using SAS for Windows (SAS Institute Inc., Cary, Nc, USA). The overall non-adjusted disease prevalence associations (Yates corrected χ^2 and RR) were performed using NCSS 2000 and PASS 2000 (Jerry Hintze ©2001). Vaccine efficacy (VE) was expressed as 1-Relative Risk*100. Weight gain as a sub-clinical effect, was analysed by ANOVA using NCSS 2000. The effect of vaccine on weight gain was analysed by multiple regression, adjusting for sex, year and month with a random effect for the individual animal using SAS.

5.3 Results

No vaccination site lesions or other untoward responses were observed following vaccination.

5.3.1 Non-infected herd (Farm 4)

There were no titres in control deer in Year 1. Titres to Hardjobovis and Pomona in control deer in Year 2 were the result of colostral antibody from residual titres in hinds given a bivalent vaccine more than two years before the trial, while titres to Copenhageni are considered to be cross-reactivity to Pomona.

Mean seroprevalences and GMTs (±95% CI) for each serovar following vaccination are presented in Figure 5.1. Similar patterns were observed for serovars Hardjobovis and Pomona. In Year 1, antibodies were detected in approximately 20% of deer at day 15, 80% at day 28 and 100% at day 90. Seroprevalence decreased to 80% day 120. In Year 2, the seroprevalence pattern was similar, although there were persistent titres in some control animals to both serovars. In Year 1, GMTs increased from zero at the time of vaccination to approximately 24 and 48 for Hardjobovis and Pomona, respectively, at day 15 and remained

relatively stable thereafter for at least 120 days. In Year 2, a similar pattern occurred, but titres for Hardjobovis persisted for 12 months compared with 10 months for Pomona.

Seroprevalence for Copenhageni rose to 90% at day 15 and remained around 70% until day 60, falling rapidly thereafter. In Year 2, the seroprevalence was similar to that of Pomona. The GMT for serovar Copenhageni showed a similar pattern to both Hardjobovis and Pomona.

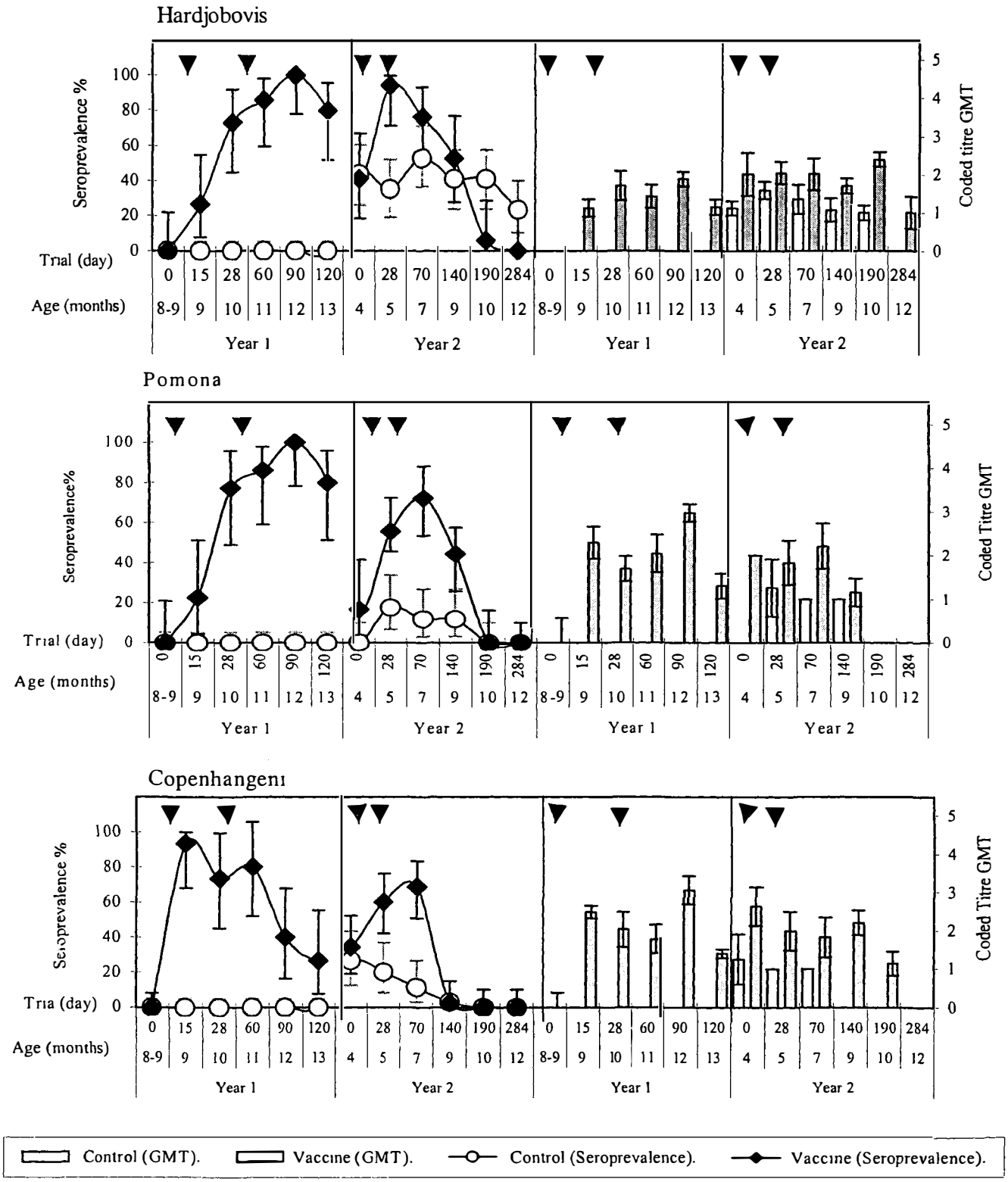


Figure 5.1. Seroprevalence (left) and coded GMT (right) (±95% CI) of each serovar in vaccinated and non-vaccinated control deer on the non-infected Farm (Farm 4) in Years 1 and 2. (Vaccination times represented by arrows).

5.3.2 Infected herds (Farms 1-3)

5.3.2.1 Hardjobovis

Mean seroprevalences and GMTs (±95% CI) for serovar Hardjobovis following are presented in Figure 5.2. On Farm 1, antibodies were detected in 57.5% (GMT 1:24) of deer at day 0 (6-7 months of age). One month after the vaccine sensitiser, seroprevalence in vaccinated animals rose to>90% (GMT 1:48) and remained between 90% and 100% until day 80. The peak reciprocal GMT (1:96) was recorded at day 50. Seroprevalence in the vaccinated group decreased to 60% (GMT 1:36-1:40) by day 120 post vaccination and remained in the same range for the last two sampling points at day 165 and 255. Seroprevalence in the control group was initially similar that of vaccinated deer, decreasing to 7.5% (GMT 1:24) at day 28. Control group seroprevalence rose to 22.5% at day 50 and persisted >20% for the next two sampling periods and then rose to 40% and 57.5%, similar to the vaccinated groups for the last two sampling periods. Reciprocal GMTs were below 1:48 at all times (Figure 5.2).

Hardjobovis seroprevalences on Farm 2 Year 1 showed a similar pattern to those observed in Farm 1 although the initial seroprevalence was 10%. Twenty-eight days after receiving the sensitiser, seroprevalence rose to approximately 70% and remained around that range for the next three sampling periods. The peak reciprocal GMT (1:72) was observed at day 50. Seroprevalence decreased to 33.3% at day 120, and rose to 72% by day 165. In control deer, seroprevalence for the first four sampling periods were ≤20% and GMTs were >1:30). At the final two sampling points seroprevalence rose to 32.4% (GMT 1:64), and 47.5% (GMT 1:50), respectively (Figure 5.2).

On Farm 2 Year 2 the trial began at 4 months of age. Seroprevalence for the first four sampling periods was >95% for both vaccinated and control deer with GMT ranging from 1:45 to 1:69. Seroprevalence later decreased below 10% and GMTs were 1:59 and 1:69 in vaccinated and control groups, respectively. At the final sampling, seroprevalence was 55.0% with GMTs of 1:29 and 1:41 in the control and vaccinated animals, respectively (Figure 5.2).

On Farm 3 both vaccinated and control animals had similar seroprevalences throughout the trail. At day 0 (8 months old) seroprevalence was 22% (GMT 1:27). One month later seroprevalence was >95% (GMT 1:45 in vaccinates and 1:38 in controls) and at the final sampling period seroprevalence was 60% (GMT 1:37) and 72.1% (GMT 1:84) for vaccine and controls groups, respectively (Figure 5.2).

5.3.2.2 Pomona

Mean seroprevalences and GMTs (±95% CI) for serovar Pomona are presented in Figure 5.3. On Farm 1, no antibodies were detected in the control animals throughout. In vaccinated animals seroprevalence was 15% (GMT 1:24) at Day 0. One month after the vaccine sensitiser the seroprevalence in vaccinated animals rose to >95% and remained elevated in the same range. Reciprocal GMT peaked at 50 days No titres were recorded at the final sampling.

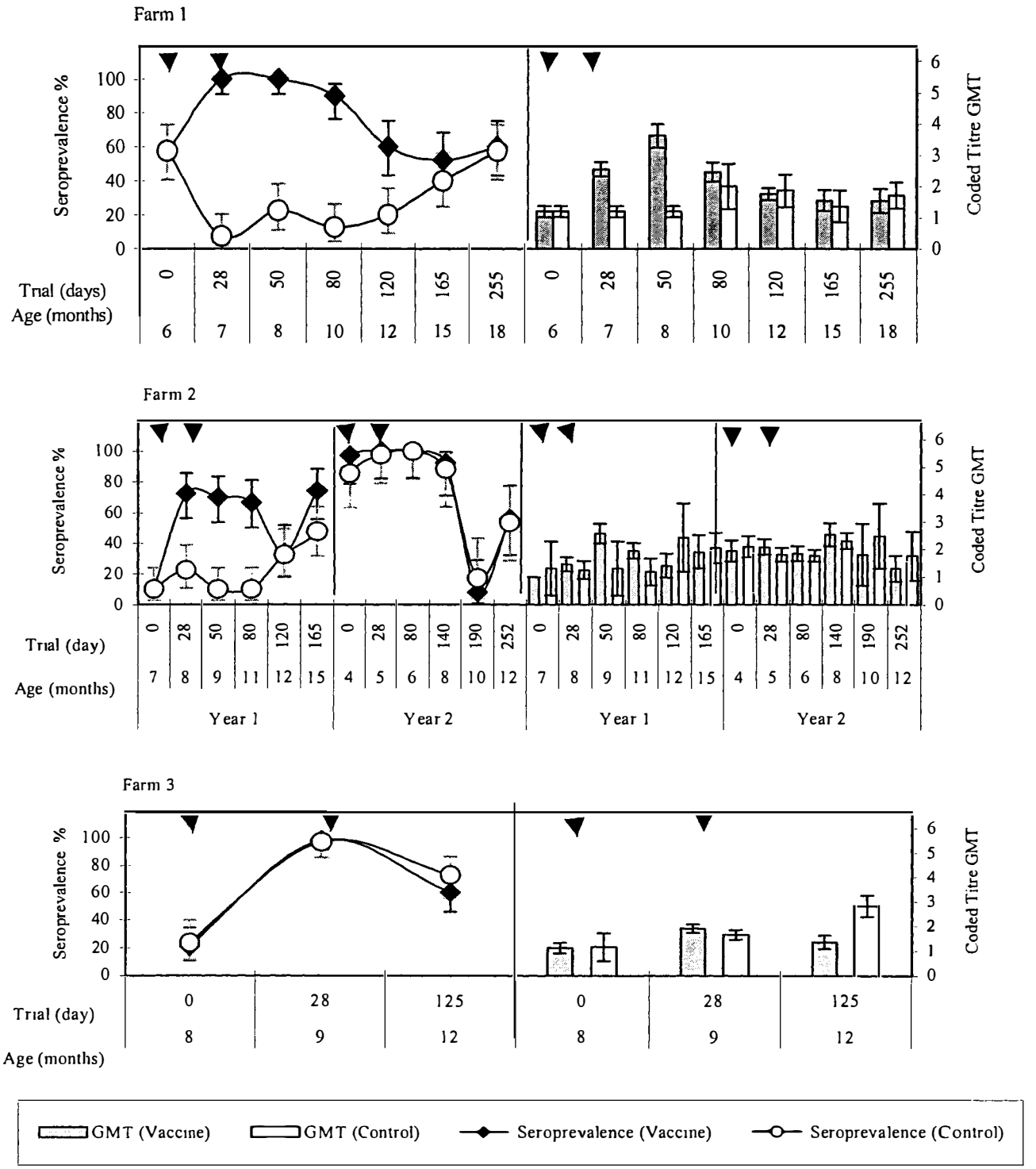


Figure 5.2 Seroprevalence (left) and coded GMT (±95% CI) (right) of serovar Hardjobovis in vaccinated and non-vaccinated control deer on each farm by sampling day and age. (Vaccination represented by arrows).

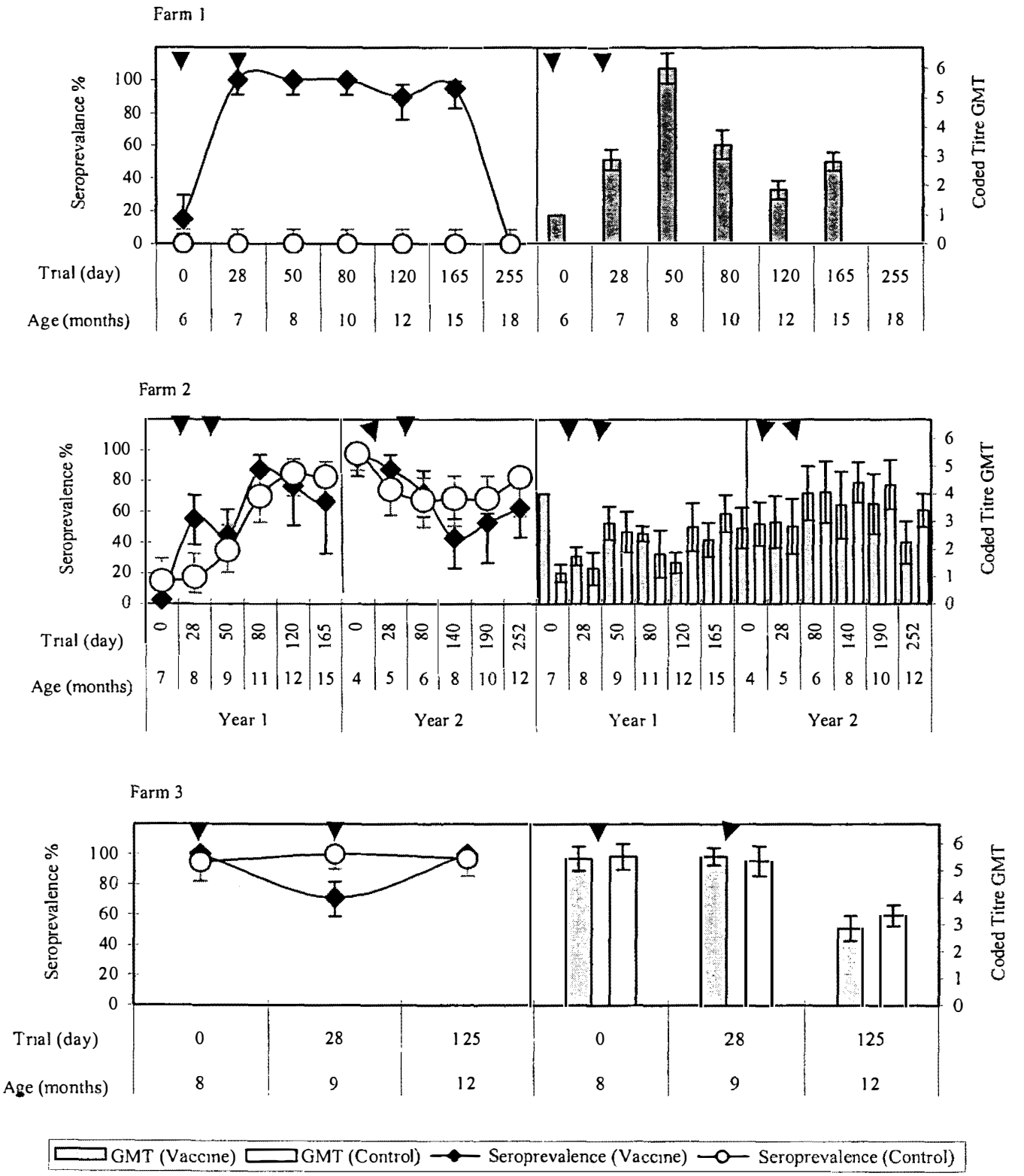


Figure 5.3 Seroprevalence (left) and coded GMT (±95% CI) (right) of serovar Pomona in vaccinated and non-vaccinated control deer on each farm by sampling day and age. (Vaccination times represented by arrows).

Seroprevalence of Pomona on Farm 2 Year 1 at Day 0 for the vaccine group was 2.5% (GMT 1:192) and 55% (GMT 1:40.3) 28 days later, decreased to 45% (GMT 1:92) by day 50. Seroprevalence peaked at day 80 decreased at the last two sampling periods. Control animals had an initial seroprevalence of 15% - 17.5% for first two sampling periods thereafter rising to about 80% at day 120. GMTs varied. On Farm 2 Year 2 the initial seroprevalence for both groups was 95% (GMT 1:81) and decreased similarly over the next three sampling periods. Seroprevalence rose from day 140. GMTs tended to increase to1:254 and 1:241 at day 190, and fell slightly at day 252. On Farm 3 both vaccinated and control animals had similar seroprevalences thought the trail. At day 0 seroprevalence was >95% with a GMTs of 1:514-1:544. At days 28 and 125 vaccinated and control animals had a seroprevalence of 71.4% and 100%, respectively. Seroprevalence in the control animals remained >97% (Figure 5.3).

5.3.2.3 Copenhageni

Mean seroprevalences and GMTs (±95% CI) for serovar Copenhageni are presented in Figure 5.4.

On Farm 1, no Copenhageni antibodies were detected in the control animals. Seroprevalences in vaccinated animals were 0% and 5.0% (GMT 1:24) at days 0 and 28, respectively. At days 50 and 80 seroprevalence rose to 100% (GMT of 1:249.8-1:121). Seroprevalence subsequently decreased to 10% (GMT 1:24).

Copenhageni seroprevalence on Farm 2 Year 1 was initially zero. After vaccination, seroprevalence rose to 80% (GMT 1:70) and 92.5% (1:126) on days 28 and 50, respectively. Seroprevalence and GMT decreased for the next three sampling periods. In control deer, seroprevalence remained low to day 50, thereafter rising moderately. GMTs initially remained below those of vaccinated deer, but were similar from day 80. On this farm in Year 2, the initial seroprevalence for the vaccinated group was 60%, increasing to 97.3% at day 28, thereafter falling steadily to undetectable by day 190. Seroprevalence in control animals was 29.2% and 69.2% at days 1 and 28, respectively, falling thereafter to zero by day 190 the GMTs were variable but generally higher in control animals.

On Farm 3 both vaccinated and control animals had similar seroprevalences at day 0 with seroprevalence in vaccinated deer rising to approximately 50% thereafter, and remaining low in control animals. The GMT ranged from 1:29-1:35.

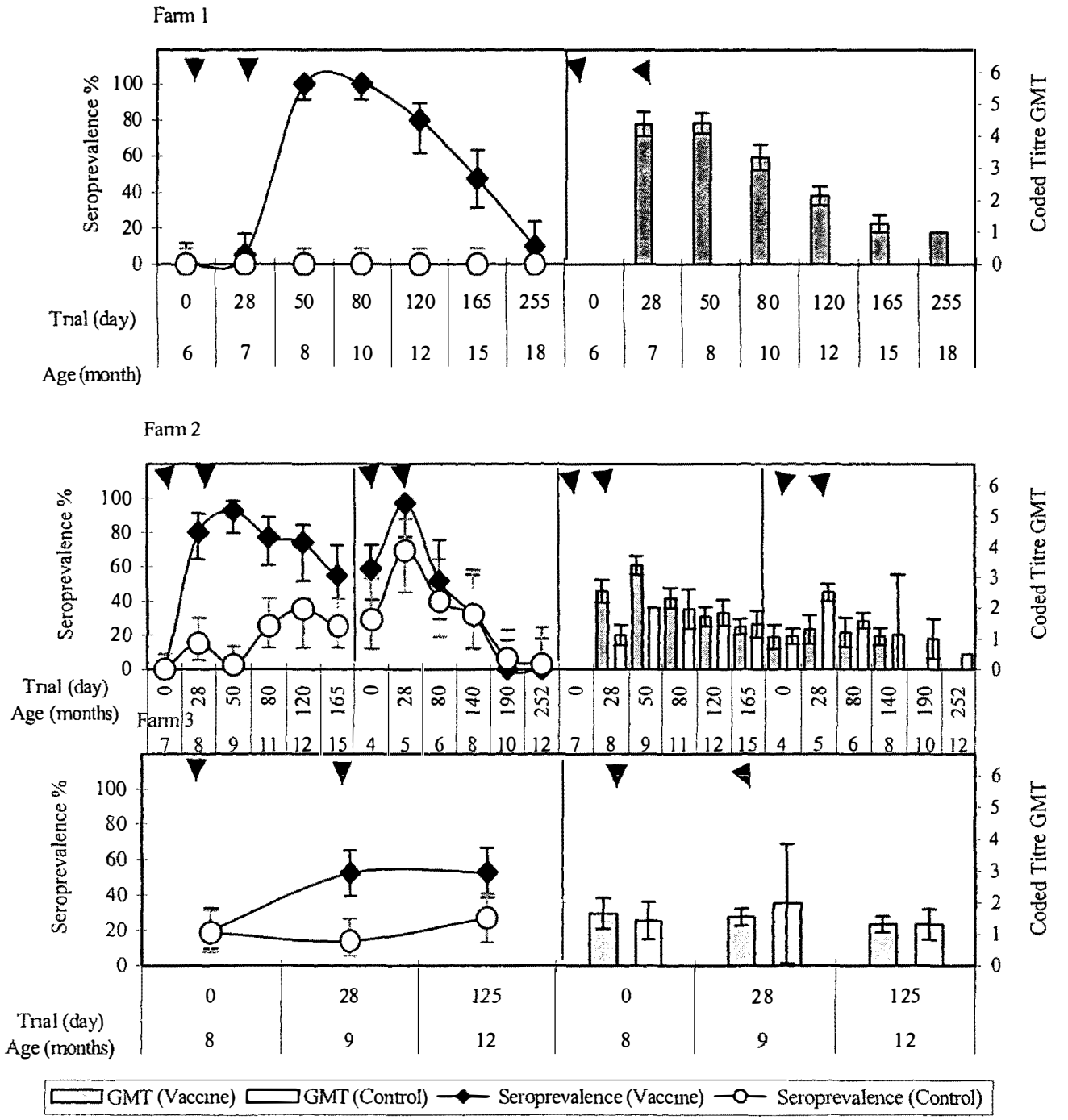


Figure 5.4 Seroprevalence (left) and coded GMT (±95% CI) (right) of serovar Copenhageni in vaccinated and non-vaccinated control deer on each farm by sampling day and age. (Vaccination times represented by arrows).

5.3.2.4 Titre response

To compare overall vaccine titres in infected and control groups on infected farms the pooled geometric means within treatment were calculated for the first 5 sampling points regardless of the age or time of year that they began. Later samplings were not used because of the likely greater confounding effect of natural challenge. Data are presented in Figure 5.5. The reciprocal GMTs for serovar Hardjobovis in vaccinated deer were 1:34-1:106 while reciprocal GMTs for Hardjobovis in control deer ranged from 1:34-1:54. For serovar Pomona, reciprocal GMTs were1:94-1:343 and 1:51-1:130 for vaccinated and control deer, respectively. For serovar Copenhageni reciprocal GMTs were 1:29-1:177 and 1:29-1:48 in vaccinated and control deer, respectively. The highest titre for all serovars was at day 50, 4.5 weeks after booster vaccination.

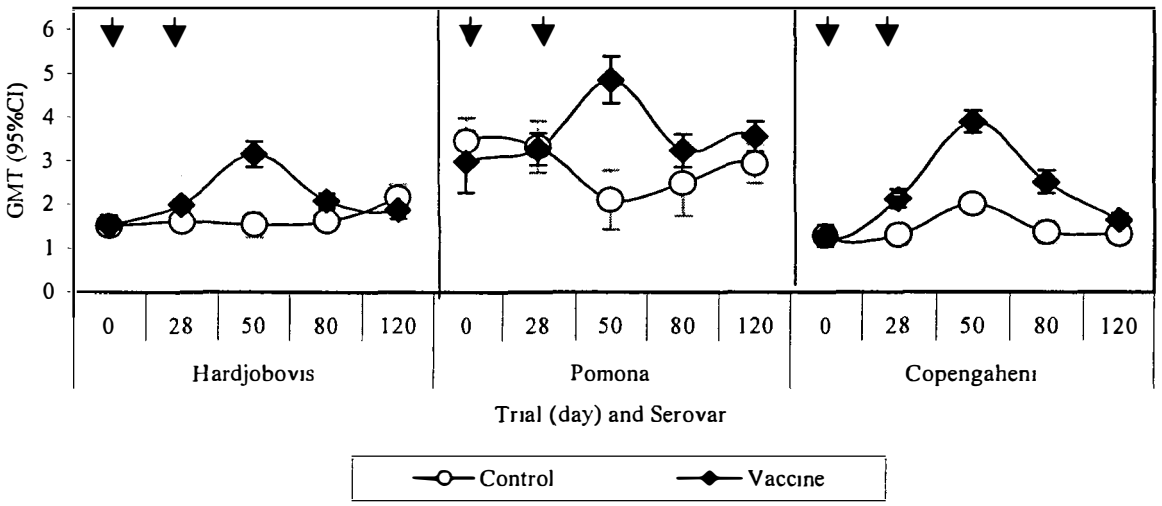


Figure 5.5 Mean coded GMT (±95% CI) of combined titres from infected farms for each serovar from vaccinated and non-vaccinated control deer (Vaccination times represented by arrows).

5.3.3 Urine shedding and culture

Urine DFM and culture results are presented in Table 5.2. An average of three (range 0-5) urine samples were collected per hind. Five hundred and twenty eight urine samples subject to DFM and culture from 185 hinds from Farms (1), (2) and (3) were included in the statistical analysis. Fifty five percent of samples were obtained from vaccinated animals with 31.0% positive under DFM. Of 252 samples from control and 292 samples from vaccinated animals, 38.4 and 24.6%, respectively, were DFM positive (Yates corrected χ 2 of 11.4 (p= 0.0007)).

Thirty urine samples were culture positive to Leptospira, 22 were typed either as Hardjobovis and/or Pomona, and none were positive for Copenhageni. Nineteen were from control and 11 from vaccinated animals. Twenty-three of 169 (13.6%) DFM positive urine samples and seven of 375 (1.8%) DFM negative urine samples were culture positive (Yates corrected χ^2

28.6.94 (p< 0.0001). The relative risk of obtaining a positive culture from a DFM positive urine sample was 7.48 (3.05-18.4) (p< 0.0001). No urine samples were positive for sv Copenhageni.

Vaccine efficacy for the urine shedding based on the urine DFM results were estimated as 1-Realtive Risk. The RR was estimated with the model regression analysis for the response variable DFM to all urine darkfield for females, adjusting for the repeated samples at the individual level, and initial serology, for the. The model vaccine, Farm*Year, sex, seroprevalence at day zero was significant (p >0.001) with a Relative Risk of 0.56 and a VE (reduction in number of animals shedding) of 44.0%.

Table 5.2. Urine DFM and culture results for vaccinated and control deer on each farm.

Farm (Year)	Group	No. of samples	Positive				Typed cultures			Not typed
			DFM		Culture		Hardjobovis	Pomona	*Hardjobovis* & Pomona	
			(n)	%	(n)	%				
1(1)	Vaccine	85	8	9.4	0	0				
	Control	69	18	26.0	0	0				
2 (1)	Vaccine	63	9	14.2	1	1.5				1
	Control	55	13	23.6	5	9.0	5			
2 (2)	Vaccine	95	25	26.3	3	3.1				3
	Control	88	34	38.6	14	15.9	5	3	2	4
3 (2)	Vaccine	49	30	61.2	3	6.1		3		
	Control	40	32	80.0	4	10.0		4		
Total	Vaccine	**292**	**72**	**24.6**	**7**	**2.3**	**0**	**3**	**0**	**4**
Total	Control	**252**	**97**	**38.4**	**21**	**8.3**	**10**	**7**	**2**	**4**
Total		**544**	**169**	**30.4**	**30**	**5.5**	**10**	**10**	**2**	**8**

5.3.4 Kidney lesions

One hundred and fifty four kidneys were collected from the animals sent to slaughter, 84 from vaccinates and 70 from controls, and 39, 30 and 85 from Farms 1-3, respectively. Fifty seven percent of kidneys showed moderate to severe chronic lymphoplasmacytic interstitial nephritis (white spots). Lesions varied from a few foci (2-4 mm in diameter) to diffuse involvement of more than 80% of the renal parenchyma (See Figures 8.5-8.8 in Chapter 8). No difference in gross lesions between vaccinated and control deer were observed (67.8% and 62.8%, respectively, (Yates corrected χ^2 0.14 (p=0.7)). Microscopic lesions were recorded in 74% of 158 kidneys, with 84% in both vaccinated and control animals. Kidneys were available from 10 deer from Farm 4. No lesions were evident.

5.3.5 Kidney culture

The proportions of culture positive kidneys on each farm are presented in Table 5.3. There were no cultures for Copenhageni. Positive cultures were obtained from 23.8% of vaccinated deer compared with 52.9% of non-vaccinated animals. Twenty four percent of cultures were from non-vaccinated animals and 12.9% from vaccinated animals (Yates corrected χ^2 12.6, p=0.0003) The relative risk of not obtaining a positive culture from the kidney samples of the control, compared with the vaccinated animals was 0.45 showing an overall crude estimate of vaccine efficacy of 55.0 % using this measure. However, when vaccine efficacy was estimated based on positive culture for kidney and urine combined, adjusting for repeated

samples and serology at day zero, the model vaccine, farm*year, sex, seroprevalence at day zero was not significant (p= 0.64), the RR was 0.87 and the VE was 12.2%.

Table 5.3. Kidney culture results for vaccinated and control deer on each farm.

Farm	Group	No.sampled	Culture Positive		Typed cultures			Not Typed
			(n)	Prevalence% (95% CI)	Hardjobovis	Pomona	Hardjobovis & Pomona	
1	Vaccine	19	2	1.0 (0.0-26.0)	2			
	Control	20	1	0.5 (0.01-31.7)	1			
2	Vaccine	14	2	14.2 (0.02-42.8)	2			
	Control	16	14	87.5 (41.0-89.0)	5	3	4	2
3	Vaccine	51	16	31.13 (18.0-42.9)		6	8	2
	Control	34	22	64.7 (47.0-80.3)	2	4	0	16
Total	**Vaccine**	**84**	**20**	**23.8 (15.2-34.3)**	**4**	**6**	**8**	**2**
	Control	**70**	**37**	**52.8 (40.6-64.9)**	**8**	**7**	**4**	**18**
Total		**154**	**57**	**37.0 (29.4-45.2)**	**12**	**13**	**12**	**20**

5.3.6 Relationship between DFM and urine and kidney culture

Data of "live *Leptospira* index" for each farm are presented in Figure 5.6. The "live *Leptospira* Index" was positive in 24.7% of vaccinated and 26.5% of control animals. Regression analysis of the variable "live *Leptospira* index" using the model vaccine, Farm*Year, sex, seroprevalence at Day 0, adjusting for the repeated samples at the individual level, and initial serology was significant with a p >0.001. The overall RR was 0.63, indicating a VE of 37.1%. The interaction Farm*Year was significant with an estimated VE for Farm 1 of 57% (p=0.08), Farm 2 Year 1 35% (p=0.34), Farm 2 Year 2 45% (p=0.0036) and 4.6% (p=0.7) for Farm 3.

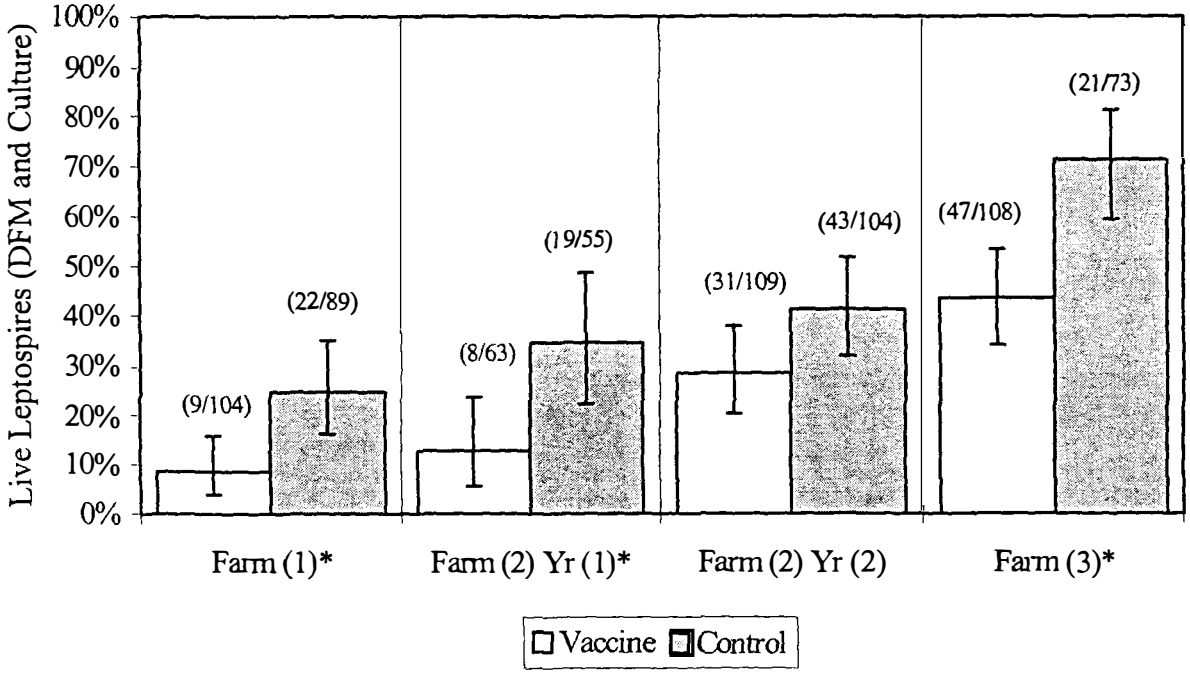


Figure 5.6. Proportion of "live *Leptospira* index" positive deer in vaccinated and non-vaccinate groups on infected farms. * = Significant difference.

5.3.6.1 Shedding time

The average shedding time was 47 days (median 7 days) for control animals, and 19 days (median of 0 days) in vaccinated animals. Vaccine efficacy was estimated using the sum of shedding days to produce a RR of 0.56 (4226.5 vs. 7453 days vaccine and control groups) (Wilcox two sample test, one side hypothesis, p=0.0001). By reduction in shedding time in vaccinates compared with controls, VE was 44.0%.

5.3.7 Production outcomes

5.3.7.1 Weight gain

Multiple regression analysis of the effect of vaccination on weight gain, adjusting for sex, year and month, accounting for the random effect of individual animal and interactions, showed there was no difference between vaccinated and control groups in the non-infected farm (Farm 4) (p=0.94). Weights were not available from Farms 1 and 3. Mean weight gain data related to subclinical infection status and "live *Leptospira* index" on Farm 2 are presented in Figure 5.7. There was a 3.71 kg higher weight gain in non-vaccinated animals without evidence of infection (ANOVA F-ratio 4.41, p =0.037), but there was no significant difference in vaccinated animals.

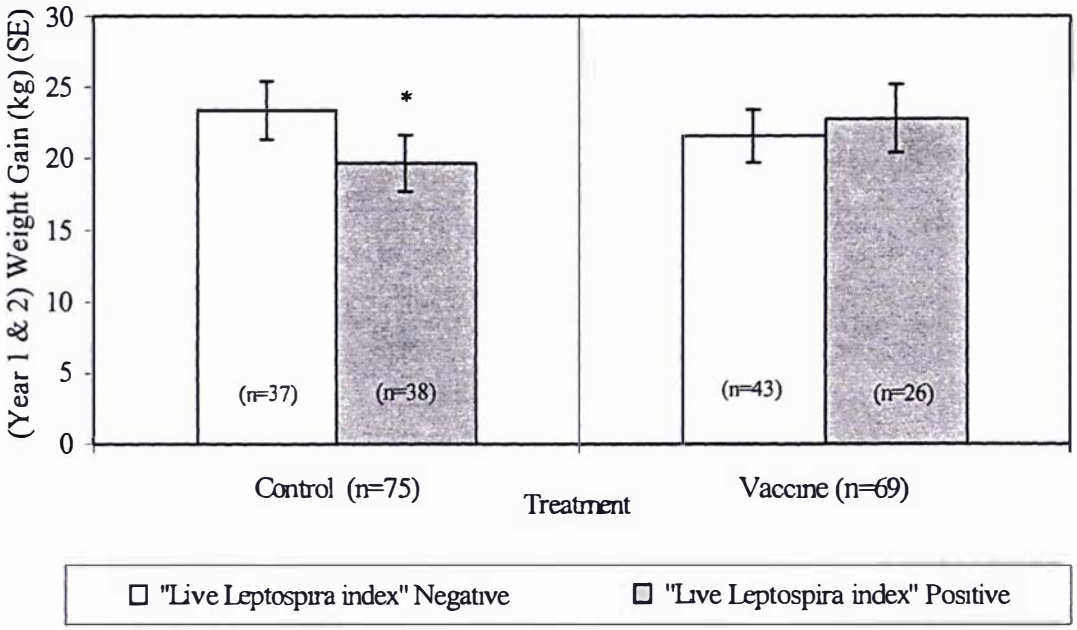


Figure 5.7 Mean weight gain (kg ± SE) from March to September (both years pooled) in "live *Leptospira* index" positive and negative deer from vaccinated and control groups on Farm 2.

5.3.7.2 Weaning percentage

No difference in weaning percentage was observed between first-calving and older hinds. The weaning percentage was significantly higher in vaccinated hinds (97% (94/97)) than in controls (88% (82/93)) (Yates corrected χ^2 of 4.1, p= 0.04), relative risk of 1.10).

5.4 Discussion

This is the first longitudinal study of a leptospiral vaccine in commercially farmed deer. A non-infected herd was used to establish the base-line response without interference of challenge since serology cannot distinguish between vaccine and natural infection-derived antibody. Infected herds were used to establish the serological response and efficacy in protecting deer, and ultimately humans, from infection in an environment found typically on commercial deer farms. It has shown that vaccination produces antibodies in non-infected deer against the three stains investigated and that vaccination enhances immunity in infected herds, despite continued natural challenge, resulting in a reduction in the proportion of animals shedding leptospiral organisms in urine, and the proportion of kidneys that were culture positive. No untoward response to vaccination was observed. Vaccination had no effect on weight gain in non-infected deer. This research also showed sub-clinical effects of leptospirosis for the first time in deer with a lower weight gain observed in infected animals that were not vaccinated. Vaccination was also associated with a higher weaning rate. The latter two observations need to be replicated because data permitted analysis on only one farm.

Immunological protection from leptospirosis is serovar specific and bacterins should contain those serovars enzootic to the country in which they are to be used (Bolin et al 1989b). Vaccines are evaluated by their ability to produce protective immunity, requiring the vaccine agent to mimic as closely as possible the antigenic challenge of natural infection (Babiuk et al 2002; Zinkernagel 2003). The commercial vaccine used for this study showed significant protection in cattle in New Zealand (Marshall et al 1982). Results from the present study suggest that strains in the trivalent vaccine used are homologous with those causing natural infection of farmed deer in New Zealand since immunological responses in non-infected deer were similar to those of natural challenge.

The trivalent vaccine containing Copenhageni was chosen for this study because of dual Hardjobovis and Copenhageni cultures from a herd previously experiencing a clinical outbreak reported by Flint et al (1986), serological evidence from Wilson et al (1998) and Flint et al (1988a) and serological evidence from an outbreak on one farm (Walker, pers comm.).

Anamnestic responses were not observed following booster vaccination in infected herds, but were observed in the non-infected herd. Titres in vaccinated animals waned after 120 days after vaccination, but were boosted concurrent with an increase in seroconversion in control animals as a response to increasing natural challenge. Similar anamnestic responses have been reported in cattle and sheep with natural or experimental infectious challenge with Hardjobovis (Tripathy et al 1976; Marshall et al 1979a,b; Ris and Hamel 1979, 1982; Hancock et 1984).

The results from this study in deer are consistent with those observed in cattle and pigs in many countries (Radostitis et al 1999; OSH 2001). Combined Hardjobovis-Pomona bacterins have been used in New Zealand since the late 1970s giving significant protection against infection and leptospiruria caused by Hardjobovis in cattle and sheep and abortion and disease in bovine calves caused by Pomona (Marshall et al 1979 a, b; Flint and Liardet 1980; Mackintosh 1981). In New Zealand, vaccination has been used successfully to prevent leptospirosis in the face of outbreaks on deer farms (Dean et al 2005).

The peak GMT for the three serovars in the vaccine was observed at day 50 after the sensitiser vaccine dose, with similar magnitudes while the peak response in the non-infected farm was observed at day 70. Wilson and Schollum (1984) reported the peak antibody response for Hardjobovis and Pomona 56 days after the sensitiser. Flint (1987 unpublished) reported the peak antibody responses to the trivalent vaccine used here after 40 to 50 days. In similarly vaccinated cattle, peak antibody responses occurred 56- 70 days after sensitiser (Tripathy et al 1976; Mackintosh et al 1980d,e; Broughton et al 1984; Hancock et al 1984; Bolin et al 1989b; Palit et al 1996; Bolin et al 2001).

5.4.1 Non-Infected herd

The two-year trials on a non-infected herd (Farm 4), determined by absence of kidney lesions, negative urine DFM and kidney culture, evaluated the immunological response in one-year old farmed deer to vaccination without interference of a natural challenge. Data from adult hinds on this farm are reported in the evaluation of maternal antibody effects presented in Chapter 6. Vaccine responses to the three serovars involved in both years were classic

humoral responses, as seen in other mammals (Tizard 1995). The overall pattern was that seroprevalence for Hardjobovis was more persistent than for Pomona and Copenhageni.

In Year 1, some trace maternal antibody was detected in some deer as a result of residual titres in dams following vaccination at least two years earlier. Only seronegative animals were chosen for vaccination to avoid potentially confounding effects. The study began at 8-months of age since animals were weaned late because of the drought so vaccine response could be followed for only 120 days. In Year 2 animals were vaccinated at 3-4 months of age, including some with maternal antibody titres and sampled until 12 months of age (284 days).

In the present study Hardjobovis and Pomona seroprevalence and GMTs after vaccination with "Leptavoid-3" followed a pattern similar to that reported by Wilson and Schollum (1984) in a small trial involving 4 initially seronegative adult hinds using a bivalent vaccine ("Leptavoid-2" Welcome NZ Ltd). That study showed a response to both the sensitiser and booster doses with titres generally persisting for 5-6 months. In the only other study of vaccination responses after leptospiral vaccination in a non-infected herd, Wilson et al (2001) reported a rise in mean titre to Pomona but not Hardjobovis 28 days after a sensitiser dose of an alternative vaccine. Three weeks after a booster, titres to Hardjobovis were observed, but mean titres were higher for Pomona. The reciprocal GMTs for both serovars observed in that study were also similar to those reported here. Seroprevalence and duration of titres in deer here are also similar to those reported in cattle after similar vaccines (Marshall et al 1979b; Ris and Hamel 1979; Mackintosh et al 1980d,e; Allen et al 1982; Broughton et al 1984; Hodges and May 1987; Bolin et al 1989b; Bolin et al 2001). However, comparison of GMTs is not meaningful because different dilutions and cut points were used by various researchers.

The reciprocal GMTs to Copenhageni were in the same range as for Pomona. A similar pattern of seroprevalence and GMT to this serovar using the same vaccine brand was described by Flint (1987 unpublished) in deer and Palit et al (1996) in cattle.

In Year 2 some animals were seropositive at 3 months of age because of colostral antibody from residual vaccination titres from their dams. Colostral antibody to Hardjobovis, Pomona and Copenhageni persisted for 12, 9 and 7 months, respectively, in some animals. Copenhageni titres were the result of cross-reactions to Pomona since the vaccine used in the previous history of the farm did not contain this serovar. Colostral antibody associated with vaccination against leptospires, *Brucella abortus* and Rinderpest in cattle (Hellstrom 1978) persisted up to 6 months. Colostrum-derived antibodies to clostridium toxins persisted up to ten months in cattle (Tizard 1995), and leptospira Pomona antibodies persisted up to 20 weeks in pigs (Bolt 1990).

5.4.2 Infected farms

In the first year, to minimise the influence of the natural cycle of challenge, only forty weaners per farm were vaccinated. Farm 2 Year 1 was initially infected only with serovar Hardjobovis. Pomona was introduced early in the study, most likely by bulls brought onto the farm since grazing and water were shared. In Year 2, the owner vaccinated all weaners and other deer not in the trial group, and cattle, on the recommendation of his veterinary practitioner, given that research and vaccination in Year 1 suggested that losses due to leptospirosis were occurring on the farm, and climatic, environmental and management risk factors were high. On Farm 3, initially only 40 deer in the study mob were vaccinated, but one

month later all other deer and the cattle on the property were vaccinated. This was to reduce the risk of a possible clinical outbreak with serovar Pomona since the high seroprevalence and high titres, combined with cold and very wet conditions, and a death confirmed as leptospirosis in a rising 1-year-old deer in another mob, suggested the risk of an outbreak was high. While these decisions were justified on animal welfare and production grounds, they may have altered the transmission dynamics of leptospirosis. These events are not atypical of the reality of conducting trials on commercial farms.

Serovars Hardjobovis and Pomona, but not Copenhageni, were confirmed by culture in these herds, thus serological results for the two former serovars represent a composite of vaccine and natural challenge.

For Hardjobovis, the pattern of seroconversion in vaccinated and non-vaccinated control animals differed between years with seroconversion in control animals different from vaccinated animals in Year 1, Farms 1 and 2, but not in Year 2, Farms 2 and 3. Evidence suggests a higher challenge in Year 2 as is known to occur with high rainfall and humidity (Elder 1986; Hellstrom 1978) compared with Year 1 in which a drought was experienced. Thus, vaccination appeared to enhance immune responses, measured by seroconversion rate and titre in the face of a low natural challenge, but not in the face of high challenge. The GMTs to serovar Hardjobovis were similar in vaccinated and non-vaccinated deer, ranging between 1:24 to 1:96. Despite lack of detectable difference in immune response, urine shedding and the "live *Leptospira* index", including serovars Hardjobovis and Pomona, were lower in vaccinated animals. In Hardjobovis infected cattle herds, similar variation in relationships between seroconversion and GMTs were also observed in vaccinated and non-vaccinated animals between years and studies (Tripathy et al 1976; Marshall et al 1979b; Dufree and Allen 1980; Mackintosh et al 1980d; Allen et al 1982).

Seroprevalence and GMT for Pomona in response to vaccination in Farm 1, in the absence of infection by this serovar, showed a similar profile to that described for Farm 4 above. On Farms 2 and 3, seroprevalence in both vaccinated and non-vaccinated animals was generally similar. However, on Farm 2 Year 1, there was initially an apparent response to vaccination above controls when the seroprevalence was low, shortly after introduction of Pomona infection to the herd. Thus a vaccination response was detectable when seroprevalence was low, but not then seroprevalence was high, similar to the pattern for Hardjobovis. The GMTs to Pomona on Farms 1 and 2, Year 1 were similar. However, in Year 2 on Farm 2, when infection with Pomona was well established, and on Farm 3, GMTs were high. In vaccinated cattle challenged one year later with serovar Pomona, Huhn et al (1975) demonstrated an anamnestic response to infection of similar magnitude to that observed in control animals. In that study, culture was subsequently positive in 3.2% and 63.4 % of kidneys from vaccinated and non-vaccinated cattle, respectively. Reduction of urine shedding and kidney culture was observed after vaccination of deer in this study.

Persistent antibody titres with low GMTs after vaccination as seen for Hardjobovis have been reported previously in deer (Wilson and Schollum 1984; Flint 1987 unpublished) and cattle (Ris and Hamel 1979; Allen et al 1982; Broughton et al 1984; Little et al 1992a,b). This pattern is typical of those reported for endemic infections with host-adapted serovars (Faine et al 1999; Hathaway et al 1981a,b). The low agglutinating titres following either vaccination or infection with Hardjobovis have been attributed to its lipopolysaccharide composition which appears to induce a strong and long lasting cell-mediated immune response (Naiman et al 2001; Naiman et al 2002; Brown et al 2003).

Titres to Pomona and Copenhageni in vaccinated animals without challenge had shorter duration but a higher antibody titre than to Hardjobovis. Similar results were observed in deer by Flint (1987 unpublished) and Wilson and Schollum (1984). In cattle, Allen et al (1982) and Palit et al (1996) reported short-term seroprevalence and lower titres to Pomona than those observed for Hardjobovis, similar to those reported by Mackintosh et al (1980b). Wilson et al (2001) reported relatively low antibody responses to serovars Hardjobovis and Pomona.

On Farm 2 GMTs to Pomona were higher in Year 2 when infection was well established, ranging between 1:96 and 1:384 compared with 1:48 to 1:96 the previous year, and GMTs on Farm 3 were higher. These findings concur with other reports describing active infectious challenge or outbreaks in deer (Ferris et al 1960; Trainer et al 1961; Reilly et al 1962a,b; Farley et al 1984 and 1986, Dean et al 2005). High GMTs have been reported in Pomona outbreaks and following artificial challenge in cattle (Te Punga and Bishop 1953; McDonald and Rudge 1957; Hellstrom 1978; Mackintosh 1981d).

Higher GMTs to Copenhageni were detected on Farm 1 showing a classic vaccination response, compared with sustained titres on Farms 2 and 3 which were a composite response to vaccination and natural challenge with Pomona, causing cross reactivity.

5.4.3 Urine shedding, "live *Leptospira* index" shedding time and kidney lesions

Reduction but not elimination of the "live *Leptospira* index" and reduction of the average shedding time are consistent with observations from cattle and pigs in the face of on-going natural (Marshall 1979b; Mackintosh et al 1980d,e ; Cargill et al 1981; Allen et al 1982; Hodges et la 1985: Leonard et al 1992, 1993) and experimental challenge (Tripathy et al 1976; Broughton et al 1984; Marshall et al 1979a; Marshall et al 1982; Bolt 1990; Bolin et al 1989b, Bolin et 2001). Vaccination of only a small proportion of the herd or study group in this research does not permit the evaluation of the full efficacy of the vaccine since the rate of challenge would be higher from non-vaccinated herd-mates than would be the case if all animals in the study mob and farm were vaccinated. Nevertheless, vaccine efficacy, when estimated by urine DFM observations, LLI or shedding interval, demonstrated a reduction between 37.1% and 44.0% in vaccinated animals. It has been shown in Cattle that vaccination of all animals in a herd on a long-term basis can eliminate or almost eliminate infection by incrementally increasing herd-level immunity because younger vaccinated animals are resistant to infection and the whole-herd challenge diminishes with time (Mackintosh et al 1980d; Broughton et al 1984; Little et al 1992b). Vaccinated animals that do become infected may experience lighter infection, shed at a lower level and have a shorter duration of leptopsiruria, thus reducing the degree of challenge on herd-mates as shown in cattle by Mackintosh et al (1980d).

Observations here were in young deer. It is possible that vaccination of older deer may not result in an equivalent reduction in urine shedding since Hancock et al (1984) reported a reduction in urine shedding in cattle that were vaccinated before 22 months of age but no reduction when animals were vaccinated after their first calving. However, the improvement in reproductive performance on Farm 2 suggests that vaccination may be at least partially effective in reducing shedding and/or *in utero* transmission in older animals. Radostitis et al (1999) and Bolin and Prescott (1999), report that protection from both the disease and re-infection can persist for a least a year in cattle. This reinforces the likely importance of vaccination of deer early in the first year of life, preferably before any infection occurs,

combined with annual booster vaccination, if reduction of herd and human risk are to be achieved.

No differences in kidney pathology were observed between vaccinated and non-vaccinated control deer, although there were significantly fewer cultures from vaccinated animals on Farms 2 and 3, which may have been associated with a relatively high infection rate with Pomona, especially in non-vaccinates. Evidence suggests that colonisation of the kidney, with subsequent lesions is prevented by the presence of antibodies before rather than after infection (Cargill et al (1981). It is possible that very heavy challenge may have resulted in the number of leptospires reaching the kidney in the bacteraemic phase in the presence of circulating antibody. Those kidneys with lesions probably developed them during colonization. However, the leptospires may have been prevented from establishing tubal colonisation and shedding by the presence of antibody, promoted by vigorous host antibody response and opsonisation. Reduction of kidney lesions in animals vaccinated prior to experimental challenge has been reported in cattle, sheep and pigs (McDonald and Rudge 1957; Huhn et al 1975; Marshall et al 1979a,b ; Cargill et al 1981; Bolin et al 1989b). Trypathy et al (1976) reported a reduction of the severity and number of kidney lesions in vaccinated cattle following challenge with serovar Hardjo compared with non-vaccinated animals, but not total elimination of pathology. Bolin et al (1989a) reported kidney colonization and lesions in vaccinated pregnant cows challenged with serovar Hardjobovis. Most of the kidneys collected for pathological examination in the present study were from the dual infected farms in the second year, when there was evidence of a continuing high leptospiral challenge particularly with serovar Pomona prior to vaccination. It is likely that a reduction in kidney lesions would be seen in subsequent years if whole herd vaccination was implemented, since vaccination reduces shedding, and therefore environmental challenge should reduce over time. There were no kidney lesions in kidneys from the uninfected farms.

While this study evaluated only one vaccine, it is possible that the efficacy of other formulations may be different in deer. However, the vaccine used here was reported in cattle in the UK and Australia (Bolin and Prescott 1999) to be similar to "Leptoshield" (Pfizer NZ Ltd) which is licenced for deer in New Zealand. The efficacy of that and other vaccines needs to be tested in deer.

5.4.4 *Production outcomes*

A weight-gain difference was observed between deer with and without evidence of infection in unvaccinated deer on Farm 2. Those without infection grew at the same rate as vaccinated deer regardless of infectious state. This suggests that a weight gain response could be expected when vaccination is practised in infected herds. Given the greater kidney lesion severity associated with Pomona, it is likely that the response was largely due to protection against this serovar. However, an effect of Hardjobovis alone on growth cannot be discounted and needs to be evaluated. Evidence presented in Chapter 3 suggests that infection with Pomona may not persist on farms, and given that about 17% of farms have this serovar (Chapter 7) it would appear that many farms would be exposed to it on a sporadic and possibly cyclical basis. It is suggested by Thrusfield (1995), that 80% sustained herd immunity should be sufficient to limit infectious cycles. Thus while it cannot be expected that all herds vaccinated would experience a weight gain response in the short term, it is likely that many herds would over time. One study in ruminants reported no difference in weight gain following vaccination in beef cows (Fava et al 2004), but no other studies have investigated the relationship between infection status and weight gain as undertaken in this study.

There was no difference in weight gain associated with vaccination on Farm 3. However, the seroprevalence for Pomona due to natural infection was >90% before vaccination began in July. On Farm 2 Year 2 vaccination started in March. Although seroprevalence was then high, it is likely to be largely attributable to colostral antibody, so it probable that infection had not become well established in those animals. Thus, to achieve a weight gain response, it is likely that early vaccination would be required. Power analysis using a one-sided two-sample t-test indicated that a sample of 37-38 animals/group was needed to achieve 94% power (alpha <0.05) to detect the difference of 3.7 kg reported here.

The effect of subclinical infections on growth and production in domestic animals may be associated with catabolic effects induced by cytokines, immune cell proliferation and antibody production, together with tissue damage, kidney failure and loss of protein through kidney damage (Tizard 1995, Morilla 1986).

On Farm 2 Year 2 vaccinated hinds were almost four times more likely to wean a calf, giving an 11% higher weaning rate, with the effect likely to be associated with Pomona. Since hinds were diagnosed pregnant early in gestation and no abortions were observed, it is likely that higher perinatal survival in progeny of vaccinated hinds was responsible for the difference. This could be due to reduced *in utero* infection and/or protection by colostral antibody early in life. Serovar Pomona in domestic ruminants has been associated with late abortion, stillbirth and clinical outbreaks of haemolytic syndrome and acute death in young animals (Te Punga and Bishop 1953; McDonald and Rudge 1957; Amatredjo et al 1975; Hellstrom 1978; Mackintosh 1981; Ellis 1994; Vermunt et al 1994; Radostits et al 1999). Stillbirth and weak neonates have been reported due to Hardjoprajitno in pigs and cattle (Ellis 1994; Heath and Johnson 1994). It is likely that sporadic mortalities due to acute leptospirosis would be undetected on commercial deer farms. There is a lesser association between Hardjobovis and clinical disease or reproductive wastage (Holroyd 1980; Thiermann 1983; Bolin et al 1989a). Colostral antibody is protective against neonatal leptospiral infection particularly with serovar Pomona in bovine calves (McDonald and Rudge 1957; Amatredjo et al 1975). Hanson et al (1972) reported reduction of abortion and stillbirths in cattle herds infected with serovar Hardjo, and Holroyd and Smith (1976) and Holroyd (1980) reported higher weaning rates in vaccinated cattle. Replication of the production outcomes reported here is required.

Ellis (1984) proposed that it is difficult to determine the overall production losses associated with leptospirosis because there are too many confounding variables. However, while it may not be possible to expect or predict production responses in all situations, such responses are not the only justification for vaccination, since human occupational safety and animal welfare protection are important, but are difficult to evaluate purely in economic terms (Wilson et al 2005).

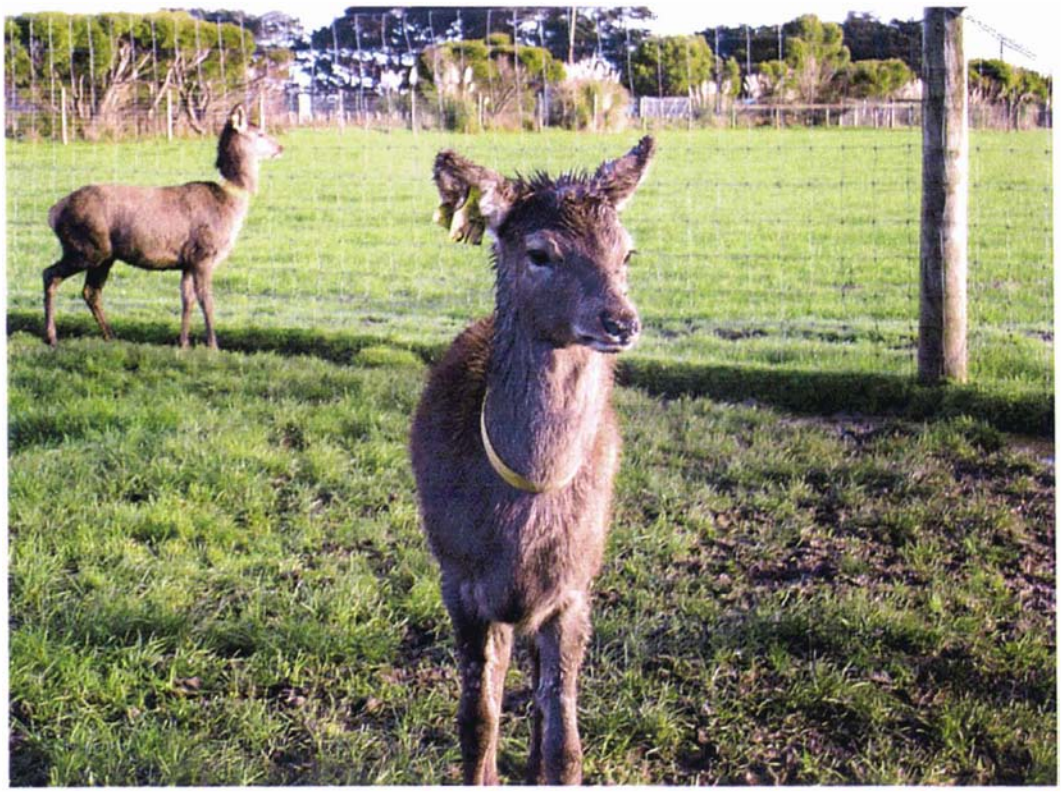
5.5 Conclusions

- The commercial trivalent vaccine used in this research produced an antibody response, and reduced leptospiruria and kidney lesions, therefore reducing the risk of leptospiral infections and disease in deer, and transmission to other livestock species and man.

- Results suggest that early vaccination of young animals and annual revaccination of all stock classes would be necessary to achieved effective herd immunity and reduce risk of disease and transmission.
- Vaccination reduced reproductive loss and may produce a growth response in some circumstances. The impact of production losses associated with leptopsirosis may be under-estimated on New Zealand deer farms.

Chapter 6

Serological responses to a Trivalent Leptospiral Vaccine in pregnant hinds and progeny



6.1 Introduction

Vaccination of the dam in late pregnancy is intended to provide protection of the offspring early in life (Tizard 1995; Morein et al 2002). In ungulates, colostral immunoglobulins are essential for the protection of new-born since placental transfer of antibodies does not occur (Salmon 1999; Kehrli and Harp 2001). However, passive immunity can compromise immuno-responsiveness to vaccination of progeny early in life, largely by maternal antibodies neutralising vaccine antigens (Barrington and Parish 2001). Schollum and Marshall (1985) and Bolt (1995) proposed that the optimum timing of leptospiral vaccination of young cattle and pigs was when maternal antibody waned but before exposure to significant natural challenge. Little is known about passive immunity in farmed deer.

Hibma and Griffin (1988) reported high antibody titres to keyhole limpet haemocyanin in 5-week-old progeny of hinds immunised 22 weeks pre-calving, demonstrating passive transfer of immunity. Mackintosh et al (2001) demonstrated a classical reduction of titres to presumed passively acquired antibody from 1 day to 12 weeks after birth from dams given a *Yersinia pseudotuberculosis* vaccine. Those authors also reported a reduced antibody response to vaccination of progeny at six and nine weeks after birth compared with those vaccinated at 12 weeks, suggesting that maternally derived antibody interfered with vaccination when given at an early age in deer. Studies reported in Chapters 3 and 4 indicate that farmed deer may be exposed at a young age to leptospiral infection, possibly before weaning. Thus, maternally derived antibody may be an important consideration in a vaccination programme for leptospirosis, both in relation to protection of the newborn and potential interference with subsequent vaccination aimed at active immunity.

This study investigated the humoral response to a trivalent commercial leptospiral vaccine given to hinds in both infected and non-infected herds, passive immunity in their progeny and subsequent active immunity from 12 weeks of age, to establish the pattern of maternally derived antibody and possible interference with vaccination of progeny.

6.2 Materials and methods

6.2.1. Farms, animals and management

Farm 2, infected with both Hardjobovis and Pomona, and Farm 4, not infected, involved with studies reported in Chapters 4 and 5, were used in this study. Animals and management are described in those chapters. Adult hinds >2-years of age confirmed pregnant by rectal ultrasound early in gestation and their progeny were used.

6.2.2 Trial design

6.2.2.1 Hinds

A summary of experimental design is presented in Table 6.1. The number of hinds chosen was determined firstly to achieve the minimum number of weaners required (see section 6.2.2.2), but also to fit with management requirements on the properties.

On Farm 2, for one production cycle (2003-4), 200 dams were randomly allocated to control and vaccinated groups and managed together until calving. Sensitiser and booster vaccination was given on October 23 and November 21, 2003, respectively. Thirty from each group were randomly selected for blood sampling on those occasions and on February 6, 2004 (day 110). Farm 4 was used for two production cycles (2003-5). In year one, 80 hinds were randomly allocated to two groups with sensitiser vaccination of one group on October 25 and a booster on November 24, 2003 as for Farm 2. In year two, half of the vaccinated and control hinds from the previous year received one vaccination on October 23, 2004. All hinds were sampled on those days and on February 14, 2004 and February 13, 2005 (Table 6.1) Vaccinated and control groups were separate during calving on both farms to allow accurate identification of progeny with respect to vaccination status of their dam, and to eliminate the confounding effect of cross-suckling. However, on Farm 4 Year 2, it was not possible to separate hinds vaccinated and non-vaccinated in the previous year, thus data from progeny were related only to the vaccination status of dams in that year only.

Table 6.1. Summary of trial design and vaccination and sampling dates for hinds.

Farm/Yr	Group	No. Hinds		Sampling (Month/Year and Day after vaccination)					
		Per group	Sampled	Oct-03	Nov-03	Feb-04	Oct-04	Nov-04	Feb-05
				Day 0	Day 28	Day 100	Day 0	Day 30	Day 113
2	Vaccinated	100	30	BS, V	BS, B	BS			
	Control	100	30	BS	BS	BS			
4, Yr 1	Vaccinated	40	40	BS, V	BS, B	BS			
	Control	40	40	BS	BS	BS			
4, Yr 2	Vaccinated*	40	40				BS, B	BS	BS
	Control*	40	40				BS	BS	BS

* Half from each of vaccinated and control groups Year 1, BS = blood sample, V = sensitiser vaccination, B = booster vaccination

6.2.2.2 Progeny

A summary of experimental design is presented in Table 6.2. The response variable was difference of 80% in seroconversion between vaccinated and non-vaccinated animals. Power analysis showed a sample size of 12 should achieve 80% power to detect an effect size (W) of 0.8 (80%) using a one degree of freedom Chi-Square Test with a significance level (alpha) of 0.05 (NCSS 2000 and PASS 2000 (Jerry Hintze ©2001)). A final minimum group size of 15 progeny was proposed since some progeny can die or be misplaced on commercial farms. In Year 1, half of the progeny from vaccinated and control hinds from both properties were randomly allocated to

vaccinated and control groups, balanced by sex where possible. Numbers sampled are shown in Table 6.2. All 71 progeny from Farm 4, and 80 progeny from Farm 2 were used in Year 1, and 76 progeny on Farm 4 were used in Year 2. They were sampled at approximately 6-week intervals from February 6-14 Year 1 (2004) (day –28) when hinds were sampled, until approximately 12-months of age. The final sampling was conducted on-farm at day 252 (November) for those deer retained, and at slaughter on day 284 (December) for the remainder. Data for these final samples are combined for presentation and analysis. Progeny on Farm 4 in Year 2 (2005) were blood sampled in mid-February and mid-March. Weaning dates were 15 February on Farm 2, and 16 February and 1-3 March on Farm 4, Years 1 and 2, respectively. Sensitiser vaccination was given to progeny Year 1 on March 15 and 16, on Farms 1 and 2, respectively.

6.2.2.3 Vaccination, sampling and serology

The vaccine, vaccination and sampling, sample handling and storage, and the MAT used are described in Chapter 2. Vaccine batch 7762D1A was used in Year 1, and batch 7762 A1 was used for the booster in Year 2, Farm 4.

6.2.3 Statistical analysis

Descriptive data are expressed as antibody prevalence with 95% CI and geometric mean of the coded titres with a 95% confidence interval (95% CI). All titres ≥1:24 were included for the statistical analysis. A generalised mixed logistic regression model adjusting the effect of vaccination on sero-status within sampling day and farm for sex and for the correlation of repeated sampling of individual animals was used for multifactorial analysis and evaluated on the basis of the overall model χ^2. The relative risk (RR) and Wald-χ^2 for individual effects were the criteria to explore the humoral immune response associations using NCSS 2000 and PASS 2000 software (Jerry Hintze ©2001).

Table 6.2 Summary of trial design and vaccination and sampling dates for progeny.

Month						Feb	Mar	Apr	May-Jun	Jul-Aug	Sep	Nov-Dec
Day						-28	0	28	70-80	140	190	258-284
Farm	Year	Vaccine		Sex	No.							
		Hind	Progeny									
2	1	No	No	♂	10	BS	BS	BS	BS	BS	BS	BS
			No	♀	10	BS	BS	BS	BS	BS	BS	BS
			Yes	♂	10	BS	BS, V	BS, B	BS	BS	BS	BS
			Yes	♀	10	BS	BS, V	BS, B	BS	BS	BS	BS
		Yes	No	♂	10	BS	BS	BS	BS	BS	BS	BS
			No	♀	10	BS	BS	BS	BS	BS	BS	BS
			Yes	♂	10	BS	BS, V	BS, B	BS	BS	BS	BS
			Yes	♀	10	BS	BS, V	BS, B	BS	BS	BS	BS
4	1	No	No	♂	11	BS	BS	BS	BS	BS	BS	BS
			No	♀	5	BS	BS	BS	BS	BS	BS	BS
			Yes	♂	8	BS	BS, V	BS, B	BS	BS	BS	BS
			Yes	♀	9	BS	BS, V	BS, B	BS	BS	BS	BS
		Yes	No	♂	12	BS	BS	BS	BS	BS	BS	BS

			No	♀	8	BS	BS	BS	BS	BS	BS	BS
			Yes	♂	9	BS	BS, V	BS, B	BS	BS	BS	BS
			Yes	♀	9	BS	BS, V	BS, B	BS	BS	BS	BS
	2	No	No	♂	12	BS	BS					
			No	♀	25	BS	BS					
		Yes	No	♂	26	BS	BS					
			No	♀	13	BS	BS					

BS =Blood sample, V = Sensitiser vaccination, B = Booster vaccination

6.3 Results

Raw data by sampling period, farm, deer age, class, sex, treatment group and serological titre to each serovar are presented in Appendix 2.

6.3.1 Seroprevalence and GMT in Hinds

6.3.1.1. Year 1

Mean seroprevalence (±95% CI) and GMT data for each serovar in hinds studied in Year 1 are presented in Figure 6.1. Seroprevalence for Hardjobovis and Pomona on both farms was high before vaccination and remained above 60%. The pattern was similar for both vaccinated and control groups. Seroprevalence for Copenhageni was initially low, increased to >80% in response to sensitiser vaccination, and fell by day 110. Seroprevalence in control groups remained low. There was an increase in GMT 28 days after sensitiser to approximately 1:48 and 1:96 for Hardjobovis and Pomona, respectively. Responses to Copenhageni were lower.

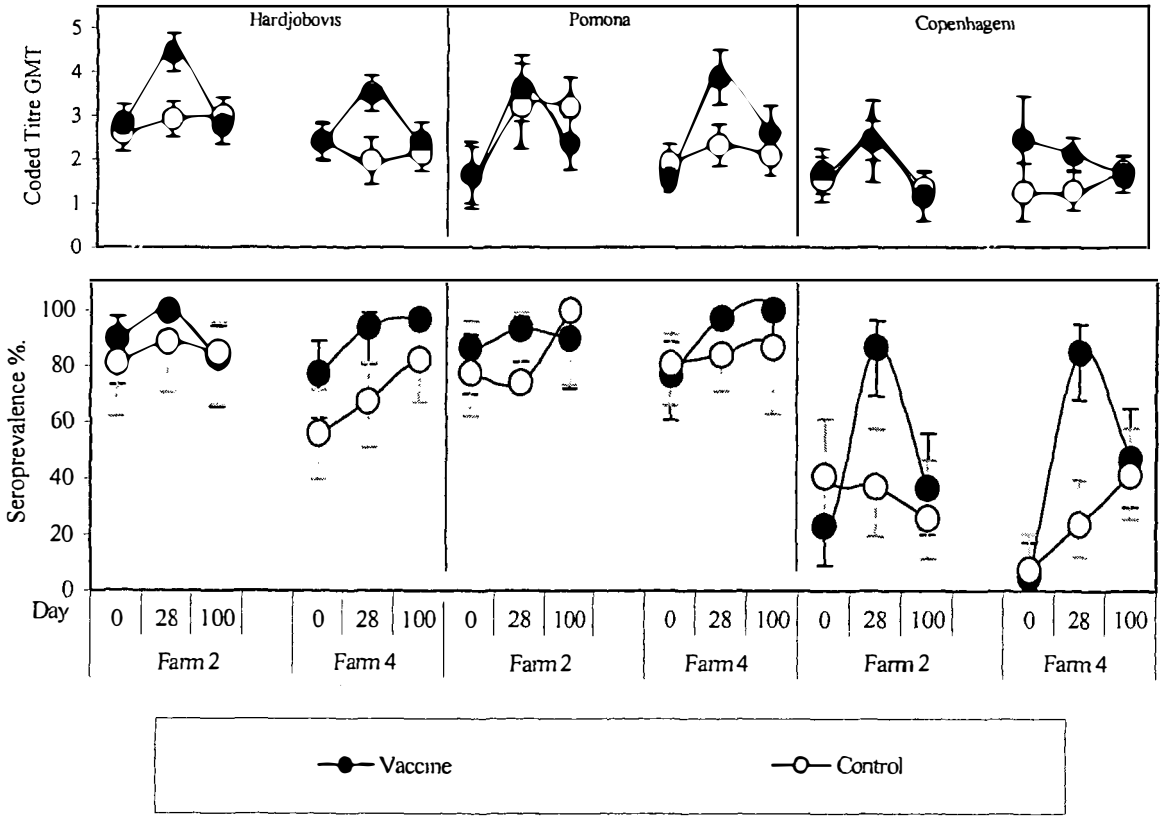


Figure 6.1. Geometric mean of the coded titre (top) and seroprevalence (bottom) (±95% CI) of Hardjobovis, Pomona and Copenhageni in vaccinated and control hinds on days 0 (vaccinated, October), 28 (booster, November) and 100 (February) on Farms 2 and 4 in Year 1.

Results of logistic regression analyses are presented in Table 6.3. There was a difference in seroprevalence between farms for Hardjobovis at titre cut point ≥24. There were differences between vaccinated and control animals at cut point ≥24, and between days at both cut points for serovars Pomona and Copenhageni.

Table 6.3. Summary of regression analyses of seroprevalence in hinds for all serovars at different titre cut points.

Serovar	Titre Cut point	Model χ^2 (p-value)	Effect variable χ^2 (p-value) within the model		
			Farm	Vaccine	Day
Hardjobovis	≥1:24	40.6 (p< 0.001)	21.3 (p< 0.001)	15.5 (p< 0.001)	0.97 (p=0.3)
	≥1:48	30.0 (p< 0.001)	8.5 (p=0.03)	19.3 (p< 0.001)	0.91 (p=0.3)
Pomona	≥1:24	14.2 (p=0.002)	0.0 (p=0.9)	3.7 (p=0.05)	8.7 (p=0.03)
	≥1:96	13.0 (p= 0.004)	0.75 (p=0.3)	3.08 (p=0.07)	9.1 (p=0.002)
Copenhageni	≥1:24	20.8 (p< 0.001)	3.26 (p=0.07)	12.7 (p= 0.003)	4.5 (p=0.03)

6.3.1.2. Year 2

Seroprevalence and GMT data for hinds in Year 2 for Farm 4 are presented in Figure 6.2. Titres to serovar Hardjobovis and Pomona, but not Copenhageni, were detected in October in both previously vaccinated and control hinds. Regression analyses showed no significant differences between vaccinated and control groups or day for Hardjobovis or Pomona, but vaccination produced significantly higher seroprevalence to Copenhageni at each cut point (≥1:24 : χ^2 =10.2, p=0.001; ≥1:48 : χ^2 =14.4, p<0.001, and ≥1:96, χ^2 =6.8, p=0.008). A similar response in GMT was observed to that in Year 1.

In Year 2 on Farm 4, while there were no differences in seroprevalence between hinds vaccinated pre-calving that season alone, there was significantly higher seroprevalence in the previous year in vaccinated animals for Hardjobovis (χ^2=14.7, p<0.001) and Pomona (χ^2=40.0, p<0.001) but not Copenhageni.

Data for seroprevalence and GMT in progeny of vaccinated hinds in Years 1 and 2 are presented in Figures 6.3 and 6.4, respectively.

On Farm 2, seroprevalence to Hardjobovis and Pomona was high in February, 28 days before vaccination and remained high in both vaccinated and control progeny with a temporary fall at Day 190 (Sep). Seroprevalence to Copenhageni was high in February, low in March, rising in April in both groups, followed by a steady decline. The seroprevalence patterns in progeny of control and vaccinated hinds were similar for all serovars. On Farm 2, seroprevalence in progeny of both vaccinated and control

hinds was high for all serovars, but seroprevalence for Copenhageni tended to fall, while Hardjobovis and Pomona remained high.

In February seroprevalence in progeny of vaccinated hinds on Farm 4 was generally higher than in progeny of control hinds for all serovars, but by mid-March, seroprevalence had fallen. Following vaccination on Farm 4, seroprevalence increased until Days 70- 80 or 140 whereas seroprevalence in progeny of non-vaccinated hinds tended to fall.

Regression analysis showed no difference in the overall pattern of seroprevalence for any serovar in progeny from vaccinated and control hinds, with or without vaccination. Individual corrected Yates χ^2 analyses showed there was a significantly higher seroprevalence for serovars Hardjobovis and Pomona in progeny from vaccinated compared with control hinds on Farm 4 in February Year 1 (97.2% vs. 60.4%, χ^2 14.6, $p< 0.0001$) and (95.0% vs. 72.0%, $\chi^2=5.87$, $p<0.01$), respectively (Figure 6.5). Seroprevalence for Copenhageni was significantly lower for the first two sampling periods in the control progeny of Farm 4 (70.0% vs. 45.7% ($\chi^2=24.9$ $p<0.0001$) and 12.1% and 14.1% ($\chi^2=7.2$ ($p<0.007$), respectively, (Figure 6.5).

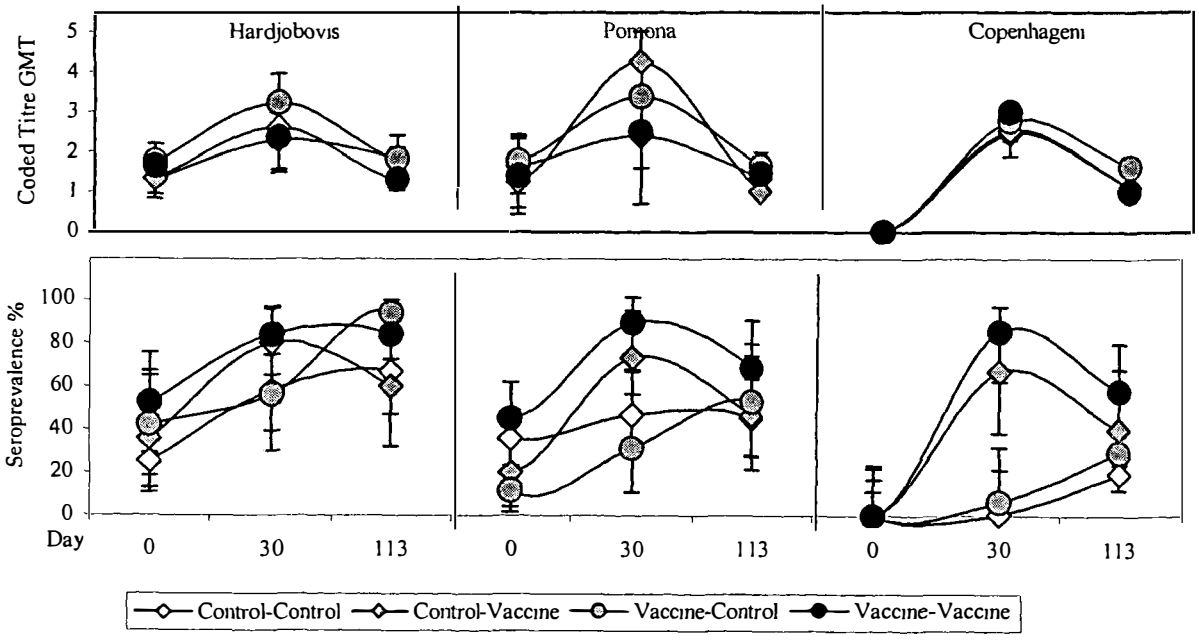


Figure 6.2. Geometric mean of the coded titre (top) and seroprevalence (bottom) (±95% CI) of Hardjobovis, Pomona and Copenhageni in control and vaccinated hinds given a booster on day 0 (October), and sampled day 30 (November) and 113 (February) on Farm 4 Year 2. Note, half of boosted and control hinds had received vaccination in the previous season (Figure 6.1).

Geometric mean titres within each serovar on Farm 2 followed a similar pattern in progeny of vaccinated and control hinds, and were similar in vaccinated and non-vaccinated weaners. GMTs to Pomona were higher than for the other serovars. The

pattern of GMT was similar on Farm 4 for each serovar, regardless of vaccination status of dam or vaccination. The GMT for serovars Pomona and Copenhageni fell to zero toward the end of Year 1. GMTs ranged between 1:24 and 1:96 for Hardjobovis, 0 to 1:96 for Pomona on Farm 4, and up to 1:384 on Farm 2 and 0 to 1:192 for serovar Copenhageni, with an outlier on Farm 4 Day 140 (June).

The GMT for Pomona on Farm 2 was significantly higher than for other serovars on both farms. There was no difference in GMT between vaccinated and control progeny from either vaccinated or control hinds.

On Farm 4 Year 2, only progeny of vaccinated hinds had titres. Seroprevalence of Hardjobovis was 80% in both February and March (GMT 31.1) while seroprevalence to Pomona fell from 47.5% (GMT 24) to 7.5% % (GMT 24), and that for Copenhageni fell from 25% to 17.5% (GMTs 24).

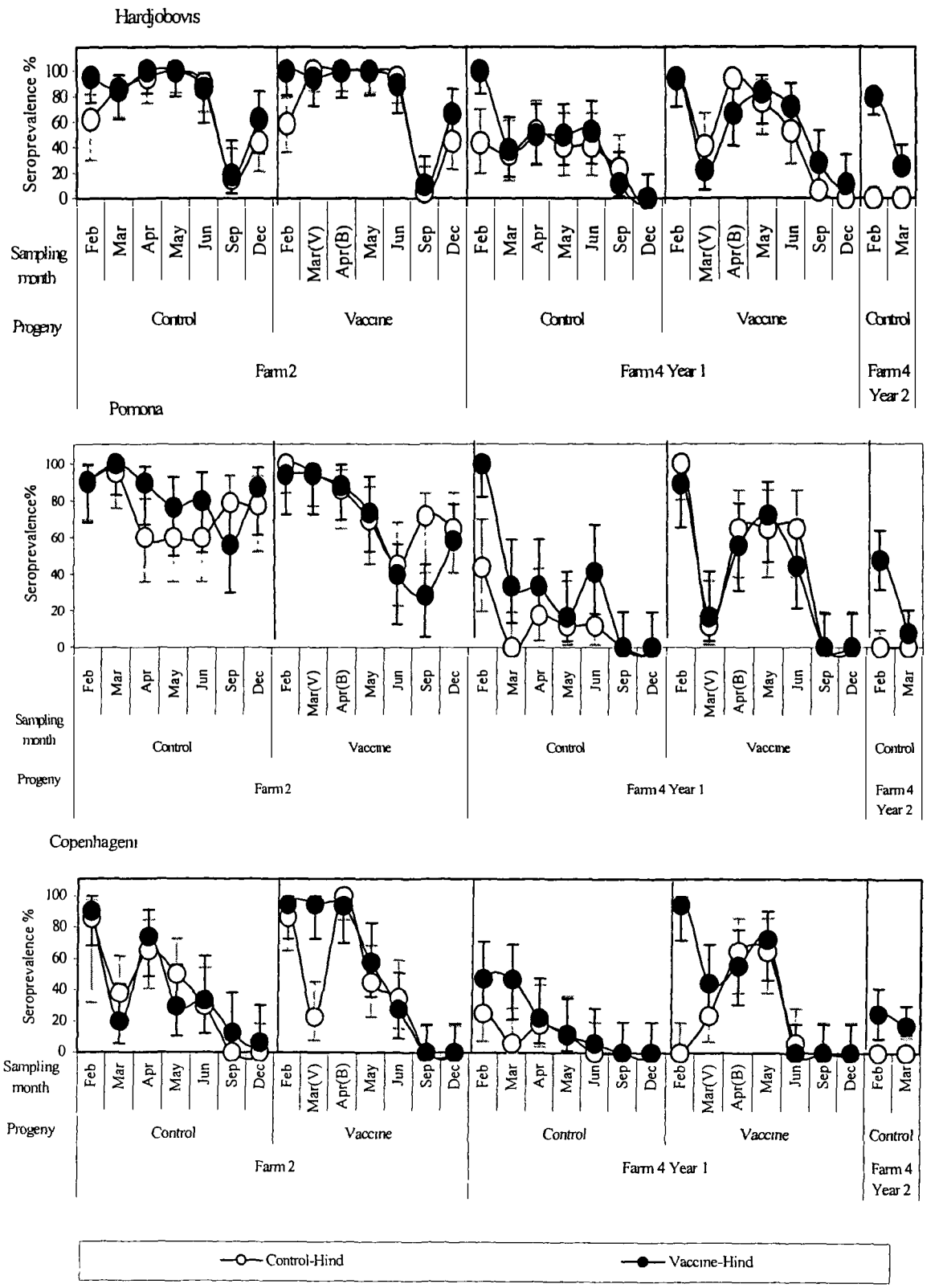


Figure 6.3. Seroprevalence (±95% CI) in vaccinated and control progeny of vaccinated and control hinds from 28 days before vaccination (February) and up to 284 days thereafter. (V= Vaccine in March, B= Booster in April).

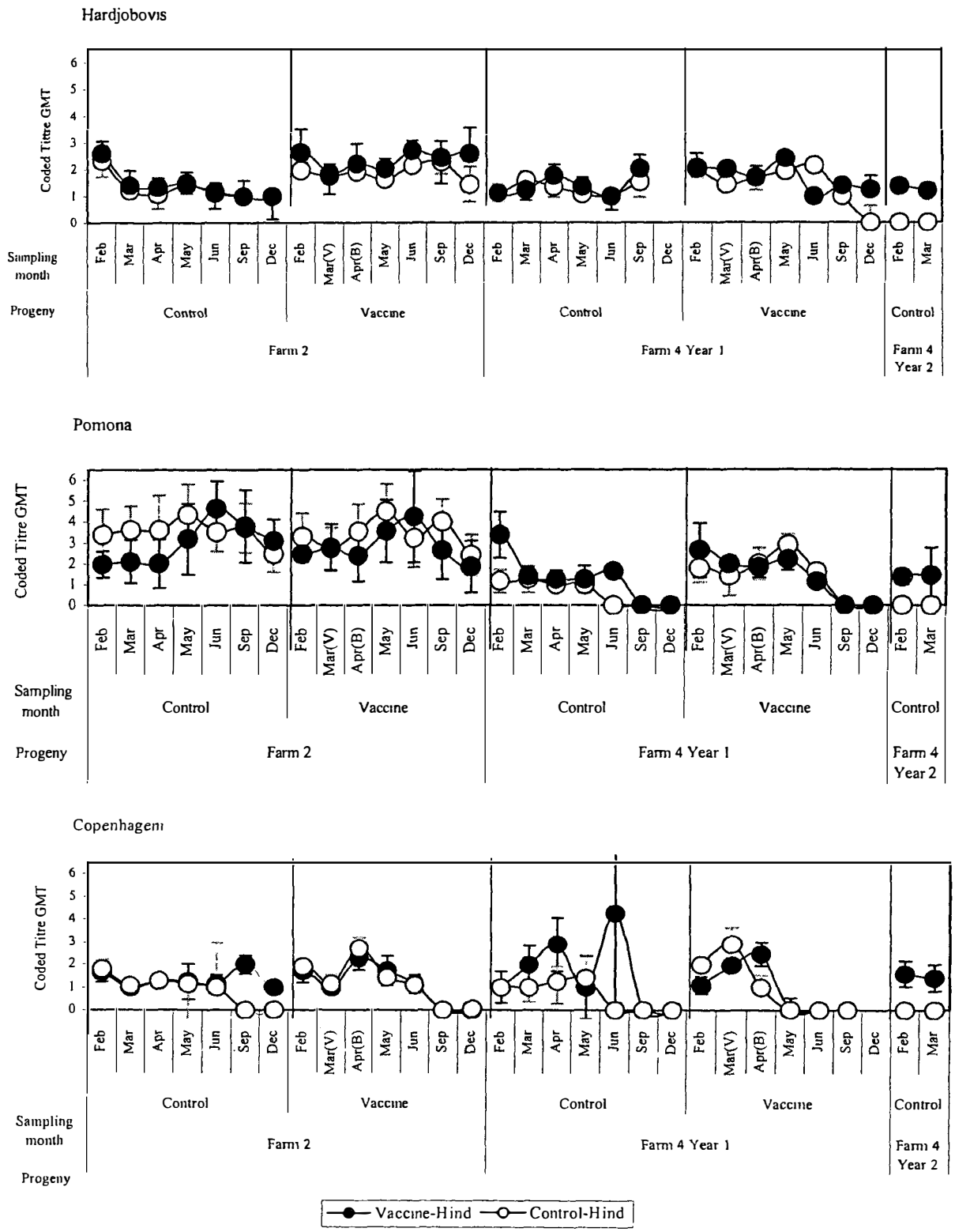


Figure 6.4 Coded geometric mean titre (GMT) (±95% CI) in vaccinated and control progeny of vaccinated and control hinds from 28 days before vaccination (February) and up to 284 days thereafter. (V= Vaccine in March, B= Booster in April).

6.3.3 Relationships between serology of dams and progeny

Seroprevalence and GMT data from dams in Feb, and progeny in February and March were used to investigate relationships between the serology of dams and progeny as presented in Figure 6.5. It should be noted that data in this figure are drawn from relevant points in Figures 6.1 to 6.4.

In hinds, the seroprevalence and GMT for all three serovars were not different between vaccinated and control groups in Year 1, within or between farms, or between vaccinated and control groups Year 2, Farm 4. Seroprevalence to Copenhageni was lower than for Hardjobovis and Pomona.

Table 6.4. Summary of regression analyses (χ^2 and p-value) and Relative Risk (95%CI) for the relationship between the response variable, seroprevalence in progeny at different titre cut points, and the effect variables hind (vaccinated or control), farm, and day in Year 1.

Response variable Serovar (Titre cut point)	Regression χ^2 (p)	Effect variables	Estimated χ^2 (p) in the regression	Relative Risk (95% CI)
Hardjobovis ≥1:24	43.4 (p<0.001)	Hind Farm Day	7.9 (p<0.001) 25.1 (p<0.001) 10.3(p<0.001)	1.6 (1.1-2.2) 2.4 (1.8-3.0)
Hardjobovis ≥1:48	58.8 (p<0.001)	Hind Farm Day	10.4 (p<0.001) 26.7 (p<0.001) 22.6 (p<0.001)	1.6 (1.1-1.9) 2.1 (1.7-2.6)
Hardjobovis ≥1:96	33.9 (p<0.001)	Hind Farm Day	11.6 (p<0.001) 3.2 (p=0.07) 18.9 (p<0.001)	1.6 (1.2-2.0) 1.3 (0.9-1.8)
Pomona ≥1:24	147.5 (p<0.001)	Hind Farm Day	5.9 (p<0.01) 62.4 (p<0.001) 42.0 (p<0.001)	1.6 (1.1-1.7) 5.0 (4.3-5.3)
Pomona ≥1:48	102.0 (p<0.001)	Hind Farm Day	3.8 (p=0.04) 60.3 (p<0.001) 42.0 (p<0.001)	1.3 (1.0-1.7) 3.2 (2.6-3.5)
Pomona ≥1:96	60.1 (p<0.001)	Hind Farm Day	0.34 (p=0.56) 42.2 (p<0.001) 13.8 (p<0.001)	1.0 (0.6-1.9) 2.5 (2.0-2.9)
Copenhageni ≥1:24	79.6 (p<0.001)	Hind Farm Day	16.8 (p<0.001) 19.7 (p<0.001) 42.8 (p<0.001)	1.8 (1.4-2.3) 1.9 (1.4-2.2)
Copenhageni ≥1:48	94.0 (p<0.001)	Hind Farm Day	5.6 (p=0.01) 16.0 (p<0.001) 46.1(p<0.001)	1.5 (1.0-2.9) 2.0 (1.5-2.5)

In progeny on Farm 2, the seroprevalence to Hardjobovis and Pomona was high in February and remained high to March. On Farm 4, Years 1 and 2, seroprevalence to all three serovars in progeny fell from February to March, as did seroprevalence to Copenhageni on Farm 2. Seroprevalence to Hardjobovis and Pomona in progeny of vaccinated hinds in Year 1 was similar to that of both vaccinated and control dams, while seroprevalence to Copenhageni was higher in progeny than dams in February, particularly from vaccinated dams. A similar pattern was observed on Farm 4, Year 2,

for Pomona and Hardjobovis but seroprevalence to Copenhageni was lower. No titres to any serovar were recorded from the offspring of non-vaccinated hinds.

Data for regression analysis of seroprevalence in progeny at different titre cut points performed on data for Year 1 are presented in Table 6.4. The seroprevalence to all three serovars at all titre cut points tested, except Pomona at ≥96, in progeny of vaccinated hinds were significantly higher than in progeny of control hinds, with a RR for seropositivity in progeny related to seropositivity in the dam ranging from 1.0 to 1.8. Seroprevalence was significantly higher in progeny on Farm 2 for all serovars at all cut points except Hardjobovis at ≥96. The RR showed that progeny on the farm with infection would be 1.3 to 5.0 times as likely to be seropositive than progeny on the non-infected farm. Relative risk were particularly high for Pomona. Seroprevalence in progeny was lower in March for all serovars.

Regression analyses of data for Farm 4 Year 2, (Table 6.5) demonstrated a significant difference in seroprevalence of progeny of hinds vaccinated pre-calving for all serovars at all titre cut points analysed, with RR indicating that progeny of vaccinated hinds were 2.7 to 5.4 times as likely to be seropositive than progeny of non-vaccinated hinds.

Table 6.5. Summary of regression analyses (χ^2 and p-value) and Relative risk (95%CI) for the relationship between the response variable seroprevalence to serovars Hardjobovis, Pomona and Copenhageni at different titre cut points, and the effect variables hind (vaccinated or control) and day on Farm 4 Year 2.

Response variable Serovar (Titre cut point)	Regression χ^2 (p)	Effect variables	Estimated χ^2 (p) in the regression	Relative risk (95% CI)
Hardjobovis ≥1:24	87.3 (p<0.001)	Hind Day	58.6 (p<0.001) 19.6(p<0.001)	5.4 (3.8-7.2)
Hardjobovis ≥1:48	53.1 (p<0.001)	Hind Day	20.0 (p<0.001) 28.4 (p<0.001)	2.5 (1.7-3.2)
Hardjobovis ≥1:96	52.6 (p<0.001)	Hind Day	15.4 (p<0.001) 24.7 (p<0.001)	2.7 (1.7-3.6)
Pomona ≥1.24	165.0 (p<0.001)	Hind Day	26.5 (p<0.01) 54.7(p<0.001)	3.3 (2.4-3.9)
Pomona ≥1:48	67.9 (p<0.001)	Hind Day	18.2 (p<0.001) 42.0 (p<0.001)	2.7 (1.8-3.4)
Pomona ≥1:96	43.3 (p<0.001)	Hind Day	13.4 (p<0.001) 18.0 (p<0.001)	2.8 (1.7-3.7)
Copenhageni ≥1:24	46.7 (p<0.001)	Hind	33.5 (p<0.001)	3.5 (2.6-4.3)

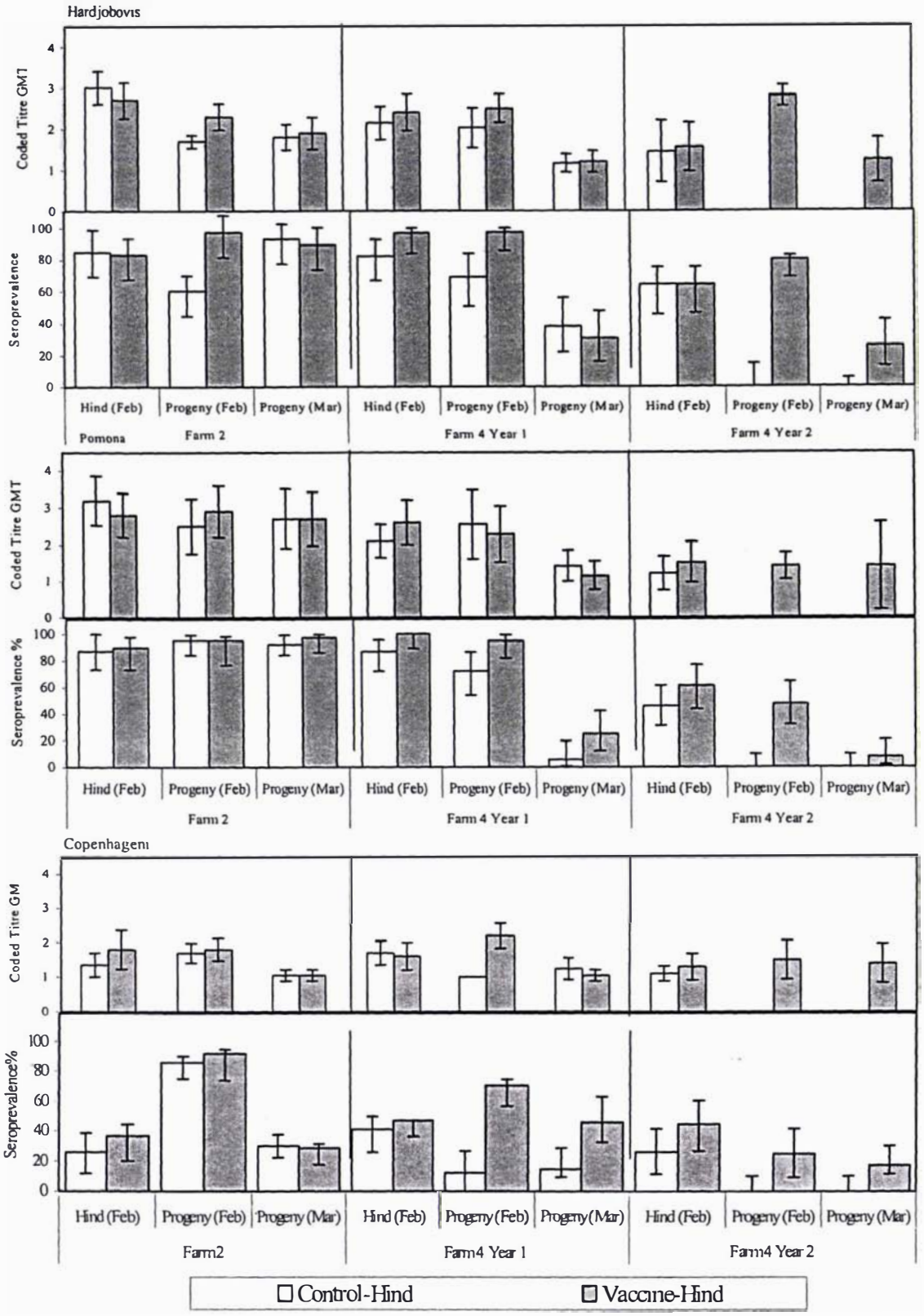


Figure 6.5 Geometric mean of the coded titre (above) and seroprevalence (below) (±95% CI) of vaccinated and control hinds in March and progeny in February and March.

6.4 Discussion

This study showed a classic humoral response to leptospiral vaccination in pregnant adult hinds and progeny, and transfer of naturally and/or vaccine-acquired antibody via colostrum similar to that reported for other mammals (Tizard 1995). It also showed no detectable maternally derived antibody interference with vaccination of progeny at about 12-16 weeks of age. Similar responses were observed to vaccination in progeny of vaccinated and non-vaccinated hinds with and without natural infection and vaccination produced antibody responses similar to those following natural challenge.

Both infected and non-infected herds were chosen for this study to allow observation of serological responses to vaccine in deer with or without exposure to natural challenge with Hardjobovis and Pomona. Most deer herds in New Zealand have evidence of leptospiral infection but about 18% do not (Wilson et al 1998; Ayanegui-Alcérreca et al 2006a; and Chapter 7). In the commercial farming environment, it is likely that non-infected herds may be exposed to infection through introduction of deer from other properties (Dean et al 2005), or from other livestock species increasingly being farmed in conjunction with deer (Mackintosh and Wilson 2005; and Chapter 7). Further, it is likely that introduction of infection to an immunologically naïve herd could result in serious disease, particularly with Pomona, because there would be no herd immunity. Thus, should the deer industry or individual farmers implement vaccination as a strategy to prevent leptospirosis infection in deer and/or risk to humans, vaccination would apply equally to infected and non-infected herds.

This study was designed to test maternal response to vaccination, antibody transfer to progeny and possible interference with vaccination. Vaccination at 12 weeks of age was chosen based on previous data suggesting that deer can be exposed to infection early in life (Chapters 3 and 4), data from other studies and species, and as a pragmatic time in relation to normal farming practice. It is uncommon for deer farmers to handle young deer before 12 weeks of age because of risk of injury to young deer, and vaccination programmes are more likely to be adopted if they fit with accepted management practices. Mackintosh et al (2001) showed interference with a vaccine against *Yersinia pseudotuberculosis* in deer vaccinated earlier than, but not at or after that age. Palit et al (1991) reported resistance to challenge with Hardjobovis in progeny of vaccinated cows vaccinated from 4-18 months of age with the highest humoral response in those vaccinated at 18-weeks. Gillespie and Kenzy (1958) demonstrated an effective vaccine response in calves vaccinated at 6-8 months of age in which 8% became infected after challenge with Pomona, compared with 76% of those vaccinated at 1-2 months of age. However, Schollum and Marshall (1985) reported poor serological responses in 3-month-old bovine calves with maternal antibody compared with those vaccinated at 6 months of age.

Newborn ungulates acquire immunoglobulins via colostrum ensuring relatively constant concentrations for the first 3-6 weeks of life (Salmon 1999; Bolt 1990). Approximately 80% of the immunoglobulin in colostrum is IgG1 with an estimated half-life of 16-days in bovine calves resulting in more than 97% being catabolised at 80 days (Hellstrom 1978). Colostral antibody associated with vaccination against leptospires, Brucella abortus and Rinderpest in cattle (Hellstrom 1978) persisted up to

6 months. Colostrum-derived antibodies to clostridium toxins persisted up to ten months in cattle (Tizard 1995), and leptospira Pomona antibodies persisted up to 20 weeks in pigs (Bolt 1990). The rapid reduction in seroprevalence of all three serovars from February to March in progeny on Farm 4 is consistent with the expectation when there is no natural challenge, and is similar to the pattern described for maternally derived antibody to *Yersinia pseudotuberculosis* reported by Mackintosh et al (2001). A similar pattern was observed for Copenhageni on Farm 2, infected with Hardjobovis and Pomona. This study showed persistence of maternally derived antibody for 6-9 months depending on serovar in the non-infected herd. Antibody to Hardjobovis and Pomona did not wane on the infected farm where there was no difference between vaccinated and control progeny, suggesting persistent natural challenge. McDonald and Rudges (1957) and Hellstrom (1978) reported the persistence of maternal antibodies in calves for 100-140 days.

For Farm 4, antibody was presumed to be from the dam. The apparent increase in seroprevalence in April was likely due to the sensitivity of the MAT since all titres were included. However, those differences were not significant and the GMT remained relatively constant over that period.

While maternally derived antibody should protect the progeny, a widely acknowledged consideration in production animal systems is the direct interaction between those antibodies and antigens in a vaccine. Timing of the initiation of a vaccination programme in relation to decay of maternally derived antibody is critical in cattle (McDonald and Rudge 1957; Schollum and Marshall 1985; Palit et al 1991). Most vaccine programs begin at 2-4 months of age (Tizard 1995; Barrington and Parish 2001). Evidence of interference of maternally derived antibodies with vaccination at 8 to 12 weeks of age has been recognised in dogs and cats infected with canine Parvovirus, canine adenovirus and feline paleukopenia (Pollock and Carmichael 1982; Carmichael et al 1983; Scott 1970). Timing of vaccination must balance risk of exposure against risk of colostral antibody interference. Bolt (1990) suggested that the first vaccination should be given between 8 to 10 weeks of age in pig herds infected with *Leptospira* Pomona, which is before exposure to infection generally at 12 to 14 weeks of age. There appeared to be no interference by colostral antibody with antibody production following vaccination of deer in this study in March at about 12 weeks of age. Responses to vaccination of progeny in the infected herd were of similar magnitude and duration to those in the non-infected herd.

Colostral antibody originates from naturally and/or vaccine stimulated immunity. Pre-calving vaccination produced enhanced passive immunity in progeny in this study, probably as a result of a combination of natural and vaccine stimulated antibody in the hind. It probably also involved active immunity in progeny exposed to infection at an early age, since seroprevalence did not decrease from February to March in deer on the infected farm, but did in the non-infected herd.

The seroprevalence and GMT in progeny in this study at about 8 and 12 weeks of age were similar to those in dams at 8 weeks. Seroprevalence and GMT for leptospiral colostral titres in young bovine calves and pigs are generally equivalent to or greater than those of their dams in the first few weeks of life (McDonald and Rudges 1957; Hellstrom 1978; Bolt and Marshall 1995).

Evidence of protection by maternally derived immunity against neonatal leptospiral infection has been reported by McDonald and Rudge (1957) in 1-2 month old bovine calves from vaccinated dams, challenged with Pomona. Colostral antibodies are believed to be protective up to 100 days in cattle with resistance to natural or experimental infection in calves (Hanson et al 1972). The observation of a higher weaning rate on Farm 2 (see Chapter 5) suggests that colostral immunity may have been protective in newborn and young deer on that farm. Results from Chapter 3 suggest infection occurs at this age in some herds.

Vaccination elevated seroprevalence to Hardjobovis and Pomona in a similar manner in adult hinds in both infected and non-infected herds. It is possible that the response in the non-infected herd was due to prior vaccination several years earlier, although the same response was observed in sero-negative 8-month-old deer as reported in Chapter 5. Seroprevalence and titres of similar duration and magnitude to those observed in the present study have been reported in vaccinated cattle exposed to natural challenge with serovar Hardjobovis (Mackintosh et al 1980d; Allen et al 1982). Seroconversion to Copenhageni in vaccinated animals was similar to a primo-humoral response, with a high antibody rise of short duration (Tizard 1995), because there was no apparent prior natural or vaccine challenge with that serovar. Titres to Copenhageni in the non-vaccinated hinds were probably due to cross-reaction with Pomona from natural infection (Farm 2) or prior vaccination (Farm 4) as discussed in Chapters 3 and 8.

This study showed a response in pregnant adult hinds to both the sensitiser and booster doses with titres persisting for at least a 100 days in more than 40% and in 20-40% of animals after one year without natural challenge. Of the twenty control-control hinds on that farm (Year 2) that had not been vaccinated for at least four years, 20 to 40% remained seropositive, demonstrating that titres may persist for some time following vaccination. In the only other longitudinal study of vaccination responses after vaccination with a similar leptospiral vaccine (Wilson and Schollum 1984), titres persisted for at least 12 months in some deer. In adult cattle detectable antibodies were present up to 5 years after cessation of a 5-year vaccination programme in a herd initially exposed to a natural challenge with serovar Hardjo (Little et al 1992a).

There was a higher seroprevalence in progeny of vaccinated hinds in both infected and non-infected herds in Year 1 when hinds received a two-dosage vaccine schedule, than in progeny of vaccinated hinds from farm 4 in year 2, receiving only one booster. The lower seroprevalence and GMT in progeny of non-vaccinated hinds on Farm 4 in Year 2 compared with Year 1 may demonstrate the importance of immunological memory in contributing to maternally derived antibody in progeny (Amadtredjo et al 1975) by annual vaccination of previously sensitised and boosted hinds.

6.5 Conclusions

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

provide a degree of immunity to these specific strains of leptospirosis. The vaccine produced similar seroprevalence and GMT patterns to those resulting from natural infection in hinds and progeny and a sustained antibody response in progeny. Maternally derived antibody did not interfere with response to vaccination of progeny at about 12 weeks of age in infected or non-infected herds. These data suggest that optimum immunity from vaccination would require annual booster vaccination of previously immunised hinds, followed by vaccination of progeny at about 12 weeks of age.

Chapter 7

Regional seroprevalence of Leptospirosis on deer farms in New Zealand.



7.1 Introduction

Most publications on leptospirosis in farmed deer in New Zealand describe clinical outbreaks (Ayanegui-Alcérreca et al 2006 b, this thesis), while some describe serological surveys. Williams (1985) reported "positive reactions" to Hardjobovis and Copenhageni in four of 10 herds and "weak reactors" on 9 of 10 properties in the Nelson region. Flint et al (1988a) surveyed nine South Island and an unspecified number of North Island herds, with an overall seroprevalence of 28.6% for Hardjobovis, 2.7% for Pomona and 16.6% for Copenhageni. Seroprevalence in 53 herds in the lower North Island was 73.6% for Hardjobovis, 41.5% for Pomona and 11.3% for Copenhageni (Wilson et al 1998). Reactivity to serovars Australis, Ballum, Bratislava and Tarassovi were reported by the latter authors. Reichel (1999) tested either one or two samples from 417 farms throughout New Zealand claiming an overall seroprevalence of 9.8 for Hardjobovis, 1.4% for Pomona and 0.2 for Copenhageni. While these reports generally suggest that infection was widespread, they fail to provide definitive data of national or all-region prevalence of infection.

New Zealand deer industry concern about leptospirosis prompted research into the epidemiology of infection on deer farms (Chapters 3 and 4) and evaluation of the efficacy of a vaccine and a vaccination regime (Chapters 5 and 6). Knowledge of the national and regional distribution of leptospiral infections is essential to underpin strategies for control at farm, regional and national levels. The present regional seroprevalence survey was performed to establish the distribution of infection by serovars Hardjobovis, Pomona and Copenhageni in farmed deer herds regionally throughout New Zealand. Results from 2016 samples from red and red x wapiti deer from 110 farms are reported.

7.2 Materials and methods

7.2.1 Study design

The optimum number of herds required was established using the formula $n = Z^2\alpha (pq)/L^2$ for estimating proportions, with the desired precision assuming an estimated 50% herd prevalence for Hardjobovis and 10% for Pomona based on published herd prevalence data (Wilson et al 1998) combined with data from herd screening to identify suitable herds for studies presented in Chapter 3 and 4. Assumptions used for this determination are presented in Table 7.1.

A prevalence of 10% was chosen as indicative of herd infection. The number samples required from each herd was calculated on FreeCalc (Modified binomial approximation) based on diagnostic test sensitivity and specificity according to Faine et al (1999) and OIE (2000), taking population and average herd size into account (Official Agricultural Production Census, Statistics New Zealand 2002), to provide 95% confidence and 80% power. Assumptions used for this determination are presented in Table 7.1.

Based on the number of herds reported per-region in the Official Agricultural Production Census (Statistics New Zealand 2002), regions were grouped in blocks as in Table 7.2, and the number of herds required per region were estimated by number of herds per block/total Number of Herds* Number of herds required. A herd was considered positive if there were at least 3 seroreactors with a titre of ≥1:24 for Hardjobovis and ≥1:48 for Pomona and Copenhageni. Different titre cut points were chosen for the latter two serovars to increase the herd-based specificity. A higher number of seroreactors within each herd was also chosen to indicate infection.

Table 7.1 Summary of assumptions, and procedures, for estimating the number of herds and the number of samples per herd required to estimate herd seroprevalence for serovars Hardjobovis and Pomona using the formula n= $Z^2\alpha$ (pq)/L^2 (see Table) for estimating proportions.

Parameter	Hardjobovis ≥ 1:24	Pomona ≥ 1:48
Estimated herd prevalence (p)	50%	10 %
Allowable error (L^2)	10%	5%
Calculation	$((1.96)^2$* 0.50* (1-0.50)/$(0.1)^2$)	$((1.96)^2$* 0.10* (1-0.10)/$(0.05)^2$)
Number of herds (rounded)	**97**	**134**
Average herd size	350	350
Sensitivity	80%	95%
Specificity	95%	95%
Within herd prevalence	30%	80%
Number of samples per herd	**16**	**3**

Table 7.2. Number of herds calculated and actually sampled, by region, and sample source (Farm or DSP).

Region	Number of herds		
	Calculated	Sampled	Source
North Island Northland/Auckland	5	7	Farm
Waikato	12	3	Farm
Bay of Plenty/Gisborne	12	10	Farm
Hawkes Bay	10	7	5 Farm, 2 DSP
Taranaki/Manawatu/Wanganui/Wellington	19	15	7 Farm, 8 DSP
WestCoast/Nelson/Malborough	9	20	5 Farm, 15 DSP
Canterbury	31	19	16 Farm, 3 DSP
Otago	14	15	Farm
Southland	22	15	Farm
Total	**134**	**110**	

7.2.2 Farms, blood samples and serology

Samples from 80 herds were collected on selected farms based on the presence of suitable animals and facilities, and the owner's willingness to participate. Candidate farmers were recruited by co-operating veterinary practitioners in each area, with some farmers in the Manawatu and Hawkes Bay being recruited by the researcher. Samples were collected and handled as described in Chapter 2, with those collected by veterinary practitioners being couriered fresh to Massey University for processing. In addition, samples from 30 herds were collected from Deer Slaughter Premises (DSP) at Hokitika, Feilding and Hastings as described in Chapter 2. Data from DSP samples were also used for the analysis of risk of leptospiral shedding described in Chapter 8. Animals in this study were from farms with no history of vaccination of deer against Leptospira. Red and red x wapiti deer were sampled. Sampling took place between March 2003 and February 2005, with the majority collected in the latter part of 2004.

Twelve to twenty deer were randomly selected from each herd for blood sampling. Most deer sampled on-farm were hinds 9- to 30-months of age while most sampled in DSPs were males in the same age range. Serology for Hardjobovis, Pomona and Copenhageni was undertaken using the method described in Chapter 2.

7.2.3 Questionnaire

A questionnaire was constructed (Appendix 3b) that included demographic, farm, management and animal information relevant to the epidemiology of leptospirosis. Veterinary practitioners were asked to complete questionnaires on the day that the samples were collected on-farm, and 73 of 80 were received. For herds sampled at slaughter, DSP managers contacted the owners and the seven who agreed to participate were contacted by telephone for questionnaire completion.

7.2. 4 Statistical analyses

Descriptive data were expressed as prevalence (proportions) with a 95% confidence interval (95%CI). There were no differences in data from samples collected at the slaughterhouses and farms thus data from both sources were equivalent. The McNemar χ^2 test was used to test agreement between seroprevalence of Hardjobovis and Pomona. Logistic regression models based on χ^2, giving Odds Ratios for the associations between serology and likelihood ratios for the questionnaire variable and geographical regions were analysed using NCSS 2000 and PASS 2000 (Jerry Hintze ©2001).

7.3 Results

Raw data by farm, age, sex, titre and region are presented in Appendix 3. Data from 2016 samples were collected from 110 herds as described in Table 7.2.

7.3.1 Seroprevalence

Data is presented in Figure 7.1. A total of 1226 (60.8%, 58.6-63.0 CI) individual samples and 90 herds (81.8%, 73.3-88.5 CI) were positive for Leptospira. For Hardjobovis alone, individual animal and herd seroprevalence was 54.2% (52.0-56.4 CI) and 61.3% (51.5-70.5), respectively. For Pomona alone, individual animal and herd seroprevalence was 1.8% (1.3-2.5 CI) and 3.6% (0.9-9.1 CI), respectively. Dual seroreactivity to both Hardjobovis and Pomona was 6.6% (5.6-7.9) and 16.4% (9.9-24.8 CI) at the individual animal and herd levels, respectively. Thus overall, 60.8% (58.6-62.9 CI) of individuals and 77.7% (69.3-85.5 CI) of herds were seropositive for Hardjobovis, and 8.4% (7.3-9.7 CI) of individuals and 20% (11.5-26.7 CI) of herds were seropositive to Pomona. For Copenhageni 1.2% (0.8-1.8 CI) of individual animals were seropositive but no herds were seropositive, using the criteria described above.

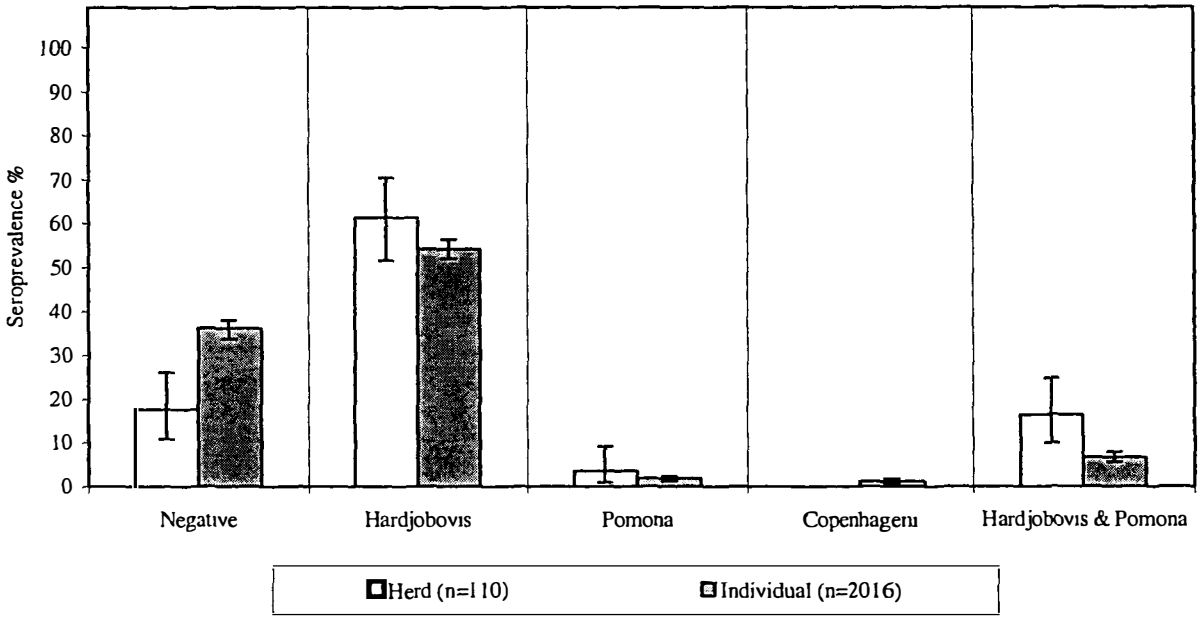


Figure 7.1. Herd and overall individual animal level seroprevalence (± 95% CI) of Hardjobovis and Pomona alone or in combination and individual animal seroprevalence for Copenhageni.

The distribution of within-herd seroprevalence for Hardjobovis and Pomona, individual and dual infections combined, is presented in Figure 7.2. For Hardjobovis, seroprevalence ranged from 0-100% with a mean within-herd seroprevalence in positive herds of 68.1%. For Pomona, seroprevalence ranged form 0 to 100% with a mean within-herd seroprevalence in positive herds of 39.8%. The distribution pattern in dual infected herds shows varying combinations of high and low seroprevalence of each serovar, demonstrating that infection by each serovar was independent. The herd prevalence of each serovar by region is presented in Figure 7.3 and the location of farms surveyed and the herd status is presented in Figure 7.4. Hardjobovis was detected in herds from all regions while Pomona was detected in all but two regions. Seroprevalence within region for Hardjobovis alone ranged from 16.6 to 100%, for Hardjobovis and Pomona combined from 0 to 50%, and for Pomona alone from 0 to 13%.

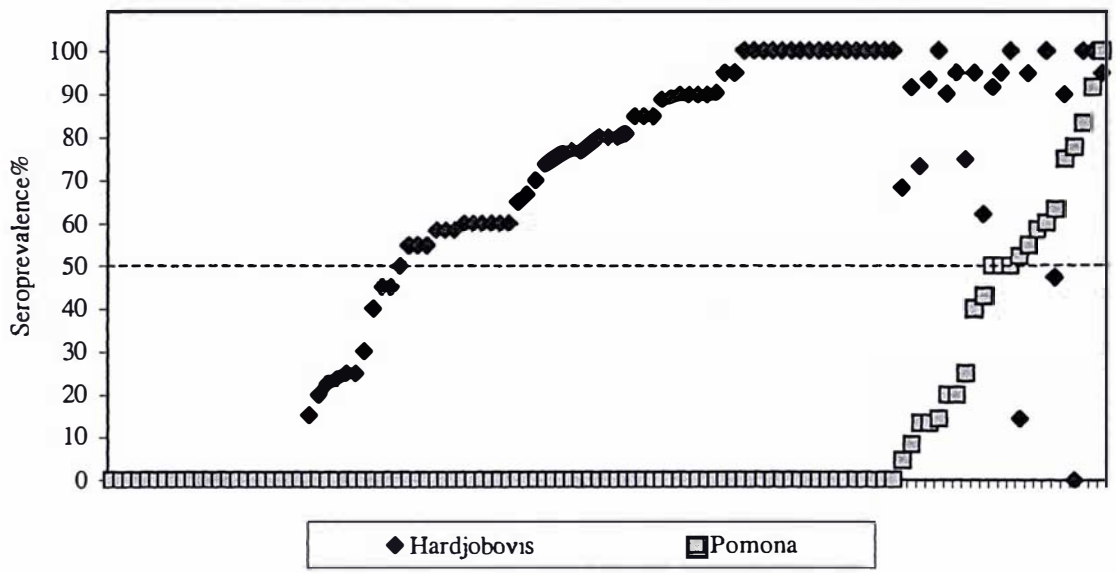


Figure 7.2. Distribution of within-herd seroprevalence for Serovars Hardjobovis and Pomona alone or in combination, ranked by seroprevalence to Pomona.

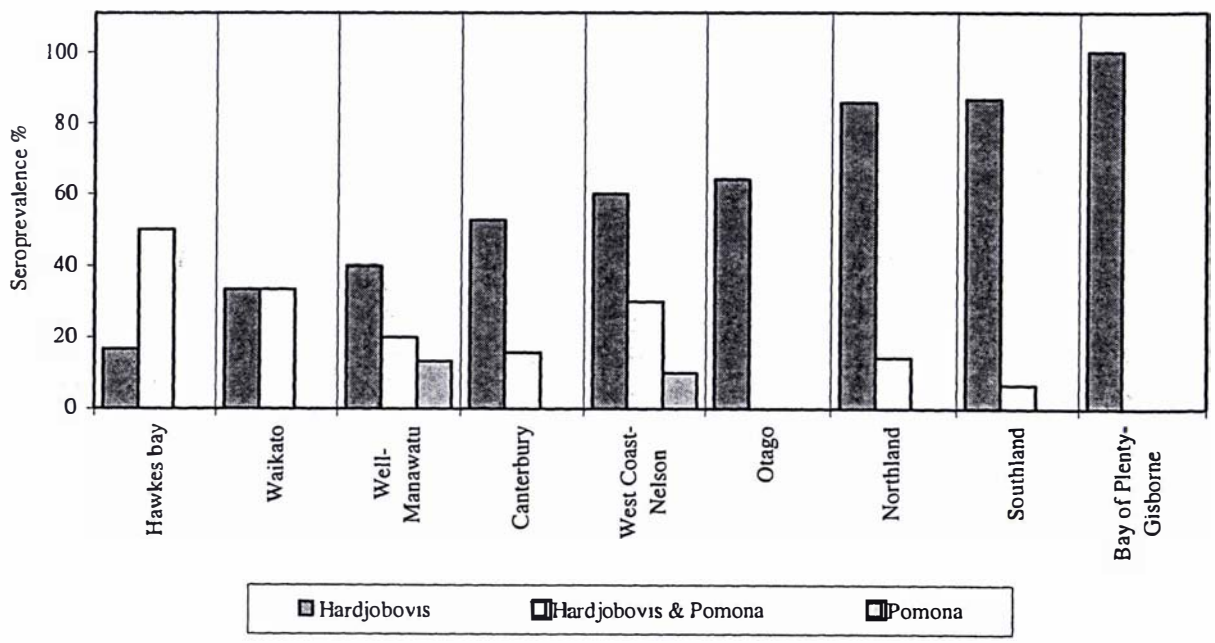


Figure 7.3. Herd seroprevalence for Hardjobovis and Pomona individually or combined in each region ranked by Hardjobovis.

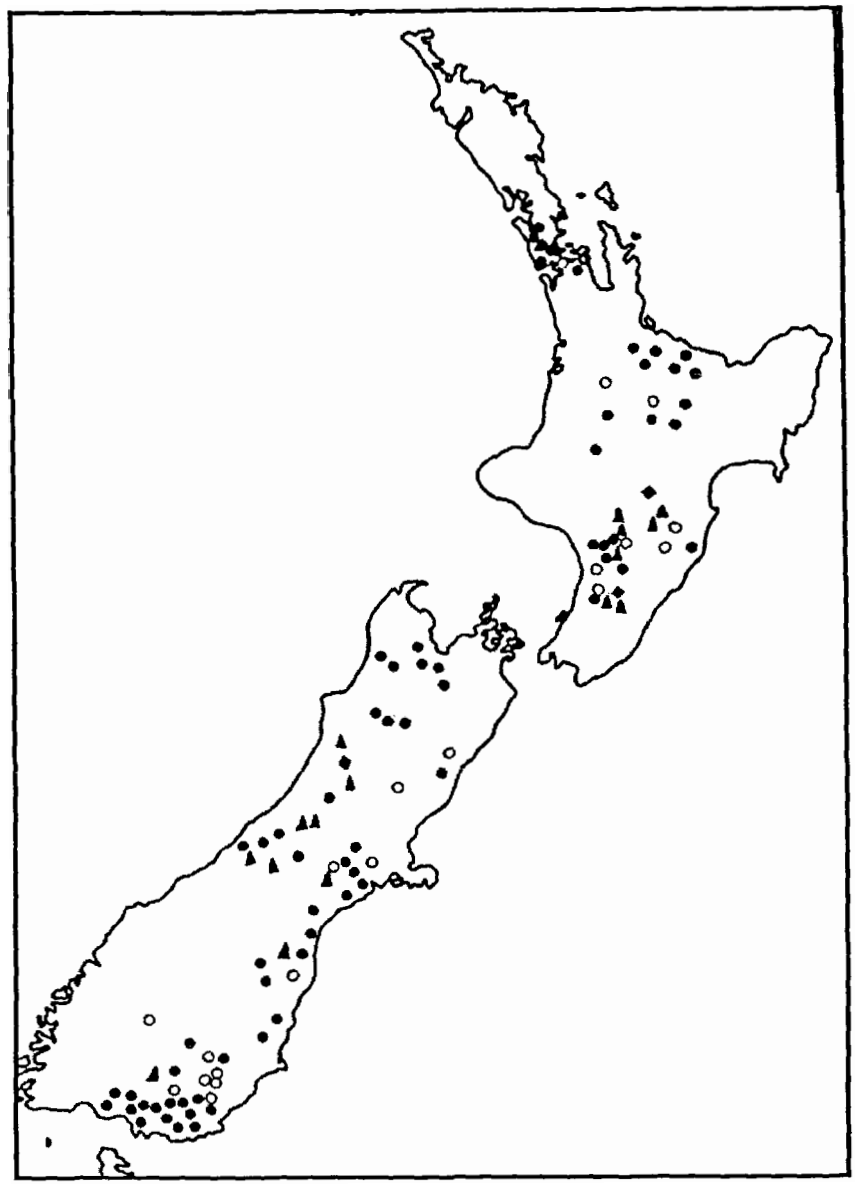


Figure 7.4. New Zealand regional survey: approximate distribution of leptospirosis in farmed deer, Serovar Hardjobovis 62.0% (●), Pomona 3.7% (♦), Hardjobovis and Pomona 17.6% (▲), Negative farms 16.7%.

Regression analysis of the prevalence of herds seropositive overall and to Hardjobovis and Pomona alone showed no difference between regions. There was a regional difference (χ^2 5.09, p=0.02) in the prevalence of herds with dual Hardjobovis and Pomona infection. There was no association between seroprevalence for Hardjobovis and Pomona in dual infected herds.

7.3.2 Questionnaire

Herds in this survey ranged from 62 to 5800 deer, farmed either as deer alone (21.25%) or in conjunction with other species, predominantly beef and sheep, with some dairy cattle, goats and pigs. Nine of 80 farmers reported an outbreak or clinical

symptoms of leptospirosis over the previous five years, with three diagnosed at the time as being Hardjobovis and Pomona combined, one Pomona alone and 5 Hardjobovis alone. In relation to previous diagnoses in herds, the serological evidence from this survey showed one negative herd that had been positive to Hardjobovis in the past, three herds that had remained Hardjobovis positive, four that were reported as only Hardjobovis in the past yet were positive to both Hardjobovis and Pomona in the present survey, and one that was reported positive to both serovars previously, but only Hardjobovis in the present survey. One farm from this survey reported abortion, but the serovar was not confirmed at the time although this farm was Hardjobovis positive in this survey. Farmers reported symptoms in all age groups.

7.4 Discussion

This study has shown that leptospiral infection on deer farms is widespread throughout New Zealand with 82% of herds infected. Hardjobovis was the predominant serovar at the herd and individual level, but Pomona was present in 20% of herds. No evidence was found for herd infection with Copenhageni.

Serological surveys are the conventional method for determining presence or absence of infection, and prevalence at individual animal, herd and population levels. Most studies of leptospirosis in animal populations employ serology because it is highly specific at the serovar level across host species, and is considered to constitute evidence of infection if performed in non-vaccinated animals and interpreted at appropriate titre cut points (Faine et al 1992, 1999).

The number of herds sampled, despite being below the number estimated provided sufficient power for robust conclusions because seroprevalence for Hardjobovis and Pomona was higher than that used in calculations. Considerable difficulty was experienced in recruiting farms in some areas, particularly Waikato. It was necessary to adopt a convenience rather than the preferred random herd selection methodology because of legal difficulties in randomly identifying herds through the Deer Industry New Zealand list because of the Privacy Act and logistical difficulties in contacting and recruiting participants. Animals of both sexes were considered acceptable because previous analyses (Chapters 3 and 4) showed no sex differences within age categories.

Animals 9–30-months of age were chosen because results from Chapters 3 and 4 indicated that this age group had the highest seroprevalence in infected herds. This optimised the sensitivity of the serology result at the herd level, which was the primary unit of interest for the present study. Bahaman et al (1984b) and Little et al (1992a,b) reported the highest seropervalence in 1- and 2-year-old cattle in dairy herds.

Copenhageni has been reported on deer farms in New Zealand (Flint et al 1988a; Wilson et al 1998), but at low herd and animal seroprevalence suggesting sporadic occurrence and there is evidence of limited clinical significance. To estimate the infection rate with this serovar would require large numbers of herds and animals. The sample size and number of herds required for this survey were therefore calculated based on Hardjobovis and Pomona which were the most prevalent serovars reported in previous studies in farmed deer in New Zealand (Ayanegui-Alcérreca et al 2006a)

and in other studies presented in this thesis. However, serology included Copenhageni. While titres to serovars Australis, Ballum, Bratislava and Tarassovi have been reported in deer in New Zealand (Flint et al 1988a; Wilson et al 1998), these serovars were not included in the present survey because of the lack of culture evidence linking these serovars to infection in deer. However, the significance of titres to those serovars in deer needs to be established.

For this study, cut points of 1:24 and 1:48 were used for Hardjobovis and Pomona, respectively, to provide the optimum sensitivity and specificity of determination of infection at the herd level, particularly with Hardjobovis, based on considerations discussed by Faine et al (1999) and Bolin and Prescott (1999). Other authors reporting serological surveys in deer either use different titre cut points and questionable study design (Reichel 1999), lack sufficient detail to permit conclusions (Williams 1985, Flint et al 1988a), or were from selected regions (Wilson et al 1998) making some direct comparisons difficult and preventing conclusions about regional and national seroprevalence. However, seroprevalence data presented here is broadly consistent with other valid publications in deer and cattle.

The overall seroprevalence for Hardjobovis was 77.7% and 60.8% at the herd and animal levels, respectively, using a titre cut point of 1:24. Flint et al (1988a) reported individual animal seroprevalence of 29% using a titre cut point of 1:20. That study gave only a partial description of the origin of data, preventing calculation of herd prevalence. Wilson et al (1998) reported a herd seroprevalence of 73.6% (n=622 deer from 53 farms) with a cut point of 1:96, but when all titres $\geq$1:24 were included, as used in this study, the herd seroprevalence was 81.1% and the individual animal seroprevalence was 63.7%.

The predominance of serovar Hardjobovis in this study is similar to findings from a national survey in dairy cattle in New Zealand in which Hellstrom (1978) showed that 81% of herds and 60% of individuals were seropositive. Mackintosh (1981) showed that 38% of herds and 66.3% of individuals were seropositive in the North Island. Cordes et al (1982), sampling 7,500 cows in 250 dairy herds in the North Island, reported 12.5% herd seroprevalence at a titre cut point of 1:200, and 88% if all titres $\geq$1:20 were included. Bahaman et al (1984b) showed a 66% seroprevalence of Hardjobovis in 800 dairy cows tested in the Taranaki district and Lake (1973) reported 18% seroprevalence of Hardjobovis in 890 samples from cattle, predominantly dairy, in herds linked to clinical leptospirosis in humans, where Hardjobovis was the predominant serovar.

In this study the overall seroprevalence for Pomona was 20% and 8.4% at the herd and animal levels, respectively, using a titre cut point of 1:48. Flint (1988a) reported 2.8% individual animal seroprevalence for Pomona at a cut point of 1:40, while Wilson et al (1998) reported 41.5% of herds and 12% of individual animals were seropositive at a cut point of 1:96, including some with dual infections with Hardjobovis but when all titres $\geq$1:48 were included, as used in this study, the herd seroprevalence was 47.1 % and the individual animal seroprevalence was 17.3%. In New Zealand dairy cattle, Hellstrom (1978) reported 36% of herds and 18% of individuals were seropositive, Mackintosh (1981) showed that 21.2% of herds and 5.2% of individuals were seropositive, Bahaman et al (1984b) reported 80% of 10

herds and 4% of individuals were seropositive and Cordes et al (1982) reported 6.0% of herds and 3.5% of individuals were seropositive to Pomona.

Concurrent Hardjobovis and Pomona infections were observed in 16.4% of herds and 6.6% of individuals. Studies in New Zealand in cattle (Hellstrom 1978; Mackintosh 1981; Cordes et al 1982), sheep (Dorjee et al 2005), pigs (Ryan 1978) also report single or dual-serovar infections in some herds and flocks.

No herds in this survey and only 1.2% of animals were seropositive for Copenhageni at cut point 1:48. Flint (1988a) reported 16.7% individual animal seroprevalence with a titre cut point of 1:40 and Wilson et al (1998) reported 11.3% seroprevalence at the herd level, where five herds showed titres ≥1:96 in more than one animal, in the absence of titres to Pomona. Some cross-reactivity is known to occur between Pomona and Copenhageni (Ryan 1978; Hellstrom 1978). It would appear that this serovar is not particularly significant in deer at the industry level, although sporadic infection can occur (Flint et al 1986). Evidence that deer can be infected with serovar Copenhageni was shown by Inglis (1984) who reported six of 10 deer with titres ≥1:100, and Flint et al (1986) subsequently isolated Copenhageni from urine of four of 27 deer on the same property. In cattle, using titre cut point of 1:24, Hellstrom (1978) reported 6% of herds and 2% of individuals were seropositive and Mackintosh (1981) showed that 26.9% of herds and 4.4% of individuals were seropositive.

Reichel (1999) reported that the national seroprevalence was 9.8 and 1.4% for Hardjobovis and Pomona respectively, noting significant disparity with data from Wilson et al (1998), and suggesting that further research was required to establish robust data for the prevalence of leptospirosis on New Zealand deer farms. The present results are consistent with those of Flint et al (1988a) and Wilson et al (1998). This confirms that estimates of Reichel (1999) underestimated nation-wide seroprevalence because a research design selecting only one or two animals from each herd lacks sufficient power to accurately estimate prevalence. Furthermore, a titre cut point of 1:100 was used, which alone would under-estimate herd level infection (Chapter 3), particularly when a small number of samples per herd is used. Results from the present study also refute the suggestion by Reichel (1999) that the prevalence reported by Wilson et al (1998) was high because herds were clustered in the lower North Island where leptospirosis had a higher prevalence. This survey showed no difference between islands.

There was insufficient power in data from the questionnaire to warrant statistical analysis of possible risk factors for leptospirosis on survey farms because of the high seroprevalence and the limited number of questionnaires. However, it is notable that 11% of farmers believed they had experienced leptospirosis in their deer in the previous 5 years, including those of all ages but most commonly in weaners, with both Hardjobovis and Pomona involved. This observation supports the proposition (Chapter 3) that losses associated with leptospirosis on deer farms may be more common than widely believed.

7.5 Conclusions

It is concluded that the seroprevalence of leptospirosis is high and evenly distributed in farmed deer herds thought New Zealand, similar to earlier data from dairy cattle prior to introduction of a vaccination programme. At the herd and individual animal levels the most prevalent serovar was Hardjobovis with some individual and dual infections with Pomona. There was little evidence of infection with Copenhageni. These results confirm that risk of disease in deer and exposure of infection to humans and other livestock is widespread, suggesting that control of the disease needs to be considered at the national rather than regional level.

Chapter 8

Relationship between Leptospira serology, kidney lesions and culture



8.1 Introduction

Leptospirosis is an important emerging disease globally in animals and humans and is considered one of the most widespread zoonosis (Plank and Dean, 2000). In New Zealand the number of human cases of leptospirosis peaked at 875 in 1971, falling rapidly to approximately 200 in 1982, and declining steadily to approximately 80 in 1998 (Thornley et al 2002). The reduction was largely in dairy farm workers attributed to a dairy cattle vaccination strategy promoted for animal health and human occupational health and safety purposes (Marshall and Manktelow, 2002). However, there is growing evidence for and concern about leptospirosis in humans associated with deer, both on farms and at slaughterhouses (Wilson et al 2005). Between 1999 and 2002, the number of cases doubled (Baker and Lopez 2004). Of 355 cases between 2001-3, 27% and 37.5% of cases were in farm workers other than dairy, and meat workers, respectively (Baker and Lopez 2004). Thornley et al (2002) reported five cases of leptospirosis in humans associated with deer. More recently Bell (2005) reported leptospirosis in eight of 38 deer slaughterhouse workers at risk in Southland, New Zealand, over a two-year period and Brown (2005) reported two severe cases at another deer slaughter premise confirming that deer slaughterhouse workers are at significant risk of leptospirosis.

Focal chronic interstitial nephritis, characterised mainly by white spots in kidneys, can be a common finding in cattle, swine, and sheep at slaughter (Baker et al 1989; Uzal et al 2002; Dorjee et al 2005). Wilson et al (1998) described various kidney lesions, including a high incidence of white spots, in apparently normal deer at slaughter, and cultured leptospiral organisms from approximately 10 of 202 randomly selected kidneys. These findings confirmed that deer slaughterhouse workers were at risk of leptospirosis.

This chapter sets out to provide evidence of risk of exposure to slaughterhouse workers of contracting leptospirosis from deer based on data collected for epidemiological studies elsewhere in this thesis combined with more detailed analysis than that published from the original serological, kidney lesion and culture data derived from the study of Wilson et al (1998).

8.2 Materials and Methods

This study reports data from 1164 non-vaccinated red and red x wapiti farmed deer selected at random from 82 lines at three Deer Slaughter Premises (DSP) in New Zealand in two sampling periods.

8.2.1 Animals and sampling

Period 1, 1992-1993: Data for this period is from blood and kidney samples collected from 622 deer (predominantly one-year-old) from 53 herds (n = 10-12 samples/herd, selected randomly) from the southern North Island of New Zealand, slaughtered at a Feilding DSP, as described by Wilson et al (1998). While that publication reported

serological data in detail, kidney lesion data were reported only in summary form. Original data were supplied by the senior author.

Period 2, 2003-2004: Note: all samples reported here were collected either for herd screening to identify suitable herds for studies presented in Chapter 4, or for the regional seroprevalence survey reported in Chapter 7. Blood and kidney samples were collected from 220 deer from 18 herds in Westland (Hokitika DSP) predominantly one-year-old stags and 125 animals from the southern North Island (Feilding DSP). In addition, blood and kidneys were collected from 197 deer from the three herds at the conclusion of epidemiological and vaccination studies presented in Chapters 4-6 (n= 83, 39 and 75, Farms 1, 2 and 3, respectively), collected at either the Feilding or Hastings DSPs.

Owners of herds sampled in period 1 were surveyed by postal questionnaire (Wilson et al 1998), to confirm that no herds had been vaccinated. Owners of herds sampled at the Hokitika DSP in period 2 were phoned by the DSP veterinarian for confirmation of vaccination status confirming that one herd (21) had been vaccinated. Data from this herd were not included in this study. Herds from Farms 1, 2 and 3, in period 2 were used for epidemiological and vaccination studies presented in Chapters 4-6, but the data from vaccinated deer excluded in statistical analyses.

8.2.2 Blood collection and handling

Ten ml of blood was collected by free flow at the beginning of the carcass processing line directly into an open 10ml glass tube without anti-coagulant (Vacutainer ®). The serum was aliquoted into two 1.5ml plastic storage tubes which were labelled and stored frozen for serology, as described in Chapter 2.

8.2.3 Serology

Serological techniques are described in Chapter 2. Titre cut points used for various analyses are described in the text as appropriate.

8.2.4 Kidney collection and gross lesion description

Both kidneys from each deer were collected from the inspection tray into clean and dry plastic bags, identified, and matched to the carcass and animal identification numbers. Each kidney was examined for description of gross pathology. Macroscopic lesions were categorised, by the absence (0) or presence (1) of white spots and scaring, or red spots, when either one or both of the kidneys had 10% or more of the surface involved. While lesions in kidneys from the study in Period 1 had been scored numerically 1-6, for Period 2, different people collected kidneys and the scoring system was not consistent. Therefore, for the purpose of this analysis, kidney lesions were categorised as present (yes) or not (no). The range of lesions are presented in Figures 8.5 to 8.8

8.2.5 Histopathology

One kidney from each deer in herds from Farms 1-3, from period 2, were subjected to histological examination, after preparation using standard histological methods and stained with haemotoxylin and eosin (H & E) as described in Chapter 2. The kidneys were examined randomly between farms. Lesions were described and a score of 0 (no), or 1 (yes) was given based on the description of multi-focal interstitial nephritis consistent with leptospirosis (Prescott 1993).

8.2.6 Culture

The second kidney from the animals sampled at slaughter from Farms 1-3 (n= 197) were collected, in addition to kidneys from 16 additional animals from the same farms that were submitted for necropsy at Massey University with one confirmed as leptospirosis. Thus, data from 213 animals were available for the analyses involving culture positive deer. The culture technique is described in Chapter 2.

8.2.7 Statistical analysis

Descriptive data analysis was expressed as prevalence (proportions) and geometric mean of the titres (GMT) (using coded titres with back-transformation as described in Chapter 2, with a 95% confidence interval (95%CI). An individual animal was considered seropostive at ≥1:24 for Hardjobovis and ≥ 1:48 for Pomona and Copenhageni. To establish herd prevalence, the same definitions for a seropositive herd as those described in Chapter 7 were applied. Associations between kidney lesion classification (gross and histological), and whether or not the deer were seropositive using the above definitions were investigated on data from all animals from the 82 herds using the Mantel-Haenszel Chi-Square (χ^2 test in SAS for Windows (SAS Institute Inc., Cary, NC, USA). The analyses were performed separately using titre cut points of 1:24, 1:48 and 1:96, for all serovars. Associations between serology, kidney lesions (gross and histopathology) and culture for 197 animals from the herds on Farms 1-3 were performed using the same cut points.

A logistic regression analysis was performed to test for differences in seroprevalence between periods and slaughter plants, adjusting for possible confounding effects of period and DSP. Logistic regression was also used to test for differences in kidney lesions between periods and slaughter plants with herd as a random effect. Fixed effects in the model were tested by the likelihood ratio test. Logistic regression analysis (SAS) was used to estimate sensitivity, specificity, and prevalence of kidney lesions as an indicator in infection. The previous estimates were used to calculate the Relative Risk ratio (RR), Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of kidney spots (gross pathology). .

The Yates corrected Chi Square (χ^2 for the difference in the prevalence using NCSS-PASS and Mantel-Haenszel Relative Risk ratios and Prevalance ratios for kidney lesions in relation to serology from the 197 animals on Farms 1-3 were analysed ($p \leq 0.05$) in Episheet 2002 (Rothman © 2002). Diagnostic test agreement for those tests on samples from animals on Farms 1-3 was investigated using the Kappa value (Dohoo et al 2003).

8.3 Results

Raw data by sampling period, farm number, deer age and sex, serological titre to each serovar, lesion status, histological score and culture result are presented in Appendix 3.

8.3.1 Serology

No differences in seroprevalence was observed between the three DSPs sampled in Period 2 (χ^2 2.56, p= 0.15), data were pooled for presentation and analysis.

Data for individual animal and herd seroprevalences are summarised in Figure 8.1. Overall seroprevalence in the 1164 samples, all serovars combined, was 74.14% (71.5-76.6% CI), while the herd prevalence was 84.1% (74.7-91.3%). Titres for Hardjobovis and Pomona ranged from 0 to 1:3072 while those to Copenhageni ranged from 0 to 1536. Hardjobovis was the most prevalent serovar with 79.3% (68.9-87.4 CI) of herds seropositive and 67.3% (64.6-70% CI) of individual animals seropositive, with a reciprocal GMT of 88.32. Serovar Pomona was detected in 40.2% (29.6-51.9 CI) of herds and 25.2% (22.8-27.9% CI) of individuals, with a reciprocal GMT of 84.7. Three herds (3.4%, 0.8-10.3) and 4.6% (3.5-6.0% CI) of individuals were positive to Copenhageni with a reciprocal GMT of 32.88.

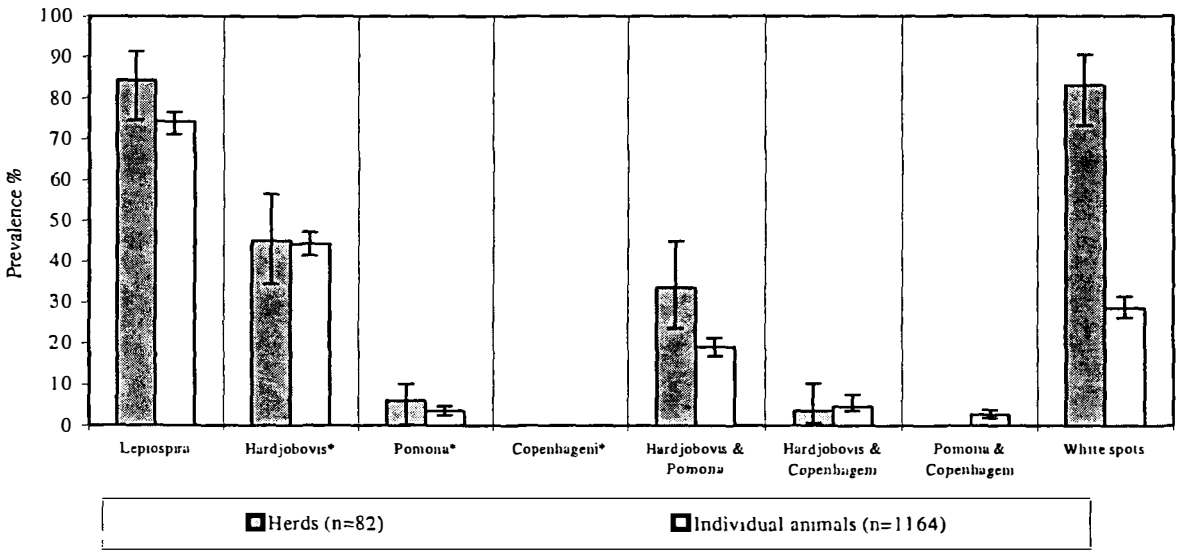


Figure 8.1. Herd and individual animal seroprevalence (±95%CI), of serovars Hardjobovis, Pomona and Copenhageni, first column (*Leptospira*) overall serology to either serovar,*= positive titre only to serovar Hardjobovis, Pomona or Copenhageni, the dual positive titres for the different combinations of serovars, final column prevalence of kidney lesions (white spots).

Dual seropositivity to serovars Hardjobovis and Pomona was observed in 28 herds (34.1% of total), dual infections Hardjobovis and Copenhageni was observed in 3 herds (3.6% of the total). Thirty-four herds (41.4%) were positive only to serovar Hardjobovis, five herds (6.1%) were positive to Pomona alone. Copenhageni-only infected herds were not observed. Three herds were positive to Copenhageni independent of titres to Pomona with titres ranging up to 1:1536 (mode 1:96).The within herd prevalence in those herds was between 50 to 100%. In the other herds where animals showed titres to both those serovars, the titres to sv Copenhageni and Pomona ranged up to 1:96 (mode of 1:24).

Titres to Hardjobovis were apparently independent of titres to Pomona (Figure 8.2). Titres to Pomona and Copenhageni were not independent, except for the three herds with the dual infection Hardjobovis-Copenhageni. With the exception of the three herds where prevalence was above 50% and independent of serovar Pomona (period 1, Figure 8.2) the within-herd titre prevalence for Copenhageni was approximately 20% or less and was always lower than the seroprevalence of Pomona.

Regression analyses were done to compare the seroprevalence of Hardjobovis and Pomona, between year, age group and sex, with the model period (1992, 2004), Sex (Female =0, Male =1)*Age (1=weaner, 2= yearling and 3= Adult). The analyses were done only using the data from the randomly selected herds, excluding Farms 1, 2 and 3. Overall Hardjobovis seroprevalence was higher in Period 2 (79.3%, 57.7-89.3 CI) than in Period (71.5% (53.2- 94.1 CI) (p=0.06). Seroprevalence was lower in male weaners (54.2%, 37.4-50.2 CI) than in yearling (86.5%, 65.8-94.4) (p=0.002) and adults (80.8%, 66.2-90.0 CI) (p= 0.01). There were no differences between age groups in females, but numbers were low. Seroprevalence in weaner females (74.6%, 58.0-86.3 CI) in 2004 was significantly higher (p=0.010) than males (66.2%, 49.0-80.0 CI), but yearlings (92.5% males, 92.9% females) and adults (80.8% males, 81.0% females) had a similar seroprevalence. Pomona results indicated that seroprevalence tended to be lower in Period 1 (13.29%, 9.9-34.8 CI) than in Period 2 (29.6%, 14.2-51.4) (p=0.06). There was no difference in overall seroprevalence between weaners (17.4%, 8.4-33.0 CI) yearlings (28.1%, 13.1- 49.9) and adults (25.4% 14.2-41.4).

8.3.2 Gross kidney lesions

It was not possible to distinguish whether red spot lesions were associated with pathology, or whether they were artefact associated with electrical stunning. Thus, presentation of results will be confined to white spots only. White spots were usually observed in both kidneys of deer from 73.3% (62.2-82.4 CI) of herds and in one or both kidneys of (28.7%, 26.2-31.5% CI) of individual deer (Figure 8.1)

No difference in lesion prevalence was observed between samples from the three DSPs sampled in Period 2 (χ^2=1.78, p= 0.18) so data was pooled for presentation and analyses.

The prevalence of white spots on kidneys was higher in deer seropositive to Hardjobovis or Pomona in period 2 (34.7%) than in period 1 (27.02 %)when analysed at any titre cut-points ($p \leq 0.05$). For Pomona positive deer, a higher

proportion had white spots on kidneys in Period 2 (45.8%) than in Period 1 (32.11 %), ($p<0.05$).

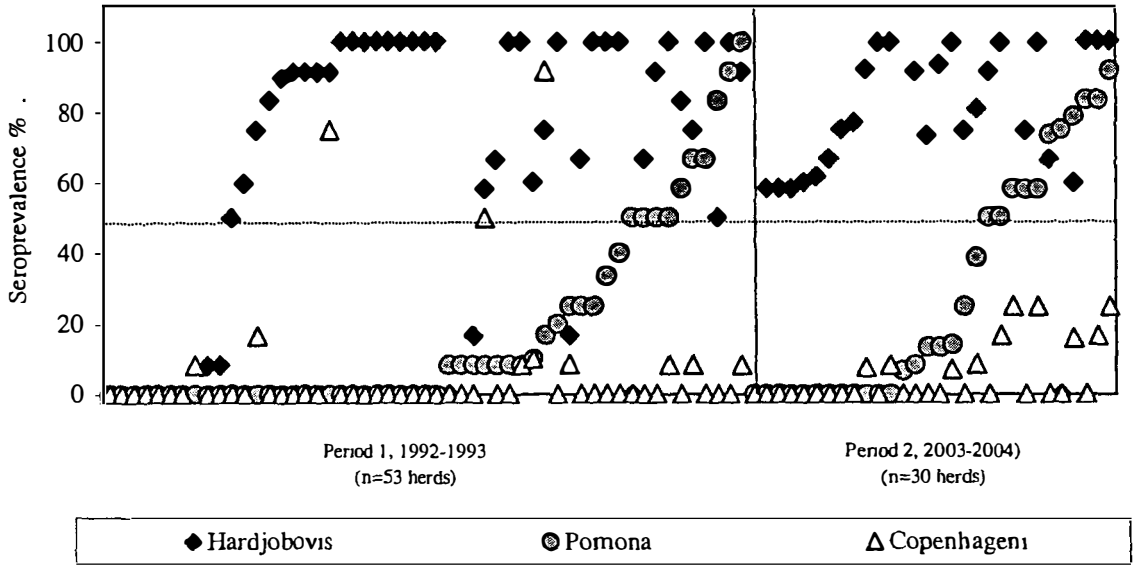


Figure 8.2. Within-herd seroprevalence distribution of deer positive to serovars Hardjobovis (≥1:24), Pomona (≥1:48), and Copenhageni (≥1:48) in Periods 1 and 2 showing herds positive to a single or multiple serovar, sorted by order of seroprevalence to Pomona followed by Hardjobovis.

8.3.2 Association between kidney lesions and serology

Samples from all deer were used for this analysis. The seroprevalence of deer with and without white spots in their kidneys that were seropositive, either to single serovars or in combination, at various titre cut-points, along with the χ^2 statistic, relative risks ratios, probabilities and confidence intervals are presented in Table 8.1.

The seroprevalence of deer with white spots on their kidneys was higher than for those without such lesions for both serovars Hardjobovis and Pomona and all titre cut-points and combinations ($p<0.01$– $p<0.001$). The relative risk of deer having white spots on their kidneys when seropositive to Hardjobovis was 1.08, and for Pomona, 1.6. White-spotted kidneys were not significantly associated with Copenhageni serology. A linear association between white spots and serology was not found. Thus an animal with a kidney lesion was more likely to be seropositive.

Associations between kidney spots and serology included all data from all the sampled herds. Hardjobovis seroprevalence was 82.4% and 76.2% in animals with and without white spots on kidneys, respectively. The sensitivity of white-spotted kidney as a predictor of Hardjobovis seropositivity was 25.5%, the specificity was 81.1%, the PPV was 82.4%, and the NPV was 23.8% (Table 8.1). Pomona seroprevalence was 33.1% and 20.5% in animals with and without white-spotted kidneys, respectively. The sensitivity of white-spotted kidney as a predictor of Pomona seropositivity was 35.1%, the specificity was 78.0%, the PPV was 33.1% and the NPV was 79.5% (Table 8.1).

Table 8.1 Summary of regression analyses for relationships between white spot kidney lesions and serology, histopathology and culture. (95%CI in brackets)

Response variable (white spots %)	Effect variable (prevalence (%) within response variable)		Kidney White Spots				
			Sensitivity %	Specificity %	Predictive value %		Relative Risk
					Positive	Negative	
35.1 (30.4-35.9)	Seroprevalence Hardjobovis ≥ 1:24 (n=1164)		25.5 (20.6-31.1)	81.1 (11.9-)	82.4	23.8	1.08 (0.9-1.1) p=0.15
	Neg	76.2 (67.7-83.1)					
	Pos	82.4 (71.8-89.6)					
3.5 (2.5-4.9)	Seroprevalence Pomona ≥ 1:48 (n=164)		35.1 (25.6-46.0)	78.0 (17.7	33.1	79.5	1.61 (1.1-2.1) p=0.01
	Neg	20.5 (14.8-27.8)					
	Pos	33.1 (22.6-45.7)					
56.4 (49.1-63.4)	Histology (n=197)		39.7	81.2	74.8	48.9	1.46 (1.3-1.5) p<0.0001
	Neg	51.1 (48.9-53.4)					
	Pos	74.8 (70.1-79.0)					
29.6 (26.4-32.8)	Culture (n=197)		31.1	78.2	28.0	80.6	1.44 (1.1-1.7) p<0.0001
	Neg	19.4 (17.5-21.4)					
	Pos	28.0 (23.9-32.6)					

8.3.3 Association between white spots, serology, histopathology and culture.

This analysis was performed only on the 197 deer from Farms 1-3 from which data for lesions, serology, histopathology and culture were available for individual deer. It should be noted that Farm 2 was evaluated over two years, with the original serological status being Hardjobovis alone. However, part way through year 1, serological analyses detected introduction of Pomona to the property. Farm 1 was seropositive for Hardjobovis only and Farm 3 was seropositive for both Pomona and Hardjobovis.

The prevalence of white spots, histological and serological results are presented in Figure 8.3. The seroprevalence in culture positive deer, either to single serovars or in combination at various titre cut-points, with or without white spots on their kidneys along with χ^2 values, relative risks ratios, probabilities and confidence intervals are presented in Table 8.3.

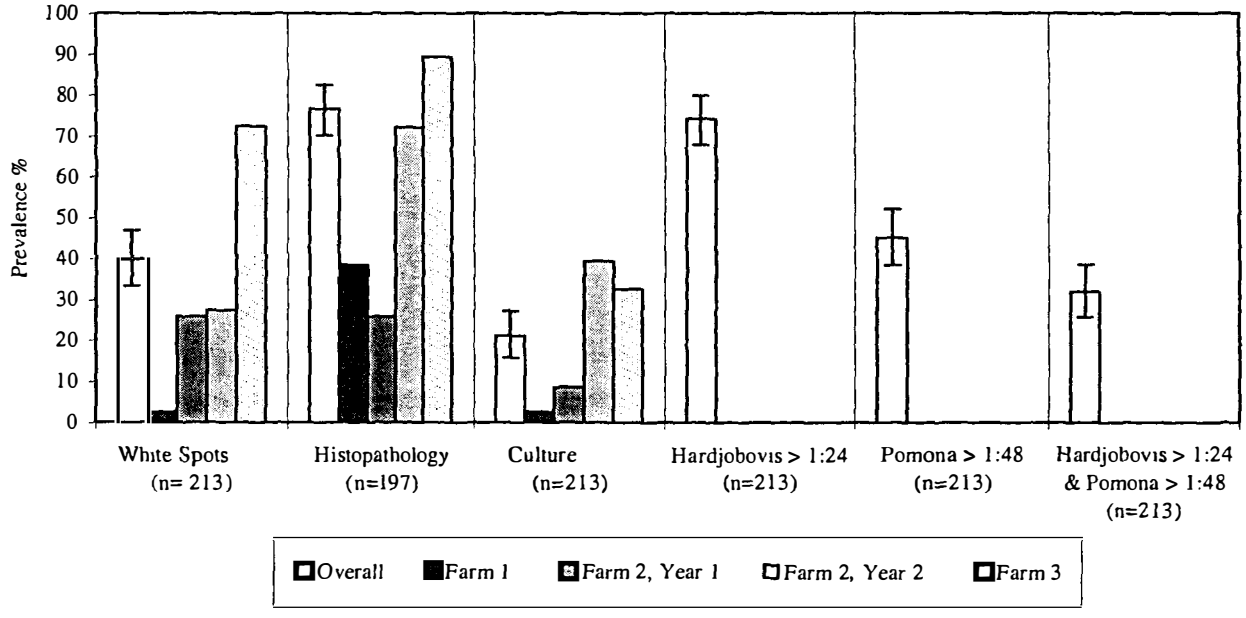


Figure 8.3. Prevalence of kidneys with white spots, histopathological lesions, culture positive, and overall seroprevalence (±95% CI) for the three farms from which data are derived. (The total number of kidneys used for each diagnostic procedure are in brackets).

Eighty percent of the animals from these three farms showed titres to *Leptospira* (serovars Hardjobovis, Pomona or Copenhageni). Eighty-one animals presented with white spot lesions in the kidneys (41.1%, 34.2-48.3 CI) (Figures 8.5 to 8.8), and 111 (56.3%, 41.9-63.4 CI) had interstitial nephritis lesions histopathologically (Figures 8.9 and 8.10). There was a higher proportion of deer with kidney lesions, both gross and histological, in deer from Farms 2 and 3, on which active infections with serovar Pomona were evident, compared with Farm 1 upon which only serovar Hardjobovis

was present. Leptospires were isolated after a minimum of 6 weeks in 45/213 (21.16 %, 15.8- 27.2) kidneys cultured (Figure 8.3). The culture prevalence from Farm 1 was 2.5% (1/39 Hardjobovis). From Farm 2 in year one, 8.6% (5/58) of kidneys were culture positive, and in year 2, 39.3% (13/33) were culture positive, with dual infections both years. Farm 3 had 31.3% (26/83) of kidneys culture positive with dual infections. Of the 45 cultures 13 were Hardjobovis, 8 were Pomona, 6 were dual Hardjobovis and Pomona based on high titres to both serovars and 2 were positive leptospiral cultures that did not react to any of the specific antisera from the reference panel. The other 16 were positive but the cultures did not adapt and grow sufficiently to be typed.

The proportion of seropositive deer with lesions that were culture positive was higher ($p<0.01$) than the proportion without lesions that were culture positive (31.5 vs. 15%) when leptospiral titre cut points were ≥1:24, with a corresponding Relative Risk of 2.15 (Table 8.3). Two cultures were from animals with negative serology and with kidney lesions, and the remaining four cultures were from animals without lesions or titres. For deer positive for Hardjobovis alone or in combination with Pomona, there was a significantly higher prevalence of kidney lesions in the culture positive animals ($p<0.01$ and $p=0.009$, respectively). The prevalence ratios of a positive culture from the white spotted group ranged from 1.9 to 2.1, depending on serological titre combination, and all relative risk ratios were significant. However, for Pomona alone (titres ≥1:48), there was no difference in the prevalence of kidney lesions between culture positive and negative deer.

Table 8.3 Proportion of deer that were serologically positive with white spots on kidneys that were also culture positive, and a summary of the Yates corrected Chi-Square (χ^2) test for the alternative hypothesis that seroprevalence in culture positive deer with white spot lesions in the kidney (Ws(+), C(+)) is higher than in culture negative deer with white spot lesions in the kidneys (Ws(+), C(-)). The relative risk for each comparison (95% CI) is included.

Serology	Seroprevalence % (95% CI)		χ^2	Prevalance ratios (p) and (95% CI).
	Ws (+), C (+)	Ws (-), C (+)	Ws (+), C (+) > Ws (-), C (+)	
Overall	32.3 (21.7-43.8)	15 (8.6-23.5)	5.9 (p< 0.01)	2.1 (p< 0.008) (1.4 –3.95)
Hardjobovis (≥ 1:24)	30.3 (18.8-44.1)	12.3 (7.7- 23.0)	6.6 (p= 0.009)	2.1 (p< 0.017) (1.21-4.98)
Pomona (≥1:48)	34.0 (22.7-47.7.4)	20.0 (8.4-36.9)	1.5 (p< 0.20)	1.7 (p= 0.07) (0.77-3.64)
Hardjobovis (≥ 1:24) and Pomona (≥1:48)	33.3 (19.6-49.5)	19.2 (6.6-39.4)	0.9 (p< 0.3)	1.9 (p< 0.013) (1.4 –3.9)
Hardjobovis (≥ 1:24) or Pomona (≥1:48)	32.4 (21.7-43.8)	14.9 (8.6-23.5)	5.8 (p< 0.01)	1.9 (p< 0.13) (1.4 –3.9)

In additional analyses, the MH RR comparing kidney culture prevalence with and without histopathology lesions was 2.90 (1.1-6.66) ($p<0.0001$). A culture positive

prevalence of 33.3% was observed when white spots, histopathology and serology were positive, versus an 8.4% culture positive prevalence when pathological lesions and serology were negative. The relative risk ratio was 3.9 (1.3-11.8) (p =0.002). A kidney culture was 2.9 (1.1-7.5) times as likely to be positive when histopathology was positive (27.0%), than when histopathology was negative (8.4%) (p<0.001). Likewise a kidney culture was 2.06 (1.0-4.2) as likely to be positive when white spots were present in the kidney (30.5%), than when there were no white spots (14.8%) (p= 0.006). The culture prevalence was 3.17 (1.6-6.2) times as high in kidneys with macroscopic or microscopic lesions (46.9%), than when there were no macroscopic or microscopic lesions (14.8%) (p< 0.001).

Histopathology lesion prevalence was 51.1% and 74.8% in animals with and without white spotted kidneys, respectively. The sensitivity of white-spotted kidney as a predictor of histopathological lesions was 39.7%, the specificity was 81.2%, the PPV was 74.8% and the NPV was 48.9% (Table 8.1). The sensitivity of white-spotted kidney as a predictor of positive culture was 19.4%, the specificity was 28.0%, the PPV was 31.1% and the NPV was 80.6%. The RR of a white spotted kidney having histopathological lesions was 1.46, and for positive culture was 1.44.
Measuring the agreement between the four diagnostic procedures used to predict leptospiral microorganisms indicated moderate agreement between kidney lesions and histopathology (Kappa= 0.403 (0.28- 0.52CI)). These tests showed poor agreement between being serology and culture positive.

8.3.4 Relationship between titre and kidney lesion and culture status

The reciprocal GMTs in animals negative to the three tests ranged from 43.4 to 80.7. Those positive ranged from 49.3 to 142.7. The reciprocal GMTs of *Leptospira* overall and Hardjobovis in the three diagnostic procedures were not different and remained low. For animals with serovar Pomona, the reciprocal GMT was higher in the positive animals (range 99.2 to 142.7) compared with the negative animals (range 60.9 to 80.7), with the highest GMT in culture positive animals.

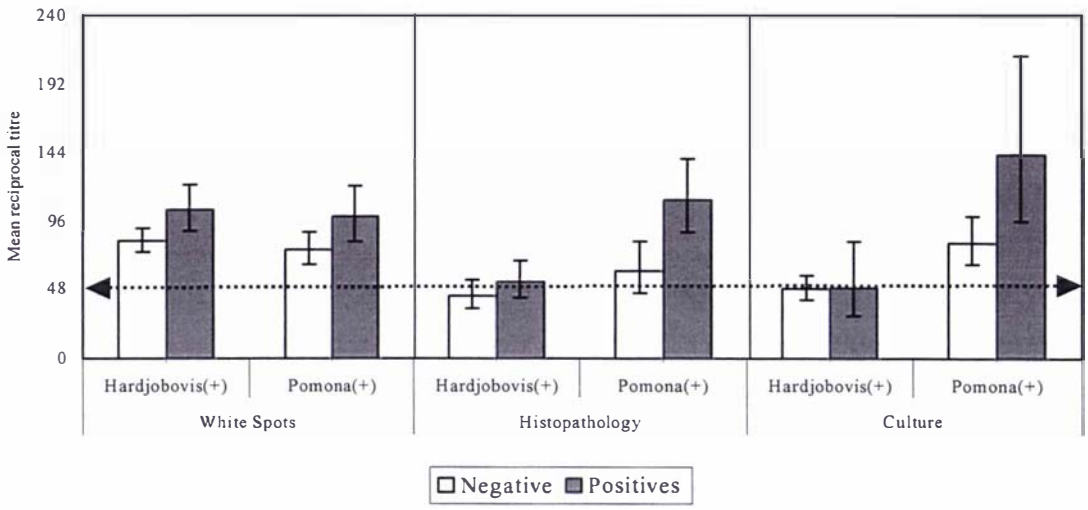


Figure 8.4 Reciprocal geometric mean titre (GMT) (± 95% CI) of titres for all serovars combined, or Hardjobovis and Pomona individually, in deer that were kidney gross lesion, histological lesion or culture positive or negative.



Figure 8.5 Kidneys without lesions.



Figure 8.6 Congested kidney, scaring on the kidney surface and cortex.

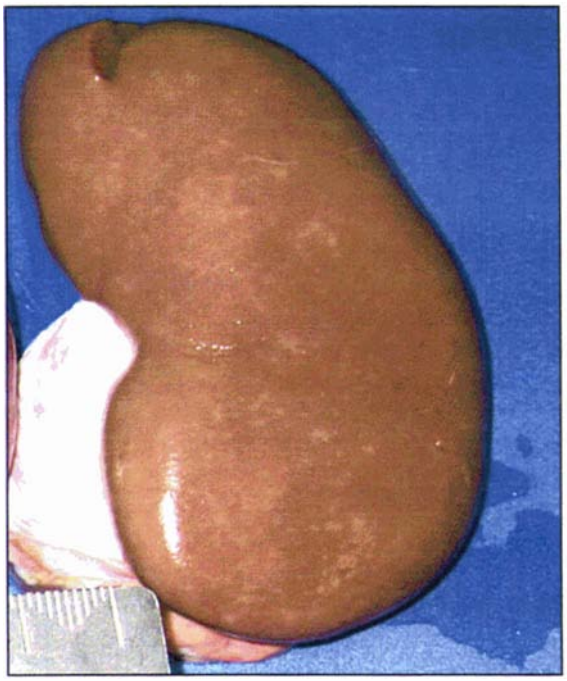


Figure 8.7 White spots on approximately 20% of the kidney surface.

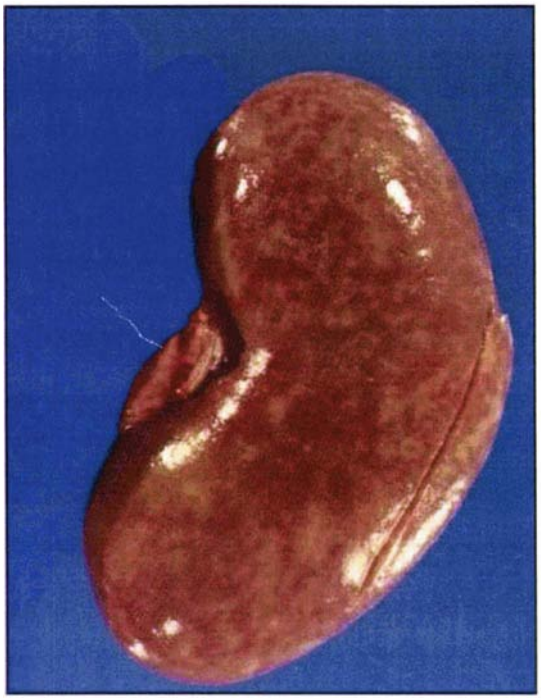


Figure 8.8 White spots on approximately 40-50% of the kidney surface.

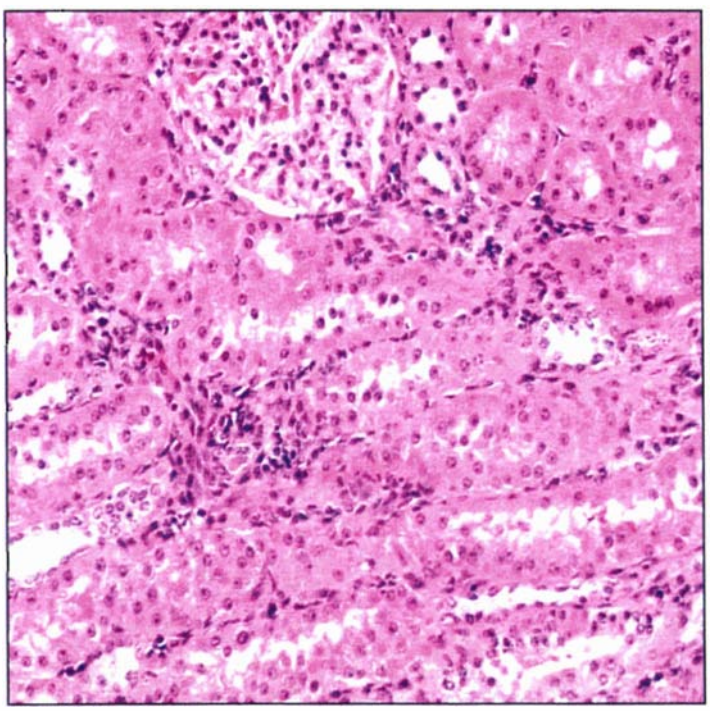


Figure 8.9 Focal Interstitial inflammatory cell infiltration of seropositive adult hind to Hardjobovis and with white spotted kidneys. H&E .

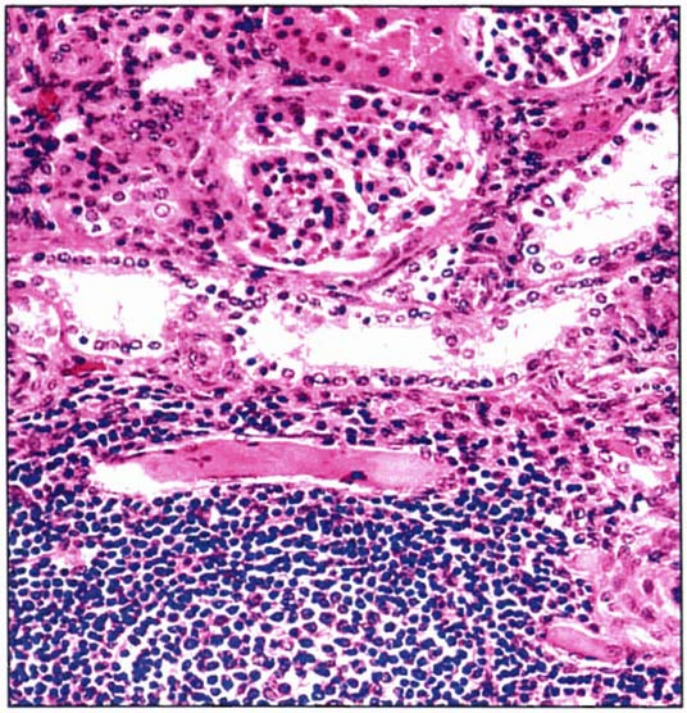


Figure 8.10 Interstitial nephritis, mononuclear cell infiltration in the cortico medular area of a white spotted kidney from a seropositive Hardjobovis and Pomona weaner. H&E.

8.4 Discussion.

This is the first investigation to test relationships between various potential means for diagnosis of leptospirosis and the risk that a deer was culture positive, and therefore potentially a risk to humans exposed to kidneys from deer. Pooling data from several studies, including those from two periods 10 years apart, provided a valid opportunity to test relationships between serology, gross and histopathological lesions and culture. The analyses were performed only for serovars Hardjobovis and Pomona because there were few herds with titres to Copenhageni and there was no culture evidence of infection. The study showed that a high proportion of deer at slaughterhouses were seropositive, particularly to serovar Hardjobovis. More than half of the herds had deer with kidney lesions, predominantly white spots, indistinguishable between serovars Pomona and Hardjobovis, and there was a higher risk of kidney lesions in animals seropositive to both. There was a higher probability of a kidney being culture positive when lesions were present.

8.4.1 Seroprevalence

Almost 85% of herds and 73% of individual deer were seropositive to Hardjobovis and/or Pomona giving a seroprevalence and distribution within age category similar to that observed from other surveys presented in this thesis (Chapters 3 and 7). A full discussion of seroprevalence is presented in those chapters.

8.4.2 Gross pathology and serology

White spot lesions observed in the kidneys in this study are similar to multifocal chronic interstitial nephritis (MCIN) lesions reported in other species (Prescott 1992). These lesions and positive serology are commonly observed in cattle, pigs and sheep in New Zealand and other countries and while not pathognomonic, are generally regarded as a indication of leptospirosis (Amatredjo et al 1976; Peet et al 1983;Gregoiré et al 1987; Baker et al 1989; Chappel et al 1998; Yener and Keles 2001; Dorlet et al 2002; Dorjee et al 2005). In deer, chronic and acute interstitial nephritis has been reported in the kidneys of infected deer with clinical leptospirosis (Trainer et al 1961; Fairley et al 1984; Wilson and McGhie 1993). The analysis of association between kidney lesions and culture in this paper was performed on samples collected from known infected farms collected in period 2. The lesions observed were similar to those reported by Wilson et al (1998) from samples described in period 1 of this study.

White spots were observed in kidneys of more than 70% of the herds and 28.7% of individuals. A significantly higher seroprevalence was observed for Hardjobovis and/or Pomona when kidneys presented white spots. A higher RR ratio of being seropositive was observed in animals with kidney lesions with the highest ratio being in animals with titres ≥1:96, particularly for serovar Pomona.

No direct correlation could be established between seropositivity and MCIN lesions, because despite higher seroprevalence in animals with kidney lesions, titres were also present in animals without lesions. Similar findings have been reported in cattle and pigs (Gregoiré et al 1987; Skilbeck et al 1988; Uzal et al 2002, Boqvist et al 2003).

The positive relationship between a high GMT and kidney lesions observed here is consistent with recent or active infection described in pigs and cattle (Michna 1970; Black et al 2001). Boqvist et al (2003) suggested that leptospires in the kidney may result in MCIN and white spots even if antibody titres are low, either because antibodies are declining after the initial stage of infection, or that the infection is in the early stage, prior to expression of high titres. White spots develop between one and three weeks post-infection (Uzal et al 2002). Many authors report a poor correlation between seropositivity and kidney lesions such as reported in pigs and cattle (Uzal et al 2002; Boqvist et al 2003) despite lesions being definitively associated with acute and chronic leptospiral infections in the kidney. Some lesions observed represent permanent tissue damage due to previous but resolved infection (Jones et al 1987; Skilbeck et al 1988; Baker et al 1989; Faine et al 1999), sometimes with persistent antibody titres.

8.4.3 Pathology, culture and serology

Significantly higher seroprevalence was observed in animals with cultures and kidney lesions compared with animal culture positive but without kidney lesions with a RR of two. The RR increased to three when both macroscopic and microscopic lesions were observed concurrently. This finding is consistent with reports indicating that high prevalence of interstitial nephritis is indicative of leptospiral infection and therefore risk to humans as reported from cattle (White et al 1982; Thiermann 1983; Kingscote 1985a) and pigs (Baker et al 1989; Scanziani 1991).

The histopathological lesions in this study were similar to those reported in adult cattle and in pigs (Michna and Cambell 1969; Amatredjo et al 1976; Gregoiré et al 1982; Silbeck et al 1988; Prescott 1992). Lesions were described as focal or multifocal tubulointestitial nephritis characterised by a mononuclear infiltrate ranging from mild to severe. Similar histological findings have been reported in natural and experimental clinical cases in deer (Ferris et al 1960; Trainer et al 1961; Reilly et al 1962; Fairley et al 1984; Wilson and McGhie 1993.

The 21.1% culture prevalence reported here was from three known infected herds. Wilson et al (1998) reported a 4.9% positive culture rate from 202 kidneys sampled at random from 53 herds. In both studies, both serovars were isolated with 42% of cultures being Hardjobovis 16% being Pomona and 11% dual (based on high titres to both serovars), while 11% of cultures could not be typed. Serovar Hardjobovis was also predominant in cattle (White et al 1982) where culture was positive in 36% of 226 animals with 55% identified as Hardjo, 3.6% as Pomona, and 3.6% as Balcanica. Thiermann (1983) reported 6.3% of 204 cattle were culture positive with 54% being Hardjo, 23% being Pomona and 23% other serovars, and Gregoiré et al (1987) reported 29% isolation from 120 kidneys with 77% being Hardjobovis and 23% being Pomona.

Chappel et al (1992) compared various diagnostic procedures for porcine leptospirosis. Kidney lesions were clearly associated with infection with a 23.5% culture rate in kidneys with lesions, but with 7.5% in kidneys without lesion. Forty four percent of kidneys with white spots had MAT titres $\geq$ 1:1024 similar to the relationship in deer in this study, particularly with serovar Pomona. Prescott et al (1987) and Kingscote (1985b) also reported a higher culture rate from cattle with high titres with and without lesions. The relative pattern with more lesions and cultures from animals with high titres to Pomona than to Hardjobovis is consistent with reports in cattle, in which Hardjobovis is considered to be the host adapted species and Pomona an accidental infection (White et al 1982; Thiermann 1983; Gregoiré et al 1987), similar to the relationship proposal in the farmed deer in New Zealand.

8.4.4 Agreement between diagnostic techniques

The present study confirmed a positive relationship between several diagnostic criteria and kidney culture result, suggesting that these are useful measures to predict the likelihood of a kidney being culture positive and hence a risk to humans, particularly meat workers, exposed to deer kidneys. While no diagnostic criteria were totally sensitive or specific, when the proportion of deer in a line and the severity or frequency of lesions was taken into account, there appears to be a higher probability that some kidneys in the line would be culture positive (Wilson et al 1998). Combining pathology and serology for Hardjobovis resulted in a low sensitivity and a moderate specificity of kidney lesions as a predictor of serological status. This serovar:lesion relationship in deer is similar to that observed in cattle, in which only mild kidney pathology is observed in response to infection with this host-adapted serovar, as discussed in Chapters 3 and 4. Thus, gross pathology as the sole indicator of infection with Hardjobovis is of limited value. However, the high PPV (>80%), and a low NPV (<30%) indicates that an animal with white spots in its kidney has a

higher probability of being seropositive. Similar observations appear not to have been studied in other species.

Kidney gross lesion as a predictor of seroprevalence alone had similar sensitivity and specificity for Pomona and Hardjobovis. The lower PPV and the higher NPV that a deer with kidney lesions would be seropositive to Pomona compared with Hardjobovis is due to the lower prevalence of infection with Pomona consistent with the expectation described by (Dohoo et al 2003). The PPV and NPV for the relationship between kidney culture and gross pathology were similar to those between gross pathology and seropostivity for Pomona, while the relationships between histological lesions and gross lesions were similar to the relationship between Hardjobovis serology and gross lesions. These relationships are likely to be associated with low culture prevalence due to the fastidious nature of the microorganism and/or that lesions are more commonly associated with a chronic infection rather than an active renal shedder state (Michna 1970), particularly for Pomona.

Combining pathology and serology improved the ability to predict kidney culture, but is unlikely to be practical in a slaughterhouse situation. Thus for practical reasons, kidney lesions alone would be used as the sole predictor of infectious status with leptospirosis despite it not being the most sensitive predictor, but it is likely that the PPV would be higher if the severity of lesions, and the within-line prevalence of lesions were considered in parallel. Since there was insufficient power in the observations reported here to investigate this suggestion, further research would be needed for confirmation.

The best agreement was histopathology and gross pathology. Baker et al (1989) and Chappel et al (1992) in pigs, and White (1982) and Gregoiré et al (1987) in cattle, reported a better agreement between serology and culture, than culture and white spots. Scanziani (1991) and Boqvist et al (2003) in pigs and Yener and Keles (2001) in cattle suggested that other diagnostic procedures such as the immunofluorescence and immunoperoxidase of the kidney tissue can be a better indicator of the presences of bacteria in kidney tissue. Since *Leptospira* is a fastidious microorganism with a low isolation rate and with long incubation periods, culture alone underestimates the risk of exposure to this microorganism of humans at-risk such as meat industry workers.

8.4.5 Possible Risk of exposure to humans

Humans are an accidental host to leptospires and transmission is usually through contact with infected urine or kidneys. The risk evaluated in this chapter was based only on kidney culture. In New Zealand, as in other extensive farming countries with temperate climates, most of the human cases are linked to agricultural practices and animal populations in the ecosystem (domestic or wildlife) (Milner et al 1980a,b; Prescott et al 1992; Levett, 2001). The epidemiology of human leptospirosis in New Zealand has changed in the last three decades. Previously most leptospiral cases were in dairy and pig farmers and slaughterhouse workers (Wilks 1997). During 2001-3, only 10% of human cases were in dairy workers while 27% were in farm workers other than dairy, and 37.5% were in meat workers (Baker and Lopez 2004). Data of Wilks (1997) and Baker and López (2004), combined shows 42.7% of human cases

were Hardjobovis and 33.5% Pomona which are the predominant serovars reported in this thesis in deer in New Zealand and internationally, as reviewed earlier in this thesis. Thornely et al (2002) reported five clinical disease cases in humans associated with deer, Bell (2005) reported eight of 38 deer slaughterhouse workers affected over two years and Brown (2005) described two similar cases.

While >70% of individual deer in this study were seropositive and >70% of herds contained at least one deer with kidney lesions, the culture rate varies between herds. The three known infected herds presented in this study yielded cultures from 21.1% of kidneys overall, while 4.9% of kidneys sampled at random from 53 herds in period one were culture positive. From farm 1, infected only with Hardjobovis, one kidney (2.5%) was culture positive, while from farm 2 with Hardjobovis infection and recent introduction of Pomona, five (8.6%) were culture positive in year 1, and 13 (39.3 %) were positive the following year, and from Farm 3 with an active dual infection with serovars Hardjobovis and Pomona, 26 (31.3%) of kidneys were culture positive. This demonstrates that the rate of exposure varies within and between herds with different presentations of leptospiral infection, with a higher risk of exposure when deer were from farms with recent clinical or subclinical outbreaks. This is evidence that farmed deer pose a leptospirosis risk to meat workers similar to that reported for other animal industries (Wilks 1997; Dowd 2003; Dorjee et al 2005). Using data in this chapter, a stochastic risk analysis estimated that with the procedures used in the Feilding deer slaughter premise, that eviscerators and inspectors were on average at risk of exposure to infected kidneys from nine (3-19 CI) carcasses per day, and offal handlers were exposed to 4 (1-10 CI) (Heuer et al 2005). It is likely that exposure to infected urine would increase the overall risk of transmission to workers.



Figure 8.11 A deer carcass at the Feilding DSP shortly after sticking, demonstrating voidance of urine, placing workers at risk of direct contact.

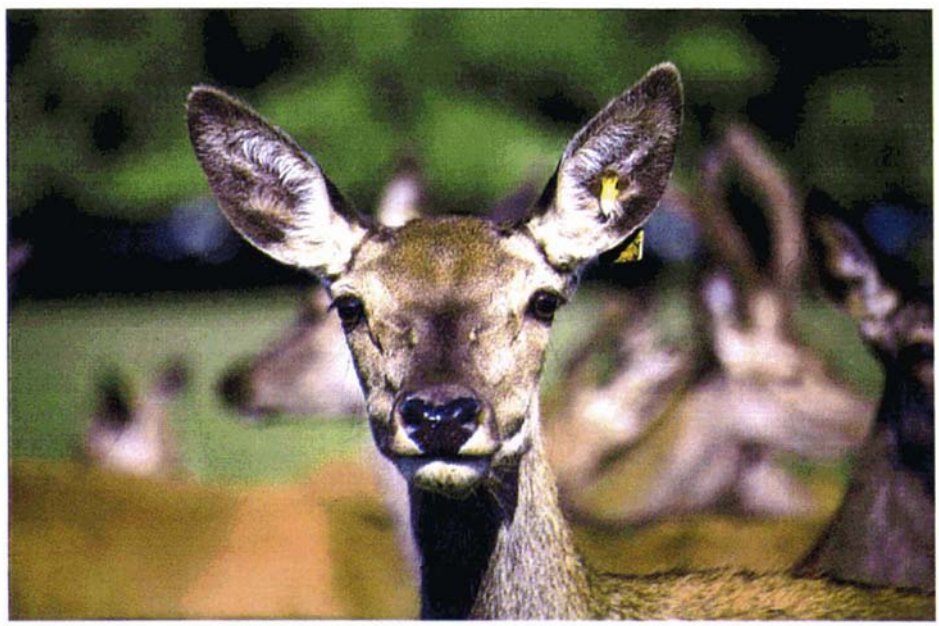
8.5 Conclusions.

- The bacteriological, pathological and serological findings reported here indicate that leptospiral infections are common in slaughtered farmed deer in New Zealand.
- The presence of white spots in the kidneys of farmed deer at slaughter was associated with a higher probability of having culture positive kidneys.
- There was a higher probability of kidney culture from seropositive deer, but some seronegative deer had culture positive kidneys
- There was a positive relationship between macroscopic or microscopic pathological findings and kidney culture
- The highest probability of a kidney being culture positive was when serology and pathology were combined as a diagnostic test particularly when Pomona was involved.
- There is a significant risk of exposure to people working in areas of the processing plants that can come into contact with kidneys additional to the risk of contact with urine *per se.*
- While serology, gross and histopathology are useful indicators that kidney culture may be positive, particularly at the herd level, evidence is presented that shows there is a risk, albeit lower, that kidneys without lesions and from serologically negative deer may carry leptospires in their kidneys.
- If no deer from a line of deer have kidney lesions, it could be assumed that there is a lower risk of the kidneys being infected.

- The daily risk of exposure to humans in slaughterhouses would vary between and within herds depending on the leptospiral infection status in the herd and the number of deer carcass handled daily by each worker.

Chapter 9

General discussion



9.1 Introduction

Studies presented in this thesis were designed to investigate the epidemiology of leptospirosis in farmed deer, its regional and national seroprevalence, the efficacy of a vaccine for control, including the influence of maternally derived antibody, and analysis of the risk of exposure to humans. The study employed serology, bacteriology and pathology as determinants of infection and vaccine efficacy in a natural infection environment and under commercial farming practices. This chapter discusses the combined results from this research and their implications, addresses some of the limitations experienced when employing this research methodology, proposes areas for further research, provides recommendations for a vaccination programme and makes suggestions for industry to consider in relation to how to respond to the findings of this research.

9.2 Serovars, serology and bacteriology

This study concentrated on serovars Hardjobovis, Pomona and Copenhageni largely because review of the literature (Ayanegui-Alcérreca et al 2006) indicated culture evidence in deer for only these serovars in New Zealand. While positive culture evidence is the definitive evidence of infection in a population (Faine et al 1999; WHO 2001), serology was chosen as the primary study tool because at the population level there is a high correlation between serology and infection and is considered a reliable tool, being regarded as the "gold standard" for epidemiological studies (OIE 2000). Serology permits identification to the serovar level, and is the most logistically applicable tool for studies such as those reported here. Culture was performed to validate serology findings, to confirm urine Dark Field Microscopy observations and to evaluate vaccine responses and risk of exposure to humans. Culture confirmed serovars Hardjobovis and Pomona only. When combined with serological evidence, this suggests that infection with Copenhageni is not common in farmed deer. Serovars Tarassovi, Balcanica and Ballum have been identified by culture in New Zealand in other animal species and Australis and Canicola have been identified in humans (Midwinter 1999; Marshall and Manktelow 2002). Serological reports for these serovars in deer have been presented by Flint et al (1988) and Wilson et al (1998). Study of those serovars was beyond the scope and resources for this study when balanced against the likely importance of the serovars studied, although further research is needed to establish their significance. Duplicate sera from all samples collected during this study have been stored in a serum bank to allow such studies.

9.3 Epidemiology

Study design allowed analysis at the herd and individual animal level for both the intensive longitudinal studies (Chapters 3 and 4) and the regional seroprevalence survey (Chapter 7). Evidence for infection was found for serovars Hardjobovis and Pomona.

The availability of the serum bank from a previous longitudinal study containing samples from deer of both sexes and three age-groups over a 2-year period (Audigé 1995) provided a unique opportunity to establish substantial serological evidence of infection and its cycle on commercial deer farms. These data (Chapter 3) defined age- and sex-related seroprevalence contributing to the design of subsequent studies, which accordingly focussed largely on animals in the first year of life. In addition, re-sampling of a number of those herds during this

study provided useful information on persistence of infection, also helping to define host:serovar relationships.

Combination of data from that study and the individual farm studies in Chapter 4 allow robust conclusions to be drawn about the epidemiology of infection on commercial deer farms. It is notable that the seroprevalence and seroconversion patterns to both Hardjobovis and Pomona in deer shown here closely resemble those shown by research in dairy cattle in New Zealand in the late 1970s, prior to implementation of a vaccination programme (Hellstrom 1978; Mackintosh 1981; Cordes et al 1982).

For Hardjobovis, an endemic cycle of infection was shown with the highest seroprevalence in deer at 1-2 years of age. Dark field microscopy evidence showed that infection can occur by three months of age, with generally increasing seroprevalence through the first year of life. Hardjobovis infection is characterised by high seroprevalence, low titres, absence of clinical evidence, mild kidney lesions but high urine shedding and kidney culture rates. It appears that infection with this serovar is persistent, since evidence was maintained for at least two years in all infected herds in Chapters 3 and 4, and all but one herd re-sampled (n 7) 10 years later (Chapter 3) remained seropositive. The pattern of seroconversion appeared to be influenced by climate as is known for leptospirosis, but this research did not specifically set out to study the influence of climate. This study was conducted during climatic extremes from severe drought to severe flooding, with a higher seroprevalence during the wet year, but this may have been confounded by stage of the infection cycle, particularly for Pomona.

For Pomona, an epidemic cycle of infection was apparent, with no year or age relationship. Infection was characterised by high prevalence within infected herds, high titres, clinical and subclinical evidence of disease, severe kidney lesions, but a lower culture rate from kidneys and urine than for Hardjobovis. In a longitudinal study involving three farms, it was fortuitous that infection with Pomona occurred on Farm 2 early in the study, permitting observation of a developing infection. Farm 3 was heavily infected with Pomona before observations commenced, and data suggested that active infection persisted for at least 12 months from initial screening. Data also suggested that infection was not as persistent as for Hardjobovis given that the regional survey (Chapter 7) showed about 20% of herds (n = 110) were infected with this serovar, and repeat sampling of some herds after 10-12 years (Chapter 3) showed the same prevalence but with infection in different herds. This indicates that infection with Pomona is apparently sporadic, sometimes causing clinical and/or subclinical disease, and possibly being self-limiting. Further longitudinal studies would be needed to fully characterise infection with this serovar, including risk factors for occurrence of disease outbreaks.

The regional survey (Chapter 8) provided evidence of infection with both serovars throughout New Zealand with the most prevalent serovar being Hardjobovis, but with a significant proportion of herds demonstrating Pomona and dual infection.

Thus, epidemiological evidence confirms that infection with leptospirosis is widespread on deer farms throughout New Zealand with a high rate of shedding likely on many properties, as suggested in smaller and more localised earlier surveys by Flint et al (1988) and Wilson et al (1998). Therefore, the risk of clinical disease, particularly associated with Pomona in deer and transmission of either serovar to humans (summarised in Chapter 8) is significant. While the number of reports *per se* of clinical disease associated with leptospirosis in deer in New Zealand is not high (Wilson and McGhie 1993; Fairley et al 1984 and 1986, Flint et al 1986,

Dean et al 2005; Ayanegui-Alcérreca et al 2006a), those reports do not permit estimates of disease prevalence. Since the summary of veterinary diagnostic reports by Wilson and McGhie (1993), clinical leptospirosis has been recorded in laboratory reports of deer diseases in New Zealand more commonly than any other disease (Ayanegui-Alcérreca et al 2006a). Given the nature of infection demonstrated by studies in this thesis, particularly for Pomona, and that investigation of disease and mortalities on deer farms is not common (Mackintosh and Wilson 2003; Wilson 2004), it is the author's belief that since sudden death caused by leptospirosis can be readily confused with other causes, the clinical expression of leptospirosis on deer farms in New Zealand is under-estimated. Of possibly more significance is its subclinical influence, possibly of either serovar, as a contributor to outbreaks of other disease, such as yersiniosis, other individual disease syndromes as observed on Farm 3 during this study, or reduction of growth and reproductive rates. After vaccination, the disease and mortality incidence rate declined sharply on Farm 3 on which infection with Pomona appeared to be particularly active, and a reduction in the number and severity of kidney lesions in deer from that farm compared with previous years was reported (unsolicited) from deer slaughter premises. Similar observations were recorded on Farm 2. More and accurate data are needed on the causes of clinical and subclinical disease on deer farms, and the role that leptospirosis plays.

9.4 Host serovar relationship

Observations from the epidemiological studies in Chapters 3, 4 and 7 allow host:serovar relationships to be proposed, based on conventions described by Hathaway (1978) combining seroprevalence, titre range, duration of infection and severity of infection. Hardjobovis is a host-adapted serovar in deer at animal and population levels, similar to that serovar in cattle (Hellstrom 1978), Pomona and Tarassovi in pigs (Ryan 1978: Bolt 1990), Balcanica in possums (Hathaway (1978) and Ballum and Copenhageni in rats (Hathaway 1978; Broukei 1977).

Pomona is a non-host adapted serovar in deer at the individual animal level, but deer can serve as hosts at the herd and population level, similar to observations in cattle in New Zealand (Hellstrom 1978, Mackintosh 1981). From reviewed evidence combined with data from this thesis, it is proposed that Copenhageni is also a non-host adapted species in deer, as in cattle (Hellstrom 1978).

9.5 Zoonotic risk

This is the first substantial study quantifying the risk of exposure from deer to humans. Urine shedding was demonstrated on study farms from an early age with the highest rate of shedding toward 12 months of age, the age at which most deer are slaughtered. At any one time during this study, up to 40% of urine samples were positive for leptospires. About 70% of non-vaccinated deer shed leptospires in urine at some time during the study in their first year of life. The actual rate would be higher because the techniques used here are not 100% sensitive, but are regarded as 100% specific. Similar shedding patterns have been observed in cattle (Hellstrom 1978; Mackintosh et al 1980). A significant proportion of kidneys were culture positive, indicating frequent exposure to humans handling carcasses.

Combining kidney lesion and culture results from studies in Chapter 4 with previous data (Wilson et al 1998) allowed evaluation of the risk of exposure of humans to leptospires from deer described in Chapter 8. Data show that handlers of live animals and organs after slaughter are at risk of exposure. Risk varied considerably between herds, since the regional survey (Chapter 7) showed about 18% of herds were not infected, and the within-herd prevalence varied greatly depending on serovar and stage of infection (Chapter 4). Evidence from Thornley et al (2002), Bell (2005) and Brown (2005) confirms the risk to deer slaughterhouse workers. Workers on farms would be at risk from contact with urine, particularly during procedures such as checking the sex at marking, rectal ultrasound scanning and artificial breeding, and where yard flooring is wet, predisposing to splashes.

Human leptospirosis is "hyper-endemic" in New Zealand (Crump et al 2001; Thornley et al 2002). There is an obligation under Occupational Safety and Health legislation in New Zealand to manage known risks and this has been addressed by the dairy and pig industries. It is probable that action may be required by the deer industry now that the risks have been quantified by data in this thesis. However, to keep this in perspective, despite similar shedding rates in non-vaccinated dairy cattle to those observed here in deer, the relative on-farm risk of transmission from dairy cattle is higher than in deer because of the higher frequency of animal handling, and the design of milking sheds predisposing to contamination with urine.

While a preliminary risk analysis of exposure to workers in one DSP has been done comparing risk to sheep slaughterhouse workers (Dorjee et al 2006), further research is needed both to quantify exposure in different DSPs, since systems differ, as well as at different points in the processing chain.

9.6 Vaccination

Vaccination is an internationally recognised tool for the control of leptospirosis aimed at reducing the renal carrier state as a means of limiting the cycle of infection (Ellis 1984; Bolin and Prescott 1999; Faine et al 1999). In New Zealand, research in cattle and pigs demonstrated that long-term vaccination programmes are effective in reducing shedding and infection rates as reviewed by Marshall and Manktelow (2002) and Dowd (2003). Vaccination of dairy cattle in New Zealand began in the early 1980s (Marshall and Manktelow 2002) when the disease rate in humans was high (Mackintosh et al 1982). Since then, the number of human cases of leptospirosis has fallen from almost 600 to less than 100 (Baker and Lopez 2004), with only 10% of recent cases associated with dairy workers. The number of cases in farm workers other than dairy, and in meatworkers mainly handling animal types not commonly vaccinated (sheep, beef and deer), increased in the years 1999 to 2003. If notified human cases were regarded as a monitor of infection in animals, these data show vaccination of livestock to be highly effective in reducing the public health risk of this disease. While antibiotic, management and environmental means for control of leptospirosis are effective in intensive animal industries (Bolin and Prescott 1999), in pastoral systems, vaccination is the most effective option (Marshall et al 1979; Mackintosh et al1980; Little et al 1992). Thus, significant focus was given by this research to evaluate a candidate vaccine in deer.

The conventional method for evaluation of vaccine efficacy is challenge, comparing infection and disease response in vaccinated and non-vaccinated animals to artificial infection in a

controlled environment. Efficacy is defined by protection of 80% of animals from infection based on kidney colonisation, shedding and clinical disease and/or mortality (Thrusfield 1995; Bolin et al 1989). Those studies usually employ high challenge doses and routes of administration that may not reflect natural challenge, animals are challenged uniformly and are immunologically naïve before vaccination. Furthermore, challenge strains are commonly laboratory-adapted rather than field strains and sometimes are not from the same species as the test species. These circumstances vary significantly from the natural infection in pastoral farming situations. There is growing animal welfare and ethical concern about challenge studies for vaccine evaluation. The Agricultural Compounds and Veterinary Medicines Group of the New Zealand Food Safety Authority allows extension of label claims to additional species for some vaccines, based on sero-equivalence alone (http://www.nzfsa.govt.nz/acvm/publications/stasndad-guidelines/vaccine.pdf 2004), and this is understood to be the basis for the licensing of leptospiral vaccines for deer and/or cattle in New Zealand ("Leptavoid-3" (Hardjobovis, Pomona and Copenhageni), "Leptavoid-2" (Hardjobovis and Pomona) (Schering-Plough Animal Health Ltd) which was the vaccine used in the research presented in this thesis, "Leptoshield" Pfizer Animal Health trivalent and bivalent vaccine (which is the CLS technology of "Spirovac" from Pfizer UK), and the 7-in-1 multivalent combination "Ultravac 7-in-1") (Hardjobovis, Pomona and *Cl. perfringens* type D, *Cl. tetani*, *Cl. septicum*, *C. chauvei* and *Cl. novyi* type B) Pfizer Animal Health NZ (Index of Veterinary Specialties, New Zealand 2005).

This study confirmed that serological responses to the vaccine in infected and non-infected deer herds were similar to those observed in cattle using the same vaccine. One unpublished challenge trial (Schering-Plough Animal Health, 1985, pers. comm.) using the vaccine studied in the present research, demonstrated that none of 14 vaccinated deer and 6/16 (37.5%) of non-vaccinated controls showed evidence of shedding. No similar challenge study has been done with Pomona, and no evaluation has been reported in the face of natural challenge in deer.

In field trials involving ongoing natural infection, it is not expected that the convention of 80% reduction of infection would be achieved in response to the first vaccination cycle. There is no opportunity for comparable immuno-naïve control animals used in challenge studies because of continued natural challenge and, possible prior natural immunity. However, in practice, the application of a vaccine is in a commercial farming situation where it is intended to reduce and possibly eliminate infection over a period of time through sustained herd-based immunity, with primary protection from challenge *per se* being targeted for young animals. Leptospiral vaccines are not fully effective in eliminating the carrier state of already infected animals and may not completely protect all animals from infection in the face of continuing challenge (Hancock et al 1984). In the long term, reduction of the number of animals infected, the number shedding and consequently environmental challenge should diminish if a sustained immunity of >80% is achieved in a herd. Combined evidence from New Zealand and the United Kingdom from dairy cattle (Broughton et al 1984; Little et al 1992) demonstrated that in Hardjobovis infected closed herds, when herd immunity was sustained by vaccination for at least 5 years in herds, infection could be eliminated, as confirmed by lack of seroconversion either in introduced seronegative non-vaccinated sentinel animals or patterns of continued low seroprevalence and low titres over time in vaccinated replacement progeny typical of non-infected non-challenged animals.

Thus, field evaluation was chosen as the study method for the vaccine used in this thesis to establish the pattern of response to first vaccination in a natural challenge situation

experienced in normal farming conditions with the aim of providing data to allow prediction of the long term expectation of the vaccine in that environment. The reduction of urine shedding, kidney and urine culture (Chapter 5) is strong evidence that the vaccine is behaving in deer in a manner similar to that reported in cattle and pigs (Marshall et al 1979; Marshall et al 1982; Hancock et al 1984; Hodges et al 1985; Bolt and Marshall 1990). This is supported by the growth and reproduction responses observed in Chapter 5. The similarity with cattle permits tentative extrapolation that sustained and appropriately applied vaccination of deer herds may result in continued reduction of shedding and possibly elimination of infection in closed deer herds without environmental exposure from neighbouring non-vaccinated livestock. This hypothesis is proposed for testing (see Section 9.10)

This study was not designed to evaluate vaccine efficacy at the herd level. Only a small proportion of the animals in the study herds were vaccinated in an endeavour to minimise disruption of the natural course of infection, since these herds were used simultaneously for studies of the epidemiology of infection. Thus, vaccinated animals on Farm 1 throughout, and on Farms 2 and 3 early in the trials, faced significant ongoing challenge, which would not occur had all animals on the farms been vaccinated at the start of the trials. On Farms 2 and 3, a whole-farm vaccination programme was adopted as the study progressed because of the risk of disease outbreaks, but the long-term impact of this decision was beyond the scope and timing of this study.

Difficulties in recruiting farms because of pressures upon farmers in the face of severe drought and then floods, determined that the preferred vaccination start date for progeny of early March was not possible on all farms, further reducing the chances of showing the full effectiveness of the vaccine, since a higher proportion of animals were infected by the time vaccination was given. Thus, it is recognised that this experimental design does not allow the full potential of the vaccine to be established. Despite these constraints, a significant reduction in infection was observed.

Responses to vaccination in young and adult animals on infected farms, coupled with studies of the influence of maternally derived antibody (Chapter 6), allows a vaccination regime to be proposed:

- In herds not currently vaccinating, all deer should be given a sensitiser vaccine at a time to fit normal management practices that permits a booster dose to be given four weeks later.
- Once the herd has been vaccinated as above, in following annual production cycles, young deer should be sensitised at about 12 weeks of age, early in March, followed by a booster four weeks later. Initially an additional booster should be given at 8-10 months of age to animals being retained on the farm, since antibody waned from about that time, and in preparation for the next infection cycle. As herd immunity becomes established, the need for this additional booster may be re-evaluated based on further research (see below). Adult hinds should be boosted annually within 4 weeks of calving, along with first calving hinds, to enhance maternally derived antibody, and stags should be boosted annually at a convenient time, such as at velvet removal or Tb testing. The latter are to ensure maintenance of herd immunity.Long-term research is needed to validate this proposed programme.

Effective control will only be achieved if other sources of infection are managed. Newly introduced deer are a known risk of infection (Wilson and McGhie 1993; Dean et al 2005)

and should be vaccinated prior to introduction to vaccinated herds. Antibiotic treatment (Streptomycin, 25 mg/kg, or Oxytetracycline 10mg/kg) would be advised to further reduce risk of introduction of leptospires. Environmental challenge from neighbouring farms must be considered. A recent survey (Castillo-Alcala et al 2006) and the regional survey questionnaire in Chapter 7 showed 85% and 79% of deer, respectively, were farmed in association with sheep and/or cattle. Infection of beef cattle with Hardjobovis and Pomona in New Zealand is known (Dowd 2003), and recent evidence showing infection with those serovars in sheep has been presented (Dorjee et al 2004; West et al 2004). However, the role of each species in mixed-species farming is not known and should be the subject of further research, as should the role of vaccination of various species.

9.7 Production responses

The growth and reproduction responses observed in this thesis require replication using appropriate methodology. However, both of those responses are plausible. In this study and elsewhere (Wilson et al 1998), the severity of kidney lesions observed in some animals in herds heavily infected, particularly with Pomona, was substantial. While clinical losses were not always observed or reported, it is likely that metabolic function could have been affected, particularly during the establishment phase of infection, resulting in reduction of growth rate and food conversion efficiency. While it has not been proven that abortion or neonatal losses result from leptospiral infections in deer, these are well known in cattle (Ellis 1984, 1994) and pigs (Ellis 1999). Abortion and neonatal losses are difficult to detect by direct observation in commercial farmed deer herds. It should be noted that these responses appear to be associated with Pomona. Given that infection with this serovar is relatively prevalent, and that infection is sporadic, it is probable that a high proportion of deer farms may be at risk of production losses associated with this serovar over time.

9.8 Proposed industry action

It is likely that the deer industry will need to address the issues of leptospiral infection of deer based upon data presented in this thesis, as discussed above. A number of options have been proposed by the researchers as summarised by Wilson et al (2005) ranging from industry-wide vaccination supported formally by industry and the veterinary profession, targeted control programmes on farms, through to management of risk at DSPs. It is the opinion of the author that the most effective and logistically feasible option to reduce risk to both animals and humans is vaccination of all herds as above, with a vaccine with demonstrated efficacy in deer. However, adoption of vaccination could take a number of years to significantly reduce the incidence, depending on the rate of farmer uptake. Dowd (2003) estimated it would take 7-9 years to achieve a real reduction in sheep, although adoption of vaccination in the dairy industry in the early 1980s resulted in a rapid reduction of human cases over 3-4 years (Baker Lopez, 2004).

9.9 Study limitations

Limitations associated with conducting research on commercial deer farms including identification of candidate farms, recruitment, timing of events, management and climate, and ability to produce substantial data of production responses have been discussed above, as has the need to confine this study to serovars Hardjobovis, Pomona and Copenhageni.

This study focussed solely on deer despite the presence of other susceptible livestock on Farms 2 and 3, which may well have been involved with the epidemiology of infection on those properties, because it was beyond the logistical and financial capability of the project to extend observations, and lack of facilities for handling beef bulls for sampling. However, infection of those species and the role of the environment and grazing management in the spread of infection are well known, requiring no replication.

The studies in Chapter 4 were concentrated on young animals, although adult hinds were studied on Farm 2. While justification for this has been presented above, further information could have been gathered from sampling older animals. However, significantly robust data from older animals were available from the longitudinal study in Chapter 3 involving 15 farms, and thus do not require replication. Cohort groups could not be studied for long periods sufficient to make robust conclusions about shedding persistence beyond nine months. However, indirect evidence suggests shedding for significantly longer periods.

Vaccination studies (Chapters 5) were confined to the first 19 months of life or less and involved only a small sub-sample within infected herds rather than whole-herd vaccination, as discussed above. Kidney pathology observations were constrained by the number of animals that each farmer sent to slaughter, limiting statistical power and therefore the ability to detect differences in kidney lesions between vaccinated and control animals. This was confounded by the fact that many animals were infected before vaccination commenced, particularly on Farm 3 when vaccination commenced in July, thus lesions would have been evident before vaccination in many animals as shown in some necropsies performed on that property. However, robust data on shedding were produced, sufficient to show significant effects and to demonstrate similarities with the vaccine in other species, allowing defensible proposals on the likely outcome of whole herd vaccination. In studies of the influence of maternal immunity (Chapter 6), colostrum and earlier blood samples could not be collected because of both the risk to animals and due to the fact that they were involved in other research simultaneously, requiring compromise in experimental design.

Random identification and recruitment of farms for the regional survey (Chapter 7) was not possible for reasons discussed above, and it was impossible to achieve the calculated numbers of farms in some regions because of the inability of veterinary practitioners to fulfil requests of the researcher. It was beyond the time and financial resources of the project for the researcher to undertake sampling at distant locations. However, in the event, the number of farms was sufficient in most regions and overall, because the seroprevalence was higher for both Hardjobovis and Pomona than used for estimation of sample size required for that study.

Analysis of risk of exposure to people presented in Chapter 8 was opportunistic, harnessing data from this thesis and other studies. Better estimates would be possible with a study designed specifically to address the question. Alternatively, more substantial conclusions would have been possible had more data been available from randomly selected animals for bacteriology.

The DFM technique for detection of urine shedding, coupled with the low culture rate from urine and the low sensitivity of culture *per se*, resulted in under-estimation of shedding rates because the sensitivity of those tests is not 100%. Utilisation of other diagnostic techniques such as immunofluorescence or PCR may have improved the sensitivity of detection of

infection in kidney and urine samples. However, those techniques would require validation in deer.

9.10 Further research

While this study has provided significant data on the epidemiology of leptospirosis in farmed deer and the likely effectiveness of a vaccine, it has identified several areas for further research:

- Long-term evaluation of whole-herd vaccination in terms of shedding, kidney lesions, serological profiles, other laboratory-based techniques as below, and use of introduced sentinel animals
- The interaction between deer and other livestock species in multi-species farming systems and the most appropriate control strategies for those situations
- Determination of the significance in deer of serological evidence of infection with serovars not studied in this research,
- Whether there are deer-adapted strains of *Leptospira* and whether leptospires in deer are utero-trophic, or can be found in semen and reproductive organs in the male
- Validation of means of diagnosis such as PCR, immunofluorescence, immunoperoxidase and cellular immunity as supplements or alternatives to serology to permit evaluation of vaccine effectiveness
- Evaluation of effects of leptospirosis on expression of disease and production losses including growth, body condition, foetal and neonatal survival, and productive lifespan. Evaluation of acute phase protein, kidney and liver function tests could be useful markers.
- Further data to quantify the risk of exposure to humans on-farm and at DSPs, including its presence in other organs
- Further longitudinal studies to fully characterise infection with Pomona, including risk factors for occurrence of disease outbreaks.

Chapter 10

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10. References

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Appendix 1 Raw data

CHAPTER 3
(Serum Bank)

Appendix 1 General data (No =Sample number, Farm= Farm identification number, Animal identification (A ID), Age/Sex (a=adult, y=yearling, w=weaner, h=hind, s=stag), MAT results by serovar-(titre)and sampling month (Mar.= March, Jun.=June, Sep.=September, Dec. December).

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
1	1	27	ah						0							0							0	
2	1	40	ah		0				192			0				0			0				0	
3	1	60	ah						96							0							0	
4	1	108	ah						24							0							0	
5	1	810	ah						384	48						0	0						0	0
6	1	4864	ah	0	0	0					0	0	0					0	0	48				
7	1	4978	ah	0	24	96			48	48	0	0	24			48	96	0	0	24			0	48
8	1	b37	ah	0	192	0					48	384	0					0	0	0				
9	1	B71	ah	0							0							0						
10	1	b80	ah	0							0							0						
11	1	b9	ah	48							0							0						
12	1	kt/27	ah						384	384						0	0						0	48
13	1	o78	ah						192	96						0	0						0	0
14	1	y526	ah	0	0	0			24		0	0	0			0		0	0	0			0	
15	1	y708	ah			0							0							0				
16	1	y870	ah			192			96	24			0			0	0			0			0	0
17	1	y974	ah	0							0							0						
18	1	21	as						24							0							0	
19	1	820	as						384	96						24	24						0	0
20	1	936	as						384							0							0	
21	1	4602	as		48	24			96	0		48	0			0	0		0	0			0	0
22	1	4704	as		48	48						0	0						0	0				
23	1	4822	as		96	768						192	0						0	0				
24	1	b70	as	0	48	48			48	24	0	0	0			0	0	0	0	24			0	0
25	1	y90/47	as						24	48						0	0						0	0
26	1	y920	as		96	96			48	48		0	0			0	0		0	0			0	24
27	1	20	wh						0							0							0	
28	1	25	wh						0							0							0	
29	1	46	wh						384							0							0	
30	1	54	wh						0							0							0	
31	1	2501	wh						768	48						0	0						0	0
32	1	2524	wh						0	96						0	0						0	0
33	1	2527	wh						192	96						0	0						0	0

	General data			Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
34	1	2544	wh						0							0							0	
35	1	2547	wh						24	192						0	0						0	0
36	1	4180	wh		0	192			24			0	0			0			0	0			0	24
37	1	4182	wh		0	0			48	0		0	0			0	0		0	0			0	0
38	1	y163	wh		0	0			0	0		0	0			0	0		0	0			0	0
39	1	y181	wh		0	0				0		48	0				0		0	0				0
40	1	26	ws						0							0							0	
41	1	30	ws						192							0							0	
42	1	49	ws						0							0							0	
43	1	1127	ws		0	24		96	0	192		0	0		0	0	48		0	0		48	0	0
44	1	2009	ws						24	96						0	0						0	0
45	1	2010	ws					0	0	96					0	24	0					0	0	0
46	1	2012	ws						0	96						0	24						0	0
47	1	2037	ws						24	96						0	0						0	0
48	1	2044	ws						96	48						0	0						0	0
49	1	4112	ws		0	0		0	1536			0	0		0	0			0	0		48	0	
50	1	4123	ws		0	0		0	0	192		0	0		0	24	0		0	0		48	0	0
51	1	y119	ws		0	0		0	0	96		0	0		0	0	0		0	0		192	0	24
52	1	y142	ws		0	24		0	3072	192		384	0		0	24	0		24	0		96	0	0
53	1	y152	ws		0	0			0	0		0	0			0	0		0	0			0	0
54	1	17	yh						1536							0							0	
55	1	106	yh						192							0							0	
56	1	184	yh						0							0							0	
57	1	4062	yh		0	0			96			0	0			0			0	0			0	
58	1	4066	yh		0	0						0	0						192	0				
59	1	y054	yh		0	0						48	0						96	0				
60	1	y084	yh		96	192						0	0						0	48				
61	1	27	ys						0							0							0	
62	1	48	ys						96							0							0	
63	1	4010	ys		0	0						0	0						0	0				
64	1	y022	ys	24	0	0					0	0	0					0	0	0				
65	1	y028	ys	0	0	0					0	0	0					0	0	0				
66	1	y032	ys	0	0	0					0	0	0					0	0	0				

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992			Pomona-1993			Copenhageni-1992					Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
67	1	y038	ys	0	0	0			0	48	0	0	0			0	0	0	0	0			0	0
68	2	0 038	ah			96		0	96	192			24		0	0	24			0		48	0	0
69	2	952	ah					0							0							24		
70	2	g10	ah					96	384	48					0	96	0					0	0	0
71	2	g22	ah			48							0							0				
72	2	g58	ah						192	192						384	48						0	0
73	2	g63	ah						48	24						0	0						0	0
74	2	g68	ah			24			96	0			24			0	0			0			0	0
75	2	r53	ah					0							0							0		
76	2	r55	ah					0							0							24		
77	2	w016	ah			96		0	48	192			0		0	0	24			0		24	0	0
78	2	w112	ah			48							0							0				
79	2	w145	ah			24		0	96	384			0		0	0	24			0		24	0	48
80	2	y16	ah			48			48	1536			0			24	0			0			0	0
81	2	y255	ah			48							0							48				
82	2	y55	ah			96							0							0				
83	2	b22	wh					0	96	0					0	0	0					24	0	0
84	2	b48	wh					0	48	0					0	0	48					48	0	0
85	2	b60	wh					0	0	0					0	0	0					192	0	24
86	2	b63	wh					24	0	0					48	0	0					0	0	0
87	2	b97	wh					24	0	0					0	0	0					0	0	96
88	2	y11	wh			768							0							48				
89	2	y131	wh			48							0							0				
90	2	y25	wh			768		0	768	3072			0		0	0	0			0		0	0	0
91	2	y38	wh			384			192	192			48			0	0			0			0	48
92	2	y5	wh			0		0					0		0					0		0		
93	2	b131	ws					48	24	0					0	0	0					0	0	0
94	2	b16	ws					96							0							0		
95	2	b164	ws						0	0						0	24						0	0
96	2	b61	ws					48							0							24		
97	2	b91	ws					192	48	0					0	384	0					24	0	0
98	2	y187	ws	48		384					0		24					0		24				
99	2	y24	ws	24		48					0		0					0		0				

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
100	2	y32	ws	96		0					0		0					0		0				
101	2	y37	ws	24		0					0		0					0		0				
102	2	y77	ws	24		24					0		0					0		0				
103	2	r1	yh	192				0			0				48			0				48		
104	2	r109	yh	96		384		0			0		24		0			0		0		0		
105	2	r28	yh	96		96					0		24					0		24				
106	2	r43	yh	192		24					0		24					0		0				
107	2	r51	yh	384		96		0			24		0		0			0		0		0		
108	2	y113	yh						96	48						0	0						0	0
109	2	y123	yh						96	48						0	0						24	0
110	2	y149	yh						24	0						0	0						0	0
111	2	y17	yh						48	192						0	0						0	0
112	2	y183	yh					48		48					0		0					0		24
113	2	y21	yh					0	96	0					0	0	0					0	0	0
114	2	y22	yh					0	192	384					0	48	0					0	0	0
115	2	y47	yh					0							0							0		
116	2	y82	yh					0	48	0					0	0	0					48	0	0
117	2	y91	yh					48	96	96					0	0	0					48	24	48
118	2	r14	ys			0							24							0				
119	2	r29	ys			96							48							0				
120	2	r33	ys			96							24							0				
121	2	r65	ys			96							0							0				
122	2	r86	ys			96							0							0				
123	3	63	ah	48	96	0					0	0	24					0	0	0				
124	3	93	ah						0	192						0	0						0	48
125	3	98	ah						0	0						0	0						0	0
126	3	9905	ah	384	384	192					0	0	0					0	0	0				
127	3	p21	ah						0	0						0	0	0					0	0
128	3	w504	ah	48		96			48	96	0		0			0	0			0			0	0
129	3	y503	ah	48	192	96					0	0	0					0	0	0				
130	3	y70	ah	0		0					0		0					0		0			0	
131	3	y930	ah	0		0					0		0					0		0				
132	3	9123	as						0	0						0	0						0	0

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
133	3	r822	as		48	48			24	24		0	0			0	0		0	0			0	0
134	3	r966	as		96	96						0	0						0	0				
135	3	R967	as		384	192						0	0						0	0				
136	3	r968	as		48	384						0	0						0	0				
137	3	r969	as		768	96			96	96		0	0			0	0		0	0			0	0
138	3	r974	as		96	192						0	0						0	0				
139	3	y1706	as		0	0	0		0	0		24	0	0		0	0		0	0	0		0	0
140	3	y703	as	0	0	24	0		0	48	0	0	0	0		0	0	0	24	24	0		0	0
141	3	y705	as		0	0						24	0						0	0				
142	3	104	wh		0	0	0		0	0		48	0			0	0		0	0			0	0
143	3	116	wh	48	0	0	0				24	0	0	24				0	0	0	0			
144	3	118	wh	0		0	0				24		0	24				0		0	0			
145	3	122	wh	48	0	0	0				0	48	0	0				0	96	0	0			
146	3	124	wh	48	0	0	0				0	0	0	0				0	0	0	0			
147	3	215	wh						0	0						0	0						0	0
148	3	o209	wh						48	0						0	24						0	0
149	3	o210	wh						96	0						0	24						48	0
150	3	o218	wh						0	0						0	0						0	0
151	3	o220	wh						0	0						0	0						0	0
152	3	126	ws	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
153	3	127	ws	384	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	48
154	3	128	ws	24	0	24	0	0	0	0	24	0	24	24	0	0	0	0	0	0	0	24	0	0
155	3	129	ws		0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	24	0	0
156	3	130	ws	24	0	96		0		0	0	0	0		0		0	0	0	0		0		0
157	3	o231	ws						48	0						0	0						0	0
158	3	o234	ws						0	0						0	24						0	0
159	3	o237	ws						0	0						0	0						0	0
160	3	o243	ws						0	0						0	0						24	0
161	3	o249	ws						0	0						0	0						0	0
162	3	o101	yh						0	0						0	0						96	48
163	3	o108	yh						0	0						0	0						48	0
164	3	o109	yh						48	0						0	24						0	24
165	3	o118	yh						0	0						0	0						0	0

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
166	3	p15	yh	0	0						0	0						0	0					
167	3	p21	yh	0	0						0	0						0	0					
168	3	p36	yh	0	0						0	0						0	96					
169	3	p40	yh	0	192				0	0	0	0				0	0	0	0				0	0
170	3	o130	ys						0	0						0	0						0	0
171	3	p82	ys	48	48	192	96				24	0	0	24				0	0	0	0			
172	4	0 08	ah	0	0	24					0	0	0					0	0	0				
173	4	120	ah	24	0	0			24	0	96	0	0			0	0	0	0	0			0	0
174	4	127	ah	0	0	0				0	0	0	0					0	0	0				
175	4	g7	ah						0	0						0	0						0	0
176	4	y27	ah						0	0						0	0						0	0
177	4	y37	ah						24	0						0	0						0	0
178	4	y43	ah					0	0	0					0	0	0					0	0	0
179	4	y6	ah	24	96	96		0	24	48	0	96	0		0	0	0	0	0	0		0	24	0
180	4	83	wh					0	0	0					0	0	0					0	0	0
181	4	90	wh					96	0	0					0	0	0					0	0	0
182	4	94	wh						0	0						0	0						0	0
183	4	95	wh					0	0	0					0	0	0					24	0	0
184	4	132	wh					0	24	0					0	48	0					24	0	48
185	4	166	wh	0	0	0	0				0	48	0	0				0	48	0	0			
186	4	1129	wh	0	0	24	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0
187	4	g42	wh	0	0	24	0				24	0	0	0				0	48	0	0			
188	4	y59	wh	0	0	48	0	0	0	0	0	0	0	24	0	0	0	0	24	0	0	48	0	24
189	4	y62	wh		0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	0	0
190	4	106	ws					0	0	0					0	0	0					0	0	0
191	4	115	ws					0	24	0					0	0	0					0	0	0
192	4	119	ws					0	0	0					0	48	96					0	0	0
193	4	122	ws		0	24		0	24	0		0	0		0	0	0		0	0		24	24	0
194	4	123	ws					0	24	0					0	0	0					48	0	0
195	4	1156	ws		0	48						48	0						0	0				
196	4	1165	ws		0	24						0	0						0	0				
197	4	1j2	ws			0							0							0				
198	4	g49	ws		0	0						0	0						0	0				

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992			Pomona-1993			Copenhageni-1992				Copenhageni-1993			
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
199	4	y33	ws	48	0	24					0	0	0					0	0	0				
200	4	129	yh						48	0						48	48						0	0
201	4	143	yh	24	0	24		0		0	0	0	0		0		0	0	0	0		24		0
202	4	149	yh	0	0	24					0	0	0					0	0	0				
203	4	154	yh					48							0							0		
204	4	155	yh					0	0	0					0	0	0					24	0	0
205	4	165	yh					0							0							0		
206	4	g17	yh	0	0	0			0	0	0	0	0			0	0	0	0	0			0	0
207	4	w49	yh						48	48						48	0						0	0
208	4	y126	yh						0	0						0	0						0	0
209	4	y146	yh	0	24	48					0	0	48					0	24	0				
210	4	y41	yh		0	48		0	48	0		48	48		0	0	0		0	0		0	0	48
211	4	y53	yh					0	0	0					0	0	0					0	0	0
212	4	y56	yh						24	0						0	0						48	48
213	4	y64	yh					0	0	0					0	0	0					0	0	24
214	4	y70	yh						0	0						0	0						0	0
215	5	459	ah			96							0							0				
216	5	y23	ah			768	192		192				0	0		0				0	0		0	
217	5	y64	ah		192	0						0	0						0	0				
218	5	y71	ah			0	0		48				48	0		48				0	0		0	
219	5	y77	ah		0	192	384		48	96		0	0	0		0	0		0	0	0		0	0
220	5	0 014	as					192	48						0	0						0	0	
221	5	0 014	as			96	96						384	0						0	0			
222	5	0 034	as			24							192							0				
223	5	0 037	as		192	1536						0	0						0	0				
224	5	0 043	as	48	768	96	384				48	0	0	0				0	0	0	0			
225	5	0 54	as		96	384						0	0						0	0				
226	5	50	as				96							0							0			
227	5	o33	as						96	96						0	0						0	0
228	5	w10	as						192							0							0	
229	5	w11	as						96							0							0	
230	5	w55	as						0	0						0	0						0	0
231	5	w8	as	0	0			0			0	0			0			24	0			0		

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
232	5	WE3	AS				96	48						0	96						0	0		
233	5	91	wh						0	0						0	0						0	0
234	5	98	wh						48	192						0	24						0	0
235	5	99	wh						24	0						0	0						0	0
236	5	923	wh						48	0						0	0						0	0
237	5	924	wh						96	0						0	0						0	0
238	5	w24	wh		0	24	0	0	3072	768		0	0	0	48	0	0		24	0	0	0	0	0
239	5	w27	wh	24	0	48			24	48	0	0	24			0	0	0	0	0			0	0
240	5	w3	wh		24	192	0		96	0		0	24	0		0	0		24	0	0		0	0
241	5	w6	wh		0	768			0	768		0	48			0	0		0	0			0	0
242	5	b22	ws	0	0	192		0			48	48	0		48			0	0	0		24		
243	5	b23	ws	0	0	48	96	0			0	24	0	0	0			0	0	0	0	0		
244	5	b274	ws	96	0						0	0						0	0					
245	5	b3	ws	96	0	192	0	0			24	48	0	0	24			0	0	0	0	0		
246	5	b32	ws	96	0	96	96	0		192	0	0	0	0	0		0	0	0	24	0	0		0
247	5	b35	ws	24		48					0		0					0		0				
248	5	r14	ws						48							24							0	
249	5	r15	ws						24							24							0	
250	5	r18	ws						48							0							0	
251	5	r24	ws						48	96						24	24						0	0
252	5	r42	ws						96	768						48	0						0	0
253	5	b130	yh		0	24						0	0						0	0				
254	5	b1e	yh	192		24	24				0		0	0				0		0	0			
255	5	b2e	yh	96	0	192			96	768	24	24	0			24	0	0	0	0			0	0
256	5	b40	yh	48	24	3072			768	192	0	48	0			0	24	0	0	0			0	0
257	5	b13	ys	96			0	0	0		0			0	0	0		0			0	192	0	
258	5	b16	ys				0	0	48	192				0	96	24	0				0	0	0	0
259	5	b20	ys						48	0						0	0						0	0
260	5	w14	ys	0	0	384					0	48	48					0	24	48				
261	5	w16	ys	96	24	96		0			0	0	0		48			0	0	0		24		
262	5	w1e	ys	48	0	24				192	0	0	0				24	0	24	24				0
263	5	w2e	ys		0	24	0					48	0	0					0	0	0			
264	6	927	ah						24	0						0	0						0	0

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
265	6	9809	ah		0	0			0	0		48	0			0	0		0	0			0	0
266	6	b21	ah						0	0						0	0						0	0
267	6	b4	ah						24	0						0	0						0	0
268	6	bl48	ah						0	0						0	0						0	0
269	6	g12	ah		24	48						0	0						0	0				
270	6	g3	ah		0							0							0					
271	6	o15	ah		48	24			48	96		24	96			0	0		0	0			0	0
272	6	o16	ah		24	24			48	96		0	24			24	48		0	0			0	0
273	6	y20	ah		96	96						0	24						0	0				
274	6	y646	ah						48	48						0	0						0	0
275	6	b947	as		48	384						0	24						0	0				
276	6	b110	as		192	96						24	48						0	0				
277	6	g804	as		384	96						0	0						0	0				
278	6	r2	as	0	0	0	48	0			0	0	0	0	24			0	0	0	0	0		
279	6	r52	as		96	24						0	0						0	0				
280	6	r687	as	24	48						0	0						0	0					
281	6	102	wh	0	0						0	0						0	0					
282	6	o104	wh	48	24						0	0						0	0					
283	6	w12	wh						0	0						0	0						0	0
284	6	w43	wh						24	0						0	0						0	0
285	6	w51	wh						0	0						0	0						0	0
286	6	w56	wh						0	0						0	0						0	0
287	6	w61	wh						24	48						0	0						0	0
288	6	y100	wh	96	24	0	24		0	0	0	0	0	0		0	0	0	24	0	0		0	0
289	6	y103	wh	3172		0	0		0	0	0		0	0		0	0	0		0	0		0	0
290	6	y104	wh	48		0	0		0	0	0		48	0		0	0	0		0	0		0	0
291	6	y128	wh			24	24						0	0						0	0			
292	6	y136	wh			0	0						0	0						0	0			
293	6	y161	wh		0							0							0					
294	6	y55	wh		0							0							0					
295	6	y56	wh		0							0							0					
296	6	y58	wh		0							0							0					
297	6	2	ws	96							0							0						

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
298	6	945	ws		0				0	0		0				0	0		0				0	0
299	6	955	ws		0							0							0					
300	6	b6	ws						0	0						0	0						0	0
301	6	b66	ws						24	0						192	96						0	0
302	6	o115	ws		24							0							0					
303	6	r 3	ws	24	24	0	24	0			0	0	0	0	0			0	0	0	0	24		
304	6	R 5	ws	48	0	0	0				0	0	24	0				0	0	0	0			
305	6	r1	ws	48		24	0	0			24		0	0	48			0		0	0	24		
306	6	r4	ws	48	24	0	24	0			0	0	0	0	96			0	0	0	0	0		
307	6	141	yh		48							24							0					
308	6	b17	yh	96	0	0					0	0	0					0	0	0				
309	6	b274	yh	0	48						0	0						0	0					
310	6	b28	yh	96	24	0					48	0	0					0	0	0				
311	6	b42	yh		24	0						24	96						0	0				
312	6	b45	yh		0	0			96	0		0	48			48	0		0	0			0	24
313	6	y146	yh						0	0						0	0						0	0
314	6	r18	ys						48	48						24	48						96	0
315	6	r23	ys						0	0						0	0						0	0
316	6	r26	ys						0	0						24	0						0	0
317	6	r27	ys	0				0	0	0	0				48	0	0	0				0	0	0
318	6	r926	ys						48	96						24	48						0	0
319	6	w11	ys		24	0	0					0	0	0					0	0	0			
320	6	w13	ys						0	0						0	0						0	0
321	6	w43	ys		0	0	0		24	0		0	24	0		0	0		0	0	0			0	0
322	6	w44	ys		0	0	0					0	24	24					0	0	0			0
323	6	w45	ys	0	0	0	48		0	0	0	0	0	0		0	0	0	0	0	0		0	0
324	6	w55	ys	0	0	0	0				0	0	0	0				0	0	0	0			
325	6	y136	ys						24	0						0	0						0	0
326	7	92	ah	0	0	0					48	0	0					0	0	192				
327	7	122	ah	24							0							0						
328	7	g3	ah	0		0					0		0					0		48				
329	7	o113	ah	0	0	48					0	0	0					0	0	0				
330	7	y41	ah	0		24			0	0	48		0			0	0	0		0			0	0

General data				Hardjobovis-1992				Hardjobovis-1993				Pomona-1992			Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
331	7	y90	ah						0							0							0	
332	7	940	as	48							0							0						
333	7	r10	as	0	0	48		0	24	0	0	0	0		48	0	0	0	0	48		0	0	0
334	7	r116	as	0	96	0					24	0	0					0	0	0				
335	7	r12	as	0	0	24		0	0	0	96	0	0		0	0	24	0	0	0		0	24	0
336	7	r17	as					0	96	0					24	0	0					0	0	0
337	7	r19	as					0	0	0					0	0	0					0	0	0
338	7	r2	as	0		24					0		0					0		0				
339	7	r3	as					0	0	0					48	0	0					0	0	0
340	7	r53	as			96							0							48				
341	7	y22	as			0			48	0			0			0	0			48			0	0
342	7	y57	wh		0				48	0		0				0	0		0				0	0
343	7	y69	wh						0	0						0	0						0	0
344	7	y72	wh						0	0						0	24						0	0
345	7	y73	wh						0	0						0	0						0	0
346	7	y74	wh						48	0						0	0						0	0
347	7	y75	wh						0	0						0	0						0	0
348	7	654	ws						96							0							0	
349	7	655	ws						48							0							0	
350	7	657	ws						96							0							0	
351	7	674	ws						0	0						0	0						0	0
352	7	675	ws						0	0						0	0						0	0
353	7	973	ws						24	0						0	0						0	0
354	7	141	yh		24	24						0	0						0	0				
355	7	751	yh		0	0						0	0						0	0				
356	7	p29	yh		0	48						0	0						0	48				
357	7	p39	yh		0	0			48	0		0	0			0	0		0	96			0	0
358	7	y48	yh		0	48			0	0		0	0			0	0		0	0			0	0
359	7	y51	yh						0	0						0	0						0	0
360	7	y55	yh						0	0						0	0						0	0
361	7	y56	yh						24	0						0	24						0	0
362	7	y61	yh						48	0						0	0						0	0
363	7	660	ys						96							0							0	

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
364	7	662	ys						0	0						0	0						0	0
365	7	931	ys	0	0	0					0	0	0					0	0	0				0
366	7	955	ys					0	24	0					48	0	0					24	0	0
367	7	957	ys		0	0		0	48	0		0	0		0	0	0		0	0		0	0	0
368	7	958	ys					0	0	0					0	0	0					0	0	0
369	7	962	ys					0		0					0		0					0		0
370	7	g42	ys	0		24					48		0					0		48				
371	7	o171	ys	24	0	24					0	0	0					0	0	24				
372	7	o45	ys	0		96					0		0					0		96				
373	8	18	ah	192							0							0						
374	8	95	ah	384							0							0						
375	8	140	ah	48							0							0						
376	8	913	ah	96							24							0						
377	8	oy55	ah	48	24	48				48	24	0	0				0	0	0	0				0
378	8	pap209	ah	192	96	96			384	96	0	0	0			0	0	0	0	0			0	0
379	8	pap254	ah		24	48				0		0	0				0		0	0				0
380	8	phwl40	ah		48	48			96	384		0	0			0	0		0	24			0	0
381	8	r84	ah						96							24							0	
382	8	r843	ah	192	96	192			384	96	0	0	0			0	0	0		24			0	0
383	8	y21	ah	0		96					0		0					0	0	0				
384	8	2	wh		0	24	1536			384		0	48	96			48		0	24	0			0
385	8	20	wh		0	48	96		24	0		0	48	0		0	0		0	0	0		0	0
386	8	63	wh		0	24	192		192	24		0	0	24		0	0		0	0	0		0	0
387	8	67	wh						768	48						0	0		0		0		0	0
388	8	85	wh		0	48	0					0	0	0					0	0	0			
389	8	87	wh		0	48	0		48	96		0	0	48		0	0		0	0	0		0	0
390	8	b27	wh						192	0						0	24						0	0
391	8	b29	wh						24	0						0	0						0	0
392	8	b33	wh						48	192						0	0						0	0
393	8	b44	wh						0	0						0	0						0	0
394	8	b72	wh						96	0						0	0						0	0
395	8	144	ws		0	0	768					0	0	48					0	0	24			

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
396	8	153	ws		0	48	192					0	0	48					0	0	0			
397	8	155	ws		0	192	384					0	0	48					0	0	0			
398	8	218	ws		0	0		192	3072	384		0	0		0	0	0		0	0		0	0	0
399	8	219	ws		0	48	384	192	384	384		0	0	48	96	0	0		0	0	0	0	0	0
400	8	b165	ws						48	0						0	0						0	0
401	8	b169	ws						48	0						0	0						0	0
402	8	b216	ws						0	24						0	0						0	0
403	8	b231	ws						0	0						0	0						0	0
404	8	p1	yh		48	96	384		768	48		0	0	0		0	0		0	0	0		0	0
405	8	p10	yh		24	48			192	48		0	0			0	0		0	0			0	0
406	8	p22	yh		96	0			96	96		0	0			0	24		0	0			0	0
407	8	p25	yh		96	384			384			0	0			0			0	0			0	
408	8	p44	yh		192	192			384			0	0			0			0	0			0	
409	8	w16	yh						96	384						0	0						0	0
410	8	138	ys	192							0							0						
411	8	141	ys					1536	96	96					24	0	0					48	0	0
412	8	146	ys	192				192			0				0			0				0		
413	8	183	ys	384							0							24						
414	8	195	ys					384	1536	96					0	0	0					0	0	0
415	8	206	ys	384					24	0	0					0	0	0					0	0
416	8	215	ys						96	0						0	0						0	0
417	9	b185	ah		192	192						0	0						0	0				
418	9	w155	ah		192	0			96	192		0	24			0	0		0	0			0	0
419	9	w265	ah						768	1536						0	0						0	0
420	9	w397	ah		0	0						0	0						0	0				
421	9	w471	ah		48	0			96	384		0	0			0	0		0	0			0	0
422	9	y157	ah	48	384	48					24	24	0					0	0	0				
423	9	48	as		24	0	192					0	0	0					0	0	0			
424	9	109	as		24		0					48		0					48		0			
425	9	286	as		96	0		96				48	24		48				0	0		0		
426	9	288	as		192	192						0	0						0	0				
427	9	942	as		48	24		0	96			24	0		0	0			0	0		0	0	
428	9	b143	as						96							0							24	

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
429	9	b174	as						96	1536						0	0						0	24
430	9	b421	as						96	384						0	0						0	0
431	9	r89	as						192	384						24	0						24	0
432	9	y2	as	0		192					0		0					0		0				
433	9	y365	as						0	0						0	0						0	0
434	9	911	wh						48	0						0	0						0	0
435	9	920	wh						0	0						0	0						24	0
436	9	929	wh						0	0						0	0						0	24
437	9	930	wh						24	0						0	0						0	0
438	9	932	wh						0	0						0	0						192	0
439	9	r18-91	wh	0	0	0	96				0	0	0	0				0	0	0	0			
440	9	r35-91	wh	48	24	24	96		96	96	0	24	0	0		0	0	0	24	0	0		24	0
441	9	r37-91	wh	384	0	192	384		96	48	0	24	24	0		0		0	0	0	0		0	
442	9	r3-91	wh	48	24	0	384		1536	1536	24	48	48	0		24	24	0	0	48	0		48	0
443	9	r9-91	wh		0	0	192			48		0	0	0			0		0	0	0			0
444	9	954	ws						48	0						0	0						0	24
445	9	967	ws						0							0							0	
446	9	982	ws						48	0						0	0						0	0
447	9	r48-91	ws	0	0	24	192	48	384	768	24	0	0	0	96	0	0	0	24	0	0	0	0	0
448	9	r57-91	ws	24	0	24		384	384	3072	0	0	0		24	24	24	0	0	0		0	0	0
449	9	r62-91	ws			0	192	48	384	96			0	0	24	0	0			0	0	0	0	0
450	9	r66-91	ws		0	0		0	0			0	0		48	0			0	0		0	0	
451	9	r75-91	ws	48	0	96	384	192			48	0	24	24	192			48	0	0	0	0		
452	9	b274	yh	24	96	0					0	0	0					0	24	0				
453	9	b465	yh	0	192	24					0	0	0					0	0	0				
454	9	o293	yh						96	48						0	0						0	0
455	9	r17	yh						96	192						0	48	0					0	48
456	9	y12	yh	0	384	24			384	192	24	0	0			0	0	0	0	0			0	0
457	9	y16	yh	0	24	24					48	0	0					0	0	24				
458	9	0 028	ys		384	0						0	0						0	0				
459	9	0 029	ys		48	0						0	0						0	0				
460	9	b148	ys		96	192		96				0	192		0				0	0		0		
461	9	b494	ys	0	24	96			192	0	0	48	0			48	48	0	0	0			24	0

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
462	9	b70	ys	0	96	48					0	48	0					0	0	0				
463	9	r61	ys						384	384						0	0						0	0
464	9	r69	ys						768	1536						0	48						24	0
465	9	y24	ys		192	0						0	0						0	48				
466	10	170	ah		0	0						0	0						0	0				
467	10	981	ah	24	0	48			0	0	24	0	24			0	0	0	0	0			0	0
468	10	7219	ah						0	0						24	24						0	0
469	10	8155	ah		0	96						0	0						0	0				
470	10	8157	ah		0	0			48	0		0	0			0	0		48	24			0	0
471	10	9084	ah						0	0						0	0						0	0
472	10	105	as	24	0				0	0	0	0				24	0	0	0				0	0
473	10	865	as	48	48	0	192				48	48	0	0				0	0	0	0			
474	10	893	as	0	0	48	0			0	0	0	0	0			0	0	0	0	0			0
475	10	91125	as		0	24	768					0	0	0					0	0	0			
476	10	9-124	as		192	24						48	0						0	0				
477	10	153	wh	0		192	0		0	0	0		0	0		0	0	0		0	0		0	0
478	10	154	wh			96							0							0				
479	10	188	wh		0	48	0		24			48	0	0		0			0	0	0		0	
480	10	216	wh		0	24	0		0	0		0	0	0		0	0		0	0	0		0	0
481	10	232	wh	24	0	384	0			384	0	0	0	0			0	0	24	0	48			0
482	10	2013	wh						0	0						0	24						0	0
483	10	2061	wh						0	0						0	0						0	0
484	10	2094	wh						0	0						0	0						0	0
485	10	2122	wh						0	0						0	0						0	0
486	10	2135	wh						0	0						0	0						0	0
487	10	137	ws	0	0	48	48	0	0	0	0	0	48	24	0	0	0	0	0	0	0	0	0	0
488	10	144	ws	0	0	48	0	0	0	0	24	0	0	24	24	0	0	0	0	0	0	0	0	0
489	10	148	ws	0	0	24	0	0	0	0	24	0	0	24	0	0	0	0	0	0	0	0	0	0
490	10	156	ws	24	0	48	0		0	0	0	24	0	0		0	0	0	0	0	0		0	0
491	10	263	ws	0	0	0	0		0	0	24	0	0	48		0	0	0	0	0	24		0	0
492	10	2012	ws						0	0						0	0						0	0
493	10	2027	ws						0	0						0	0						0	0
494	10	2043	ws						0	0						0	0						0	0

	General data			Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
495	10	2067	ws						24	0						0	0						0	0
496	10	2125	ws						0	0						0	0						0	0
497	10	0 045	yh		0	96			0	0		0	0			0	0		0	0			0	0
498	10	0 071	yh		0	24						0	0						24	0				
499	10	0 081	yh		0	48						0	0						0	0				
500	10	0 084	yh		0	48	0					24	0	0					0	0	48			0
501	10	1184	yh						48	24						0	0						0	0
502	10	1232	yh						0	0						0	0						0	0
503	10	0.017	ys	48	0	0			0	0	0	0	48			0	0	0	0	0			0	0
504	10	0 062	ys		0	96			0	0		0	0			0	0		24	0			0	0
505	10	0 66	ys			96	0						0	0						0	0			
506	10	123	ys	0	0	48	0				0	24	0	0				0	0	0	0			
507	10	148	ys		0	0	0		0	0		0	0	0		0	0		0	0	0		0	0
508	11	0 011	ah			0							0							0				
509	11	40	ah	96							0							0						
510	11	160	ah	48							48							0						
511	11	127/4	ah	384							48							0						
512	11	86/40	ah							96							24							0
513	11	89/91	ah							192							24							0
514	11	g2	ah			0							0							0				
515	11	g3	ah		24	0						48	0						24	0				
516	11	o810	ah	192						96	48						24	0						0
517	11	y108	ah	192						768	24						24	0						0
518	11	y22	ah		0	0						0	0						0	0				
519	11	y41	ah			48							0							24				
520	11	0 012	as			192							0							0				
521	11	33	as		96			96	96	0		0			48	48	0		0			0	0	0
522	11	47	as				0	48		48				0	0		0				0	0		0
523	11	920	as		0							0							0					
524	11	949	as		24			192				0			0				0			0		
525	11	9y17	as							48							0		0					0
526	11	r10	as		48	0						48	0						0	0				
527	11	r12	as			48							0							0				

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
528	11	r2	as		24	0						0	0						0	0				
529	11	r3	as			0							0							0				
530	11	y24	as				768							48							0			
531	11	y33	as				96							0							0			
532	11	117	wh				0							0							0			
533	11	120	wh				384							0							0			
534	11	9220	wh							48							0							24
535	11	9221	wh							0							0							0
536	11	9246	wh							768							0							0
537	11	9254	wh							0							0							0
538	11	y55	wh			0			48	384			0			0	0			0			0	0
539	11	y56	wh			0	48						0	0						0	0			
540	11	y58	wh			0			384	192			0			0	0			0			0	0
541	11	y61	wh		24	24						0	0						0	0				
542	11	655	ws			0	0						0	0						0	0			
543	11	658	ws			48							0							0				
544	11	923	ws							0							0							0
545	11	957	ws			192							0							0				
546	11	962	ws			0							0							0				
547	11	9225	ws							384							0							24
548	11	9226	ws							0							0							0
549	11	9249	ws							0							0							0
550	11	g60	ws			0	0			0			0	0			0			0	0			0
551	11	20	yh							1536							0							0
552	11	1172	yh	24							0							0						
553	11	1175	yh	24							24							0						
554	11	o17	yh							384							0							0
555	11	o6	yh							48							0							0
556	11	p39	yh			0							0							0				
557	11	y173	yh	24							24							0						
558	11	y38	yh	48							0							0						
559	11	y43	yh		0	0						0	0						24	0				
560	11	y48	yh			96	192						0	0						0	0			

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
561	11	y51	yh			192							0							0				
562	11	y57	yh	0		0					24		0					0		0				
563	11	18	ys	0							48							0						
564	11	27	ys		768		192	48		0		0		0	0		0		24		0	0		0
565	11	32	ys				0	384	1536	3072				0	0	0	0				0	24	24	0
566	11	36	ys				0	384	48	96				0	24	0	0				0	0	24	0
567	11	48	ys				384	384	96					0	24	0					0	0	0	
568	11	52	ys					192	384	192					0	0	0					24	0	0
569	11	136	ys							0							0							0
570	11	148	ys							192							0							0
571	11	152	ys				0	384						0	24						0	0		
572	11	171	ys			0							0							0				
573	11	941	ys			24							0							0				
574	11	g31	ys			0				768			0				0			0				24
575	11	g42	ys			0							0							0				
576	11	g45	ys			96							0							0				
577	11	y10	ys	10							0							0						
578	11	y16	ys	24							0							0						
579	11	y25	ys	24							0							0						
580	11	y67	ys	48							0							0						
581	13	0 022	AH		24	192						0	24						0	0				
582	13	190/y2	ah						384	96						0	0						0	0
583	13	b35	ah		24	96						24	0						0	0				
584	13	b93	ah		0	0						24	0						0	0				
585	13	go12	ah	24	96	192	96				0	0	96	0				0	0	0	0			
586	13	w30	ah	768	96	192					0	0	0					0	0	0				
587	13	y161	ah	48	24	96					0	0	24					0	0	0				
588	13	y709	ah		24	48						0	48						0	0				
589	13	y807	ah	96	96	96					0	0	0					0	0	0				
590	13	y832	ah	3072	96	96	24				0	0	0	0				0	0	48	0			
591	13	y90	ah						192	0						0	0						0	0
592	13	9021	as						48	96						0	24						0	0
593	13	r606	as				48		96					48		0					0		0	

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
594	13	127	wh						192	96						0	0						0	96
595	13	1215	wh						384	192						0	0						0	0
596	13	1222	wh						48	0						0	0						0	0
597	13	1259	wh						48	96						0	0						0	0
598	13	1265	wh						48	192						0	0						0	0
599	13	y130	wh		1536	384	192		192	192		0	0	0		0	0		0	48	0		0	0
600	13	y143	wh		48	192	96		48	0		0	0	24		0	0		0	24	0		0	0
601	13	y154	wh		24	24	96		384	24		0	0	0		0	0		24	0	0		48	0
602	13	y163	wh		0	768	192		384	48		24	24	0		0	0		0	96	0		0	0
603	13	y176	wh		0	24	192		192	96		0	0	0		0	0		0	0	0		0	0
604	13	9215	ws						48	96						0	0						0	0
605	13	9246	ws						0	0						0	0						0	0
606	13	9248	ws						24	384						0	0						0	0
607	13	9260	ws						3072	1536						0	0						0	0
608	13	c26	ws						96	192						0	0						0	24
609	13	g121	ws		0	96	96	48	384	192		0	48	0	24	48	0		0	0	0	0	0	0
610	13	g125	ws		0	96	96	48	96	48		0	0	0	24	0	0		0	0	0	0	0	0
611	13	g146	ws	96	24	96	96	24	48	46	0	0	0	0	48	0	0	0	0	0	0	0	0	0
612	13	g170	ws		0	96	384	192	384	768		0	0	0	24	0	0		0	0	0	0	0	24
613	13	g189	ws		0	0	24	96		96		0	0	0	0		0		0	48	0	0		0
614	13	1130	yh						768	96						0	24						0	0
615	13	1176	yh						96	48						0	0						0	0
616	13	y003	yh	48	96	384	96				24	0	0	0				0	24	0	0			
617	13	y012	yh		192	192	48					0	24	0					0	0	0			
618	13	y027	yh		96	0						24	0						0	0				
619	13	y039	yh	48	192	48					0	0	0					0	0	0				
620	13	y041	yh		96	384	96					0	0	0					0	0	0			
621	13	y047	yh		384	384	48					0	0	0					0	0	0			
622	13	906	ys	0	1536	96	96		96	24	0	0	0	0		0	0	0	0	0	0		0	0
623	13	9189	ys						96	192						0	0						0	0
624	13	g029	ys	24	1536	1536	192				48	48	0	0				0	0	0	0			
625	13	g038	ys	1536	96	192	96		48	192	48	0	24	0		0	0	0	0	0	0		0	0
626	13	g40	ys	384	384	384			768	192	0	0	0			0	0	0	0	0			0	0

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
627	14	477	ah			96							0							0				
628	14	484	ah			96							3072							24				
629	14	513	ah			0							0							0				
630	14	516	ah			48							0							0				
631	14	243-89	ah			384							0							0				
632	14	244-13	ah	96		24					24		0					0		0				
633	14	518-85	ah	0		48					0		192					0		0				
634	14	526-88	ah	0		0					0		0					0		0				
635	14	572-84	ah	48		48					0		48					0		0				
636	14	110-89	as	0		24					0		0					0		0				
637	14	142-89	as	24		0	48				0		0	48				0		0	96			
638	14	158-89	as			0							0							0				
639	14	159-89	as			0							0							0				
640	14	197-89	as			24	0						0	24						0	0			
641	14	228-89	as	96		24	0				24		0	0				0	48	0	0			
642	14	267-89	as	48		96	0				0		0	0				0		0	0			
643	14	268-89	as	96		48					24		24					0		0				
644	14	472-87	as			48							0							0				
645	14	j92-89	as			0							0						48	24				
646	14	150-91	ws			96	0						0	24						24	0			
647	14	159-91	ws	0		48	0				0		0	0				0		0	0			
648	14	186-91	ws			0							24						24	24				
649	14	449-91	ws	0		0					0		0					0		0				
650	14	465-91	ws			24							0						0	48				
651	14	493-87	ws			192						96	384							48				
652	14	513-91	ws			24	0						384	24						0	24			
653	14	514-91	ws			24	0						48	0						0	0			
654	14	515-91	ws			24	0						384	0						0	0			
655	14	517-91	ws			0							0							0				
656	14	p16091	ws			0							24						0	0				
657	15	o106	ah						384	384						0	0						0	0
658	15	w09	ah	0	0	24			48	48	24	24	0			0	0	0	0	0			0	0
659	15	w24	ah	384	384	0			384	384	96	24	0			384	48	0	0	0			0	48

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
660	15	w29	ah	192	48	0	48				768	0	0	0				0	0	0	0			
661	15	w81	ah		192	0			192	24		0	24			48	384		0	24			24	0
662	15	22	as	96	48	192					48	0	0					0	0	0				
663	15	43	as	96	48	24					48	0	0					0	0	0				
664	15	48	as					3072	3072	768					48	3076	768					24	0	24
665	15	56	as		96		384	96	96	192		24		0	48	384	192		0		0	0	0	0
666	15	71	as					0	48	48					48	48	48					0	0	0
667	15	920	as	48	48	24	48				48	48	0	48				0	0	0	0			
668	15	933	as	48	96	24	48				768	0	0	768				0	0	0	0			
669	15	936	as	192	192	24	48				768	0	0	768				0	0	0	0			
670	15	1077	as				0	0	192	192				0	96	1536	768				0	0	0	0
671	15	w38	as			48	48						0	96						0	0			
672	15	w7	as				384							768							0			
673	15	428	wh		0	0						0	0						0	0				
674	15	431	wh		0	24						192	48						0	0				
675	15	434	wh		0	0						0	24						0	0				
676	15	435	wh	48	24	0					24	0	0					0	0	0				
677	15	896	wh	0	0	0					0	24	0					96	192	0				
678	15	1023	wh				0		96	0				0		0	0				0		0	48
679	15	1042	wh				0		0	384				0		0	768				0		0	0
680	15	1058	wh				0		24	0				0		768	1536				0		24	48
681	15	2002	wh						384	384						0	3072						0	24
682	15	2003	wh						96	192						0	768						0	0
683	15	2023	wh						0	0						0	768						0	48
684	15	2031	wh						0	48						0	3072						0	96
685	15	2034	wh						96	0						0	3072						0	384
686	15	430	ws		0	0						0	0						0	0				
687	15	433	ws		0	0	48					0	0	0					0	0	0			
688	15	436	ws		0	0	192					24	0	0					0	0	0			
689	15	437	ws		0	0						48	0						0	0				
690	15	893	ws		0	0						24	24						0	0				
691	15	2144	ws						24	192						0	768						0	48
692	15	2149	ws						0	0						0	3072						24	96

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
693	15	2153	ws						96	48						24	1536						24	48
694	15	2154	ws						768	96						48	1436						0	0
695	15	2167	ws						384	192						0	3072						0	0
696	15	109	yh		0	96						48	0						0	0				
697	15	904	yh		0	0						0	0						0	0				
698	15	1030	yh						384	192						0	0						0	0
699	15	1067	yh						192	48						0	0						0	0
700	15	o100	yh		0	24						0	0						0	0				
701	15	o136	yh		0	0	0					0	0	0					96	0	0			
702	15	o94	yh		0	48			48	768		48	0			0	48		0	0			0	24
703	15	1100	ys				0	0	48	0				0	0	3072	1536				0	0	0	0
704	15	1109	ys				96	48	48	48				0	48	192	1536				0	0	48	0
705	15	1144	ys				0	0	96	24				0	0	3072	384				0	0	0	192
706	15	1151	ys	384			96	96	96	192	48			0	48	384	3072	0			0	192	0	96
707	15	o13	ys	24	0	24	0				0	0	0	24				0	0	0	0			
708	15	o18	ys	24	0	0	0		0	48	0	0	0	0		1536	3072	0	0	0	0		0	48
709	15	o27	ys	96	0	24	0				48	24	0	48				24	24	0	24			
710	15	o56	ys	96		0	384				0		0	0				0		0	0			
711	15	o70	ys	0		1536	192	384	384	192	24		0	0	48	3072	1536	24		0	0	96	0	0
712	16	1	ah		0	0			384	0		0	0			0	0		0	0	0		0	0
713	16	22	ah	48	48	0			48	0	24	0	0			0	0	0	0	0			0	0
714	16	44	ah	0	0	0					0	0	0					0	0	0			0	
715	16	46	ah		0	0			24	0		0	0			0	0		0	0			0	0
716	16	80	ah	0	0	48			48	0	48	0	0			24	0	0	0	0			0	0
717	16	126	ah						48	0						0	0						0	0
718	16	141	ah						192	384						48	0						0	0
719	16	14	as			96	384						96	0						0	0			
720	16	15	as	0	0	0	0				0	0	0	0				0	0	0	0			
721	16	36	as		24	0			48	0		0	0			0	0		0	48			24	0
722	16	40	as	24	384	24					0	0	0					0	0	0				
723	16	63	as						384	0						0	0						0	0
724	16	65	as						24	0						0	0						0	0
725	16	67	as						0	0						0	0						0	0

General data				Hardjobovis-1992				Hardjobovis-1993			Pomona-1992				Pomona-1993			Copenhageni-1992				Copenhageni-1993		
No.	Farm	A. ID	Age/Sex	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.	Mar.	Jun.	Sep.	Dec.	Mar.	Jun.	Sep.
726	16	924	wh						0	0						0	0						0	0
727	16	966	wh						0	0						0	0						0	0
728	16	974	wh						0	0						0	96						0	0
729	16	976	wh						48	0						0	0						0	0
730	16	r23	wh		0	0	0		0	0		0	0	0		0	0		0	24	48		0	0
731	16	r33	wh		0	96	0		48	0		0	24	0		0	0		0	0	0		0	24
732	16	r48	wh		192	24	0		0	0		0	0	0		0	0		0	0	0		0	0
733	16	r53	wh		0	0	0		48	0		0	0	0		0	0		0	0	0		0	0
734	16	r6	wh		0	24	0		48	0		0	24	0		0	0		0	0	0		0	0
735	16	97	ws						0	0						0	0						0	0
736	16	129	ws						48	0						0	0						0	0
737	16	912	ws						48	0						0	0						0	0
738	16	920	ws						48	0						0	0						0	0
739	16	961	ws						96	0						24	0						0	0
740	16	r10	ws	0	0	0	0	0	48	0	24	0	0	0	0	0	0	0	0	0	0	0	48	0
741	16	r27	ws		0	0	0		48	0		0	0	0		24	0		0	24	24	0	0	0
742	16	r46	ws		0	0	0	0	0	0		0	0	0	0	0	0		0	0	0	0	0	0
743	16	r51	ws	0	0	0	0	0	96	0	0	0	96	0	0	0	0	0	0	0	0	0	0	48
744	16	r71	ws		0	24	0	0	24	0		0	0	0	0	0	0		48	0	0	0	0	0
745	16	w21	yh	24	0	0					0	0	48					0	0	48				
746	16	w27	yh	48	0	192		0			0	0	0		0			0	0	24		0		
747	16	w36	yh		0	0						0	0						0	0				
748	16	w56	yh	0	0	0					0	0	0					0	0	0				
749	16	w58	yh	96	192	0					0	0	0					0	0	0				
750	16	w23	ys	48	192	0					0	0	0					0	0	24				
751	16	w32	ys		0	0						0	192						0	0				
752	16	w34	ys		0	0						0	0						0	0				
753	16	w66	ys	0	0	24					0	0	0					0	0	0				
754	16	w68	ys		0	0						0	0						0	0				

Appendix 2 raw data

Chapters 4, 5, 6
(Farm Trials)

Table 2.1a. List of abbreviations used for Appendix 2a.

Classification	Abbreviation		Meaning		Notes
Id	Id		Animal Identification Number		Farm tag number
Farm	No.		Farm number and		See Chapter 2
	Dx.		herd leptospiral status		
Year			Year of sample collection		
Sex	S		Sex,		
	F		Female		
	M		Male		
Age	Cls.	W	Animal Class	W= Weaner	3-19 month-old
		A		A= Adult	> 24 month-old
	Mt		age in months		
Vaccination	Dm		Mother		0= Negative, 1= Positive
	Wn		Progeny		
Trial	Day		Trial in days based on the sensitiser vaccine		Vaccine trial dates
	Mt		Calendar month		
Serology	Hb		Hardjobovis		MAT titre
	Pom		Pomona		
	Cop		Copenhageni		
Culture	U		Urine, if the result is parallel to DFM column the sample was cultured.		0= Negative, 1= Positive
	K		Kidney, if the result is parallel to Kidney HP or GP column the sample was cultured.		
DFM	LLI		Darkfield microscopy	"Live *Leptospira* Index"	0= Negative, 1= Positive See Chapters 4 & 5
	Ur			Urine sample	0= Negative, 1= Positive See Chapter 2
Sv.	Sv. Tp	Hb	Sv = Serovar, Tp = Typed serovar	Hardjobovis	See Chapter. 2
		P		Pomona	
		H-P		Hardjobovis and Pomona	
Kidney	HP		Histopathology		0= Negative, 1= Positive
	GP		Gross pathology		
Histopathology	FN		Focal Nephritis		0= Negative, 1= Positive
	DN		Diffuse Nephritis		
	Nphs		Neutrophils		
	L-P		Lympho-Plasmatic cell infiltration		
	GA		Glomerular Adhesions		
	Sc		Scaring		

(Chapters 4, 5 and 6) by Animal Id, farm, age, vaccine, trial, serology for each serovar, culture, darkfield microscopy, typed serovars, kidney pathology, and histopathology results.

Appendix 2 a. Raw data for the natural infection and vaccine efficiency trials. (Chapters 4, 5 and 6) by Animal Id, farm, age, vaccine, trial, serology for each serovar, culture, darkfield microscopy, typed serovars, kidney pathology, and histopathology results. (See table 2.1.a for abbreviations used in this appendix).

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney			Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
34	1	Hb	2003	F	W	6	0	1	0	5	48	0	0												
34	1	Hb	2003	F	W	7	0	1	28	6	96	48	0												
34	1	Hb	2003	F	W	8	0	1	50	7	96	3072	384	0	0	0									
34	1	Hb	2003	F	W	10	0	1	80	9	48	384	192	0	0	0									
34	1	Hb	2003	F	W	12	0	1	120	11	24	192	48	0	0	0									
34	1	Hb	2004	F	W	15	0	1	165	2	0	96	0	0	0	0									
34	1	Hb	2004	F	W	19	0	1	255	5	24	0	0	0	0	0									
35	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
35	1	Hb	2003	F	W	7	0	1	28	6	192	96	0												
35	1	Hb	2003	F	W	8	0	1	50	7	384	1536	768	0	0	0									
35	1	Hb	2003	F	W	10	0	1	80	9	192	96	192												
35	1	Hb	2003	F	W	12	0	1	120	11	0	48	48	0	0	0									
35	1	Hb	2004	F	W	15	0	1	165	2	24	0	0												
35	1	Hb	2004	F	W	19	0	1	255	5	24	0	0	0	0	0									
36	1	Hb	2003	F	W	6	0	1	0	5	48	0	0	0	0	0									
36	1	Hb	2003	F	W	7	0	1	28	6	96	96	0												
36	1	Hb	2003	F	W	8	0	1	50	7	96	3072	192	0	0	0									
36	1	Hb	2003	F	W	10	0	1	80	9	96	384	384	0	0	0									
36	1	Hb	2003	F	W	12	0	1	120	11	48	96	48	0	0	0									
36	1	Hb	2004	F	W	15	0	1	165	2	96	96	24												
36	1	Hb	2004	F	W	19	0	1	255	5	96	0	0												
38	1	Hb	2003	F	W	6	0	1	0	5	0	0	0												
38	1	Hb	2003	F	W	7	0	1	28	6	96	192	0												
38	1	Hb	2003	F	W	8	0	1	50	7	96	768	768	0	1	1									
38	1	Hb	2003	F	W	10	0	1	80	9	96	96	48	0	0	0									
38	1	Hb	2003	F	W	12	0	1	120	11	48	192	96	0	1	1									
38	1	Hb	2004	F	W	15	0	1	165	2	96	48	48	0	1	1									
38	1	Hb	2004	F	W	19	0	1	255	5	192	0	0	0	0	0									
39	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
39	1	Hb	2003	F	W	7	0	1	28	6	96	384	0												
39	1	Hb	2003	F	W	8	0	1	50	7	384	12308	384	0	0	0									
39	1	Hb	2003	F	W	10	0	1	80	9	192	768	192	0	0	0									
39	1	Hb	2003	F	W	12	0	1	120	11	96	192	48	0	0	0									
39	1	Hb	2004	F	W	15	0	1	165	2	48	384	96	0	0	0									
39	1	Hb	2004	F	W	19	0	1	255	5	48	0	0												
43	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
43	1	Hb	2003	F	W	7	0	1	28	6	192	384	0												
43	1	Hb	2003	F	W	8	0	1	50	7	384	6154	768	0	0	0									
43	1	Hb	2003	F	W	10	0	1	80	9	96	3072	1576	0	0	0									
43	1	Hb	2003	F	W	12	0	1	120	11	48	384	384	0	0	0									
43	1	Hb	2004	F	W	15	0	1	165	2	48	384	48	0	0	0									
43	1	Hb	2004	F	W	19	0	1	255	5	48	0	0	0	0	0									
45	1	Hb	2003	F	W	6	0	1	0	5	0	0	0												
45	1	Hb	2003	F	W	7	0	1	28	6	96	48	0												
45	1	Hb	2003	F	W	8	0	1	50	7	384	768	384	0	0	0									
45	1	Hb	2003	F	W	10	0	1	80	9	96	768	192	0	0	0									
45	1	Hb	2003	F	W	12	0	1	120	11	0	96	48	0	0	0									
45	1	Hb	2004	F	W	15	0	1	165	2	24	48	24	0	0	0									
45	1	Hb	2004	F	W	19	0	1	255	5	24	0	0												
46	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
46	1	Hb	2003	F	W	7	0	1	28	6	48	48	0												
46	1	Hb	2003	F	W	8	0	1	50	7	96	192	384	0	0	0									
46	1	Hb	2003	F	W	10	0	1	80	9	0	48	48	0	0	0									
46	1	Hb	2003	F	W	12	0	1	120	11	0	0	48	0	0	0									
46	1	Hb	2004	F	W	15	0	1	165	2	24	24	0	0	0	0									
46	1	Hb	2004	F	W	19	0	1	255	5	48	0	0	0	0	0									
49	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
49	1	Hb	2003	F	W	7	0	1	28	6	48	96	0												
49	1	Hb	2003	F	W	8	0	1	50	7	96	1536	192	0	0	0									
49	1	Hb	2003	F	W	10	0	1	80	9	0	768	384												
49	1	Hb	2003	F	W	12	0	1	120	11	0	192	48	0	0	0									
49	1	Hb	2004	F	W	15	0	1	165	2	0	48	0	0	1	1									
49	1	Hb	2004	F	W	19	0	1	255	5	0	0	0	0	0	0									
50	1	Hb	2003	F	W	6	0	1	0	5	0	0	0												
50	1	Hb	2003	F	W	7	0	1	28	6	48	48	0												
50	1	Hb	2003	F	W	8	0	1	50	7	96	384	192	0	0	0									
50	1	Hb	2003	F	W	10	0	1	80	9	48	96	384	0	0	0									

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney				Histopathology			
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
50	1	Hb	2003	F	W	12	0	1	120	11	0	96	0												
50	1	Hb	2004	F	W	15	0	1	165	2	48	48	0	0	0	0									
50	1	Hb	2004	F	W	19	0	1	255	5	48	0	0	0	0	0									
51	1	Hb	2003	F	W	6	0	1	0	5	24	24	0												
51	1	Hb	2003	F	W	7	0	1	28	6	48	48	0												
51	1	Hb	2003	F	W	8	0	1	50	7	96	6154	384	0	0	0									
51	1	Hb	2003	F	W	10	0	1	80	9	48	192	96												
51	1	Hb	2003	F	W	12	0	1	120	11	48	192	48	0	0	0									
51	1	Hb	2004	F	W	15	0	1	165	2	24	96	24	0	0	0									
51	1	Hb	2004	F	W	19	0	1	255	5	192	0	0												
54	1	Hb	2003	F	W	6	0	1	0	5	0	24	0												
54	1	Hb	2003	F	W	7	0	1	28	6	48	384	0												
54	1	Hb	2003	F	W	8	0	1	50	7	96	768	384	0	0	0									
54	1	Hb	2003	F	W	10	0	1	80	9	96	192	384	0	0	0									
54	1	Hb	2003	F	W	12	0	1	120	11	24	48	24	0	0	0									
54	1	Hb	2004	F	W	15	0	1	165	2	48	24	24	0	0	0									
54	1	Hb	2004	F	W	19	0	1	255	5	96	0	0	0	0	0									
55	1	Hb	2003	F	W	6	0	1	0	5	0	0	0												
55	1	Hb	2003	F	W	7	0	1	28	6	48	96	0												
55	1	Hb	2003	F	W	8	0	1	50	7	96	768	192	0	0	0									
55	1	Hb	2003	F	W	10	0	1	80	9	48	192	96	0	0	0									
55	1	Hb	2003	F	W	12	0	1	120	11	0	48	0	0	0	0									
55	1	Hb	2004	F	W	15	0	1	165	2	0	24	0	0	0	0									
55	1	Hb	2004	F	W	19	0	1	255	5	0	0	0												
56	1	Hb	2003	F	W	6	0	1	0	5	48	0	0												
56	1	Hb	2003	F	W	7	0	1	28	6	48	192	0												
56	1	Hb	2003	F	W	8	0	1	50	7	48	384	96	0	0	0									
56	1	Hb	2003	F	W	10	0	1	80	9	0	192	48												
56	1	Hb	2003	F	W	12	0	1	120	11	0	192	0	0	0	0									
56	1	Hb	2004	F	W	15	0	1	165	2	0	48	0	0	0	0									
56	1	Hb	2004	F	W	19	0	1	255	5	0	0	0	0	0	0									
58	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
58	1	Hb	2003	F	W	7	0	1	28	6	96	384	0												
58	1	Hb	2003	F	W	8	0	1	50	7	96	384	192	0	1	1									
58	1	Hb	2003	F	W	10	0	1	80	9	192	192	384	0	0	0									
58	1	Hb	2003	F	W	12	0	1	120	11	48	96	48	0	1	1									
58	1	Hb	2004	F	W	15	0	1	165	2	0	48	0	0	0	0									
58	1	Hb	2004	F	W	19	0	1	255	5	0	0	0												
59	1	Hb	2003	F	W	6	0	1	0	5	0	0	0												
59	1	Hb	2003	F	W	7	0	1	28	6	48	96	0												
59	1	Hb	2003	F	W	8	0	1	50	7	192	3072	384	0	0	0									
59	1	Hb	2003	F	W	10	0	1	80	9	192	384	384	0	0	0									
59	1	Hb	2003	F	W	12	0	1	120	11	24	192	48	0	0	0									
59	1	Hb	2004	F	W	15	0	1	165	2	0	48	0	0	0	0									
59	1	Hb	2004	F	W	19	0	1	255	5	0	0	0	0	0	0									
60	1	Hb	2003	F	W	6	0	1	0	5	24	0	0	0											
60	1	Hb	2003	F	W	7	0	1	28	6	48	48	0												
60	1	Hb	2003	F	W	8	0	1	50	7	96	1536	192	0	0	0									
60	1	Hb	2003	F	W	10	0	1	80	9	48	384	384												
60	1	Hb	2003	F	W	12	0	1	120	11	0	192	96	0	0	0									
60	1	Hb	2004	F	W	15	0	1	165	2	96	96	48	0	0	0									
60	1	Hb	2004	F	W	19	0	1	255	5	48	0	0	0	0	0									
62	1	Hb	2003	F	W	6	0	1	0	5	24	0	24												
62	1	Hb	2003	F	W	7	0	1	28	6	48	48	24												
62	1	Hb	2003	F	W	8	0	1	50	7	48	1536	192	0	0	0									
62	1	Hb	2003	F	W	10	0	1	80	9	48	1536	192	0	0	0									
62	1	Hb	2003	F	W	12	0	1	120	11	0	192	48	0	1	1									
62	1	Hb	2004	F	W	15	0	1	165	2	24	48	0												
62	1	Hb	2004	F	W	19	0	1	255	5	24	0	0	0	0	0									
63	1	Hb	2003	F	W	6	0	1	0	5	24	0	0												
63	1	Hb	2003	F	W	7	0	1	28	6	48	96	0												
63	1	Hb	2003	F	W	8	0	1	50	7	192	1536	768												
63	1	Hb	2003	F	W	10	0	1	80	9	48	384	384	0	0	0									
63	1	Hb	2003	F	W	12	0	1	120	11	0	192	48	0	0	0									
63	1	Hb	2004	F	W	15	0	1	165	2	0	24	24	0	0	0									
63	1	Hb	2004	F	W	19	0	1	255	5	0	0	0	0	1	1									
65	1	Hb	2003	F	W	6	0	1	0	5	0	24	0												
65	1	Hb	2003	F	W	7	0	1	28	6	96	192	0												
65	1	Hb	2003	F	W	8	0	1	50	7	192	384	192	0	0	0									
65	1	Hb	2003	F	W	10	0	1	80	9	48	96	96	0	0	0									
65	1	Hb	2003	F	W	12	0	1	120	11	0	48	0	0	0	0									
65	1	Hb	2004	F	W	15	0	1	165	2	0	48	0	0	0	0									
65	1	Hb	2004	F	W	19	0	1	255	5	0	0	0	0	0	0									
103	1	Hb	2003	M	W	6	0	1	0	5	0	0	0												
103	1	Hb	2003	M	W	7	0	1	28	6	96	48	0												
103	1	Hb	2003	M	W	8	0	1	50	7	384	1536	192												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
103	1	Hb	2003	M	W	10	0	1	80	9	96	96	48											
103	1	Hb	2003	M	W	12	0	1	120	11	48	96	96											
103	1	Hb	2004	M	W	15	0	1	165	2	0	48	0											
103	1	Hb	2004	M	W	19	0	1	255	5	24	0	0	1	1		Hb	1	0	1	0	0	1	1	0
104	1	Hb	2003	M	W	6	0	1	0	5	24	0	0											
104	1	Hb	2003	M	W	7	0	1	28	6	96	48	0											
104	1	Hb	2003	M	W	8	0	1	50	7	384	6154	384											
104	1	Hb	2003	M	W	10	0	1	80	9	48	192	96											
104	1	Hb	2003	M	W	12	0	1	120	11	48	192	48											
104	1	Hb	2004	M	W	15	0	1	165	2	24	48	0											
104	1	Hb	2004	M	W	19	0	1	255	5	48	0	0	0	0			0	0	0	0	0	0	0	0
105	1	Hb	2003	M	W	6	0	1	0	5	0	0	0											
105	1	Hb	2003	M	W	7	0	1	28	6	96	24	0											
105	1	Hb	2003	M	W	8	0	1	50	7	768	768	768											
105	1	Hb	2003	M	W	10	0	1	80	9	192	48	96											
105	1	Hb	2003	M	W	12	0	1	120	11	48	96	96											
105	1	Hb	2004	M	W	15	0	1	165	2	96	192	24											
105	1	Hb	2004	M	W	19	0	1	255	5	24	0	24	0	0			0	0	0	0	0	0	0	0
106	1	Hb	2003	M	W	6	0	1	0	5	0	0	24											
106	1	Hb	2003	M	W	7	0	1	28	6	48	96	24											
106	1	Hb	2003	M	W	8	0	1	50	7	48	192	48											
106	1	Hb	2003	M	W	10	0	1	80	9	48	24	48											
106	1	Hb	2003	M	W	12	0	1	120	11	0	48	0											
106	1	Hb	2004	M	W	15	0	1	165	2	24	0	0											
106	1	Hb	2004	M	W	19	0	1	255	5	24	0	0											
107	1	Hb	2003	M	W	6	0	1	0	5	24	0	0											
107	1	Hb	2003	M	W	7	0	1	28	6	192	192	0											
107	1	Hb	2003	M	W	8	0	1	50	7	384	1536	768											
107	1	Hb	2003	M	W	10	0	1	80	9	96	48	96											
107	1	Hb	2003	M	W	12	0	1	120	11	48	96	96											
107	1	Hb	2004	M	W	15	0	1	165	2	48	48	48											
107	1	Hb	2004	M	W	19	0	1	255	5	24	0	0	1	1		Hb	1	1	1	0	0	1	0	0
108	1	Hb	2003	M	W	6	0	1	0	5	0	0	0											
108	1	Hb	2003	M	W	7	0	1	28	6	96	48	0											
108	1	Hb	2003	M	W	8	0	1	50	7	96	384	192											
108	1	Hb	2003	M	W	10	0	1	80	9	48	48	96											
108	1	Hb	2003	M	W	12	0	1	120	11	48	0	0											
108	1	Hb	2004	M	W	15	0	1	165	2	24	48	0											
108	1	Hb	2004	M	W	19	0	1	255	5	0	0	0											
109	1	Hb	2003	M	W	6	0	1	0	5	0	0	0											
109	1	Hb	2003	M	W	7	0	1	28	6	48	384	0											
109	1	Hb	2003	M	W	8	0	1	50	7	96	96	96											
109	1	Hb	2003	M	W	10	0	1	80	9	24	48	48											
109	1	Hb	2003	M	W	12	0	1	120	11	24	24	48											
109	1	Hb	2004	M	W	15	0	1	165	2	24	48	0											
109	1	Hb	2004	M	W	19	0	1	255	5	0	0	0	0	0			0	0	0	0	0	0	0	0
110	1	Hb	2003	M	W	6	0	1	0	5	24	24	0											
110	1	Hb	2003	M	W	7	0	1	28	6	96	96	0											
110	1	Hb	2003	M	W	8	0	1	50	7	96	384	384											
110	1	Hb	2003	M	W	10	0	1	80	9	48	96	192											
110	1	Hb	2003	M	W	12	0	1	120	11	0	0	0											
110	1	Hb	2004	M	W	15	0	1	165	2	0	24	24											
110	1	Hb	2004	M	W	19	0	1	255	5	0	0	24	0	0			0	1	0	0	0	0	0	0
111	1	Hb	2003	M	W	6	0	1	0	5	24	0	0											
111	1	Hb	2003	M	W	7	0	1	28	6	192	96	0											
111	1	Hb	2003	M	W	8	0	1	50	7	192	1536	384											
111	1	Hb	2003	M	W	10	0	1	80	9	192	48	48											
111	1	Hb	2003	M	W	12	0	1	120	11	48	0	192											
111	1	Hb	2004	M	W	15	0	1	165	2	0	48	24											
111	1	Hb	2004	M	W	19	0	1	255	5	24	0	0	0	0			0	1	0	0	0	0	0	0
112	1	Hb	2003	M	W	6	0	1	0	5	0	0	0											
112	1	Hb	2003	M	W	7	0	1	28	6	96	192	0											
112	1	Hb	2003	M	W	8	0	1	50	7	96	192	192											
112	1	Hb	2003	M	W	10	0	1	80	9	48	192	192											
112	1	Hb	2003	M	W	12	0	1	120	11	24	96	48											
112	1	Hb	2004	M	W	15	0	1	165	2	0	48	0											
112	1	Hb	2004	M	W	19	0	1	255	5	0	0	0	0	0			0	1	0	0	0	0	0	0
113	1	Hb	2003	M	W	6	0	1	0	5	48	24	0											
113	1	Hb	2003	M	W	7	0	1	28	6	48	96	0											
113	1	Hb	2003	M	W	8	0	1	50	7	192	384	192											
113	1	Hb	2003	M	W	10	0	1	80	9	48	192	192											
113	1	Hb	2003	M	W	12	0	1	120	11	48	48	24											
113	1	Hb	2004	M	W	15	0	1	165	2	0	24	24											
113	1	Hb	2004	M	W	19	0	1	255	5	0	0	0	0	0			0	0	0	0	0	0	0	0
114	1	Hb	2003	M	W	6	0	1	0	5	0	24	0											
114	1	Hb	2003	M	W	7	0	1	28	6	48	192	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney	Histopathology						
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
114	1	Hb	2003	M	W	8	0	1	50	7	48	96	96												
114	1	Hb	2003	M	W	10	0	1	80	9	0	24	48												
114	1	Hb	2003	M	W	12	0	1	120	11	0	24	0												
114	1	Hb	2004	M	W	15	0	1	165	2	0	24	0												
114	1	Hb	2004	M	W	19	0	1	255	5	24	0	0	0	0			1	1	1	0	0	1	1	0
115	1	Hb	2003	M	W	6	0	1	0	5	24	0	0												
115	1	Hb	2003	M	W	7	0	1	28	6	192	192	0												
115	1	Hb	2003	M	W	8	0	1	50	7	768	768	768												
115	1	Hb	2003	M	W	10	0	1	80	9	192	192	384												
115	1	Hb	2003	M	W	12	0	1	120	11	48	48	96												
115	1	Hb	2004	M	W	15	0	1	165	2	0	24	24												
115	1	Hb	2004	M	W	19	0	1	255	5	0	0	24	0	0			1	1	1	0	0	1	0	0
116	1	Hb	2003	M	W	6	0	1	0	5	0	0	0												
116	1	Hb	2003	M	W	7	0	1	28	6	48	48	0												
116	1	Hb	2003	M	W	8	0	1	50	7	96	384	384												
116	1	Hb	2003	M	W	10	0	1	80	9	48	48	48												
116	1	Hb	2003	M	W	12	0	1	120	11	0	192	48												
116	1	Hb	2004	M	W	15	0	1	165	2	0	48	0												
116	1	Hb	2004	M	W	19	0	1	255	5	0	0	0	0	0			0	0	0	0	0	0	0	0
117	1	Hb	2003	M	W	6	0	1	0	5	48	0	0												
117	1	Hb	2003	M	W	7	0	1	28	6	48	96	0												
117	1	Hb	2003	M	W	8	0	1	50	7	192	768	96												
117	1	Hb	2003	M	W	10	0	1	80	9	24	192	96												
117	1	Hb	2003	M	W	12	0	1	120	11	0	96	24												
117	1	Hb	2004	M	W	15	0	1	165	2	48	24	24												
117	1	Hb	2004	M	W	19	0	1	255	5	48	0	0	0	0			1	0	1	0	0	1	0	0
118	1	Hb	2003	M	W	6	0	1	0	5	24	0	0												
118	1	Hb	2003	M	W	7	0	1	28	6	48	48	0												
118	1	Hb	2003	M	W	8	0	1	50	7	96	384	96												
118	1	Hb	2003	M	W	10	0	1	80	9	24	192	96												
118	1	Hb	2003	M	W	12	0	1	120	11	48	48	96												
118	1	Hb	2004	M	W	15	0	1	165	2	0	48	24												
118	1	Hb	2004	M	W	19	0	1	255	5	24	0	0	0	0			1	0	1	0	0	1	1	0
119	1	Hb	2003	M	W	6	0	1	0	5	24	0	0												
119	1	Hb	2003	M	W	7	0	1	28	6	48	96	0												
119	1	Hb	2003	M	W	8	0	1	50	7	384	768	384												
119	1	Hb	2003	M	W	10	0	1	80	9	192	48	48												
119	1	Hb	2003	M	W	12	0	1	120	11	48	96	24												
119	1	Hb	2004	M	W	15	0	1	165	2	0	48	48												
119	1	Hb	2004	M	W	19	0	1	255	5	24	0	0	0	0			1	0	1	0	0	0	1	0
120	1	Hb	2003	M	W	6	0	1	0	5	48	0	0												
120	1	Hb	2003	M	W	7	0	1	28	6	192	48	0												
120	1	Hb	2003	M	W	8	0	1	50	7	384	6154	384												
120	1	Hb	2003	M	W	10	0	1	80	9	96	96	96												
120	1	Hb	2003	M	W	12	0	1	120	11	48	48	48												
120	1	Hb	2004	M	W	15	0	1	165	2	0	96	24												
120	1	Hb	2004	M	W	19	0	1	255	5	48	0	24	0	0			0	0	0	0	0	0	0	0
121	1	Hb	2003	M	W	6	0	0	0	5	24	0	0												
121	1	Hb	2003	M	W	6	0	1	0	5	0	0	0												
121	1	Hb	2003	M	W	7	0	0	28	6	0	0	0												
121	1	Hb	2003	M	W	7	0	1	28	6	96	384	0												
121	1	Hb	2003	M	W	8	0	0	50	7	0	0	0												
121	1	Hb	2003	M	W	8	0	1	50	7	384	1536	192												
121	1	Hb	2003	M	W	10	0	0	80	9	0	0	0												
121	1	Hb	2003	M	W	10	0	1	80	9	96	192	192												
121	1	Hb	2003	M	W	12	0	0	120	11	0	0	0												
121	1	Hb	2003	M	W	12	0	1	120	11	48	96	96												
121	1	Hb	2004	M	W	15	0	0	165	2	0	0	0												
121	1	Hb	2004	M	W	15	0	1	165	2	48	24	0												
121	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0			1	0	1	0	0	1	1	0
122	1	Hb	2003	M	W	6	0	0	0	5	0	0	0												
122	1	Hb	2003	M	W	6	0	1	0	5	0	0	0												
122	1	Hb	2003	M	W	7	0	0	28	6	0	0	0												
122	1	Hb	2003	M	W	7	0	1	28	6	48	48	0												
122	1	Hb	2003	M	W	8	0	0	50	7	48	0	0												
122	1	Hb	2003	M	W	8	0	1	50	7	1536	384	384												
122	1	Hb	2003	M	W	10	0	0	80	9	0	0	0												
122	1	Hb	2003	M	W	10	0	1	80	9	96	384	192												
122	1	Hb	2003	M	W	12	0	0	120	11	48	0	0												
122	1	Hb	2003	M	W	12	0	1	120	11	48	48	48												
122	1	Hb	2004	M	W	15	0	0	165	2	0	0	0												
122	1	Hb	2004	M	W	15	0	1	165	2	24	24	0												
122	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0			1	1	0	0	0	0	1	0
123	1	Hb	2003	M	W	6	0	0	0	5	0	0	0												
123	1	Hb	2003	M	W	7	0	0	28	6	0	0	0												
123	1	Hb	2003	M	W	8	0	0	50	7	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
123	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
123	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
123	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
123	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0			0	1	0	0	0	0	0	0
124	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
124	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
124	1	Hb	2003	M	W	8	0	0	50	7	48	0	0											
124	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
124	1	Hb	2003	M	W	12	0	0	120	11	48	0	0											
124	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
124	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0			0	1	0	0	0	0	0	0
125	1	Hb	2003	M	W	6	0	0	0	5	0	0	0											
125	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
125	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
125	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
125	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
125	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
125	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0			1	0	1	0	0	1	0	0
126	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
126	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
126	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
126	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
126	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
126	1	Hb	2004	M	W	15	0	0	165	2	24	0	0											
126	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0			1	0	1	0	0	1	0	0
127	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
127	1	Hb	2003	M	W	7	0	0	28	6	48	0	0											
127	1	Hb	2003	M	W	8	0	0	50	7	48	0	0											
127	1	Hb	2003	M	W	10	0	0	80	9	48	0	0											
127	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
127	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
127	1	Hb	2004	M	W	19	0	0	255	5	48	0	0	0	0			0	0	0	0	0	0	0	0
128	1	Hb	2003	M	W	6	0	0	0	5	0	0	0											
128	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
128	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
128	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
128	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
128	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
128	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0			1	1	1	0	0	1	0	0
129	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
129	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
129	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
129	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
129	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
129	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
129	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0			0	0	0	0	0	0	0	0
130	1	Hb	2003	M	W	6	0	0	0	5	0	0	0											
130	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
130	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
130	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
130	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
130	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
130	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0			0	1	0	0	0	0	0	0
131	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
131	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
131	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
131	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
131	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
131	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
131	1	Hb	2004	M	W	19	0	0	255	5	48	0	0	0	0			0	1	0	0	0	0	0	0
132	1	Hb	2003	M	W	6	0	0	0	5	48	0	0											
132	1	Hb	2003	M	W	7	0	0	28	6	96	0	0											
132	1	Hb	2003	M	W	8	0	0	50	7	96	0	0											
132	1	Hb	2003	M	W	10	0	0	80	9	192	0	0											
132	1	Hb	2003	M	W	12	0	0	120	11	96	0	0											
132	1	Hb	2004	M	W	15	0	0	165	2	192	0	0											
132	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0			1	0	1	0	0	1	1	0
133	1	Hb	2003	M	W	6	0	0	0	5	0	0	0											
133	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
133	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
133	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
133	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
133	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
133	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0			0	0	0	0	0	0	0	0
134	1	Hb	2003	M	W	6	0	0	0	5	48	0	0											
134	1	Hb	2003	M	W	7	0	0	28	6	24	0	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
134	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
134	1	Hb	2003	M	W	10	0	0	80	9	24	0	0											
134	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
134	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
134	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	1	1		Hb	1	1	1	0	0	1	1	0
135	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
135	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
135	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
135	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
135	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
135	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
135	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0			0	1	0	0	0	0	0	0
136	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
136	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
136	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
136	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
136	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
136	1	Hb	2004	M	W	15	0	0	165	2	24	0	0											
136	1	Hb	2004	M	W	19	0	0	255	5	48	0	0	0	1			0	1	1	0	0	1	1	0
137	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
137	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
137	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
137	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
137	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
137	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
137	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0		0	0	0	0	0	0	0	0	0
138	1	Hb	2003	M	W	6	0	0	0	5	24	0	0											
138	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
138	1	Hb	2003	M	W	8	0	0	50	7	24	0	0											
138	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
138	1	Hb	2003	M	W	12	0	0	120	11	24	0	0											
138	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
138	1	Hb	2004	M	W	19	0	0	255	5	24	0	0	0	0		0	0	1	1	0	1	1	0	1
139	1	Hb	2003	M	W	6	0	0	0	5	48	0	0											
139	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
139	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
139	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
139	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
139	1	Hb	2004	M	W	15	0	0	165	2	24	0	0											
139	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0		0	0	0	0	0	0	0	0	0
140	1	Hb	2003	M	W	6	0	0	0	5	0	0	0											
140	1	Hb	2003	M	W	7	0	0	28	6	0	0	0											
140	1	Hb	2003	M	W	8	0	0	50	7	0	0	0											
140	1	Hb	2003	M	W	10	0	0	80	9	0	0	0											
140	1	Hb	2003	M	W	12	0	0	120	11	0	0	0											
140	1	Hb	2004	M	W	15	0	0	165	2	0	0	0											
140	1	Hb	2004	M	W	19	0	0	255	5	0	0	0	0	0		1	1	0	0	1	1	1	1	0
201	1	Hb	2003	F	W	6	0	0	0	5	24	0	0											
201	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
201	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	1	1								
201	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0								
201	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	1	1								
201	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	0	0								
201	1	Hb	2004	F	W	19	0	0	255	5	24	0	0											
202	1	Hb	2003	F	W	6	0	0	0	5	0	0	0											
202	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
202	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0								
202	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0								
202	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0								
202	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	0	0								
202	1	Hb	2004	F	W	19	0	0	255	5	0	0	0											
203	1	Hb	2003	F	W	6	0	0	0	5	48	0	0											
203	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
203	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0								
203	1	Hb	2003	F	W	10	0	0	80	9	0	0	0											
203	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0								
203	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	0	0								
203	1	Hb	2004	F	W	19	0	0	255	5	0	0	0											
204	1	Hb	2003	F	W	6	0	0	0	5	48	0	0											
204	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
204	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0								
204	1	Hb	2003	F	W	10	0	0	80	9	0	0	0											
204	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0								
204	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	0	0								
204	1	Hb	2004	F	W	19	0	0	255	5	0	0	0											
205	1	Hb	2003	F	W	6	0	0	0	5	24	0	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
205	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
205	1	Hb	2003	F	W	8	0	0	50	7	48	0	0	0	0	0									
205	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0									
205	1	Hb	2003	F	W	12	0	0	120	11	48	0	0	0	0	0									
205	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	0	0									
205	1	Hb	2004	F	W	19	0	0	255	5	96	0	0												
206	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
206	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
206	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
206	1	Hb	2003	F	W	10	0	0	80	9	0	0	0												
206	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
206	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	0	0									
206	1	Hb	2004	F	W	19	0	0	255	5	0	0	0												
207	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
207	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
207	1	Hb	2003	F	W	8	0	0	50	7	96	0	0	0	0	0									
207	1	Hb	2003	F	W	10	0	0	80	9	48	0	0	0	0	0									
207	1	Hb	2003	F	W	12	0	0	120	11	96	0	0	0	1	1									
207	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	1	1									
207	1	Hb	2004	F	W	19	0	0	255	5	0	0	0												
208	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
208	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
208	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
208	1	Hb	2003	F	W	10	0	0	80	9	0	0	0												
208	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
208	1	Hb	2004	F	W	15	0	0	165	2	48	0	0	0	0	0									
208	1	Hb	2004	F	W	19	0	0	255	5	192	0	0												
209	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
209	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
209	1	Hb	2003	F	W	8	0	0	50	7	48	0	0	0	0	0									
209	1	Hb	2003	F	W	10	0	0	80	9	24	0	0	0	0	0									
209	1	Hb	2003	F	W	12	0	0	120	11	48	0	0	0	0	0									
209	1	Hb	2004	F	W	15	0	0	165	2	48	0	0	0	0	0									
209	1	Hb	2004	F	W	19	0	0	255	5	96	0	0												
210	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
210	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
210	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
210	1	Hb	2003	F	W	10	0	0	80	9	0	0	0												
210	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
210	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	0	0									
210	1	Hb	2004	F	W	19	0	0	255	5	96	0	0												
211	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
211	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
211	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
211	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0									
211	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
211	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	1	1									
211	1	Hb	2004	F	W	19	0	0	255	5	48	0	0												
212	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
212	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
212	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	1	1									
212	1	Hb	2003	F	W	10	0	0	80	9	0	0	0												
212	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	1	1									
212	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	0	0									
212	1	Hb	2004	F	W	19	0	0	255	5	0	0	0												
213	1	Hb	2003	F	W	6	0	0	0	5	24	0	0												
213	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
213	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
213	1	Hb	2003	F	W	10	0	0	80	9	0	0	0												
213	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
213	1	Hb	2004	F	W	15	0	0	165	2	24	0	0												
213	1	Hb	2004	F	W	19	0	0	255	5	24	0	0												
214	1	Hb	2003	F	W	6	0	0	0	5	24	0	0												
214	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
214	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
214	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0									
214	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
214	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	1	1									
214	1	Hb	2004	F	W	19	0	0	255	5	0	0	0												
215	1	Hb	2003	F	W	6	0	0	0	5	0	0	0												
215	1	Hb	2003	F	W	7	0	0	28	6	0	0	0												
215	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0									
215	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0									
215	1	Hb	2003	F	W	12	0	0	120	11	0	0	0												
215	1	Hb	2004	F	W	15	0	0	165	2	0	0	0	0	0	1									
215	1	Hb	2004	F	W	19	0	0	255	5	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
216	1	Hb	2003	F	W	6	0	0	0	5	48	0	0											
216	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
216	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0								
216	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0								
216	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0								
216	1	Hb	2004	F	W	15	0	0	165	2	192	0	0	0	1	1								
216	1	Hb	2004	F	W	19	0	0	255	5	192	0	0											
217	1	Hb	2003	F	W	6	0	0	0	5	0	0	0											
217	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
217	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	0	0								
217	1	Hb	2003	F	W	10	0	0	80	9	0	0	0											
217	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	1								
217	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	1	1								
217	1	Hb	2004	F	W	19	0	0	255	5	96	0	0											
218	1	Hb	2003	F	W	6	0	0	0	5	24	0	0											
218	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
218	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	1	1								
218	1	Hb	2003	F	W	10	0	0	80	9	0	0	0											
218	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	0	0								
218	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	1	1								
218	1	Hb	2004	F	W	19	0	0	255	5	96	0	0											
219	1	Hb	2003	F	W	6	0	0	0	5	24	0	0											
219	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
219	1	Hb	2003	F	W	8	0	0	50	7	0	0	0	0	1	1								
219	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0								
219	1	Hb	2003	F	W	12	0	0	120	11	0	0	0	0	1	1								
219	1	Hb	2004	F	W	15	0	0	165	2	24	0	0	0	1	1								
219	1	Hb	2004	F	W	19	0	0	255	5	48	0	0											
220	1	Hb	2003	F	W	6	0	0	0	5	24	0	0											
220	1	Hb	2003	F	W	7	0	0	28	6	0	0	0											
220	1	Hb	2003	F	W	8	0	0	50	7	24	0	0	0	1	0								
220	1	Hb	2003	F	W	10	0	0	80	9	0	0	0	0	0	0								
220	1	Hb	2003	F	W	12	0	0	120	11	24	0	0	0	0	0								
220	1	Hb	2004	F	W	15	0	0	165	2	48	0	0	0	1	1								
220	1	Hb	2004	F	W	19	0	0	255	5	48	0	0											
78	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
78	2	H-P	2003	M	W	8	0	0	28	7	0	0	0											
78	2	H-P	2003	M	W	9	0	0	50	8	0	0	0											
78	2	H-P	2003	M	W	11	0	0	80	10	0	24	0											
78	2	H-P	2003	M	W	12	0	0	120	11	0	48	0											
78	2	H-P	2004	M	W	15	0	0	165	2	0	192	0											
80	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
80	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
80	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
80	2	H-P	2003	F	W	11	0	0	80	10	0	24	24	0	0	0								
80	2	H-P	2003	F	W	12	0	0	120	11	0	24	0											
80	2	H-P	2004	F	W	15	0	0	165	2	192	3072	48											
83	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
83	2	H-P	2003	M	W	8	0	0	28	7	0	0	0											
83	2	H-P	2003	M	W	9	0	0	50	8	0	0	0											
83	2	H-P	2003	M	W	11	0	0	80	10	0	0	24											
83	2	H-P	2003	M	W	12	0	0	120	11	0	0	0											
83	2	H-P	2004	M	W	15	0	0	165	2	0	0	0											
84	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
84	2	H-P	2003	M	W	8	0	0	28	7	0	0	0											
84	2	H-P	2003	M	W	9	0	0	50	8	0	96	0											
84	2	H-P	2003	M	W	11	0	0	80	10	0	96	0											
84	2	H-P	2003	M	W	12	0	0	120	11	0	24	0											
84	2	H-P	2004	M	W	15	0	0	165	2	48	384	48											
85	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
85	2	H-P	2003	M	W	8	0	0	28	7	0	0	0											
85	2	H-P	2003	M	W	9	0	0	50	8	0	192	0											
85	2	H-P	2003	M	W	11	0	0	80	10	0	24	96											
85	2	H-P	2003	M	W	12	0	0	120	11	24	768	96											
85	2	H-P	2004	M	W	15	0	0	165	2	24	48	0											
86	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
86	2	H-P	2003	M	W	8	0	0	28	7	24	24	48											
86	2	H-P	2003	M	W	9	0	0	50	8	0	0	0											
86	2	H-P	2003	M	W	11	0	0	80	10	0	24	0											
86	2	H-P	2003	M	W	12	0	0	120	11	0	48	0											
86	2	H-P	2004	M	W	15	0	0	165	2	0	192	0											
87	2	H-P	2003	F	W	7	0	0	0	6	24	0	0											
87	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
87	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
87	2	H-P	2003	F	W	11	0	0	80	10	0	0	0	0	0	0								
87	2	H-P	2003	F	W	12	0	0	120	11	0	1536	48	0	1	1								

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology						
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
87	2	H-P	2004	F	W	15	0	0	165	2	24	192	0												
88	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
88	2	H-P	2003	F	W	8	0	0	28	7	48	0	0												
88	2	H-P	2003	F	W	9	0	0	50	8	0	0	0												
88	2	H-P	2003	F	W	11	0	0	80	10	0	24	0	0	0	0									
88	2	H-P	2003	F	W	12	0	0	120	11	24	96	0												
88	2	H-P	2004	F	W	15	0	0	165	2	24	192	0												
89	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
89	2	H-P	2003	M	W	8	0	0	28	7	0	0	24												
89	2	H-P	2003	M	W	9	0	0	50	8	0	0	0												
89	2	H-P	2003	M	W	11	0	0	80	10	0	1536	0												
89	2	H-P	2003	M	W	12	0	0	120	11	0	768	0												
89	2	H-P	2004	M	W	15	0	0	165	2	0	384	0												
90	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
90	2	H-P	2003	F	W	8	0	0	28	7	0	0	0	0	0	0									
90	2	H-P	2003	F	W	9	0	0	50	8	0	0	0	0	0	0									
90	2	H-P	2003	F	W	11	0	0	80	10	0	24	0	0	0	0									
90	2	H-P	2003	F	W	12	0	0	120	11	0	48	0	0	0	0									
90	2	H-P	2004	F	W	15	0	0	165	2	0	24	0												
91	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
91	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
91	2	H-P	2003	M	W	9	0	0	50	8	0	768	0												
91	2	H-P	2003	M	W	11	0	0	80	10	0	192	48												
91	2	H-P	2003	M	W	12	0	0	120	11	0	192	0												
91	2	H-P	2004	M	W	15	0	0	165	2	0	192	0												
92	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
92	2	H-P	2003	F	W	8	0	0	28	7	48	96	24												
92	2	H-P	2003	F	W	9	0	0	50	8	0	0	0												
92	2	H-P	2003	F	W	11	0	0	80	10	0	3072	192	0	0	0									
92	2	H-P	2003	F	W	12	0	0	120	11	24	1536	48	0	1	1									
92	2	H-P	2004	F	W	15	0	0	165	2	96	384	96												
93	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
93	2	H-P	2003	F	W	8	0	0	28	7	24	0	0												
93	2	H-P	2003	F	W	9	0	0	50	8	0	0	0												
93	2	H-P	2003	F	W	11	0	0	80	10	0	24	0	0	0	0									
93	2	H-P	2003	F	W	12	0	0	120	11	1536	24	24	1	1	0	Hb								
93	2	H-P	2004	F	W	15	0	0	165	2	192	48	0	1	1	1	Hb								
94	2	H-P	2003	F	W	7	0	0	0	6	96	24	0												
94	2	H-P	2003	F	W	8	0	0	28	7	0	0	24												
94	2	H-P	2003	F	W	9	0	0	50	8	0	24	0	0	0	0									
94	2	H-P	2003	F	W	11	0	0	80	10	24	0	0	0	0	0									
94	2	H-P	2003	F	W	12	0	0	120	11	0	768	48	0	0	0									
94	2	H-P	2004	F	W	15	0	0	165	2	0	96	0	0	0	0									
95	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
95	2	H-P	2003	F	W	8	0	0	28	7	0	0	0												
95	2	H-P	2003	F	W	9	0	0	50	8	0	0	0												
95	2	H-P	2003	F	W	11	0	0	80	10	0	0	0	0	0	0									
95	2	H-P	2003	F	W	12	0	0	120	11	0	0	0												
95	2	H-P	2004	F	W	15	0	0	165	2	0	0	0												
96	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
96	2	H-P	2003	F	W	8	0	0	28	7	0	0	0												
96	2	H-P	2003	F	W	9	0	0	50	8	0	96	0	0	0	0									
96	2	H-P	2003	F	W	11	0	0	80	10	0	24	96	0	0	0									
96	2	H-P	2003	F	W	12	0	0	120	11	48	24	0	0	1	1									
96	2	H-P	2004	F	W	15	0	0	165	2	48	192	48	0	1	0									
97	2	H-P	2003	M	W	7	0	0	0	6	0	24	0												
97	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
97	2	H-P	2003	M	W	9	0	0	50	8	0	192	0												
97	2	H-P	2003	M	W	11	0	0	80	10	0	192	0												
97	2	H-P	2003	M	W	12	0	0	120	11	48	192	0												
97	2	H-P	2004	M	W	15	0	0	165	2	96	192	0												
98	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
98	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
98	2	H-P	2003	M	W	9	0	0	50	8	0	24	0												
98	2	H-P	2003	M	W	11	0	0	80	10	24	0	0												
98	2	H-P	2003	M	W	12	0	0	120	11	768	1536	96												
98	2	H-P	2004	M	W	15	0	0	165	2	24	384	0												
99	2	H-P	2003	M	W	7	0	0	0	6	0	24	0												
99	2	H-P	2003	M	W	8	0	0	28	7	24	24	0												
99	2	H-P	2003	M	W	9	0	0	50	8	0	0	0												
99	2	H-P	2003	M	W	11	0	0	80	10	0	24	0												
99	2	H-P	2003	M	W	12	0	0	120	11	0	24	0												
99	2	H-P	2004	M	W	15	0	0	165	2	0	24	0												
100	2	H-P	2003	M	W	7	0	0	0	6	0	24	0												
100	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
100	2	H-P	2003	M	W	9	0	0	50	8	0	96	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
100	2	H-P	2003	M	W	11	0	0	80	10	0	24	0											
100	2	H-P	2003	M	W	12	0	0	120	11	0	24	0											
100	2	H-P	2004	M	W	15	0	0	165	2	0	24	0											
101	2	H-P	2003	M	W	7	0	0	0	6	0	48	0											
101	2	H-P	2003	M	W	8	0	0	28	7	0	0	0											
101	2	H-P	2003	M	W	9	0	0	50	8	0	24	0											
101	2	H-P	2003	M	W	11	0	0	80	10	0	0	0											
101	2	H-P	2003	M	W	12	0	0	120	11	0	24	48											
101	2	H-P	2004	M	W	15	0	0	165	2	0	96	24											
102	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
102	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
102	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
102	2	H-P	2003	F	W	11	0	0	80	10	0	48	0	0	0	0								
102	2	H-P	2003	F	W	12	0	0	120	11	0	0	24	0	0	0								
102	2	H-P	2004	F	W	15	0	0	165	2	48	24	24	0	1	1								
103	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
103	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
103	2	H-P	2003	F	W	9	0	0	50	8	24	0	0	0	1	1								
103	2	H-P	2003	F	W	11	0	0	80	10	0	0	24	0	0	0								
103	2	H-P	2003	F	W	12	0	0	120	11	24	1536	96	0	1	1								
103	2	H-P	2004	F	W	15	0	0	165	2	48	96	24	1	1	0	Hb							
104	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
104	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
104	2	H-P	2003	F	W	9	0	0	50	8	0	0	0	0	0	0								
104	2	H-P	2003	F	W	11	0	0	80	10	0	0	0	0	0	0								
104	2	H-P	2003	F	W	12	0	0	120	11	384	24	0	0	1	0								
104	2	H-P	2004	F	W	15	0	0	165	2	384	24	0	1	1	1	Hb							
105	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
105	2	H-P	2003	F	W	8	0	0	28	7	0	0	24											
105	2	H-P	2003	F	W	9	0	0	50	8	0	0	0	0	0	0								
105	2	H-P	2003	F	W	11	0	0	80	10	0	0	0	0	0	0								
105	2	H-P	2003	F	W	12	0	0	120	11	24	96	0	0	1	0								
105	2	H-P	2004	F	W	15	0	0	165	2	48	0	0	0	1	0								
106	2	H-P	2003	F	W	7	0	0	0	6	24	0	0											
106	2	H-P	2003	F	W	8	0	0	28	7	0	24	0											
106	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
106	2	H-P	2003	F	W	11	0	0	80	10	0	24	0	0	0	0								
106	2	H-P	2003	F	W	12	0	0	120	11	384	3072	48	0	1	1								
106	2	H-P	2004	F	W	15	0	0	165	2	96	3072	0											
107	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
107	2	H-P	2003	F	W	8	0	0	28	7	0	24	0											
107	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
107	2	H-P	2003	F	W	11	0	0	80	10	0	0	0	0	0	0								
107	2	H-P	2003	F	W	12	0	0	120	11	0	24	0	0	0	0								
107	2	H-P	2004	F	W	15	0	0	165	2	96	192	0											
108	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
108	2	H-P	2003	M	W	8	0	0	28	7	48	48	0											
108	2	H-P	2003	M	W	9	0	0	50	8	0	48	0											
108	2	H-P	2003	M	W	11	0	0	80	10	0	24	0											
108	2	H-P	2003	M	W	12	0	0	120	11	0	192	0											
108	2	H-P	2004	M	W	15	0	0	165	2	0	192	0											
109	2	H-P	2003	F	W	7	0	0	0	6	24	0	0											
109	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
109	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
109	2	H-P	2003	F	W	11	0	0	80	10	0	24	0	0	0	0								
109	2	H-P	2003	F	W	12	0	0	120	11	0	1536	0											
109	2	H-P	2004	F	W	15	0	0	165	2	0	384	0											
110	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											
110	2	H-P	2003	F	W	8	0	0	28	7	24	0	0											
110	2	H-P	2003	F	W	9	0	0	50	8	96	0	0	0	1	1								
110	2	H-P	2003	F	W	11	0	0	80	10	24	0	0	0	0	0								
110	2	H-P	2003	F	W	12	0	0	120	11	0	24	24	0	1	1	Hb							
110	2	H-P	2004	F	W	15	0	0	165	2	0	96	0	0	0	0								
111	2	H-P	2003	M	W	7	0	0	0	6	0	0	0											
111	2	H-P	2003	M	W	8	0	0	28	7	0	0	0											
111	2	H-P	2003	M	W	9	0	0	50	8	0	192	0											
111	2	H-P	2003	M	W	11	0	0	80	10	0	24	48											
111	2	H-P	2003	M	W	12	0	0	120	11	0	3072	96											
111	2	H-P	2004	M	W	15	0	0	165	2	0	3072	48											
112	2	H-P	2003	F	W	7	0	0	0	6	0	24	0											
112	2	H-P	2003	F	W	8	0	0	28	7	0	0	0											
112	2	H-P	2003	F	W	9	0	0	50	8	0	0	0											
112	2	H-P	2003	F	W	11	0	0	80	10	0	24	0	0	0	0								
112	2	H-P	2003	F	W	12	0	0	120	11	384	0	24	0	1	1								
112	2	H-P	2004	F	W	15	0	0	165	2	48	0	0											
113	2	H-P	2003	F	W	7	0	0	0	6	0	0	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
113	2	H-P	2003	F	W	8	0	0	28	7	24	0	0												
113	2	H-P	2003	F	W	9	0	0	50	8	0	96	0	0	0	1									
113	2	H-P	2003	F	W	11	0	0	80	10	0	48	0	0	0	0									
113	2	H-P	2003	F	W	12	0	0	120	11	0	96	0												
113	2	H-P	2004	F	W	15	0	0	165	2	24	48	24												
114	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
114	2	H-P	2003	M	W	8	0	0	28	7	0	0	24												
114	2	H-P	2003	M	W	9	0	0	50	8	24	0	48												
114	2	H-P	2003	M	W	11	0	0	80	10	0	3072	0												
114	2	H-P	2003	M	W	12	0	0	120	11	0	1536	0												
114	2	H-P	2004	M	W	15	0	0	165	2	0	384	0												
115	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
115	2	H-P	2003	M	W	8	0	0	28	7	24	0	0												
115	2	H-P	2003	M	W	9	0	0	50	8	24	0	0												
115	2	H-P	2003	M	W	11	0	0	80	10	0	48	0												
115	2	H-P	2003	M	W	12	0	0	120	11	0	96	0												
115	2	H-P	2004	M	W	15	0	0	165	2	0	192	0												
116	2	H-P	2003	F	W	7	0	0	0	6	0	0	0												
116	2	H-P	2003	F	W	8	0	0	28	7	0	0	0	0	0	0									
116	2	H-P	2003	F	W	9	0	0	50	8	0	96	0	0	0	0									
116	2	H-P	2003	F	W	11	0	0	80	10	0	48	96	0	0	0									
116	2	H-P	2003	F	W	12	0	0	120	11	0	0	0	0	0	0									
116	2	H-P	2004	F	W	15	0	0	165	2	0	0	0	0	0	0									
117	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
117	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
117	2	H-P	2003	M	W	9	0	0	50	8	0	0	0												
117	2	H-P	2003	M	W	11	0	0	80	10	48	1536	0												
117	2	H-P	2003	M	W	12	0	0	120	11	0	1536	0												
117	2	H-P	2004	M	W	15	0	0	165	2	0	1536	0												
118	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
118	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
118	2	H-P	2003	M	W	9	0	0	50	8	0	0	0												
118	2	H-P	2003	M	W	11	0	0	80	10	0	48	0												
118	2	H-P	2003	M	W	12	0	0	120	11	0	0	0												
118	2	H-P	2004	M	W	15	0	0	165	2	0	0	0												
119	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
119	2	H-P	2003	M	W	8	0	0	28	7	0	24	0												
119	2	H-P	2003	M	W	9	0	0	50	8	0	192	0												
119	2	H-P	2003	M	W	11	0	0	80	10	0	192	48												
119	2	H-P	2003	M	W	12	0	0	120	11	384	24	48												
119	2	H-P	2004	M	W	15	0	0	165	2	48	0	24												
120	2	H-P	2003	M	W	7	0	0	0	6	0	0	0												
120	2	H-P	2003	M	W	8	0	0	28	7	0	0	0												
120	2	H-P	2003	M	W	9	0	0	50	8	0	0	0												
120	2	H-P	2003	M	W	11	0	0	80	10	0	0	0												
120	2	H-P	2003	M	W	12	0	0	120	11	0	96	0												
120	2	H-P	2004	M	W	15	0	0	165	2	0	96	0												
A17	2	H-P	2003	M	W	7	0	1	0	6	0	0	0												
a17	2	H-P	2003	M	W	8	0	1	28	7	48	24	24												
A17	2	H-P	2003	M	W	9	0	1	50	8	96	0	96												
A17	2	H-P	2003	M	W	11	0	1	80	10	24	96	48												
A17	2	H-P	2003	M	W	12	0	1	120	11	0	24	48												
A17	2	H-P	2004	M	W	15	0	1	165	2	192		24												
F522	2	H-P	2003	F	W	7	0	1	0	6	0	0	0												
F522	2	H-P	2003	F	W	8	0	1	28	7	0	48	192												
F522	2	H-P	2003	F	W	9	0	1	50	8	48	192	192	0	0	0									
F522	2	H-P	2003	F	W	11	0	1	80	10	48	48	0	0	0	0									
F522	2	H-P	2003	F	W	12	0	1	120	11	0	24	24	0	0	0									
F522	2	H-P	2004	F	W	15	0	1	165	2	384	48	24	0	0	0									
F542	2	H-P	2003	M	W	7	0	1	0	6	0	0	0												
F542	2	H-P	2003	M	W	8	0	1	28	7	48	24	96												
F542	2	H-P	2003	M	W	9	0	1	50	8	48	96	192												
F542	2	H-P	2003	M	W	11	0	1	80	10	24	192	96												
F542	2	H-P	2003	M	W	12	0	1	120	11	0														
F542	2	H-P	2004	M	W	15	0	1	165	2	0														
H804	2	H-P	2003	F	W	7	0	1	0	6	0	0	0												
H804	2	H-P	2003	F	W	8	0	1	28	7	96	0	48												
H804	2	H-P	2003	F	W	9	0	1	50	8	0	0	192												
H804	2	H-P	2003	F	W	11	0	1	80	10	0	48	192	0	0	0									
H804	2	H-P	2003	F	W	12	0	1	120	11	0	24	96	0	0	1									
H804	2	H-P	2004	F	W	15	0	1	165	2	0		0												
H838	2	H-P	2003	M	W	7	0	1	0	6	0	0	0												
H838	2	H-P	2003	M	W	8	0	1	28	7	24	0	96												
H838	2	H-P	2003	M	W	9	0	1	50	8	96	0	96												
H838	2	H-P	2003	M	W	11	0	1	80	10	48	96	48												
H838	2	H-P	2003	M	W	12	0	1	120	11		96	24												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney	Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
H838	2	H-P	2004	M	W	15	0	1	165	2		96	24											
H839	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
H839	2	H-P	2003	M	W	8	0	1	28	7	24	48	96											
H839	2	H-P	2003	M	W	9	0	1	50	8	24	24	192											
H839	2	H-P	2003	M	W	11	0	1	80	10	0	48	48											
H839	2	H-P	2003	M	W	12	0	1	120	11	48													
H839	2	H-P	2004	M	W	15	0	1	165	2	48													
1630	2	H-P	2003	M	W	7	0	1	0	6	0	192	0											
1630	2	H-P	2003	M	W	8	0	1	28	7	0	0	0											
1630	2	H-P	2003	M	W	9	0	1	50	8	48	0	0											
1630	2	H-P	2003	M	W	11	0	1	80	10	48	48												
1630	2	H-P	2003	M	W	12	0	1	120	11	0													
1630	2	H-P	2004	M	W	15	0	1	165	2	48													
1636	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
1636	2	H-P	2003	M	W	8	0	1	28	7	0	96	192											
1636	2	H-P	2003	M	W	9	0	1	50	8	0	48	384											
1636	2	H-P	2003	M	W	11	0	1	80	10	0	48	96											
1636	2	H-P	2003	M	W	12	0	1	120	11	0	48	96											
1636	2	H-P	2004	M	W	15	0	1	165	2	96		48											
J701	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
J701	2	H-P	2003	F	W	8	0	1	28	7	0	48	0											
J701	2	H-P	2003	F	W	9	0	1	50	8	0	48	96											
J701	2	H-P	2003	F	W	11	0	1	80	10	0	96	24	0	0	0								
J701	2	H-P	2003	F	W	12	0	1	120	11	0			0	0	0								
J701	2	H-P	2004	F	W	15	0	1	165	2	24													
J703	2	H-P	2003	F	W	7	0	1	0	6	24	0	0											
J703	2	H-P	2003	F	W	8	0	1	28	7	48	0	96											
J703	2	H-P	2003	F	W	9	0	1	50	8	0	0	192	0	0	0								
J703	2	H-P	2003	F	W	11	0	1	80	10	0	48	0	0	0	0								
J703	2	H-P	2003	F	W	12	0	1	120	11				1	1	1								
J703	2	H-P	2004	F	W	15	0	1	165	2	24			0	0	0								
J705	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
J705	2	H-P	2003	F	W	8	0	1	28	7	48	0	0											
J705	2	H-P	2003	F	W	9	0	1	50	8	384	48	96	0	0	0								
J705	2	H-P	2003	F	W	11	0	1	80	10	0	384	96	0	0	0								
J705	2	H-P	2003	F	W	12	0	1	120	11	24	0	48	0	1	1								
J705	2	H-P	2004	F	W	15	0	1	165	2	0	96	0	0	0	0								
J710	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
J710	2	H-P	2003	F	W	8	0	1	28	7	0	0	0											
J710	2	H-P	2003	F	W	9	0	1	50	8	48	0	48	0	0	0								
J710	2	H-P	2003	F	W	11	0	1	80	10	48	0	0	0	0	0								
J710	2	H-P	2003	F	W	12	0	1	120	11	0	24	0	0	0	0								
J710	2	H-P	2004	F	W	15	0	1	165	2	24	384	24											
J712	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
J712	2	H-P	2003	F	W	8	0	1	28	7	48	48	48											
J712	2	H-P	2003	F	W	9	0	1	50	8	96	192	192											
J712	2	H-P	2003	F	W	11	0	1	80	10	96	96	96	0	0	0								
J712	2	H-P	2003	F	W	12	0	1	120	11	48	24	0											
J712	2	H-P	2004	F	W	15	0	1	165	2		48	0											
J715	2	H-P	2003	F	W	7	0	1	0	6	24	0	0											
J715	2	H-P	2003	F	W	8	0	1	28	7	48	0	48											
J715	2	H-P	2003	F	W	9	0	1	50	8	48	0	96	0	0	0								
J715	2	H-P	2003	F	W	11	0	1	80	10	48	0	48											
J715	2	H-P	2003	F	W	12	0	1	120	11	0	24	24											
J715	2	H-P	2004	F	W	15	0	1	165	2	48	24	24	0	0	0								
J722	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
J722	2	H-P	2003	F	W	8	0	1	28	7	48	0	96											
J722	2	H-P	2003	F	W	9	0	1	50	8	48	768	0	0	0	0								
J722	2	H-P	2003	F	W	11	0	1	80	10	24	96	48	0	0	0								
J722	2	H-P	2003	F	W	12	0	1	120	11	0			0	0	0								
J722	2	H-P	2004	F	W	15	0	1	165	2	24			0	0	0								
J729	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
J729	2	H-P	2003	F	W	8	0	1	28	7	0	96	192											
J729	2	H-P	2003	F	W	9	0	1	50	8	48	0	192											
J729	2	H-P	2003	F	W	11	0	1	80	10		96	192											
J729	2	H-P	2003	F	W	12	0	1	120	11		0	96	0	0	0								
J729	2	H-P	2004	F	W	15	0	1	165	2			48	0	0	0								
J731	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
J731	2	H-P	2003	M	W	8	0	1	28	7	24	0	0											
J731	2	H-P	2003	M	W	9	0	1	50	8	0	0	48											
J731	2	H-P	2003	M	W	11	0	1	80	10	96	48	0											
J731	2	H-P	2003	M	W	12	0	1	120	11		24	0											
J731	2	H-P	2004	M	W	15	0	1	165	2		24	48											
J745	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
J745	2	H-P	2003	M	W	8	0	1	28	7	0	96	48											
J745	2	H-P	2003	M	W	9	0	1	50	8	0	0	96											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
J745	2	H-P	2003	M	W	11	0	1	80	10	0	48	192											
J745	2	H-P	2003	M	W	12	0	1	120	11	0	24	96											
J745	2	H-P	2004	M	W	15	0	1	165	2		96	24											
J748	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
J748	2	H-P	2003	M	W	8	0	1	28	7	24	0	48											
J748	2	H-P	2003	M	W	9	0	1	50	8	0	0	48											
J748	2	H-P	2003	M	W	11	0	1	80	10	0	0	48											
J748	2	H-P	2003	M	W	12	0	1	120	11														
J748	2	H-P	2004	M	W	15	0	1	165	2														
J749	2	H-P	2003	M	W	7	0	1	0	6	24	0	0											
J749	2	H-P	2003	M	W	8	0	1	28	7	0	48	96											
J749	2	H-P	2003	M	W	9	0	1	50	8	192	96	768											
J749	2	H-P	2003	M	W	11	0	1	80	10	48	96	48											
J749	2	H-P	2003	M	W	12	0	1	120	11														
J749	2	H-P	2004	M	W	15	0	1	165	2														
J757	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
J757	2	H-P	2003	M	W	8	0	1	28	7	0	24	24											
J757	2	H-P	2003	M	W	9	0	1	50	8	0	0	192											
J757	2	H-P	2003	M	W	11	0	1	80	10	0	96	48											
J757	2	H-P	2003	M	W	12	0	1	120	11	0		24											
J757	2	H-P	2004	M	W	15	0	1	165	2			24											
J758	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
J758	2	H-P	2003	M	W	8	0	1	28	7	24	0	384											
J758	2	H-P	2003	M	W	9	0	1	50	8	0	0	24											
J758	2	H-P	2003	M	W	11	0	1	80	10	48	192	192											
J758	2	H-P	2003	M	W	12	0	1	120	11	0	48	96											
J758	2	H-P	2004	M	W	15	0	1	165	2	0	0	48											
L301	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L301	2	H-P	2003	F	W	8	0	1	28	7	0	48	192											
L301	2	H-P	2003	F	W	9	0	1	50	8	96	0	192	0	1	1								
L301	2	H-P	2003	F	W	11	0	1	80	10	48	0	48	0	0	0								
L301	2	H-P	2003	F	W	12	0	1	120	11	0	48	48	0	0	0								
L301	2	H-P	2004	F	W	15	0	1	165	2	48	384	24											
L304	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L304	2	H-P	2003	F	W	8	0	1	28	7	24	24	48											
L304	2	H-P	2003	F	W	9	0	1	50	8	0	0	192	0	0	0								
L304	2	H-P	2003	F	W	11	0	1	80	10	48	0	96	0	0	0								
L304	2	H-P	2003	F	W	12	0	1	120	11	0	0	0	0	0	0								
L304	2	H-P	2004	F	W	15	0	1	165	2	48	0	0	0	0	0								
L305	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L305	2	H-P	2003	F	W	8	0	1	28	7	96	24	48											
L305	2	H-P	2003	F	W	9	0	1	50	8	192	48	0	0	0	0								
L305	2	H-P	2003	F	W	11	0	1	80	10	96	96	24	0	0	0								
L305	2	H-P	2003	F	W	12	0	1	120	11	0	24	48											
L305	2	H-P	2004	F	W	15	0	1	165	2	48	0	0											
L306	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L306	2	H-P	2003	F	W	8	0	1	28	7	96	48	192											
L306	2	H-P	2003	F	W	9	0	1	50	8	96	0	192	0	0	0								
L306	2	H-P	2003	F	W	11	0	1	80	10	96	48	192	0	0	0								
L306	2	H-P	2003	F	W	12	0	1	120	11	0	48	48	0	0	0								
L306	2	H-P	2004	F	W	15	0	1	165	2	0	48	0	0	0	0								
L307	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L307	2	H-P	2003	F	W	8	0	1	28	7	24	0	96											
L307	2	H-P	2003	F	W	9	0	1	50	8	0	0	96	0	0	0								
L307	2	H-P	2003	F	W	11	0	1	80	10	0	48	0	0	1	1								
L307	2	H-P	2003	F	W	12	0	1	120	11	0	0	0											
L307	2	H-P	2004	F	W	15	0	1	165	2	24	0	0											
L310	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L310	2	H-P	2003	F	W	8	0	1	28	7	24	24	0											
L310	2	H-P	2003	F	W	9	0	1	50	8	48	96	96	0	0	0								
L310	2	H-P	2003	F	W	11	0	1	80	10	48	192	0	0	0	0								
L310	2	H-P	2003	F	W	12	0	1	120	11	48	24	24	0	1	1								
L310	2	H-P	2004	F	W	15	0	1	165	2	48		24	0	0	0								
L313	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L313	2	H-P	2003	F	W	8	0	1	28	7	24	0	384											
L313	2	H-P	2003	F	W	9	0	1	50	8	48	192	192	0	0	0								
L313	2	H-P	2003	F	W	11	0	1	80	10	24	48	96	0	0	0								
L313	2	H-P	2003	F	W	12	0	1	120	11	24	96	0	1	1	1								
L313	2	H-P	2004	F	W	15	0	1	165	2	384	0	0	0	0	0								
L314	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
L314	2	H-P	2003	F	W	8	0	1	28	7	48	0	0											
L314	2	H-P	2003	F	W	9	0	1	50	8	192	96	96	0	0	0								
L314	2	H-P	2003	F	W	11	0	1	80	10	96	96	0	0	0	0								
L314	2	H-P	2003	F	W	12	0	1	120	11	24	48	48	0	0	0								
L314	2	H-P	2004	F	W	15	0	1	165	2	24	48	0	0	0	0								
L326	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology				
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
L327	2	H-P	2003	M	W	8	0	1	28	7	48	48	48											
L328	2	H-P	2003	M	W	9	0	1	50	8	384	384	192											
L329	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
L329	2	H-P	2003	M	W	8	0	1	28	7	24	0	48											
L329	2	H-P	2003	M	W	9	0	1	50	8	192	0	96											
L329	2	H-P	2003	M	W	11	0	1	80	10	96	48	96											
L329	2	H-P	2003	M	W	11	0	1	80	10	96	96	48											
L329	2	H-P	2003	M	W	12	0	1	120	11														
L329	2	H-P	2004	M	W	15	0	1	165	2														
L330	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
L330	2	H-P	2003	M	W	8	0	1	28	7	24	24	24											
L330	2	H-P	2003	M	W	9	0	1	50	8	48	192	96											
L330	2	H-P	2003	M	W	11	0	1	80	10	0	192	0											
L330	2	H-P	2003	M	W	12	0	1	120	11	0	0	0											
L330	2	H-P	2003	M	W	12	0	1	120	11	0	0	48											
L330	2	H-P	2004	M	W	15	0	1	165	2	0	0	0											
L331	2	H-P	2004	M	W	15	0	1	165	2	96	0	0											
L335	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
L335	2	H-P	2003	M	W	8	0	1	28	7	24	96	96											
L335	2	H-P	2003	M	W	9	0	1	50	8	48	384	192											
L335	2	H-P	2003	M	W	11	0	1	80	10	24	48	0											
L335	2	H-P	2003	M	W	12	0	1	120	11	96	96	24											
L335	2	H-P	2004	M	W	15	0	1	165	2	48	96	48											
L336	2	H-P	2003	M	W	7	0	1	0	6	24	0	0											
L336	2	H-P	2003	M	W	8	0	1	28	7	48	24	24											
L336	2	H-P	2003	M	W	9	0	1	50	8	96	0	96											
L336	2	H-P	2003	M	W	11	0	1	80	10	48	48	96											
L336	2	H-P	2003	M	W	12	0	1	120	11	96	24	24											
L336	2	H-P	2004	M	W	15	0	1	165	2	48	96	48											
M907	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
M907	2	H-P	2003	F	W	8	0	1	28	7	24	0	96											
M907	2	H-P	2003	F	W	9	0	1	50	8	96	0	384	0	0	0								
M907	2	H-P	2003	F	W	11	0	1	80	10	0	96	96	0	0	0								
M907	2	H-P	2003	F	W	12	0	1	120	11	0	96	48	0	0	0								
M907	2	H-P	2004	F	W	15	0	1	165	2	0		48	0	0	0								
M911	2	H-P	2003	F	W	7	0	1	0	6	0	0	0											
M911	2	H-P	2003	F	W	8	0	1	28	7	24	0	0											
M911	2	H-P	2003	F	W	9	0	1	50	8	0	0	96	0	1	1								
M911	2	H-P	2003	F	W	11	0	1	80	10	0	24	48	0	0	0								
M911	2	H-P	2003	F	W	12	0	1	120	11	0	0	0	0	1	1								
M911	2	H-P	2004	F	W	15	0	1	165	2	0	0	0	0	0	0								
M933	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
M933	2	H-P	2003	M	W	8	0	1	28	7	24	0	96											
M933	2	H-P	2003	M	W	9	0	1	50	8	48	0	192											
M933	2	H-P	2003	M	W	11	0	1	80	10	48	96	24											
M933	2	H-P	2003	M	W	12	0	1	120	11	24	24	48											
M933	2	H-P	2004	M	W	15	0	1	165	2	48	48	0											
M938	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
M938	2	H-P	2003	M	W	8	0	1	28	7	0	96	96											
M938	2	H-P	2003	M	W	9	0	1	50	8	48	48	384											
M938	2	H-P	2003	M	W	11	0	1	80	10	48	96	48											
M938	2	H-P	2003	M	W	12	0	1	120	11	96	24	24											
M938	2	H-P	2004	M	W	15	0	1	165	2	48	24	0											
M942	2	H-P	2003	M	W	7	0	1	0	6	0	0	0											
M942	2	H-P	2003	M	W	8	0	1	28	7	48	48	192											
M942	2	H-P	2003	M	W	9	0	1	50	8	96	192	192											
M942	2	H-P	2003	M	W	11	0	1	80	10	48	48	24											
M942	2	H-P	2003	M	W	12	0	1	120	11	192	192	48											
M942	2	H-P	2004	M	W	15	0	1	165	2	192	192	48											
100	2	H-P	2004	F	W	3	0	1	-28	2	48	3072	24	0	0	0								
100	2	H-P	2004	F	W	4	0	1	0	3	96	768	48	0	0	0								
100	2	H-P	2004	F	W	5	0	1	28	4	96	3072	96	0	0	0								
100	2	H-P	2004	F	W	7	0	1	82	6	192	96	48											
100	2	H-P	2004	F	W	8	0	1	137	7	768	0	48											
100	2	H-P	2004	F	W	10	0	1	189	9		48												
100	2	H-P	2004	F	W	12	0	1	284	11														
101	2	H-P	2004	F	W	3	0	0	-28	2	0	3072	48											
101	2	H-P	2004	F	W	4	0	0	0	3	48	384	0	1	1	1	P							
101	2	H-P	2004	F	W	5	0	0	28	4	48	3072	48	0	0	0								
101	2	H-P	2004	F	W	7	0	0	82	6	48	0	24	0	0	0								
101	2	H-P	2004	F	W	8	0	0	137	7	48	768	24	0	0	0								
101	2	H-P	2004	F	W	10	0	0	189	9	0	768	0	0	0	0								
101	2	H-P	2004	F	W	12	0	0	284	11	24	384	0	1	1	1	P							
102	2	H-P	2004	F	W	3	0	1	-28	2	0	24	0	0	0	0								
102	2	H-P	2004	F	W	4	0	1	0	3	24	48	0	0	1	1								
102	2	H-P	2004	F	W	5	0	1	28	4	24	48	24	0	0	0								

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
102	2	H-P	2004	F	W	7	0	1	82	6	24	0	24	0	0	0									
102	2	H-P	2004	F	W	8	0	1	137	7	24	24	24	0	0	0									
102	2	H-P	2004	F	W	10	0	1	189	9	0	0	0	1	1	0									
102	2	H-P	2004	F	W	12	0	1	284	11	24	24	0	1	1	1									
103	2	H-P	2004	F	W	3	0	1	-28	2															
103	2	H-P	2004	F	W	4	0	1	0	3															
103	2	H-P	2004	F	W	5	0	1	28	4															
103	2	H-P	2004	F	W	7	0	1	82	6	24	0	24												
103	2	H-P	2004	F	W	8	0	1	137	7	48	0	24												
103	2	H-P	2004	F	W	10	0	1	189	9	0	96	0												
103	2	H-P	2004	F	W	12	0	1	284	11	24	24	0												
104	2	H-P	2004	F	W	3	0	0	-28	2	24	3072	48												
104	2	H-P	2004	F	W	4	0	0	0	3	48	768	0	0	0	0									
104	2	H-P	2004	F	W	5	0	0	28	4	48	1536	48	0	1	1									
104	2	H-P	2004	F	W	7	0	0	82	6	48	0	0												
104	2	H-P	2004	F	W	8	0	0	137	7	0	24	0	0	0	0									
104	2	H-P	2004	F	W	10	0	0	189	9	0	192	0	0	0	0									
104	2	H-P	2004	F	W	12	0	0	284	11	0	192	0	0	0	1									
105	2	H-P	2004	F	W	3	0	0	-28	2	0	96	48	0	0	1									
105	2	H-P	2004	F	W	4	0	0	0	3	384	768	24	0	1	1									
105	2	H-P	2004	F	W	5	0	0	28	4	48	1536	0	0	0	0									
105	2	H-P	2004	F	W	7	0	0	82	6	48	0	24	0	1	1									
105	2	H-P	2004	F	W	8	0	0	137	7	192	384	0	1	1	1	Hb								
105	2	H-P	2004	F	W	10	0	0	189	9	48	192	0	1	1	1	Hb								
105	2	H-P	2004	F	W	12	0	0	284	11	0	24	0	0	0	0									
106	2	H-P	2004	F	W	3	0	0	-28	2	0	0	48												
106	2	H-P	2004	F	W	4	0	0	0	3	48	24	0	1	1	1	Hb								
106	2	H-P	2004	F	W	5	0	0	28	4	24	96	24	0	0	0									
106	2	H-P	2004	F	W	7	0	0	82	6	48	24	24	0	0	0									
106	2	H-P	2004	F	W	8	0	0	137	7	192	192	0	1	1	1	Hb								
106	2	H-P	2004	F	W	10	0	0	189	9	0	96	0	1	1	1	H-P								
106	2	H-P	2004	F	W	12	0	0	284	11	24	96	0	1	1	1	H-P								
107	2	H-P	2004	F	W	3	0	0	-28	2	0	48	24												
107	2	H-P	2004	F	W	4	0	0	0	3	96	96	24	0	1	1									
107	2	H-P	2004	F	W	5	0	0	28	4	24	3072	0	0	0	0									
107	2	H-P	2004	F	W	7	0	0	82	6	48	0	48	0	0	0									
107	2	H-P	2004	F	W	8	0	0	137	7	48	192	0	0	0	0									
107	2	H-P	2004	F	W	10	0	0	189	9	0	384	0												
107	2	H-P	2004	F	W	12	0	0	284	11	0	0	0	0	1	1									
108	2	H-P	2004	F	W	3	0	1	-28	2	0	96	96	0	0	0									
108	2	H-P	2004	F	W	4	0	1	0	3	48	1536	0												
108	2	H-P	2004	F	W	5	0	1	28	4	24	768	192	0	0	0									
108	2	H-P	2004	F	W	7	0	1	82	6	24	0	0	0	0	0									
108	2	H-P	2004	F	W	8	0	1	137	7	24	48	0	0	0	0									
108	2	H-P	2004	F	W	10	0	1	189	9	0	96	0												
108	2	H-P	2004	F	W	12	0	1	284	11	0	48	0	0	0	0									
109	2	H-P	2004	F	W	3	0	1	-28	2	48	3072	24												
109	2	H-P	2004	F	W	4	0	1	0	3	24	96	0	0	0	0									
109	2	H-P	2004	F	W	5	0	1	28	4	96	3072	192	0	0	0									
109	2	H-P	2004	F	W	7	0	1	82	6	48	0	0												
109	2	H-P	2004	F	W	8	0	1	137	7	0	1536	0	1	1	1									
109	2	H-P	2004	F	W	10	0	1	189	9	0	1536	0	0	0	0									
109	2	H-P	2004	F	W	12	0	1	284	11	24	384	0	0	0	0									
111	2	H-P	2004	F	W	3	0	1	-28	2	0	96	0	0	0	0									
111	2	H-P	2004	F	W	4	0	1	0	3	48	1536	0												
111	2	H-P	2004	F	W	5	0	1	28	4	96	48	96	0	0	0									
111	2	H-P	2004	F	W	7	0	1	82	6	96	0	0	1	1	0									
111	2	H-P	2004	F	W	8	0	1	137	7	96	768	24												
111	2	H-P	2004	F	W	10	0	1	189	9	0	768	0	0	0	0									
111	2	H-P	2004	F	W	12	0	1	284	11	0	192	0	0	0	0									
131	2	H-P	2004	F	W	3	1	1	-28	2	96	96	48	0	0	0									
131	2	H-P	2004	F	W	4	1	1	0	3	24	96	24	0	1	1									
131	2	H-P	2004	F	W	5	1	1	28	4	96	48	48	0	0	0									
131	2	H-P	2004	F	W	7	1	1	82	6	48	3072	0	0	0	0									
131	2	H-P	2004	F	W	8	1	1	137	7	24	0	0	0	0	0									
131	2	H-P	2004	F	W	10	1	1	189	9	0	0	0												
131	2	H-P	2004	F	W	12	1	1	284	11	24	0	0												
132	2	H-P	2004	F	W	3	1	1	-28	2	96	96	96	0	0	0									
132	2	H-P	2004	F	W	4	1	1	0	3	24	96	0	0	0	0									
132	2	H-P	2004	F	W	5	1	1	28	4	48	24	0	0	0	0									
132	2	H-P	2004	F	W	7	1	1	82	6	48	768	24	0	0	0									
132	2	H-P	2004	F	W	8	1	1	137	7	96	0	0	1	1	0									
132	2	H-P	2004	F	W	10	1	1	189	9	0	0	0	0	0	0									
132	2	H-P	2004	F	W	12	1	1	284	11	24	24	0	0	0	0									
133	2	H-P	2004	F	W	3	1	1	-28	2	24	96	48	0	0	0									
133	2	H-P	2004	F	W	4	1	1	0	3	48	48	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Porn	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
133	2	H-P	2004	F	W	5	1	1	28	4	48	24	24	0	0	0									
133	2	H-P	2004	F	W	7	1	1	82	6	24	768	0												
133	2	H-P	2004	F	W	8	1	1	137	7	0	0	0												
133	2	H-P	2004	F	W	10	1	1	189	9	0	0	0	0	1	1									
133	2	H-P	2004	F	W	12	1	1	284	11	24	24	0	0	1	1									
134	2	H-P	2004	F	W	3	1	0	-28	2	96	384	48												
134	2	H-P	2004	F	W	4	1	0	0	3	48	384	0	0	0	0									
134	2	H-P	2004	F	W	5	1	0	28	4	24	24	24	0	1	1									
134	2	H-P	2004	F	W	7	1	0	82	6	24	0	0	0	0	0									
134	2	H-P	2004	F	W	8	1	0	137	7	48	96	0	0	0	0									
134	2	H-P	2004	F	W	10	1	0	189	9	0	24	0	1	0	1									
134	2	H-P	2004	F	W	12	1	0	284	11	24	96	0	1	1	1	P								
135	2	H-P	2004	F	W	3	1	1	-28	2	96	96	48												
135	2	H-P	2004	F	W	4	1	1	0	3	96	96	0												
135	2	H-P	2004	F	W	5	1	1	28	4	48	24	24	0	0	0									
135	2	H-P	2004	F	W	7	1	1	82	6	96	48	24												
135	2	H-P	2004	F	W	8	1	1	137	7	0	96	0	1	1	0									
135	2	H-P	2004	F	W	10	1	1	189	9	0	24	0	0	0	0									
135	2	H-P	2004	F	W	12	1	1	284	11	48	24	0	0	0	0									
136	2	H-P	2004	F	W	3	1	0	-28	2	48	192	384	0	0	0									
136	2	H-P	2004	F	W	4	1	0	0	3	48	96	0	0	0	0									
136	2	H-P	2004	F	W	5	1	0	28	4	48	192	0	0	0	0									
136	2	H-P	2004	F	W	7	1	0	82	6	48	384	0	0	0	0									
137	2	H-P	2004	F	W	3	1	0	-28	2	48	384	96												
137	2	H-P	2004	F	W	4	1	0	0	3	96	768	0												
137	2	H-P	2004	F	W	5	1	0	28	4	24	96	24												
137	2	H-P	2004	F	W	7	1	0	82	6	24	24	0	0	0	0									
137	2	H-P	2004	F	W	10	1	0	189	9	0	768	48	0	0	0									
137	2	H-P	2004	F	W	12	1	0	284	11	48	192	0	0	0	0									
138	2	H-P	2004	F	W	3	1	0	-28	2	0	96	48												
138	2	H-P	2004	F	W	4	1	0	0	3	24	24	0	0	0	0									
138	2	H-P	2004	F	W	5	1	0	28	4	96	24	24	0	1	1									
138	2	H-P	2004	F	W	7	1	0	82	6	48	1536	0	0	1	1									
138	2	H-P	2004	F	W	8	1	0	137	7	48	1536	0	0	0	0									
138	2	H-P	2004	F	W	10	1	0	189	9	0	3072	96												
138	2	H-P	2004	F	W	12	1	0	284	11	0	1536	24	0	0	0									
139	2	H-P	2004	F	W	3	1	0	-28	2	48	96	48												
139	2	H-P	2004	F	W	4	1	0	0	3	96	3072	24	0	0	0									
139	2	H-P	2004	F	W	5	1	0	28	4	48	24	24												
139	2	H-P	2004	F	W	7	1	0	82	6	24	0	24												
139	2	H-P	2004	F	W	8	1	0	137	7	0	0	24												
139	2	H-P	2004	F	W	10	1	0	189	9	0	0	0	1	1	1									
139	2	H-P	2004	F	W	12	1	0	284	11	0	0	0	1	1	1	Hb								
142	2	H-P	2004	F	W	3	1	1	-28	2	96	48	48	0	0	0									
142	2	H-P	2004	F	W	4	1	1	0	3	48	48	0	0	1	1									
142	2	H-P	2004	F	W	5	1	1	28	4	96	48	192	0	0	0									
142	2	H-P	2004	F	W	7	1	1	82	6	48	24	24												
142	2	H-P	2004	F	W	8	1	1	137	7	24	0	0	0	0	0									
142	2	H-P	2004	F	W	10	1	1	189	9	0	0	0	0	0	0									
142	2	H-P	2004	F	W	12	1	1	284	11	0	0	0	0	0	0									
144	2	H-P	2004	M	W	8	1	1	137	7	96														
144	2	H-P	2004	M	W	10	1	1	189	9	0														
144	2	H-P	2004	M	W	12	1	1	284	11	24	24	0												
158	2	H-P	2004	M	W	3	1	0	-28	2	48	0	96												
158	2	H-P	2004	M	W	4	1	0	0	3	24	96	0												
158	2	H-P	2004	M	W	5	1	0	28	4	48	24	48												
158	2	H-P	2004	M	W	7	1	0	82	6	48	24	0												
158	2	H-P	2004	M	W	8	1	0	137	7	48	1536	0												
158	2	H-P	2004	M	W	10	1	0	189	9	0	96	0												
158	2	H-P	2004	M	W	12	1	0	284	11	24	96	0	1	1		P	1	0	1	0	0	1	1	1
166	2	H-P	2004	M	W	3	1	1	-28	2	192	48	192												
166	2	H-P	2004	M	W	4	1	1	0	3	24	96	0												
166	2	H-P	2004	M	W	5	1	1	28	4	48	24	48												
166	2	H-P	2004	M	W	7	1	1	82	6	48	1536	0												
166	2	H-P	2004	M	W	8	1	1	137	7	48	0	0												
166	2	H-P	2004	M	W	10	1	1	189	9	0	0	0												
166	2	H-P	2004	M	W	12	1	1	284	11	192	24	0												
167	2	H-P	2004	M	W	3	1	1	-28	2	48	96	96	1	1		Hb	0	0	0	0	0	0	0	0
167	2	H-P	2004	M	W	4	1	1	0	3	24	24	0												
167	2	H-P	2004	M	W	5	1	1	28	4	24	48	48												
167	2	H-P	2004	M	W	7	1	1	82	6	24	3072	0												
167	2	H-P	2004	M	W	8	1	1	137	7	96	0	0												
167	2	H-P	2004	M	W	10	1	1	189	9	0	0	0												
167	2	H-P	2004	M	W	12	1	1	284	11	0	24	0	0	0			0	1	0	0	0	0	0	0
169	2	H-P	2004	M	W	3	1	0	-28	2	96	96	96												
169	2	H-P	2004	M	W	4	1	0	0	3	24	48	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLl	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
169	2	H-P	2004	M	W	5	1	0	28	4	48	96	0												
169	2	H-P	2004	M	W	7	1	0	82	6	48	96	0												
169	2	H-P	2004	M	W	8	1	0	137	7	48	0	0												
169	2	H-P	2004	M	W	10	1	0	189	9	0	48	0												
169	2	H-P	2004	M	W	12	1	0	284	11	48	48	0	1	1		Hb	1	0	1	0	0	1	0	0
170	2	H-P	2004	M	W	3	1	0	-28	2	24	96	96												
170	2	H-P	2004	M	W	4	1	0	0	3	48	24	0												
170	2	H-P	2004	M	W	5	1	0	28	4	48	24	0												
170	2	H-P	2004	M	W	7	1	0	82	6	48	3072	0												
170	2	H-P	2004	M	W	8	1	0	137	7	48	0	0												
170	2	H-P	2004	M	W	10	1	0	189	9	48	0	0												
170	2	H-P	2004	M	W	12	1	0	284	11	0	0	0	1	1		H-P	1	1	1	0	0	1	0	0
172	2	H-P	2004	M	W	3	1	0	-28	2	96	384	96												
172	2	H-P	2004	M	W	4	1	0	0	3	192	96	0												
172	2	H-P	2004	M	W	5	1	0	28	4	48	24	24												
172	2	H-P	2004	M	W	7	1	0	82	6	24	3072	0												
172	2	H-P	2004	M	W	8	1	0	137	7	48	768	0												
172	2	H-P	2004	M	W	10	1	0	189	9	0	768	0												
172	2	H-P	2004	M	W	12	1	0	284	11	0	192	0	1	1		H-P	1	1	1	0	0	1	1	1
174	2	H-P	2004	M	W	3	0	1	-28	2	48	48	96												
174	2	H-P	2004	M	W	4	0	1	0	3	24	96	0												
174	2	H-P	2004	M	W	5	0	1	28	4	24	48	48												
174	2	H-P	2004	M	W	7	0	1	82	6	24	0	0												
174	2	H-P	2004	M	W	8	0	1	137	7	96	0	0												
174	2	H-P	2004	M	W	10	0	1	189	9	0	0	0												
174	2	H-P	2004	M	W	12	0	1	284	11	384	0	0	0	0			0	0	0	0	0	0	0	0
175	2	H-P	2004	M	W	3	1	0	-28	2	96	24	96												
175	2	H-P	2004	M	W	4	1	0	0	3	24	3072	0												
175	2	H-P	2004	M	W	5	1	0	28	4	48	192	24												
175	2	H-P	2004	M	W	7	1	0	82	6	24	3072	0												
175	2	H-P	2004	M	W	8	1	0	137	7	96	1536	0												
175	2	H-P	2004	M	W	10	1	0	189	9	0	1536	0												
175	2	H-P	2004	M	W	12	1	0	284	11	24	768	0	1	1			0	0	0	0	0	0	0	0
176	2	H-P	2004	M	W	3	1	1	-28	2	48	48	24												
176	2	H-P	2004	M	W	4	1	1	0	3	24	1536	0												
176	2	H-P	2004	M	W	5	1	1	28	4	48	48	96												
176	2	H-P	2004	M	W	7	1	1	82	6	48	0	24												
176	2	H-P	2004	M	W	8	1	1	137	7	48	0	0												
176	2	H-P	2004	M	W	10	1	1	189	9	0	24	0												
176	2	H-P	2004	M	W	12	1	1	284	11	48	0	0	0	0			1	1	1	0	0	1	0	0
177	2	H-P	2004	M	W	3	1	1	-28	2	96	192	192												
177	2	H-P	2004	M	W	4	1	1	0	3	24	3072	0												
177	2	H-P	2004	M	W	5	1	1	28	4	48	192	48												
177	2	H-P	2004	M	W	7	1	1	82	6	48	1536	0												
177	2	H-P	2004	M	W	8	1	1	137	7	48	24	0												
177	2	H-P	2004	M	W	10	1	1	189	9	0	0	0												
177	2	H-P	2004	M	W	12	1	1	284	11	0	0	0												
188	2	H-P	2004	M	W	3	0	0	-28	2	0	48	48												
188	2	H-P	2004	M	W	4	0	0	0	3	0	0	0												
188	2	H-P	2004	M	W	5	0	0	28	4	24	0	24												
188	2	H-P	2004	M	W	7	0	0	82	6	24	0	24												
188	2	H-P	2004	M	W	8	0	0	137	7	24	192	24												
188	2	H-P	2004	M	W	10	0	0	189	9	0	96	0												
188	2	H-P	2004	M	W	12	0	0	284	11	0	24	0	1	1			0	0	0	0	0	0	1	0
189	2	H-P	2004	M	W	3	0	0	-28	2	24	3072	96												
189	2	H-P	2004	M	W	4	0	0	0	3	192	3072	0												
189	2	H-P	2004	M	W	5	0	0	28	4	96	384	48												
189	2	H-P	2004	M	W	7	0	0	82	6	48	384	0												
189	2	H-P	2004	M	W	8	0	0	137	7	96	384	24												
189	2	H-P	2004	M	W	10	0	0	189	9	0	384	0												
189	2	H-P	2004	M	W	12	0	0	284	11	0	96	0	1	1		H-P	1	0	0	1	1	1	1	0
191	2	H-P	2004	M	W	3	0	0	-28	2	24	192	0												
191	2	H-P	2004	M	W	4	0	0	0	3	384	96	0												
191	2	H-P	2004	M	W	5	0	0	28	4	24	24	24												
191	2	H-P	2004	M	W	7	0	0	82	6	24	0	0												
191	2	H-P	2004	M	W	8	0	0	137	7	48	0	0												
191	2	H-P	2004	M	W	10	0	0	189	9	0	0	0												
191	2	H-P	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
192	2	H-P	2004	M	W	3	0	1	-28	2	0	3072	384												
192	2	H-P	2004	M	W	4	0	1	0	3	96	24	0												
192	2	H-P	2004	M	W	5	0	1	28	4	24	3072	384												
193	2	H-P	2004	M	W	3	0	0	-28	2	48	192	24												
193	2	H-P	2004	M	W	4	0	0	0	3	96	24	0												
194	2	H-P	2004	M	W	3	0	1	-28	2	0	3072	96												
194	2	H-P	2004	M	W	4	0	1	0	3	96	24	0												
194	2	H-P	2004	M	W	5	0	1	28	4	48	48	96												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
194	2	H-P	2004	M	W	7	0	1	82	6	48	48	0											
194	2	H-P	2004	M	W	8	0	1	137	7	48	96	0											
194	2	H-P	2004	M	W	10	0	1	189	9	0	96	0											
194	2	H-P	2004	M	W	12	0	1	284	11	0	96	0	0	0			1	1	1	1	1	1	1	1
196	2	H-P	2004	M	W	3	0	0	-28	2	24	3072	48											
196	2	H-P	2004	M	W	4	0	0	0	3	192	96	0											
196	2	H-P	2004	M	W	5	0	0	28	4	96	0	48											
196	2	H-P	2004	M	W	7	0	0	82	6	96	0	24											
196	2	H-P	2004	M	W	8	0	0	137	7	96	384	24											
196	2	H-P	2004	M	W	10	0	0	189	9	0	384	0											
196	2	H-P	2004	M	W	12	0	0	284	11	3072	192	0	1	1		Hb	1	1	1	1	0	0	1	1
197	2	H-P	2004	M	W	3	0	1	-28	2	0	3072	48											
197	2	H-P	2004	M	W	4	0	1	0	3	96	24	0											
197	2	H-P	2004	M	W	5	0	1	28	4	192	24	96											
197	2	H-P	2004	M	W	7	0	1	82	6	96	384	0											
197	2	H-P	2004	M	W	8	0	1	137	7	96	96	0											
197	2	H-P	2004	M	W	10	0	1	189	9	0	384	0											
197	2	H-P	2004	M	W	12	0	1	284	11	0	384	0	0	0			1	1	1	1	0	1	1	1
198	2	H-P	2004	M	W	3	0	1	-28	2	24	24	24											
198	2	H-P	2004	M	W	4	0	1	0	3	96	192	0											
198	2	H-P	2004	M	W	5	0	1	28	4	96	3072	384											
198	2	H-P	2004	M	W	7	0	1	82	6	48	48	48											
198	2	H-P	2004	M	W	8	0	1	137	7	48	192	0											
198	2	H-P	2004	M	W	10	0	1	189	9	0	384	0											
198	2	H-P	2004	M	W	12	0	1	284	11	24	192	0	1	1			1	1	1	1	0	1	1	1
199	2	H-P	2004	M	W	3	0	1	-28	2	0	1536	96											
199	2	H-P	2004	M	W	4	0	1	0	3	96	0	0											
199	2	H-P	2004	M	W	5	0	1	28	4	192	3072	192											
199	2	H-P	2004	M	W	7	0	1	82	6	96	1536	48											
199	2	H-P	2004	M	W	8	0	1	137	7	384	768	24											
199	2	H-P	2004	M	W	10	0	1	189	9	0	0	0											
199	2	H-P	2004	M	W	12	0	1	284	11	0	768	0	0	0			1	1	0	1	1	1	1	1
601	2	H-P	2004	F	W	3	0	0	-28	2	0	48	0											
601	2	H-P	2004	F	W	4	0	0	0	3	0	48	0	0	1	1								
601	2	H-P	2004	F	W	5	0	0	28	4	24	48	24	0	1	1								
601	2	H-P	2004	F	W	7	0	0	82	6	24	1536	24	0	1	1								
601	2	H-P	2004	F	W	8	0	0	137	7	48	192	24	1	1	0								
601	2	H-P	2004	F	W	10	0	0	189	9	0	24	0	0	0	0								
601	2	H-P	2004	F	W	12	0	0	284	11	0	0	0											
602	2	H-P	2004	F	W	3	0	0	-28	2	0	3072	384											
602	2	H-P	2004	F	W	4	0	0	0	3	48	24	24	0	0	0								
602	2	H-P	2004	F	W	5	0	0	28	4	48	0	0	0	0	0								
602	2	H-P	2004	F	W	7	0	0	82	6	96	768	0	0	0	0								
602	2	H-P	2004	F	W	8	0	0	137	7	96	384	24	0	0	0								
602	2	H-P	2004	F	W	10	0	0	189	9	0	0	0	0	0	0								
602	2	H-P	2004	F	W	12	0	0	284	11	0	768	0	0	0	0								
603	2	H-P	2004	F	W	3	0	0	-28	2	48	96	0											
603	2	H-P	2004	F	W	4	0	0	0	3	48	1536	0											
603	2	H-P	2004	F	W	5	0	0	28	4	24	0	24	0	0	0								
603	2	H-P	2004	F	W	7	0	0	82	6	48	0	0	0	0	0								
603	2	H-P	2004	F	W	8	0	0	137	7	48	0	0	0	0	0								
603	2	H-P	2004	F	W	10	0	0	189	9	0	1536	0	0	0	0								
603	2	H-P	2004	F	W	12	0	0	284	11	0	192	0	0	1	1								
604	2	H-P	2004	F	W	3	0	0	-28	2	0	3072	48											
604	2	H-P	2004	F	W	4	0	0	0	3	24	768	24	0	1	1								
604	2	H-P	2004	F	W	5	0	0	28	4	0	0	0	0	0	0								
604	2	H-P	2004	F	W	7	0	0	82	6	48	384	0	1	1	1								
604	2	H-P	2004	F	W	8	0	0	137	7	96	0	0											
605	2	H-P	2004	F	W	3	0	1	-28	2	0	3072	48											
605	2	H-P	2004	F	W	4	0	1	0	3	48	768	24	0	1	1								
605	2	H-P	2004	F	W	5	0	1	28	4	48	0	48	0	0	0								
605	2	H-P	2004	F	W	7	0	1	82	6	24	1536	0	0	0	0								
605	2	H-P	2004	F	W	8	0	1	137	7	96	0	0	0	0	0								
605	2	H-P	2004	F	W	10	0	1	189	9	0	1536	0											
605	2	H-P	2004	F	W	12	0	1	284	11	0	96	0	0	0	0								
607	2	H-P	2004	F	W	3	0	1	-28	2	96	48	0											
607	2	H-P	2004	F	W	4	0	1	0	3	48	24	0											
607	2	H-P	2004	F	W	5	0	1	28	4	48	24	192											
607	2	H-P	2004	F	W	7	0	1	82	6	48	3072	0											
607	2	H-P	2004	F	W	8	0	1	137	7	96	0	24											
607	2	H-P	2004	F	W	10	0	1	189	9	0	1536	0											
607	2	H-P	2004	F	W	12	0	1	284	11	0	0	0											
608	2	H-P	2004	F	W	3	0	1	-28	2	24	96	48	0	0	0								
608	2	H-P	2004	F	W	4	0	1	0	3	24	96	24	0	0	0								
608	2	H-P	2004	F	W	5	0	1	28	4	24	3072	24											
608	2	H-P	2004	F	W	7		1	82	6														

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology						
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
608	2	H-P	2004	F	W	8		1	137	7															
608	2	H-P	2004	F	W	10		1	189	9	0														
608	2	H-P	2004	F	W	12		1	284	11															
609	2	H-P	2004	F	W	3	0	0	-28	2	24	48	48												
609	2	H-P	2004	F	W	4	0	0	0	3	0	3072	24	0	1	1									
609	2	H-P	2004	F	W	5	0	0	28	4	24	3072	0	0	0	0									
609	2	H-P	2004	F	W	7	0	0	82	6	96	1536	0												
609	2	H-P	2004	F	W	8	0	0	137	7	48	48	0	0	0	0									
609	2	H-P	2004	F	W	10	0	0	189	9	0	3072	0	1	1	1									
609	2	H-P	2004	F	W	12	0	0	284	11	0	24	0	1	1	1									
610	2	H-P	2004	F	W	3	0	1	-28	2	192	192	24	0	0	0									
610	2	H-P	2004	F	W	4	0	1	0	3	48	48	0	0	0	0									
610	2	H-P	2004	F	W	5	0	1	28	4	96	96	96	0	1	1									
610	2	H-P	2004	F	W	7	0	1	82	6	24	3072	0												
610	2	H-P	2004	F	W	8	0	1	137	7	48	48	0	1	1	1									
610	2	H-P	2004	F	W	10	0	1	189	9	0	384	0	0	0	0									
610	2	H-P	2004	F	W	12	0	1	284	11	0	0	0	0	1	1									
614	2	H-P	2004	F	W	3	0	1	-28	2	192	96	24												
614	2	H-P	2004	F	W	4	0	1	0	3	96	1536	0	0	1	1									
614	2	H-P	2004	F	W	5	0	1	28	4	96	1536	48	0	1	1									
614	2	H-P	2004	F	W	7	0	1	82	6	96	3072	0	0	0	0									
614	2	H-P	2004	F	W	8	0	1	137	7	96	0	0	1	1	1									
614	2	H-P	2004	F	W	10	0	1	189	9	0	384	0	0	0	0									
614	2	H-P	2004	F	W	12	0	1	284	11	0	0	0	0	0	0									
624	2	H-P	2004	F	W	3	1	1	-28	2	48	48	24	0	1	1									
624	2	H-P	2004	F	W	4	1	1	0	3	192	0	0	0	1	1									
624	2	H-P	2004	F	W	5	1	1	28	4	192	0	96	0	1	1									
624	2	H-P	2004	F	W	7	1	1	82	6	48	24	96	0	1	1									
624	2	H-P	2004	F	W	8	1	1	137	7	96	1536	24	0	0	0									
624	2	H-P	2004	F	W	10	1	1	189	9	0	1536	0												
624	2	H-P	2004	F	W	12	1	1	284	11	24	384	0												
625	2	H-P	2004	F	W	3	1	1	-28	2	96	48	24												
625	2	H-P	2004	F	W	4	1	1	0	3	96	768	24												
625	2	H-P	2004	F	W	5	1	1	28	4	48	3072	96												
625	2	H-P	2004	F	W	7	1	1	82	6	48	384	0												
625	2	H-P	2004	F	W	8	1	1	137	7	192	384	24												
625	2	H-P	2004	F	W	10	0	1	189	9	0	24	0												
625	2	H-P	2004	F	W	12	0	1	284	11	48	384	0	0	0	0									
626	2	H-P	2004	F	W	3	1	0	-28	2	96	24	24												
626	2	H-P	2004	F	W	4	1	0	0	3	0	96	24	0	1	1									
626	2	H-P	2004	F	W	5	1	0	28	4	96	24	48	0	0	0									
626	2	H-P	2004	F	W	7	1	0	82	6	48	0	0												
626	2	H-P	2004	F	W	8	1	0	137	7	96	768	24	0	0	0									
626	2	H-P	2004	F	W	10	1	0	189	9	0	0	0	0	0	0									
626	2	H-P	2004	F	W	12	1	0	284	11	0	1536	0	0	1	1									
627	2	H-P	2004	F	W	3	1	0	-28	2	48	96	24	0	0	0									
627	2	H-P	2004	F	W	4	1	0	0	3	48	768	24												
627	2	H-P	2004	F	W	5	1	0	28	4	48	24	24												
627	2	H-P	2004	F	W	7	1	0	82	6	96	0	0	0	0	0									
627	2	H-P	2004	F	W	8	1	0	137	7	384	768	0	0	0	0									
627	2	H-P	2004	F	W	10	1	0	189	9	0	192	0	0	0	0									
627	2	H-P	2004	F	W	12	1	0	284	11	96	192	0	0	0	0									
628	2	H-P	2004	F	W	3	0	1	-28	2	24	24	24	0	0	0									
628	2	H-P	2004	F	W	4	0	1	0	3	192	24	24	0	1	1									
628	2	H-P	2004	F	W	5	0	1	28	4	48	768	48	0	0	0									
628	2	H-P	2004	F	W	7	1	1	82	6	96	0	0	0	0	0									
628	2	H-P	2004	F	W	8	1	1	137	7	384	1536	0	1	1	1									
628	2	H-P	2004	F	W	10	1	1	189	9	0	0	0	0	0	0									
628	2	H-P	2004	F	W	12	1	1	284	11	24		0	0	0	0									
629	2	H-P	2004	F	W	3	1	1	-28	2	48	24	24	0	1	1									
629	2	H-P	2004	F	W	4	1	1	0	3	48	1536	24	0	0	0									
629	2	H-P	2004	F	W	5	1	1	28	4	48	768	48	0	1	1									
629	2	H-P	2004	F	W	7	1	1	82	6	96	0	0	1	1	1									
629	2	H-P	2004	F	W	8	1	1	137	7	384		0												
629	2	H-P	2004	F	W	10	1	1	189	9	0	0	0	1	1	1									
629	2	H-P	2004	F	W	12	1	1	284	11	0	192	0	1	1	1									
630	2	H-P	2004	F	W	3	1	0	-28	2	96	96	48												
630	2	H-P	2004	F	W	4	1	0	0	3	48	48	24												
630	2	H-P	2004	F	W	5	1	0	28	4	48	0	0	0	1	1									
630	2	H-P	2004	F	W	7	1	0	82	6	48	24	0												
630	2	H-P	2004	F	W	8	1	0	137	7	96	24	0	0	0	0									
630	2	H-P	2004	F	W	10	1	0	189	9	0	0	0	0	0	0									
630	2	H-P	2004	F	W	12	1	0	284	11	384	384	0	0	0	0									
631	2	H-P	2004	M	W	3	1	0	-28	2	48	24	0												
631	2	H-P	2004	M	W	4	1	0	0	3	48	24	0	0	0			0	0	0	0	0	0	0	0
636	2	H-P	2004	M	W	3	1	0	-28	2	96	24	24												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
636	2	H-P	2004	M	W	4	1	0	0	3	96	24	0											
636	2	H-P	2004	M	W	5	1	0	28	4	96	0	0											
638	2	H-P	2004	F	W	3	1	1	-28	2	96	96	24	0	0	0								
638	2	H-P	2004	F	W	4	1	1	0	3	48	768	24											
638	2	H-P	2004	F	W	5	1	1	28	4	48		48	0	0	0								
638	2	H-P	2004	F	W	7	1	1	82	6	24	0	96	0	0	0								
638	2	H-P	2004	F	W	8	1	1	137	7	48		24	0	0	0								
638	2	H-P	2004	F	W	10	1	1	189	9	96		0	0	0	0								
638	2	H-P	2004	F	W	12	1	1	284	11	24	0	0	0	0	0								
658	2	H-P	2004	M	W	3	1	1	-28	2	192	48	24											
658	2	H-P	2004	M	W	4	1	1	0	3	1536	48	24											
658	2	H-P	2004	M	W	5	1	1	28	4		48												
658	2	H-P	2004	M	W	7	1	1	82	6	192	48	48											
658	2	H-P	2004	M	W	8	1	1	137	7	384		48											
658	2	H-P	2004	M	W	10	1	1	189	9	0		0											
658	2	H-P	2004	M	W	12	1	1	284	11	0	768	0											
659	2	H-P	2004	M	W	3	1	0	-28	2	192	48	24											
659	2	H-P	2004	M	W	4	1	0	0	3	96	24	0											
659	2	H-P	2004	M	W	5	1	0	28	4	192	384	48											
660	2	H-P	2004	M	W	3	1	1	-28	2	96	0	0											
660	2	H-P	2004	M	W	4	1	1	0	3	192	48	24											
660	2	H-P	2004	M	W	5	1	1	28	4		0												
660	2	H-P	2004	M	W	7	1	1	82	6		0	192											
660	2	H-P	2004	M	W	8	1	1	137	7														
660	2	H-P	2004	M	W	10	1	1	189	9	0	0												
660	2	H-P	2004	M	W	12	1	1	284	11	24	192	0	0	0			1	1	1	1	0	1	1	0
663	2	H-P	2004	M	W	3	1	0	-28	2	48	24	24											
663	2	H-P	2004	M	W	4	1	0	0	3	0	24	0											
663	2	H-P	2004	M	W	5	1	0	28	4	48	1536	24											
663	2	H-P	2004	M	W	7	1	0	82	6	48	1536	24											
663	2	H-P	2004	M	W	8	1	0	137	7	96	1536	0											
663	2	H-P	2004	M	W	10	1	0	189	9	0	1536	0											
663	2	H-P	2004	M	W	12	1	0	284	11	0	96	0	1	1		Hb	1	0	1	0	0	1	1	0
664	2	H-P	2004	M	W	3	1	0	-28	2	96	48	0											
664	2	H-P	2004	M	W	4	1	0	0	3	96	96	0											
664	2	H-P	2004	M	W	5	1	0	28	4	96	3072	48											
664	2	H-P	2004	M	W	7	1	0	82	6	48	24	24											
664	2	H-P	2004	M	W	8	1	0	137	7	0	1536	24											
664	2	H-P	2004	M	W	10	1	0	189	9	0	0	0											
664	2	H-P	2004	M	W	12	1	0	284	11	24	192	0	1	1		P	1	1	1	1	0	1	1	0
665	2	H-P	2004	M	W	3	1	0	-28	2	48	0	24											
665	2	H-P	2004	M	W	4	1	0	0	3	48	24	0											
665	2	H-P	2004	M	W	5	1	0	28	4	96	1536	96											
665	2	H-P	2004	M	W	7	1	0	82	6	48	1536	192											
665	2	H-P	2004	M	W	8	1	0	137	7	48	1536	48											
665	2	H-P	2004	M	W	10	1	0	189	9	96	0	0											
665	2	H-P	2004	M	W	12	1	0	284	11	48	96	0											
666	2	H-P	2004	M	W	3	1	1	-28	2	48	48	48											
666	2	H-P	2004	M	W	4	1	1	0	3	24	48	0											
666	2	H-P	2004	M	W	5	1	1	28	4	48	384	192											
666	2	H-P	2004	M	W	7	1	1	82	6	48	96	96											
666	2	H-P	2004	M	W	8	1	1	137	7	96	0	24											
666	2	H-P	2004	M	W	10	1	1	189	9	0	1536	0											
666	2	H-P	2004	M	W	12	1	1	284	11	24	0	0	0	0			0	1	0	0	0	0	0	0
668	2	H-P	2004	M	W	3	1	0	-28	2	48	96	48											
668	2	H-P	2004	M	W	4	1	0	0	3	0	48	0											
668	2	H-P	2004	M	W	5	1	0	28	4	96	48	24											
668	2	H-P	2004	M	W	7	1	0	82	6	48	48	24											
668	2	H-P	2004	M	W	8	1	0	137	7	96	96	24											
668	2	H-P	2004	M	W	10	1	0	189	9	192	0	0											
668	2	H-P	2004	M	W	12	1	0	284	11	96	96	0	1	1		P	1	1	1	0	0	1	1	0
670	2	H-P	2004	M	W	3	1	1	-28	2	192	96	48											
670	2	H-P	2004	M	W	4	1	1	0	3	0	24	24											
670	2	H-P	2004	M	W	5	1	1	28	4	48	48	48											
670	2	H-P	2004	M	W	7	1	1	82	6	48	24	24											
670	2	H-P	2004	M	W	8	1	1	137	7	192	0	0											
670	2	H-P	2004	M	W	10	1	1	189	9	48		0											
670	2	H-P	2004	M	W	12	1	1	284	11	0	0	0											
671	2	H-P	2004	M	W	3	1	1	-28	2	96	192	24											
671	2	H-P	2004	M	W	4	1	1	0	3	48	24	0											
671	2	H-P	2004	M	W	5	1	1	28	4	192	3072	192											
671	2	H-P	2004	M	W	7	1	1	82	6	96	3072	48											
671	2	H-P	2004	M	W	8	1	1	137	7	96	3072	0											
671	2	H-P	2004	M	W	10	1	1	189	9	0		0											
671	2	H-P	2004	M	W	12	1	1	284	11				0	0			1	1	1	0	0	1	1	1
683	2	H-P	2004	M	W	3	0	1	-28	2	96	24	48											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
683	2	H-P	2004	M	W	4	0	1	0	3	96	48	0											
683	2	H-P	2004	M	W	5	0	1	28	4	48	48	96											
683	2	H-P	2004	M	W	7	0	1	82	6	48	24	24											
683	2	H-P	2004	M	W	8	0	1	137	7	192	0	0											
683	2	H-P	2004	M	W	10	0	1	189	9	0		0											
683	2	H-P	2004	M	W	12	0	1	284	11	24	0	0											
684	2	H-P	2004	M	W	3	0	0	-28	2	96	96	24											
684	2	H-P	2004	M	W	4	0	0	0	3	96	48	0											
684	2	H-P	2004	M	W	5	0	0	28	4	96	384	48											
684	2	H-P	2004	M	W	7	0	0	82	6	96	96	0											
684	2	H-P	2004	M	W	8	0	0	137	7	48	0	0											
684	2	H-P	2004	M	W	10	0	0	189	9	0	0	0											
684	2	H-P	2004	M	W	12	0	0	284	11	24	48	0											
685	2	H-P	2004	M	W	3	0	0	-28	2	96	192	96											
685	2	H-P	2004	M	W	4	0	0	0	3	24	768	24											
685	2	H-P	2004	M	W	5	0	0	28	4	48	0	24											
685	2	H-P	2004	M	W	7	0	0	82	6	48	3072	48											
685	2	H-P	2004	M	W	8	0	0	137	7	48	0	0											
685	2	H-P	2004	M	W	10	0	0	189	9	0	384	0											
685	2	H-P	2004	M	W	12	0	0	284	11	384	384	0											
686	2	H-P	2004	M	W	3	0	0	-28	2	48	96	48											
686	2	H-P	2004	M	W	4	0	0	0	3	24	3072	48											
686	2	H-P	2004	M	W	5	0	0	28	4	48	24	24											
686	2	H-P	2004	M	W	7	0	0	82	6	48	48	0											
686	2	H-P	2004	M	W	8	0	0	137	7	0	0	0											
686	2	H-P	2004	M	W	10	0	0	189	9	0	0	0											
686	2	H-P	2004	M	W	12	0	0	284	11	24	0	0	1	1		H-P	1	0	1	0	0	1	1	0
687	2	H-P	2004	M	W	3	0	1	-28	2	0	96	96											
687	2	H-P	2004	M	W	4	0	1	0	3	24	384	24											
687	2	H-P	2004	M	W	5	0	1	28	4	24	0	192											
687	2	H-P	2004	M	W	7	0	1	82	6	24	384	24											
687	2	H-P	2004	M	W	8	0	1	137	7	48	0	0											
687	2	H-P	2004	M	W	10	0	1	189	9	0		0											
687	2	H-P	2004	M	W	12	0	1	284	11	48	0	0	0	0			1	0	0	1	0	1	1	0
688	2	H-P	2004	M	W	3	0	0	-28	2	48	96	48											
688	2	H-P	2004	M	W	4	0	0	0	3	24	384	0											
688	2	H-P	2004	M	W	5	0	0	28	4	24	0	0											
688	2	H-P	2004	M	W	7	0	0	82	6	24	3072	24											
688	2	H-P	2004	M	W	8	0	0	137	7	48	0	0											
688	2	H-P	2004	M	W	10	0	0	189	9	0	24	0											
688	2	H-P	2004	M	W	12	0	0	284	11	24	384	0	1	1		Hb	1	0	1	1	0	1	0	1
689	2	H-P	2004	M	W	3	0	1	-28	2	48	96	48											
689	2	H-P	2004	M	W	4	0	1	0	3	48	1536	0											
689	2	H-P	2004	M	W	5	0	1	28	4	48	0	48											
689	2	H-P	2004	M	W	7	0	1	82	6	48	1536	0											
689	2	H-P	2004	M	W	8	0	1	137	7	48	0	24											
689	2	H-P	2004	M	W	10	0	1	189	9	0		0											
689	2	H-P	2004	M	W	12	0	1	284	11	0	24	0	0	0			1	0	1	0	0	1	0	0
690	2	H-P	2004	M	W	3	0	1	-28	2	48	96	96											
690	2	H-P	2004	M	W	4	0	1	0	3	48	24	0											
690	2	H-P	2004	M	W	5	0	1	28	4	48	48	48											
690	2	H-P	2004	M	W	7	0	1	82	6	24	1536	24											
690	2	H-P	2004	M	W	8	0	1	137	7	96	0	0											
690	2	H-P	2004	M	W	10	0	1	189	9	24	0	0											
690	2	H-P	2004	M	W	12	0	1	284	11	24	24	0											
693	2	H-P	2004	M	W	3	0	1	-28	2	48	192	48											
693	2	H-P	2004	M	W	4	0	1	0	3	48	192	0											
693	2	H-P	2004	M	W	5	0	1	28	4	96	48	96											
693	2	H-P	2004	M	W	7	0	1	82	6	48	192	96											
693	2	H-P	2004	M	W	8	0	1	137	7	24	0	0											
693	2	H-P	2004	M	W	10	0	1	189	9	0	0	0											
693	2	H-P	2004	M	W	12	0	1	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
694	2	H-P	2004	M	W	3	0	0	-28	2	96	0	24											
694	2	H-P	2004	M	W	4	0	0	0	3	192	3072	24											
694	2	H-P	2004	M	W	5	0	0	28	4	24	48	0											
694	2	H-P	2004	M	W	7	0	0	82	6	24	192	24											
694	2	H-P	2004	M	W	8	0	0	137	7	24	0	0											
694	2	H-P	2004	M	W	10	0	0	189	9	384	1536	0											
694	2	H-P	2004	M	W	12	0	0	284	11	48	96	0	1	1		Hb	1	1	1	0	0	1	0	0
695	2	H-P	2004	M	W	3	0	0	-28	2	48	192	48											
695	2	H-P	2004	M	W	4	0	0	0	3	48	384	0											
695	2	H-P	2004	M	W	5	0	0	28	4	48	0	24											
695	2	H-P	2004	M	W	7	0	0	82	6	48	3072	0											
695	2	H-P	2004	M	W	8	0	0	137	7	48	768	0											
695	2	H-P	2004	M	W	10	0	0	189	9	24	1536	0											
1262	3	P-H	2004	F	W	8	0	0	0	7	24	48	0	0	1	1								

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney	Histopathology						
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
1262	3	P-H	2004	F	W	9	0	0	28	8	24	48	0	0	1	1									
1275	3	P-H	2004	M	W	8	0	0	0	7	0	384	0												
1275	3	P-H	2004	M	W	9	0	0	28	8	48	3072	0												
1275	3	P-H	2004	M	W	12	0	0	125	11	192	384	0	1	1		Hb	1	1	1	1	0	1	1	0
1276	3	P-H	2004	M	W	8	0	1	0	7	0		48												
1276	3	P-H	2004	M	W	9	0	1	28	8	96	24576	48												
1276	3	P-H	2004	M	W	12	0	1	125	11	0	768	0	1	1		P	1	1	1	0	0	1	1	0
1288	3	P-H	2004	M	W	8	0	0	0	7	0	0	24												
1288	3	P-H	2004	M	W	9	0	0	28	8	24	1536	0												
1288	3	P-H	2004	M	W	12	0	0	125	11	0	96	48												
1297	3	P-H	2004	M	W	8	0	1	0	7															
1297	3	P-H	2004	M	W	9	0	1	28	8	96		0												
1297	3	P-H	2004	M	W	12	0	1	125	11	24	384	0	0	0			1	1	1	1	0	1	1	0
1300	3	P-H	2004	M	W	8	0	1	0	7	0		48												
1300	3	P-H	2004	M	W	9	0	1	28	8	48	384	0												
1300	3	P-H	2004	M	W	12	0	1	125	11	0	192		1	1		P	0	0	0	0	0	0	0	0
1306	3	P-H	2004	F	W	8	0	1	0	7	0			0	1	1									
1306	3	P-H	2004	F	W	9	0	1	28	8		0	0	0	0	0									
1306	3	P-H	2004	F	W	12	0	1	125	11															
1421	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1421	3	P-H	2004	M	W	9	0	1	28	8	96	0	48												
1421	3	P-H	2004	M	W	12	0	1	125	11	96	768	0												
1422	3	P-H	2004	M	W	8	0	0	0	7	0	384	0												
1422	3	P-H	2004	M	W	9	0	0	28	8	24	3072	0												
1422	3	P-H	2004	M	W	12	0	0	125	11	0	96	0	0	0			1	1	1	1	0	1	1	0
1430	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1430	3	P-H	2004	M	W	9	0	1	28	8	48	192	48												
1430	3	P-H	2004	M	W	12	0	1	125	11	24	1536	0												
1434	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1434	3	P-H	2004	M	W	9	0	1	28	8	48	1536	48	0	0			1	1	1	0	0	1	0	0
1434	3	P-H	2004	M	W	12	0	1	125	11	24	1536	0												
1435	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1435	3	P-H	2004	M	W	9	0	1	28	8	24	768	0												
1435	3	P-H	2004	M	W	12	0	1	125	11	96	1536	0												
1442	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1442	3	P-H	2004	M	W	9	0	1	28	8	48	0	0												
1442	3	P-H	2004	M	W	12	0	1	125	11	24	3072	24	0	0			0	0	0	0	0	0	0	0
1444	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1444	3	P-H	2004	M	W	9	0	1	28	8	48	96	24												
1444	3	P-H	2004	M	W	12	0	1	125	11	96	1536	24	0	0			1	1	1	0	0	1	1	0
1458	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1458	3	P-H	2004	M	W	9	0	1	28	8	48	0	24												
1458	3	P-H	2004	M	W	12	0	1	125	11	0	1536	0	1	1		P	1	1	1	1	0	1	1	1
1460	3	P-H	2004	M	W	8	0	0	0	7	0	0	0												
1460	3	P-H	2004	M	W	9	0	0	28	8	24	192	0												
1460	3	P-H	2004	M	W	12	0	0	125	11	0	384	0	0	0			0	0	0	0	0	0	0	0
1463	3	P-H	2004	M	W	8	0	0	0	7	0	1536	0												
1463	3	P-H	2004	M	W	9	0	0	28	8	48	384	0												
1463	3	P-H	2004	M	W	12	0	0	125	11	24	192	0	1	1		H-P	0	1	0	0	0	0	0	0
1464	3	P-H	2004	M	W	8	0	1	0	7															
1464	3	P-H	2004	M	W	9	0	1	28	8	96		0	0	0			1	1	0	1	0	1	1	0
1464	3	P-H	2004	M	W	12	0	1	125	11	0	768	48												
1465	3	P-H	2004	M	W	8	0	0	0	7	0	768	48												
1465	3	P-H	2004	M	W	9	0	0	28	8	24	1536	0												
1465	3	P-H	2004	M	W	12	0	0	125	11	96	192	24	1	1		H-P	1	1	1	0	0	1	0	0
1467	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1467	3	P-H	2004	M	W	9	0	1	28	8	48	0	24												
1467	3	P-H	2004	M	W	12	0	1	125	11	0	1536	0	0	0			1	1	1	1	1	1	1	0
1470	3	P-H	2004	M	W	8	0	1	0	7															
1470	3	P-H	2004	M	W	9	0	1	28	8	96		48												
1470	3	P-H	2004	M	W	12	0	1	125	11		384		0	0			1	1	0	1	0	1	1	1
1473	3	P-H	2004	M	W	8	0	1	0	7	24		0												
1473	3	P-H	2004	M	W	9	0	1	28	8	48	96	24												
1473	3	P-H	2004	M	W	12	0	1	125	11		384													
1475	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1475	3	P-H	2004	M	W	9	0	1	28	8	48	192	24												
1475	3	P-H	2004	M	W	12	0	1	125	11		768													
1477	3	P-H	2004	M	W	8	0	0	0	7	0	1536	0												
1477	3	P-H	2004	M	W	9	0	0	28	8	0	6144	384												
1477	3	P-H	2004	M	W	12	0	0	125	11	48	384	0												
1478	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1478	3	P-H	2004	M	W	9	0	1	28	8	48	384	0												
1478	3	P-H	2004	M	W	12	0	1	125	11	48	768	0												
1479	3	P-H	2004	M	W	8	0	1	0	7	0		0												
1479	3	P-H	2004	M	W	9	0	1	28	8	48	0	24												
1479	3	P-H	2004	M	W	12	0	1	125	11		384													

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
1482	3	P-H	2004	M	W	8	0	1	0	7	0		0											
1482	3	P-H	2004	M	W	9	0	1	28	8	48	0	0											
1482	3	P-H	2004	M	W	12	0	1	125	11		384												
1485	3	P-H	2004	M	W	8	0	0	0	7	0	768	48											
1485	3	P-H	2004	M	W	9	0	0	28	8	48	3072	0											
1485	3	P-H	2004	M	W	12	0	0	125	11	0	96	0	0	0			1	0	1	1	0	1	1	0
1486	3	P-H	2004	M	W	8	0	1	0	7														
1486	3	P-H	2004	M	W	9	0	1	28	8	48		0											
1486	3	P-H	2004	M	W	12	0	1	125	11	48	3072	24	1	1		H-P	1	0	0	1	0	1	1	0
1488	3	P-H	2004	M	W	8	0	1	0	7														
1488	3	P-H	2004	M	W	9	0	1	28	8	48		0											
1488	3	P-H	2004	M	W	12	0	1	125	11	96	1536	24	1	1		H-P	1	1	1	1	0	1	1	0
2433	3	P-H	2004	M	W	8	0	1	0	7														
2433	3	P-H	2004	M	W	9	0	1	28	8	24		48											
2433	3	P-H	2004	M	W	12	0	1	125	11	96	768	24											
2446	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1								
2446	3	P-H	2004	F	W	9	0	1	28	8	96	192	24	0	0	0								
2446	3	P-H	2004	F	W	12	0	1	125	11	24	384	24	0	0			1	1	1	1	0	1	1	1
2488	3	P-H	2004	M	W	8	0	1	0	7	0		0											
2488	3	P-H	2004	M	W	9	0	1	28	8	24	768	0											
2488	3	P-H	2004	M	W	12	0	1	125	11		768												
2496	3	P-H	2004	F	W	8	0	1	0	7	24		0	0	1	1								
2496	3	P-H	2004	F	W	9	0	1	28	8	24	1536	48	0	0	0								
2496	3	P-H	2004	F	W	12	0	1	125	11	0	384	24	1	1		P	1	1	1	1	0	1	1	0
2500	3	P-H	2004	M	W	8	0	1	0	7	0		48											
2500	3	P-H	2004	M	W	9	0	1	28	8	96	768	24											
2500	3	P-H	2004	M	W	12	0	1	125	11	96	768	24	1	1		H-P	1	0	1	1	0	1	1	0
2528	3	P-H	2004	F	W	8	0	1	0	7	24		0	0	1	1								
2528	3	P-H	2004	F	W	9	0	1	28	8	24	384	0	0	0	0								
2528	3	P-H	2004	F	W	12	0	1	125	11	0	768	0	0	0			1	1					
2529	3	P-H	2004	F	W	8	0	0	0	7	24	768	24		1	1								
2529	3	P-H	2004	F	W	9	0	0	28	8	48	6144	24											
2529	3	P-H	2004	F	W	12	0	0	125	11	192	192	24	1	1		Pom	1	1	1	1	0	1	1	1
2543	3	P-H	2004	F	W	8	0	0	0	7	24	24	0		1	1								
2543	3	P-H	2004	F	W	9	0	0	28	8	96	192	0		1	1								
2543	3	P-H	2004	F	W	12	0	0	125	11	768	96	0											
2563	3	P-H	2004	F	W	8	0	1	0	7	24		0	0	1	1								
2563	3	P-H	2004	F	W	9	0	1	28	8	24	384	24	0	1	1								
2563	3	P-H	2004	F	W	12	0	1	125	11	0	48	24	0	0			0	0	0	0	0	0	0	0
2569	3	P-H	2004	F	W	8	0	1	0	7	24	768	0	0	1	1								
2569	3	P-H	2004	F	W	9	0	1	28	8		384		0	1	1								
2569	3	P-H	2004	F	W	12	0	1	125	11	0		0	1	1		H-P	1	1	1	0	1	1	0	0
2571	3	P-H	2004	F	W	8	0	0	0	7	48	1536	0	0	1	1								
2571	3	P-H	2004	F	W	9	0	0	28	8	24	384	0	0	0	0								
2571	3	P-H	2004	F	W	12	0	0	125	11	192	96	0											
2573	3	P-H	2004	F	W	8	0	0	0	7	0	1536	0	0	1	1								
2573	3	P-H	2004	F	W	9	0	0	28	8	48	192	0	0	0	0								
2573	3	P-H	2004	F	W	12	0	0	125	11	0	96	0	0	0			1	1	1	1	0	1	1	0
2577	3	P-H	2004	M	W	8	0	1	0	7	0		0											
2577	3	P-H	2004	M	W	9	0	1	28	8	24	384	0											
2577	3	P-H	2004	M	W	12	0	1	125	11		1536												
2584	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1								
2584	3	P-H	2004	F	W	9	0	1	28	8	24	0	24	0	0	0								
2584	3	P-H	2004	F	W	12	0	1	125	11	0	96	24	0	0			1	1	1	0	0	1	1	0
2588	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1								
2588	3	P-H	2004	F	W	9	0	1	28	8	24	6144	48	0	0	0								
2588	3	P-H	2004	F	W	12	0	1	125	11		768		0	0			1	1	1	1	0	1	1	1
2591	3	P-H	2004	F	W	8	0	1	0	7	24		0	0	1	1								
2591	3	P-H	2004	F	W	9	0	1	28	8	24	384	0	0	0	0								
2591	3	P-H	2004	F	W	12	0	1	125	11	0	384	24	0	0			1	0	1	0	1	1	1	1
2627	3	P-H	2004	F	W	8	0	0	0	7	24	1536	0	0	1	1								
2627	3	P-H	2004	F	W	9	0	0	28	8	48	1536	0	0	1	0								
2627	3	P-H	2004	F	W	12	0	0	125	11	192	768	24	0	0			0	0	0	0	0	0	0	0
2630	3	P-H	2004	M	W	8	0	1	0	7														
2630	3	P-H	2004	M	W	9	0	1	28	8														
2630	3	P-H	2004	M	W	12	0	1	125	11				1	1		H-P	1	1	1	1	0	1	1	0
2637	3	P-H	2004	M	W	8	0	1	0	7	0		0											
2637	3	P-H	2004	M	W	9	0	1	28	8	48	192	0											
2637	3	P-H	2004	M	W	12	0	1	125	11				0	0			0	1	0	0	0	0	0	0
2638	3	P-H	2004	M	W	8	0	0	0	7	0	1536	0											
2638	3	P-H	2004	M	W	9	0	0	28	8	24	768	0											
2638	3	P-H	2004	M	W	12	0	0	125	11	96	48	0	0	0			1	1	1	0	0	1	1	0
2641	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1								
2641	3	P-H	2004	F	W	9	0	1	28	8		768		0	0	0								
2641	3	P-H	2004	F	W	12	0	1	125	11	24		0	0	0			1	0	0	1	0	1	0	0
2646	3	P-H	2004	M	W	8	0	1	0	7														

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney	Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
2646	3	P-H	2004	M	W	8	0	1	0	7														
2646	3	P-H	2004	M	W	9	0	1	28	8	48		48											
2646	3	P-H	2004	M	W	12	0	1	125	11	0	384	0	1	1		P	1	1	1	0	0	1	1	0
2662	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1								
2662	3	P-H	2004	F	W	9	0	1	28	8	48	1536	0	0	0	0								
2662	3	P-H	2004	F	W	12	0	1	125	11		768	96	0	0			1	1	1	1	0	1	1	0
2665	3	P-H	2004	F	W	8	0	1	0	7	0		0	1	1	1	Pom								
2665	3	P-H	2004	F	W	9	0	1	28	8	48	768	24	0	0	0									
2665	3	P-H	2004	F	W	12	0	1	125	11	24	768	0	0	0			1	1	1	1	0	1	1	0
2668	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1									
2668	3	P-H	2004	F	W	9	0	1	28	8	48	96	0	0	0	0									
2668	3	P-H	2004	F	W	12	0	1	125	11		384		0	0			1	1	0	1	0	1	1	0
2672	3	P-H	2004	F	W	8	0	0	0	7	0	384	0	0	0	1									
2672	3	P-H	2004	F	W	9	0	0	28	8	96	384	0	0	0	0									
2672	3	P-H	2004	F	W	12	0	0	125	11	96	24	0	0	0			1	1	1	1	0	1	1	0
2677	3	P-H	2004	F	W	8	0	1	0	7	0	3072	0	0	1	1									
2677	3	P-H	2004	F	W	9	0	1	28	8	96	768	0	0	1	1									
2677	3	P-H	2004	F	W	12	0	1	125	11		384		0	0			1	1	1	1	0	1	1	1
2684	3	P-H	2004	M	W	8	0	1	0	7															
2684	3	P-H	2004	M	W	9	0	1	28	8	96		192												
2684	3	P-H	2004	M	W	12	0	1	125	11	24	3072	24												
2685	3	P-H	2004	F	W	8	0	0	0	7	24	1536	48	1	1	1	Pom								
2685	3	P-H	2004	F	W	9	0	0	28	8	48	768	0	1	1	1	Pom								
2685	3	P-H	2004	F	W	12	0	0	125	11	192	768	0	0	0			1	1	1	0	1	1	1	1
2704	3	P-H	2004	M	W	8	0	1	0	7	0		0												
2704	3	P-H	2004	M	W	9	0	1	28	8	48	24576	192												
2704	3	P-H	2004	M	W	12	0	1	125	11	24	1536	24	0	0			1	1	1	1	0	1	1	0
2706	3	P-H	2004	F	W	8	0	1	0	7	48	3072	0	1	1	1	Pom								
2706	3	P-H	2004	F	W	9	0	1	28	8		1536		0	0	0									
2706	3	P-H	2004	F	W	12	0	1	125	11	0		24	0	0			1	1	1	0	0	1	1	0
2707	3	P-H	2004	F	W	8	0	1	0	7	48		24	1	1	1	Pom								
2707	3	P-H	2004	F	W	9	0	1	28	8	48	384	0	0	0	0									
2707	3	P-H	2004	F	W	12	0	1	125	11	48	384	0	1	1		H-P	1	1	1	0	0	1	1	1
2709	3	P-H	2004	M	W	8	0	0	0	7	96	3072	0												
2709	3	P-H	2004	M	W	9	0	0	28	8	96	384	96												
2709	3	P-H	2004	M	W	12	0	0	125	11	24	0	96	1	1		H-P	1	1	0	1	0	1	1	1
2718	3	P-H	2004	F	W	8	0	0	0	7	0	384	0	0	1	1									
2718	3	P-H	2004	F	W	9	0	0	28	8	48	768	0	0	1	1									
2718	3	P-H	2004	F	W	12	0	0	125	11	96	96	24	1	1		H-P	1	1	1	1	0	1	1	1
2726	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1									
2726	3	P-H	2004	F	W	9	0	1	28	8	48	1536	48	0	0	0									
2726	3	P-H	2004	F	W	12	0	1	125	11	0	1536	48	0	0			1	0	1	0	0	1	1	1
2727	3	P-H	2004	F	W	8	0	1	0	7		3072		0	1	1									
2727	3	P-H	2004	F	W	9	0	1	28	8	48		48	0	0	0									
2727	3	P-H	2004	F	W	12	0	1	125	11	24	768	0	0	0			1	1	1	1	0	1	1	0
2737	3	P-H	2004	F	W	8	0	1	0	7	0	3072	96	0	1	1									
2737	3	P-H	2004	F	W	9	0	1	28	8	96	1536	48	0	0	0									
2737	3	P-H	2004	F	W	12	0	1	125	11		768		0	0			1	1	1	0	0	1	1	0
2741	3	P-H	2004	F	W	8	0	0	0	7	24	1536	96	0	1	1									
2741	3	P-H	2004	F	W	9	0	0	28	8	48	384	0	0	1	0									
2741	3	P-H	2004	F	W	12	0	0	125	11	24	96	0	1	1		H-P	1	1	1	0	0	1	1	0
2744	3	P-H	2004	F	W	8	0	1	0	7					0										
2744	3	P-H	2004	F	W	9	0	1	28	8	48		0												
2744	3	P-H	2004	F	W	12	0	1	125	11	0	768	0	0	0			1	1	1	0	0	1	1	1
2753	3	P-H	2004	M	W	8	0	0	0	7	0	1536	0												
2753	3	P-H	2004	M	W	9	0	0	28	8	48	384	0												
2753	3	P-H	2004	M	W	12	0	0	125	11	48	96	0	1	1		H-P	1	0	0	1	0	1	1	0
2756	3	P-H	2004	M	W	8	0	0	0	7	0	384	24												
2756	3	P-H	2004	M	W	9	0	0	28	8	48	96	0												
2756	3	P-H	2004	M	W	12	0	0	125	11	384	96	0	1	1		Pom	1	0	1	1	0	1	1	0
2757	3	P-H	2004	F	W	8	0	1	0	7		3072													
2757	3	P-H	2004	F	W	9	0	1	28	8	96		0												
2757	3	P-H	2004	F	W	12	0	1	125	11	24	1536	0	0	0			1	1	1	1	0	1	1	0
2767	3	P-H	2004	M	W	8	0	0	0	7	0	24	0												
2767	3	P-H	2004	M	W	9	0	0	28	8	48	96	0												
2767	3	P-H	2004	M	W	12	0	0	125	11	96	48	0	1	1		H-P	1	1	1	0	0	1	1	0
2768	3	P-H	2004	M	W	8	0	1	0	7															
2768	3	P-H	2004	M	W	9	0	1	28	8	96		96												
2768	3	P-H	2004	M	W	12	0	1	125	11	48	384	48	0	0			1	1	0	1	0	1	1	0
2777	3	P-H	2004	F	W	8	0	1	0	7	24	768	24												
2777	3	P-H	2004	F	W	9	0	1	28	8	24	1536	0	0	0	0									
2777	3	P-H	2004	F	W	12	0	1	125	11	0	768	24	0	0			1	1	0	1	0	1	0	0
2780	3	P-H	2004	M	W	8	0	1	0	7	0		0												
2780	3	P-H	2004	M	W	9	0	1	28	8	48	96	24												
2780	3	P-H	2004	M	W	12	0	1	125	11	0	384	0	0	0			1	1	0	1	0	1	0	0
2782	3	P-H	2004	F	W	8	0	1	0	7	0	768	96	0	1	1									

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
2782	3	P-H	2004	F	W	12	0	1	125	11	0	1536	96	0	0			1	0	1	1	0	1	1	0
2784	3	P-H	2004	F	W	8	0	1	0	7	0		0												
2784	3	P-H	2004	F	W	9	0	1	28	8	96	3072	0	0	1	1									
2784	3	P-H	2004	F	W	12	0	1	125	11	24	1536	0	1	1		H-P	1	1	1	1	0	1	1	0
2800	3	P-H	2004	F	W	8	0	0	0	7	0	768	0	1	1	1	Pom								
2800	3	P-H	2004	F	W	9	0	0	28	8	48	768	0	1	1	1	Pom								
2800	3	P-H	2004	F	W	12	0	0	125	11	0	384	0	0	0			1	1	1	1	0	1	1	0
2804	3	P-H	2004	F	W	8	0	0	0	7	0	768	0	0	1	1									
2804	3	P-H	2004	F	W	9	0	0	28	8	48	384	0	0	1	1									
2804	3	P-H	2004	F	W	12	0	0	125	11	0	192	0	1	1		Pom	1	1	1	0	0	1	0	0
2823	3	P-H	2004	M	W	8	0	0	0	7	0	768	0												
2823	3	P-H	2004	M	W	12	0	0	125	11	384	24	0	0	0			1	0	0	0	0	0	0	0
2826	3	P-H	2004	F	W	8	0	1	0	7	0		0												
2826	3	P-H	2004	F	W	9	0	1	28	8	96	384	24	0	1	1									
2826	3	P-H	2004	F	W	12	0	1	125	11	24	384	48	0	0			1	1	1	1	0	1	1	1
2841	3	P-H	2004	F	W	8	0	1	0	7	24	3072	0												
2841	3	P-H	2004	F	W	9	0	1	28	8	96	768	24												
2841	3	P-H	2004	F	W	12	0	1	125	11	24	384	24	1	1		H-P	1	0	1	0	0	1	1	0
2842	3	P-H	2004	F	W	8	0	1	0	7															
2842	3	P-H	2004	F	W	9	0	1	28	8	0			0	1	1									
2842	3	P-H	2004	F	W	12	0	1	125	11	24		24	0	0			1	1	0	1	0	1	1	1
2849	3	P-H	2004	M	W	8	0	1	0	7															
2849	3	P-H	2004	M	W	9	0	1	28	8	48		0												
2849	3	P-H	2004	M	W	12	0	1	125	11	48	1536	0	0	0			0	1	0	0	0	0	0	0
2853	3	P-H	2004	F	W	8	0	0	0	7	24	384	0	0	1	1									
2853	3	P-H	2004	F	W	9	0	0	28	8	24	768	0	0	1	1									
2853	3	P-H	2004	F	W	12	0	0	125	11	0	768	0	0	0			1	1	1	0	0	1	0	0
2902	3	P-H	2004	F	W	8	0	0	0	7	0	768	0	0	0	1									
2902	3	P-H	2004	F	W	9	0	0	28	8	48	192	24	0	1	1									
2902	3	P-H	2004	F	W	12	0	0	125	11	96	384	0	0	0			0	0	0	0	0	0	0	0
2907	3	P-H	2004	F	W	8	0	1	0	7	0		0												
2907	3	P-H	2004	F	W	9	0	1	28	8	24	48	0												
2907	3	P-H	2004	F	W	12	0	1	125	11	24	768	24	0	0			1	0	0	1	0	1	1	0
2913	3	P-H	2004	F	W	8	0	0	0	7	0	768	0	1	1	1	Pom								
2913	3	P-H	2004	F	W	9	0	0	28	8	24	1536	0	0	1	1									
2913	3	P-H	2004	F	W	12	0	0	125	11	192	96	0	0	0			1	1	1	1	0	1	1	0
2926	3	P-H	2004	F	W	8	0	0	0	7	0	1536	0	0	1	1									
2926	3	P-H	2004	F	W	9	0	0	28	8	48	768	0	0	1	1									
2926	3	P-H	2004	F	W	12	0	0	125	11	96	384	0	1	1		H-P	1	1	1	1	1	1	1	0
2940	3	P-H	2004	F	W	8	0	1	0	7	0		48	0	1	1									
2940	3	P-H	2004	F	W	9	0	1	28	8	48	3072	0												
2940	3	P-H	2004	F	W	12	0	1	125	11	24	192	96												
2957	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1									
2957	3	P-H	2004	F	W	9	0	1	28	8	48	384	96	0	0	0									
2957	3	P-H	2004	F	W	12	0	1	125	11	0	96	0	0	0			1	1	1	1	0	1	1	1
2973	3	P-H	2004	F	W	8	0	0	0	7	0	384	0	0	1	1									
2973	3	P-H	2004	F	W	9	0	0	28	8	48	192	24	0	0	0									
2973	3	P-H	2004	F	W	12	0	0	125	11	0	96	96	1	1		Hb	1	1	1	1	0	1	0	0
2977	3	P-H	2004	F	W	8	0	0	0	7	0	384	0	0	1	1									
2977	3	P-H	2004	F	W	9	0	0	28	8	96	192	0	0	1	1									
2977	3	P-H	2004	F	W	12	0	0	125	11	192	96	24	1	1		H-P	1	1	1	0	0	1	0	0
2981	3	P-H	2004	F	W	8	0	1	0	7	24		0	0	1	1									
2981	3	P-H	2004	F	W	9	0	1	28	8	24	768	0												
2981	3	P-H	2004	F	W	12	0	1	125	11	24	192	0	0	0			1	1	1	1	0	1	1	0
2985	3	P-H	2004	M	W	8	0	0	0	7	0	24576	0												
2985	3	P-H	2004	M	W	9	0	0	28	8	48	768	0												
2985	3	P-H	2004	M	W	12	0	0	125	11	0	384	0	1	1		H-P	1	1	1	1	0	1	1	0
2993	3	P-H	2004	F	W	8	0	1	0	7	0		0	0	1	1									
2993	3	P-H	2004	F	W	9	0	1	28	8	48	768	24												
2993	3	P-H	2004	F	W	12	0	1	125	11	24	768	48	0	0			0	1	0	0	0	0	0	0
3002	3	P-H	2004	M	W	8	0	1	0	7															
3002	3	P-H	2004	M	W	9	0	1	28	8															
3002	3	P-H	2004	M	W	12	0	1	125	11	48		48												
3003	3	P-H	2004	F	W	8	0	0	0	7	0	1536	0												
3003	3	P-H	2004	F	W	9	0	0	28	8	24	768	0												
3003	3	P-H	2004	F	W	12	0	0	125	11	192	192	24	1	1		H-P	1	1	1	1	0	1	1	0
3009	3	P-H	2004	F	W	8	0	0	0	7	0	768	0	0	1	1									
3009	3	P-H	2004	F	W	9	0	0	28	8	48	384	0	0	0	0									
3009	3	P-H	2004	F	W	12	0	0	125	11	48	96	24	0	1		H-P	1	1	1	1	0	1	1	1
3017	3	P-H	2004	F	W	8	0	1	0	7	0	3072	24												
3017	3	P-H	2004	F	W	9	0	1	28	8	24	384	0		0										
3017	3	P-H	2004	F	W	12	0	1	125	11	24	384	0	1	1		P	1	1	1	0	0	1	1	1
3019	3	P-H	2004	F	W	8	0	0	0	7	0	768	0	0	0	1									
3019	3	P-H	2004	F	W	12	0	0	125	11	192	96	0	1	1		H-P	1	1	1	0	0	1	1	0
3035	3	P-H	2004	F	W	8	0	0	0	7	0	1536	0												
3035	3	P-H	2004	F	W	9	0	0	28	8	48	768	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM			Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur		Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
3035	3	P-H	2004	F	W	12	0	0	125	11	192	96	0	1	1			H-P	1	1	1	1	0	1	1	0
3036	3	P-H	2004	F	W	8	0	1	0	7	0		0													
3036	3	P-H	2004	F	W	9	0	1	28	8	48	1536	0	0	0	0										
3036	3	P-H	2004	F	W	12	0	1	125	11	0	768	0													
3037	3	P-H	2004	F	W	8	0	0	0	7	0	192	0	1	1	1										
3037	3	P-H	2004	F	W	9	0	0	28	8	48	384	0	0	0	0										
3037	3	P-H	2004	F	W	12	0	0	125	11	96	96	0	0	1			H-P	1	1	1	1	0	1	1	0
3049	3	P-H	2004	F	W	8	0	1	0	7	0		24													
3049	3	P-H	2004	F	W	9	0	1	28	8	96	384	0													
3049	3	P-H	2004	F	W	12	0	1	125	11		384														
4333	3	P-H	2004	F	W	8	0	1	0	7																
4333	3	P-H	2004	F	W	9	0	1	28	8																
4333	3	P-H	2004	F	W	12	0	1	125	11																
4555	3	P-H	2004	F	W	8	0	1	0	7																
4555	3	P-H	2004	F	W	9	0	1	28	8																
4555	3	P-H	2004	F	W	12	0	1	125	11																
no tag	3	P-H	2004	M	W	9	0	1	28	8	24	3072	24													
no tag	3	P-H	2004	M	W	12	0	1	125	11	0	768	48													
143	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
143	4	Neg	2003	F	W	9	0	1	15	8	0	0	48													
143	4	Neg	2003	F	W	10	0	1	28	9	24	48	96													
143	4	Neg	2003	F	W	11	0	1	58	10	48	96	48													
143	4	Neg	2003	F	W	12	0	1	88	11	24	96	96													
143	4	Neg	2003	F	W	13	0	1	140	12	24	96	0													
144	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
144	4	Neg	2003	F	W	9	0	1	15	8	0	0	48													
144	4	Neg	2003	F	W	10	0	1	28	9	0	24	192													
144	4	Neg	2003	F	W	11	0	1	58	10	48	48	48													
144	4	Neg	2003	F	W	12	0	1	88	11	48	192	48													
144	4	Neg	2003	F	W	13	0	1	140	12	48	48	0													
145	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
145	4	Neg	2003	F	W	9	0	1	15	8	24	0	24													
145	4	Neg	2003	F	W	10	0	1	28	9	0	96	0													
145	4	Neg	2003	F	W	11	0	1	58	10	24	96	24													
145	4	Neg	2003	F	W	12	0	1	88	11	24	96	0													
145	4	Neg	2003	F	W	13	0	1	140	12	24	48	0													
146	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
146	4	Neg	2003	F	W	9	0	1	15	8	0	192	192													
146	4	Neg	2003	F	W	10	0	1	28	9	48	96	0													
146	4	Neg	2003	F	W	11	0	1	58	10	48	96	48													
146	4	Neg	2003	F	W	12	0	1	88	11	96	96	384													
146	4	Neg	2003	F	W	13	0	1	140	12	24	48	0													
147	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
147	4	Neg	2003	M	W	9	0	1	15	8	0	0	96													
147	4	Neg	2003	M	W	10	0	1	28	9	0	24	48													
147	4	Neg	2003	M	W	11	0	1	58	10	48	48	48													
147	4	Neg	2003	M	W	12	0	1	88	11	24	96	0													
147	4	Neg	2003	M	W	13	0	1	140	12	24	24	0													
148	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
148	4	Neg	2003	F	W	9	0	1	15	8	0	0	384													
148	4	Neg	2003	F	W	10	0	1	28	9	0	24	24													
148	4	Neg	2003	F	W	11	0	1	58	10	24	48	24													
148	4	Neg	2003	F	W	12	0	1	88	11	24	96	0													
148	4	Neg	2003	F	W	13	0	1	140	12	24	24	0													
149	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
149	4	Neg	2003	F	W	9	0	1	15	8	0	24	24													
149	4	Neg	2003	F	W	10	0	1	28	9	0	0	384													
149	4	Neg	2003	F	W	11	0	1	58	10	24	96	96													
149	4	Neg	2003	F	W	12	0	1	88	11	24	96	96													
149	4	Neg	2003	F	W	13	0	1	140	12	24	24	48													
150	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
150	4	Neg	2003	M	W	9	0	1	15	8	24	96	96													
150	4	Neg	2003	M	W	10	0	1	28	9	192	96	384													
150	4	Neg	2003	M	W	11	0	1	58	10	96	192	96													
150	4	Neg	2003	M	W	12	0	1	88	11	384	384	96													
150	4	Neg	2003	M	W	13	0	1	140	12	24	24	24													
151	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
151	4	Neg	2003	M	W	9	0	1	15	8	0	0	24													
151	4	Neg	2003	M	W	10	0	1	28	9	0	24	0													
151	4	Neg	2003	M	W	11	0	1	58	10	24	48	0													
151	4	Neg	2003	M	W	12	0	1	88	11	96	192	0													
151	4	Neg	2003	M	W	13	0	1	140	12	48	0	24													
152	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
152	4	Neg	2003	M	W	9	0	1	15	8	0	0	0													
152	4	Neg	2003	M	W	10	0	1	28	9	0	48	24													
152	4	Neg	2003	M	W	11	0	1	58	10	24	24	0													

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM			Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur		Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
152	4	Neg	2003	M	W	12	0	1	88	11	48	96	0													
152	4	Neg	2003	M	W	13	0	1	140	12	24	24	0													
153	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
153	4	Neg	2003	M	W	9	0	1	15	8	0	0	48													
153	4	Neg	2003	M	W	10	0	1	28	9	0	24	0													
153	4	Neg	2003	M	W	11	0	1	58	10	48	0	48													
153	4	Neg	2003	M	W	12	0	1	88	11	96	48	0													
153	4	Neg	2003	M	W	13	0	1	140	12	24	24	0													
154	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
154	4	Neg	2003	F	W	9	0	1	15	8	48	0	192													
154	4	Neg	2003	F	W	10	0	1	28	9	96	0	24													
154	4	Neg	2003	F	W	11	0	1	58	10	48	24	24													
154	4	Neg	2003	F	W	12	0	1	88	11	96	48	0													
154	4	Neg	2003	F	W	13	0	1	140	12	24	24	0													
155	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
155	4	Neg	2003	M	W	9	0	1	15	8	24	0	384													
155	4	Neg	2003	M	W	10	0	1	28	9	24	0	24													
155	4	Neg	2003	M	W	11	0	1	58	10	24	0	0													
155	4	Neg	2003	M	W	12	0	1	88	11	192	48	0													
155	4	Neg	2003	M	W	13	0	1	140	12	48	24	48													
156	4	Neg	2003	F	W	9	0	1	0	8	0	0	0													
156	4	Neg	2003	F	W	9	0	1	15	8	24	0	384													
156	4	Neg	2003	F	W	10	0	1	28	9	0	96	48													
156	4	Neg	2003	F	W	11	0	1	58	10	24	24	48													
156	4	Neg	2003	F	W	12	0	1	88	11	24	96	0													
156	4	Neg	2003	F	W	13	0	1	140	12	24	0	0													
157	4	Neg	2003	F	W	9	0	0	0	8	0	0	0													
157	4	Neg	2003	F	W	9	0	0	15	8	0	0	0													
157	4	Neg	2003	F	W	10	0	0	28	9	0	0	0													
157	4	Neg	2003	F	W	11	0	0	58	10	0	0	0													
157	4	Neg	2003	F	W	12	0	0	88	11	0	0	0													
157	4	Neg	2003	F	W	13	0	0	140	12	0	0	0													
158	4	Neg	2003	F	W	9	0	0	0	8	0	0	0													
158	4	Neg	2003	F	W	9	0	0	15	8	0	0	0													
158	4	Neg	2003	F	W	10	0	0	28	9	0	0	0													
158	4	Neg	2003	F	W	11	0	0	58	10	0	0	0													
158	4	Neg	2003	F	W	12	0	0	88	11	0	0	0													
158	4	Neg	2003	F	W	13	0	0	140	12	0	0	0													
159	4	Neg	2003	M	W	9	0	1	0	8	0	0	0													
159	4	Neg	2003	M	W	9	0	1	15	8	24	192	96													
159	4	Neg	2003	M	W	10	0	1	28	9	24	0	96													
159	4	Neg	2003	M	W	11	0	1	58	10	24	96	48													
159	4	Neg	2003	M	W	12	0	1	88	11	48	384	384													
159	4	Neg	2003	M	W	13	0	1	140	12	24	0	0													
160	4	Neg	2003	M	W	9	0	0	0	8	0	0	0													
160	4	Neg	2003	M	W	9	0	0	15	8	0	0	0													
160	4	Neg	2003	M	W	10	0	0	28	9	0	0	0													
160	4	Neg	2003	M	W	11	0	0	58	10	0	0	0													
160	4	Neg	2003	M	W	12	0	0	88	11	0	0	0													
160	4	Neg	2003	M	W	13	0	0	140	12	0	0	0													
161	4	Neg	2003	M	W	9	0	0	0	8	0	0	0													
161	4	Neg	2003	M	W	9	0	0	15	8	0	0	0													
161	4	Neg	2003	M	W	10	0	0	28	9	0	0	0													
161	4	Neg	2003	M	W	11	0	0	58	10	0	0	0													
161	4	Neg	2003	M	W	12	0	0	88	11	0	0	0													
161	4	Neg	2003	M	W	13	0	0	140	12	0	0	0													
162	4	Neg	2003	M	W	9	0	0	0	8	0	0	0													
162	4	Neg	2003	M	W	9	0	0	15	8	0	0	0													
162	4	Neg	2003	M	W	10	0	0	28	9	0	0	0													
162	4	Neg	2003	M	W	11	0	0	58	10	0	0	0													
162	4	Neg	2003	M	W	12	0	0	88	11	0	0	0													
162	4	Neg	2003	M	W	13	0	0	140	12	0	0	0													
301	4	Neg	2004	M	W	3	0	1	-28	2	192	24	0													
301	4	Neg	2004	M	W	4	0	1	0	3	24	0	24													
301	4	Neg	2004	M	W	5	0	1	28	4	48	384	384													
301	4	Neg	2004	M	W	7	0	1	66	6	96	96	96													
301	4	Neg	2004	M	W	9	0	1	137	8	24	24	0													
301	4	Neg	2004	M	W	10	0	1	189	9	0	0	0													
301	4	Neg	2004	M	W	12	0	1	284	11	0	0	0													
302	4	Neg	2004	F	W	3	0	1	-28	2	24	48	0													
302	4	Neg	2004	F	W	4	0	1	0	3	0	0	24													
302	4	Neg	2004	F	W	5	0	1	28	4	48	48	48													
302	4	Neg	2004	F	W	7	0	1	66	6	96	96	96													
302	4	Neg	2004	F	W	9	0	1	137	8	24	48	0													
302	4	Neg	2004	F	W	10	0	1	189	9	0	0	0													
302	4	Neg	2004	F	W	12	0	1	284	11	0	0	0													

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
303	4	Neg	2004	M	W	3	1	0	-28	2	192	768	0												
303	4	Neg	2004	M	W	4	1	0	0	3	0	24	0												
303	4	Neg	2004	M	W	5	1	0	28	4	24	0	0												
303	4	Neg	2004	M	W	7	1	0	66	6	48	0	0												
303	4	Neg	2004	M	W	9	1	0	137	8	24	48	0												
303	4	Neg	2004	M	W	10	1	0	189	9	0	0	0												
303	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
304	4	Neg	2004	M	W	3	0	0	-28	2	24	0	0												
304	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
304	4	Neg	2004	M	W	5	0	0	28	4	48	0	0												
304	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
304	4	Neg	2004	M	W	9	0	0	137	8	0	0	0												
304	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
304	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
305	4	Neg	2004	M	W	3	1	0	-28	2	24	24	24												
305	4	Neg	2004	M	W	4	1	0	0	3	24	0	24												
305	4	Neg	2004	M	W	5	1	0	28	4	24	0	0												
305	4	Neg	2004	M	W	7	1	0	66	6	0	0	0												
305	4	Neg	2004	M	W	9	1	0	137	8	0	0	0												
305	4	Neg	2004	M	W	10	1	0	189	9	0	0	0												
305	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
306	4	Neg	2004	M	W	3	1	0	-28	2	192	384	0												
306	4	Neg	2004	M	W	4	1	0	0	3	96	0	24												
306	4	Neg	2004	M	W	5	1	0	28	4	24	48	48												
306	4	Neg	2004	M	W	7	1	0	66	6	24	24	24												
306	4	Neg	2004	M	W	9	1	0	137	8	48	24	0												
306	4	Neg	2004	M	W	10	1	0	189	9	0	0	0												
306	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
307	4	Neg	2004	F	W	3	1	0	-28	2	192	3072	0												
307	4	Neg	2004	F	W	4	1	0	0	3	24	0	0												
307	4	Neg	2004	F	W	5	1	0	28	4	24	24	24												
307	4	Neg	2004	F	W	7	1	0	66	6	24	24	0												
307	4	Neg	2004	F	W	9	1	0	137	8	24	48	0												
307	4	Neg	2004	F	W	10	1	0	189	9	0	0	0												
307	4	Neg	2004	F	W	12	1	0	284	11	0	0	0												
308	4	Neg	2004	F	W	3	0	0	-28	2	0	24	0												
308	4	Neg	2004	F	W	4	0	0	0	3	24	0	0												
308	4	Neg	2004	F	W	5	0	0	28	4	96	24	24												
308	4	Neg	2004	F	W	7	0	0	66	6	48	24	0												
308	4	Neg	2004	F	W	9	0	0	137	8	24	24	0												
308	4	Neg	2004	F	W	10	0	0	189	9	0	0	0												
308	4	Neg	2004	F	W	12	0	0	284	11	0	0	0												
309	4	Neg	2004	M	W	3	0	1	-28	2	96	384	0												
309	4	Neg	2004	M	W	4	0	1	0	3	24	0	0												
309	4	Neg	2004	M	W	5	0	1	28	4	24	192	192												
309	4	Neg	2004	M	W	7	0	1	66	6	0	384	384												
309	4	Neg	2004	M	W	9	0	1	137	8	0	0	0												
309	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
309	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												
310	4	Neg	2004	M	W	3	1	0	-28	2	192	96	24												
310	4	Neg	2004	M	W	4	1	0	0	3	0	96	48												
310	4	Neg	2004	M	W	5	1	0	28	4	48	0	0												
310	4	Neg	2004	M	W	7	1	0	66	6	48	0	0												
310	4	Neg	2004	M	W	9	1	0	137	8	24	24	0												
310	4	Neg	2004	M	W	10	1	0	189	9	0	0	0												
310	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
311	4	Neg	2004	M	W	3	1	0	-28	2	24	24	24												
311	4	Neg	2004	M	W	4	1	0	0	3	24	96	24												
311	4	Neg	2004	M	W	5	1	0	28	4	24	0	0												
311	4	Neg	2004	M	W	7	1	0	66	6	0	0	0												
312	4	Neg	2004	M	W	3	1	1	-28	2	192	48	48												
312	4	Neg	2004	M	W	4	1	1	0	3	0	0	0												
312	4	Neg	2004	M	W	5	1	1	28	4	24	48	48												
312	4	Neg	2004	M	W	7	1	1	66	6	24	48	48												
312	4	Neg	2004	M	W	9	1	1	137	8	24	0	0												
312	4	Neg	2004	M	W	10	1	1	189	9	0	0	0												
312	4	Neg	2004	M	W	12	1	1	284	11	0	0	0												
313	4	Neg	2004	F	W	3	0	1	-28	2	48	384	0												
313	4	Neg	2004	F	W	4	0	1	0	3	24	0	0												
313	4	Neg	2004	F	W	5	0	1	28	4	24	48	48												
313	4	Neg	2004	F	W	7	0	1	66	6	24	96	96												
313	4	Neg	2004	F	W	9	0	1	137	8	48	48	24												
313	4	Neg	2004	F	W	10	0	1	189	9	0	0	0												
313	4	Neg	2004	F	W	12	0	1	284	11	0	0	0												
314	4	Neg	2004	F	W	3	0	1	-28	2	48	48	0												
314	4	Neg	2004	F	W	4	0	1	0	3	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
314	4	Neg	2004	F	W	5	0	1	28	4	24	24	24											
314	4	Neg	2004	F	W	7	0	1	66	6	48	96	96											
314	4	Neg	2004	F	W	9	0	1	137	8	48	48	0											
314	4	Neg	2004	F	W	10	0	1	189	9	0	0	0											
314	4	Neg	2004	F	W	12	0	1	284	11	0	0	0											
315	4	Neg	2004	M	W	3	1	1	-28	2	192	96	48											
315	4	Neg	2004	M	W	4	1	1	0	3	24	24	0											
315	4	Neg	2004	M	W	5	1	1	28	4	48	0	0											
315	4	Neg	2004	M	W	7	1	1	66	6	48	48	48											
315	4	Neg	2004	M	W	9	1	1	137	8	24	24	0											
315	4	Neg	2004	M	W	10	1	1	189	9	24	0	0											
315	4	Neg	2004	M	W	12	1	1	284	11	0	0	0											
316	4	Neg	2004	F	W	3	0	1	-28	2	192	48	0											
316	4	Neg	2004	F	W	4	0	1	0	3	0	0	0											
316	4	Neg	2004	F	W	5	0	1	28	4	24	0	0											
316	4	Neg	2004	F	W	7	0	1	66	6	24	0	0											
316	4	Neg	2004	F	W	9	0	1	137	8	24	0	0											
316	4	Neg	2004	F	W	10	0	1	189	9	0	0	0											
316	4	Neg	2004	F	W	12	0	1	284	11	0	0	0											
317	4	Neg	2004	M	W	3	1	0	-28	2	192	48	24											
317	4	Neg	2004	M	W	4	1	0	0	3	48	24	24											
317	4	Neg	2004	M	W	5	1	0	28	4	24	0	0											
317	4	Neg	2004	M	W	7	1	0	66	6	24	0	0											
317	4	Neg	2004	M	W	9	1	0	137	8	0	0	0											
317	4	Neg	2004	M	W	10	1	0	189	9	0	0	0											
317	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
318	4	Neg	2004	M	W	3	1	0	-28	2	24	48	24											
318	4	Neg	2004	M	W	4	1	0	0	3	0	0	24											
318	4	Neg	2004	M	W	5	1	0	28	4	0	0	0											
318	4	Neg	2004	M	W	7	1	0	66	6	0	0	0											
318	4	Neg	2004	M	W	9	1	0	137	8	0	0	0											
318	4	Neg	2004	M	W	10	1	0	189	9	0	0	0											
318	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
319	4	Neg	2004	M	W	3	1	1	-28	2	48	24	48											
319	4	Neg	2004	M	W	4	1	1	0	3	48	0	24											
319	4	Neg	2004	M	W	5	1	1	28	4	24	48	48											
319	4	Neg	2004	M	W	7	1	1	66	6	0	48	48											
319	4	Neg	2004	M	W	9	1	1	137	8	0	24	0											
319	4	Neg	2004	M	W	10	1	1	189	9	0	0	0											
319	4	Neg	2004	M	W	12	1	1	284	11	0	0	0											
320	4	Neg	2004	F	W	3	0	1	-28	2	192	24	0											
320	4	Neg	2004	F	W	4	0	1	0	3	0	48	24											
320	4	Neg	2004	F	W	5	0	1	28	4	24	48	48											
320	4	Neg	2004	F	W	7	0	1	66	6	0	48	48											
320	4	Neg	2004	F	W	9	0	1	137	8	0	0	0											
320	4	Neg	2004	F	W	10	0	1	189	9	0	0	0											
320	4	Neg	2004	F	W	12	0	1	284	11	0	0	0											
321	4	Neg	2004	F	W	3	0	1	-28	2	48	24	0											
321	4	Neg	2004	F	W	4	0	1	0	3	0	0	24											
321	4	Neg	2004	F	W	5	0	1	28	4	48	24	24											
321	4	Neg	2004	F	W	7	0	1	66	6	48	48	48											
321	4	Neg	2004	F	W	9	0	1	137	8	48	0	0											
321	4	Neg	2004	F	W	10	0	1	189	9	0	0	0											
321	4	Neg	2004	F	W	12	0	1	284	11	0	0	0											
322	4	Neg	2004	F	W	3	1	1	-28	2	96	48	192											
322	4	Neg	2004	F	W	4	1	1	0	3	24	24	24											
322	4	Neg	2004	F	W	5	1	1	28	4	0	48	48											
322	4	Neg	2004	F	W	7	1	1	66	6	48	96	96											
322	4	Neg	2004	F	W	9	1	1	137	8	24	0	0											
322	4	Neg	2004	F	W	10	1	1	189	9	0	0	0											
322	4	Neg	2004	F	W	12	1	1	284	11	0	0	0											
323	4	Neg	2004	M	W	3	1	1	-28	2	96	1532	96											
323	4	Neg	2004	M	W	4	1	1	0	3	0	0	24											
323	4	Neg	2004	M	W	5	1	1	28	4	0	24	24											
323	4	Neg	2004	M	W	7	1	1	66	6	96	24	24											
323	4	Neg	2004	M	W	9	1	1	137	8	24	0	0											
323	4	Neg	2004	M	W	10	1	1	189	9	0	0	0											
323	4	Neg	2004	M	W	12	1	1	284	11	0	0	0											
324	4	Neg	2004	F	W	3	1	1	-28	2	96	768	96											
324	4	Neg	2004	F	W	4	1	1	0	3	0	0	0											
324	4	Neg	2004	F	W	5	1	1	28	4	24	24	24											
324	4	Neg	2004	F	W	7	1	1	66	6	24	384	384											
324	4	Neg	2004	F	W	9	1	1	137	8	24	0	0											
324	4	Neg	2004	F	W	10	1	1	189	9	0	0	0											
324	4	Neg	2004	F	W	12	1	1	284	11	24	0	0											
325	4	Neg	2004	F	W	3	1	0	-28	2	96	96	24											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
325	4	Neg	2004	F	W	4	1	0	0	3	0	0	0												
325	4	Neg	2004	F	W	5	1	0	28	4	0	0	0												
325	4	Neg	2004	F	W	7	1	0	66	6	0	0	0												
325	4	Neg	2004	F	W	9	1	0	137	8	0	48	0												
325	4	Neg	2004	F	W	10	1	0	189	9	0	0	0												
325	4	Neg	2004	F	W	12	1	0	284	11	0	0	0												
326	4	Neg	2004	F	W	3	1	1	-28	2	96	48	48												
326	4	Neg	2004	F	W	4	1	1	0	3	0	0	0												
326	4	Neg	2004	F	W	5	1	1	28	4	0	96	96												
326	4	Neg	2004	F	W	7	1	1	66	6	24	96	96												
326	4	Neg	2004	F	W	9	1	1	137	8	48	0	0												
326	4	Neg	2004	F	W	10	1	1	189	9	24	0	0												
326	4	Neg	2004	F	W	12	1	1	284	11	0	0	0												
327	4	Neg	2004	F	W	3	1	1	-28	2	96	0	24												
327	4	Neg	2004	F	W	4	1	1	0	3	0	0	24												
327	4	Neg	2004	F	W	5	1	1	28	4	48	0	0												
327	4	Neg	2004	F	W	7	1	1	66	6	24	0	0												
327	4	Neg	2004	F	W	9	1	1	137	8	24	0	0												
327	4	Neg	2004	F	W	10	1	1	189	9	0	0	0												
327	4	Neg	2004	F	W	12	1	1	284	11	24	0	0												
328	4	Neg	2004	F	W	3	1	1	-28	2	192	48	48												
328	4	Neg	2004	F	W	4	1	1	0	3	0	0	24												
328	4	Neg	2004	F	W	5	1	1	28	4	48	0	0												
328	4	Neg	2004	F	W	7	1	1	66	6	24	96	96												
328	4	Neg	2004	F	W	9	1	1	137	8	24	0	0												
328	4	Neg	2004	F	W	10	1	1	189	9	24	0	0												
328	4	Neg	2004	F	W	12	1	1	284	11	0	0	0												
329	4	Neg	2004	F	W	3	1	1	-28	2	24	24	48												
329	4	Neg	2004	F	W	4	1	1	0	3	0	0	0												
329	4	Neg	2004	F	W	5	1	1	28	4	48	0	0												
329	4	Neg	2004	F	W	7	1	1	66	6	24	96	96												
329	4	Neg	2004	F	W	9	1	1	137	8	24	0	0												
329	4	Neg	2004	F	W	10	1	1	189	9	24	0	0												
329	4	Neg	2004	F	W	12	1	1	284	11	0	0	0												
330	4	Neg	2004	M	W	3	0	1	-28	2	96	24	0												
330	4	Neg	2004	M	W	4	0	1	0	3	0	0	0												
330	4	Neg	2004	M	W	5	0	1	28	4	24	96	96												
330	4	Neg	2004	M	W	7	0	1	66	6	48	192	192												
330	4	Neg	2004	M	W	9	0	1	137	8	0	96	0												
330	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
330	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												
331	4	Neg	2004	F	W	3	0	1	-28	2	192	192	0												
331	4	Neg	2004	F	W	4	0	1	0	3	0	0	0												
331	4	Neg	2004	F	W	5	0	1	28	4	48	0	0												
331	4	Neg	2004	F	W	7	0	1	66	6	24	96	96												
331	4	Neg	2004	F	W	9	0	1	137	8	0	48	0												
331	4	Neg	2004	F	W	10	0	1	189	9	0	0	0												
331	4	Neg	2004	F	W	12	0	1	284	11	0		0												
332	4	Neg	2004	M	W	3	1	1	-28	2	192	24	48												
332	4	Neg	2004	M	W	4	1	1	0	3	0	0	24												
332	4	Neg	2004	M	W	5	1	1	28	4	24	0	0												
332	4	Neg	2004	M	W	7	1	1	66	6	48	48	48												
332	4	Neg	2004	M	W	9	1	1	137	8	0	48	0												
332	4	Neg	2004	M	W	10	1	1	189	9	0	0	0												
332	4	Neg	2004	M	W	12	1	1	284	11	0	0	0												
333	4	Neg	2004	M	W	3	1	1	-28	2	96	48	48												
333	4	Neg	2004	M	W	4	1	1	0	3	0	0	0												
333	4	Neg	2004	M	W	5	1	1	28	4	24	48	48												
333	4	Neg	2004	M	W	7	1	1	66	6	24	48	48												
333	4	Neg	2004	M	W	9	1	1	137	8	48	24	0												
333	4	Neg	2004	M	W	10	1	1	189	9	0	0	0												
333	4	Neg	2004	M	W	12	1	1	284	11	0	0	0												
334	4	Neg	2004	M	W	3	1	0	-28	2	96	48	24												
334	4	Neg	2004	M	W	4	1	0	0	3	0	0	0												
334	4	Neg	2004	M	W	5	1	0	28	4	0	0	0												
334	4	Neg	2004	M	W	7	1	0	66	6	0	0	0												
334	4	Neg	2004	M	W	9	1	0	137	8	0	0	0												
334	4	Neg	2004	M	W	10	1	0	189	9	0	0	0												
334	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
335	4	Neg	2004	M	W	3	0	1	-28	2	96	24	0												
335	4	Neg	2004	M	W	4	0	1	0	3	24	0	0												
335	4	Neg	2004	M	W	5	0	1	28	4	0	48	48												
335	4	Neg	2004	M	W	7	0	1	66	6	48	96	96												
335	4	Neg	2004	M	W	9	0	1	137	8	24	0	0												
335	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
335	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
336	4	Neg	2004	F	W	3	1	0	-28	2	96	384	0											
336	4	Neg	2004	F	W	4	1	0	0	3	0	0												
336	4	Neg	2004	F	W	5	1	0	28	4	0	24	0											
336	4	Neg	2004	F	W	7	1	0	66	6	192	48	24											
336	4	Neg	2004	F	W	9	1	0	137	8	48	96	48											
336	4	Neg	2004	F	W	10	1	0	189	9	24	0	0											
336	4	Neg	2004	F	W	12	1	0	284	11	0	0	0											
337	4	Neg	2004	F	W	3	1	0	-28	2	48	96	24											
337	4	Neg	2004	F	W	4	1	0	0	3	0	0	0											
337	4	Neg	2004	F	W	5	1	0	28	4	0	0	0											
337	4	Neg	2004	F	W	7	1	0	66	6	24	0	0											
337	4	Neg	2004	F	W	9	1	0	137	8	24	0	0											
337	4	Neg	2004	F	W	10	1	0	189	9	0	0	0											
337	4	Neg	2004	F	W	12	1	0	284	11	0	0	0											
338	4	Neg	2004	F	W	3	1	0	-28	2	384	1536	0											
339	4	Neg	2004	F	W	3	1	0	-28	2	96	768	0											
339	4	Neg	2004	F	W	4	1	0	0	3	0	0	0											
339	4	Neg	2004	F	W	5	1	0	28	4	24	24	24											
339	4	Neg	2004	F	W	7	1	0	66	6	48	0	0											
339	4	Neg	2004	F	W	9	1	0	137	8	24	0	0											
339	4	Neg	2004	F	W	10	1	0	189	9	0	0	0											
339	4	Neg	2004	F	W	12	1	0	284	11	0	0	0											
340	4	Neg	2004	F	W	3	0	1	-28	2	48	24	0											
340	4	Neg	2004	F	W	4	0	1	0	3	24	0	0											
340	4	Neg	2004	F	W	5	0	1	28	4	24	0	0											
340	4	Neg	2004	F	W	7	0	1	66	6	96	48	48											
340	4	Neg	2004	F	W	9	0	1	137	8	48	24	0											
340	4	Neg	2004	F	W	10	0	1	189	9	0	0	0											
340	4	Neg	2004	F	W	12	0	1	284	11	0	0	0											
341	4	Neg	2004	F	W	3	0	0	-28	2	24	0	24											
341	4	Neg	2004	F	W	4	0	0	0	3	0	0	0											
341	4	Neg	2004	F	W	5	0	0	28	4	0	0	0											
341	4	Neg	2004	F	W	7	0	0	66	6	0	0	0											
341	4	Neg	2004	F	W	9	0	0	137	8	0	0	0											
341	4	Neg	2004	F	W	10	0	0	189	9	0	0	0											
341	4	Neg	2004	F	W	12	0	0	284	11	0	0	0											
342	4	Neg	2004	M	W	3	0	0	-28	2	24	24	0											
342	4	Neg	2004	M	W	4	0	0	0	3	0	0	0											
342	4	Neg	2004	M	W	5	0	0	28	4	0	0	0											
342	4	Neg	2004	M	W	7	0	0	66	6	0	0	0											
342	4	Neg	2004	M	W	9	0	0	137	8	0	0	0											
342	4	Neg	2004	M	W	10	0	0	189	9	0	0	0											
342	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
343	4	Neg	2004	F	W	3	0	0	-28	2	24	24	24											
343	4	Neg	2004	F	W	4	0	0	0	3	24	0	0											
343	4	Neg	2004	F	W	5	0	0	28	4	0	0	0											
343	4	Neg	2004	F	W	7	0	0	66	6	0	0	0											
343	4	Neg	2004	F	W	9	0	0	137	8	0	0	0											
343	4	Neg	2004	F	W	10	0	0	189	9	0	0	0											
343	4	Neg	2004	F	W	12	0	0	284	11	0	0	0											
344	4	Neg	2004	M	W	3	1	0	-28	2	384	1536	0											
344	4	Neg	2004	M	W	4	1	0	0	3	0	0	0											
344	4	Neg	2004	M	W	5	1	0	28	4	0	0	0											
344	4	Neg	2004	M	W	7	1	0	66	6	0	0	0											
344	4	Neg	2004	M	W	9	1	0	137	8	0	0	0											
344	4	Neg	2004	M	W	10	1	0	189	9	0	0	0											
344	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
345	4	Neg	2004	F	W	3	1	1	-28	2	24	1536	48											
345	4	Neg	2004	F	W	4	1	1	0	3	0	0	0											
345	4	Neg	2004	F	W	5	1	1	28	4	0	0	0											
345	4	Neg	2004	F	W	7	1	1	66	6	24	96	96											
345	4	Neg	2004	F	W	9	1	1	137	8	24	24	0											
345	4	Neg	2004	F	W	10	1	1	189	9	0	0	0											
345	4	Neg	2004	F	W	12	1	1	284	11	0	0	0											
346	4	Neg	2004	F	W	3	1	0	-28	2	384	768	0											
346	4	Neg	2004	F	W	4	1	0	0	3	0	0	0											
346	4	Neg	2004	F	W	5	1	0	28	4	24	0	0											
346	4	Neg	2004	F	W	7	1	0	66	6	0	0	0											
346	4	Neg	2004	F	W	9	1	0	137	8	0	0	0											
346	4	Neg	2004	F	W	10	1	0	189	9	0	0	0											
346	4	Neg	2004	F	W	12	1	0	284	11	0	0	0											
347	4	Neg	2004	M	W	3	0	1	-28	2	96	24	0											
347	4	Neg	2004	M	W	4	0	1	0	3	24	24	0											
347	4	Neg	2004	M	W	5	0	1	28	4	24	24	24											
347	4	Neg	2004	M	W	7	0	1	66	6	0	0	0											
347	4	Neg	2004	M	W	9	0	1	137	8	0	24	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLJ	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
347	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
347	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												
348	4	Neg	2004	F	W	3	1	1	-28	2	48	1536	192												
348	4	Neg	2004	F	W	4	1	1	0	3	0	0	0												
348	4	Neg	2004	F	W	5	1	1	28	4	24	0	0												
348	4	Neg	2004	F	W	7	1	1	66	6	48	96	96												
348	4	Neg	2004	F	W	9	1	1	137	8	24	0	0												
348	4	Neg	2004	F	W	10	1	1	189	9	24	0	0												
348	4	Neg	2004	F	W	12	1	1	284	11	0	0	0												
349	4	Neg	2004	M	W	3	0	0	-28	2	0	0	0												
349	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
349	4	Neg	2004	M	W	5	0	0	28	4	48	0	0												
349	4	Neg	2004	M	W	7	0	0	66	6	24	0	0												
349	4	Neg	2004	M	W	9	0	0	137	8	24	0	0												
349	4	Neg	2004	M	W	10	0	0	189	9	24	0	0												
349	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
350	4	Neg	2004	M	W	3	0	1	-28	2	0	48	0												
350	4	Neg	2004	M	W	4	0	1	0	3	0	0	0												
350	4	Neg	2004	M	W	5	0	1	28	4	24	0	0												
350	4	Neg	2004	M	W	7	0	1	66	6	0	0	0												
350	4	Neg	2004	M	W	9	0	1	137	8	0	0	0												
350	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
350	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												
351	4	Neg	2004	M	W	3	0	0	-28	2	48	0	0												
351	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
351	4	Neg	2004	M	W	5	0	0	28	4	0	0	0												
351	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
351	4	Neg	2004	M	W	9	0	0	137	8	0	0	0												
351	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
351	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
352	4	Neg	2004	F	W	3	0	0	-28	2	0	0	0												
352	4	Neg	2004	F	W	4	0	0	0	3	0	0	0												
352	4	Neg	2004	F	W	5	0	0	28	4	48	24	0												
352	4	Neg	2004	F	W	7	0	0	66	6	96	24	48												
352	4	Neg	2004	F	W	9	0	0	137	8	24	24	0												
352	4	Neg	2004	F	W	10	0	0	189	9	0	0	0												
352	4	Neg	2004	F	W	12	0	0	284	11	0	0	0												
353	4	Neg	2004	M	W	3	0	0	-28	2	48	0	0												
353	4	Neg	2004	M	W	4	0	0	0	3	24	0	0												
353	4	Neg	2004	M	W	5	0	0	28	4	24	48	48												
353	4	Neg	2004	M	W	7	0	0	66	6	24	0	24												
353	4	Neg	2004	M	W	9	0	0	137	8	24	0	0												
353	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
353	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
354	4	Neg	2004	M	W	3	0	0	-28	2	0	0	0												
354	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
354	4	Neg	2004	M	W	5	0	0	28	4	0	0	0												
354	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
354	4	Neg	2004	M	W	9	0	0	137	8	0	0	0												
354	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
354	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
355	4	Neg	2004	M	W	3	1	1	-28	2	0	0	0												
355	4	Neg	2004	F	W	4	1	1	0	3	0	0	0												
355	4	Neg	2004	F	W	5	1	1	28	4	0	0	0												
355	4	Neg	2004	F	W	7	1	1	66	6	0	0	0												
355	4	Neg	2004	F	W	9	1	1	137	8	0	0	0												
355	4	Neg	2004	F	W	10	1	1	189	9	0	0	0												
355	4	Neg	2004	F	W	12	1	1	284	11	0	0	0												
356	4	Neg	2004	F	W	4	0	0	0	3	48	0	24												
356	4	Neg	2004	F	W	5	0	0	28	4	48	0	24												
356	4	Neg	2004	F	W	7	0	0	66	6	24	0	0												
356	4	Neg	2004	F	W	9	0	0	137	8	0	0	0												
356	4	Neg	2004	F	W	10	0	0	189	9	0	0	0												
356	4	Neg	2004	F	W	12	0	0	284	11	0	0	0												
357	4	Neg	2004	M	W	3	1	1	-28	2	24	96	96												
357	4	Neg	2004	M	W	4	1	1	0	3	0	0	0												
357	4	Neg	2004	M	W	5	1	1	28	4	48	96	96												
357	4	Neg	2004	M	W	7	1	1	66	6	96	0	0												
357	4	Neg	2004	M	W	9	1	1	137	8	0	24	0												
357	4	Neg	2004	M	W	10	1	1	189	9	0	0	0												
357	4	Neg	2004	M	W	12	1	1	284	11	0	0	0												
358	4	Neg	2004	M	W	3	0	1	-28	2	96	96	0												
358	4	Neg	2004	M	W	4	0	1	0	3	48	0	0												
358	4	Neg	2004	M	W	5	0	1	28	4	24	48	48												
358	4	Neg	2004	M	W	7	0	1	66	6	24	0	0												
358	4	Neg	2004	M	W	9	0	1	137	8	0	48	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
358	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
358	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												
359	4	Neg	2004	F	W	3	1	0	-28	2	24	1536	0												
359	4	Neg	2004	F	W	4	1	0	0	3	24	0	0												
359	4	Neg	2004	F	W	5	1	0	28	4	0	0	0												
359	4	Neg	2004	F	W	7	1	0	66	6	24	0	0												
359	4	Neg	2004	F	W	9	1	0	137	8	24	48	0												
359	4	Neg	2004	F	W	10	1	0	189	9	0	0	0												
359	4	Neg	2004	F	W	12	1	0	284	11	0	0	0												
360	4	Neg	2004	M	W	3	0	0	-28	2	0	24	0												
360	4	Neg	2004	M	W	4	0	0	0	3	24	0	0												
360	4	Neg	2004	M	W	5	0	0	28	4	24	0	0												
360	4	Neg	2004	M	W	7	0	0	66	6	24	0	0												
360	4	Neg	2004	M	W	9	0	0	137	8	24	0	0												
360	4	Neg	2004	M	W	10	0	0	189	9	24	0	0												
360	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0		0	0	0	0	0	0	0	0	0
361	4	Neg	2004	M	W	3	0	0	-28	2	0	0	0												
361	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
361	4	Neg	2004	M	W	5	0	0	28	4	0	0	0												
361	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
361	4	Neg	2004	M	W	9	0	0	137	8	0	0	0												
361	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
361	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
362	4	Neg	2004	M	W	3	0	0	-28	2	0	24	24												
362	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
362	4	Neg	2004	M	W	5	0	0	28	4	24	0	0												
362	4	Neg	2004	M	W	7	0	0	66	6	48	0	0												
362	4	Neg	2004	M	W	9	0	0	137	8	24	0	0												
362	4	Neg	2004	M	W	10	0	0	189	9	24	0	0												
362	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
363	4	Neg	2004	M	W	3	1	1	-28	2	192	1536	96												
363	4	Neg	2004	M	W	4	1	1	0	3	0	0	24												
363	4	Neg	2004	M	W	5	1	1	28	4	24	96	96												
363	4	Neg	2004	M	W	7	1	1	66	6	96	0	0												
363	4	Neg	2004	M	W	9	1	1	137	8	24	48	0												
363	4	Neg	2004	M	W	10	1	1	189	9	0	0	0												
363	4	Neg	2004	M	W	12	1	1	284	11	0	0	0												
364	4	Neg	2004	M	W	3	0	1	-28	2	48	192	0												
364	4	Neg	2004	M	W	4	0	1	0	3	0	0	0												
364	4	Neg	2004	M	W	5	0	1	28	4	48	0	0												
364	4	Neg	2004	M	W	7	0	1	66	6	48	0	0												
364	4	Neg	2004	M	W	9	0	1	137	8	0	48	0												
364	4	Neg	2004	M	W	10	0	1	189	9	0	0	0												
364	4	Neg	2004	M	W	12	0	1	284	11	0	0	0												
365	4	Neg	2004	M	W	3	0	0	-28	2	0	0	0												
365	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
365	4	Neg	2004	M	W	5	0	0	28	4	0	0	0												
365	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
365	4	Neg	2004	M	W	9	0	0	137	8	0	0	0												
365	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
365	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
366	4	Neg	2004	M	W	3	1	0	-28	2	192	96	24												
366	4	Neg	2004	M	W	4	1	0	0	3	0	96	96												
366	4	Neg	2004	M	W	5	1	0	28	4	0	96	0												
366	4	Neg	2004	M	W	7	1	0	66	6	0	0	0												
366	4	Neg	2004	M	W	9	1	0	137	8	0	0	0												
366	4	Neg	2004	M	W	10	1	0	189	9	0	0	0												
366	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	1	0	1	1	0
367	4	Neg	2004	M	W	3	1	0	-28	2	192	768	0												
367	4	Neg	2004	M	W	4	1	0	0	3	24	24	48												
367	4	Neg	2004	M	W	5	1	0	28	4	0	24	24												
367	4	Neg	2004	M	W	7	1	0	66	6	0	0	0												
367	4	Neg	2004	M	W	9	1	0	137	8	24	0	0												
367	4	Neg	2004	M	W	10	1	0	189	9	24	0	0												
367	4	Neg	2004	M	W	12	1	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
368	4	Neg	2004	F	W	3	0	1	-28	2	48	24	0												
368	4	Neg	2004	F	W	4	0	1	0	3	0	0	0												
368	4	Neg	2004	F	W	5	0	1	28	4	24	0	0												
368	4	Neg	2004	F	W	7	0	1	66	6	48	0	0												
368	4	Neg	2004	F	W	9	0	1	137	8	24	24	0												
368	4	Neg	2004	F	W	10	0	1	189	9	24	0	0												
368	4	Neg	2004	F	W	12	0	1	284	11	0	0	0												
369	4	Neg	2004	M	W	3	1	1	-28	2	192	3072	48												
369	4	Neg	2004	M	W	4	1	1	0	3	48	24	48												
369	4	Neg	2004	M	W	5	1	1	28	4	0	24	24												
369	4	Neg	2004	M	W	7	1	1	66	6	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney			Histopathology				
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
369	4	Neg	2004	M	W	9	1	1	137	8	0	24	0												
369	4	Neg	2004	M	W	10	1	1	189	9	0	0	0												
369	4	Neg	2004	M	W	12	1	1	284	11	0	0	0												
370	4	Neg	2004	M	W	3	0	0	-28	2	192	192	24												
370	4	Neg	2004	M	W	4	0	0	0	3	0	0	0												
370	4	Neg	2004	M	W	5	0	0	28	4	0	0	0												
370	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
370	4	Neg	2004	M	W	9	0	0	137	8	48	0	0												
370	4	Neg	2004	M	W	10	0	0	189	9	24	0	0												
370	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
370	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
371	4	Neg	2004	M	W	3	0	0	-28	2	0	24	0												
371	4	Neg	2004	M	W	4	0	0	0	3	24	0	0												
371	4	Neg	2004	M	W	5	0	0	28	4	48	0	0												
371	4	Neg	2004	M	W	7	0	0	66	6	0	0	0												
371	4	Neg	2004	M	W	9	0	0	137	8	0	0	0												
371	4	Neg	2004	M	W	10	0	0	189	9	0	0	0												
371	4	Neg	2004	M	W	12	0	0	284	11	0	0	0	0	0			0	0	0	0	0	0	0	0
1	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
1	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
2	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
2	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
3	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
3	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
4	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
4	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
5	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
5	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
6	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
6	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
7	4	Neg	2005	M	W	3	1	0	-28	2	0	24	0												
7	4	Neg	2005	M	W	4	1	0	0	3	0	0	0												
8	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0												
8	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
9	4	Neg	2005	M	W	3	1	0	-28	2	24	24	0												
9	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
10	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
10	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
11	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
11	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
12	4	Neg	2005	M	W	3	1	0	-28	2	48	0	0												
12	4	Neg	2005	M	W	4	1	0	0	3	48	0	0												
13	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0												
13	4	Neg	2005	M	W	4	0	0	0	3	0	0	0												
14	4	Neg	2005	M	W	3	1	0	-28	2	48	24	0												
14	4	Neg	2005	M	W	4	1	0	0	3	48	0	0												
15	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0												
15	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
16	4	Neg	2005	M	W	3	1	0	-28	2	48	0	0												
16	4	Neg	2005	M	W	4	1	0	0	3	48	0	0												
17	4	Neg	2005	M	W	3	1	0	-28	2	96	96	24												
17	4	Neg	2005	M	W	4	1	0	0	3	96	24	24												
18	4	Neg	2005	F	W	3	1	0	-28	2	0	24	24												
18	4	Neg	2005	F	W	4	1	0	0	3	0	0	0												
19	4	Neg	2005	F	W	3	1	0	-28	2	24	0	0												
19	4	Neg	2005	F	W	4	1	0	0	3	24	0	0												
20	4	Neg	2005	F	W	3	1	0	-28	2	24	0	0												
20	4	Neg	2005	F	W	4	1	0	0	3	24	0	0												
21	4	Neg	2005	F	W	3	1	0	-28	2	24	0	0												
21	4	Neg	2005	F	W	4	1	0	0	3	24	0	0												
22	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0												
22	4	Neg	2005	M	W	4	0	0	0	3	0	0	0												
23	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0												
23	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
24	4	Neg	2005	M	W	3	1	0	-28	2	96	0	0												
24	4	Neg	2005	M	W	4	1	0	0	3	96	0	0												
25	4	Neg	2005	M	W	3	1	0	-28	2	48	0	0												
25	4	Neg	2005	M	W	4	1	0	0	3	48	0	0												
26	4	Neg	2005	M	W	3	1	0	-28	2	0	0	0												
26	4	Neg	2005	M	W	4	1	0	0	3	0	0	0												
27	4	Neg	2005	F	W	3	1	0	-28	2	0	0	0												
27	4	Neg	2005	F	W	4	1	0	0	3	0	0	0												
28	4	Neg	2005	F	W	3	1	0	-28	2	48	24	0												
28	4	Neg	2005	F	W	4	1	0	0	3	48	0	0												
29	4	Neg	2005	F	W	3	1	0	-28	2	0	24	0												
29	4	Neg	2005	F	W	4	1	0	0	3	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM			Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur		Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
30	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
30	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
31	4	Neg	2005	F	W	3	1	0	-28	2	24	48	24													
31	4	Neg	2005	F	W	4	1	0	0	3	24	24	24													
32	4	Neg	2005	F	W	3	1	0	-28	2	24	0	0													
32	4	Neg	2005	F	W	4	1	0	0	3	24	0	0													
33	4	Neg	2005	F	W	3	1	0	-28	2	24	0	0													
33	4	Neg	2005	F	W	4	1	0	0	3	24	0	0													
34	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
34	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
35	4	Neg	2005	M	W	3	1	0	-28	2	24	0	48													
35	4	Neg	2005	M	W	4	1	0	0	3	24	0	0													
36	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
36	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
37	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
37	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
38	4	Neg	2005	M	W	3	1	0	-28	2	96	24	0													
38	4	Neg	2005	M	W	4	1	0	0	3	96	0	24													
39	4	Neg	2005	M	W	3	1	0	-28	2	0	24	0													
39	4	Neg	2005	M	W	4	1	0	0	3	0	0	0													
40	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
40	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
41	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
41	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
42	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
42	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
43	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
43	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
44	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
44	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
45	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
45	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
46	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
46	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
47	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
47	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
48	4	Neg	2005	M	W	3	1	0	-28	2	0	96	96													
48	4	Neg	2005	M	W	4	1	0	0	3	0	96	96													
49	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
49	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
50	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
50	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
51	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
51	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
52	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
52	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
53	4	Neg	2005	F	W	3	1	0	-28	2	24	0	96													
53	4	Neg	2005	F	W	4	1	0	0	3	24	0	48													
54	4	Neg	2005	M	W	3	0	0	-28	2	0	0	0													
54	4	Neg	2005	M	W	4	0	0	0	3	0	0	0													
55	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
55	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
56	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
56	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
57	4	Neg	2005	M	W	3	1	0	-28	2	96	24	0													
57	4	Neg	2005	M	W	4	1	0	0	3	96	0	48													
58	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
58	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
59	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0													
59	4	Neg	2005	M	W	4	1	0	0	3	24	0	0													
60	4	Neg	2005	F	W	3	1	0	-28	2	0	48	0													
60	4	Neg	2005	F	W	4	1	0	0	3	0	0	0													
62	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
62	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
63	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0													
63	4	Neg	2005	M	W	4	1	0	0	3	24	0	0													
64	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
64	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
65	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0													
65	4	Neg	2005	M	W	4	1	0	0	3	24	0	0													
66	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0													
66	4	Neg	2005	F	W	4	0	0	0	3	0	0	0													
67	4	Neg	2005	M	W	3	1	0	-28	2	24	48	48													
67	4	Neg	2005	M	W	4	1	0	0	3	24	0	0													
68	4	Neg	2005	F	W	3	1	0	-28	2	0	48	0													
68	4	Neg	2005	F	W	4	1	0	0	3	0	0	0													

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology			
	No	DX	year		Cls	Mt	Drn	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
69	4	Neg	2005	M	W	3	1	0	-28	2	48	96	96												
69	4	Neg	2005	M	W	4	1	0	0	3	48	0	24												
70	4	Neg	2005	F	W	3	0	0	-28	2	0	0	0												
70	4	Neg	2005	F	W	4	0	0	0	3	0	0	0												
71	4	Neg	2005	M	W	3	1	0	-28	2	24	0	24												
71	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
72	4	Neg	2005	M	W	3	1	0	-28	2	24	24	0												
72	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
73	4	Neg	2005	M	W	3	1	0	-28	2	24	0	0												
73	4	Neg	2005	M	W	4	1	0	0	3	24	0	0												
74	4	Neg	2005	M	W	3	1	0	-28	2	96	0	24												
74	4	Neg	2005	M	W	4	1	0	0	3	96	0	0												
75	4	Neg	2005	M	W	3	1	0	-28	2	48	24	0												
75	4	Neg	2005	M	W	4	1	0	0	3	48	0	0												
76	4	Neg	2005	M	W	3	1	0	-28	2	0	24	0												
76	4	Neg	2005	M	W	4	1	0	0	3	0	0	0												
150	2	H-P	2003	F	A	>24	0		0	10	48	48	24												
150	2	H-P	2003	F	A	>24	0		28	10	96	96	24												
150	2	H-P	2004	F	A	>24	0		100	2	24	96	24												
152	2	H-P	2003	F	A	>24	1		0	10	48	48	0												
152	2	H-P	2003	F	A	>24	1		28	10	192	96	96												
152	2	H-P	2004	F	A	>24	1		100	2	96	48	24												
158	2	H-P	2003	F	A	>24	1		0	10	48	24	0												
158	2	H-P	2003	F	A	>24	1		28	10	192	96	48												
158	2	H-P	2004	F	A	>24	1		100	2	0	48	0												
232	2	H-P	2003	F	A	>24	1		0	10	0	48	0												
232	2	H-P	2003	F	A	>24	1		28	10	96	192	48												
232	2	H-P	2004	F	A	>24	1		100	2	48	24	0												
235	2	H-P	2003	F	A	>24	0		0	10	0	48	0												
235	2	H-P	2003	F	A	>24	0		28	10	0	0	0												
235	2	H-P	2004	F	A	>24	0		100	2	768	48	48												
237	2	H-P	2003	F	A	>24	1		0	10	0	48	0												
237	2	H-P	2003	F	A	>24	1		28	10	384	192	48												
237	2	H-P	2004	F	A	>24	1		100	2	192	96	0												
238	2	H-P	2003	F	A	>24	0		0	10	0	24	48												
238	2	H-P	2003	F	A	>24	0		28	10	48	24	0												
238	2	H-P	2004	F	A	>24	0		100	2	768	96	24												
246	2	H-P	2003	F	A	>24	0		0	10	384	24	48												
246	2	H-P	2003	F	A	>24	0		28	10	96	0	0												
246	2	H-P	2004	F	A	>24	0		100	2	192	96	0												
278	2	H-P	2003	F	A	>24	1		0	10	48	24	0												
278	2	H-P	2003	F	A	>24	1		28	10	192	96	48												
278	2	H-P	2004	F	A	>24	1		100	2	192	192	0												
309	2	H-P	2003	F	A	>24	0		0	10	384	0	0												
309	2	H-P	2003	F	A	>24	0		28	10	384	0	0												
309	2	H-P	2004	F	A	>24	0		100	2	48	192	0												
320	2	H-P	2003	F	A	>24	1		0	10	768	384	192												
320	2	H-P	2003	F	A	>24	1		28	10	192	1536	48												
320	2	H-P	2004	F	A	>24	1		100	2	96	48	24												
509	2	H-P	2003	F	A	>24	1		0	10	96	0	0												
509	2	H-P	2003	F	A	>24	1		28	10	384	192	0												
509	2	H-P	2004	F	A	>24	1		100	2	192	192	0												
512	2	H-P	2003	F	A	>24	0		0	10	24	24	0												
512	2	H-P	2003	F	A	>24	0		28	10	384	0	0												
512	2	H-P	2004	F	A	>24	0		100	2	192	24	0												
517	2	H-P	2003	F	A	>24	1		0	10	768	24	0												
517	2	H-P	2003	F	A	>24	1		28	10	384	384	96												
517	2	H-P	2004	F	A	>24	1		100	2	48	384	24												
523	2	H-P	2003	F	A	>24	1		0	10	96	24	0												
523	2	H-P	2003	F	A	>24	1		28	10	384	768	96												
523	2	H-P	2004	F	A	>24	1		100	2	24	24	0												
524	2	H-P	2003	F	A	>24	0		0	10	0	0	24												
524	2	H-P	2003	F	A	>24	0		28	10	384	192	96												
524	2	H-P	2004	F	A	>24	0		100	2	192	48	24												
560	2	H-P	2003	F	A	>24	0		0	10	24	0	0												
560	2	H-P	2003	F	A	>24	0		28	10	0	0	0												
560	2	H-P	2004	F	A	>24	0		100	2	0	48	0												
603	2	H-P	2003	F	A	>24	1		0	10	192	1536	96												
603	2	H-P	2003	F	A	>24	1		28	10	384	1536	96												
603	2	H-P	2004	F	A	>24	1		100	2	24	96	0												
605	2	H-P	2003	F	A	>24	0		0	10	192	0	0												
605	2	H-P	2003	F	A	>24	0		28	10	192	0	0												
605	2	H-P	2004	F	A	>24	0		100	2	384	384	0												
607	2	H-P	2003	F	A	>24	0		0	10	384	1536	192												
607	2	H-P	2003	F	A	>24	0		28	10	192	1536	96												
607	2	H-P	2004	F	A	>24	0		100	2	768	768	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
610	2	H-P	2003	F	A	>24	1		0	10	768	24	0												
610	2	H-P	2003	F	A	>24	1		28	10	768	48	96												
610	2	H-P	2004	F	A	>24	1		100	2	192	192	24												
706	2	H-P	2003	F	A	>24	1		0	10	384	24	0												
706	2	H-P	2003	F	A	>24	1		28	10	384	96	48												
706	2	H-P	2004	F	A	>24	1		100	2	192	96	24												
708	2	H-P	2003	F	A	>24	1		0	10	48	24	0												
708	2	H-P	2003	F	A	>24	1		28	10	192	96	96												
708	2	H-P	2004	F	A	>24	1		100	2	24	48	24												
805	2	H-P	2003	F	A	>24	1		0	10	96	24	0												
805	2	H-P	2003	F	A	>24	1		28	10	192	48	96												
805	2	H-P	2004	F	A	>24	1		100	2	0	0	24												
807	2	H-P	2003	F	A	>24	0		0	10	48	24	0												
807	2	H-P	2003	F	A	>24	0		28	10	48	48	0												
807	2	H-P	2004	F	A	>24	0		100	2	0	384	0												
827	2	H-P	2003	F	A	>24	0		0	10	96	24	0												
827	2	H-P	2003	F	A	>24	0		28	10	96	0	0												
827	2	H-P	2004	F	A	>24	0		100	2	96	96	0												
860	2	H-P	2003	F	A	>24	0		0	10	48	0	24												
860	2	H-P	2003	F	A	>24	0		28	10	48	24	48												
860	2	H-P	2004	F	A	>24	0		100	2	0	48	0												
861	2	H-P	2003	F	A	>24	0		0	10	48	0	0												
861	2	H-P	2003	F	A	>24	0		28	10	48	96	0												
861	2	H-P	2004	F	A	>24	0		100	2	48	768	0												
862	2	H-P	2003	F	A	>24	1		0	10	96	24	0												
862	2	H-P	2004	F	A	>24	1		28	10	192	96	96												
862	2	H-P	2004	F	A	>24	1		100	2	0	0	24												
914	2	H-P	2003	F	A	>24	0		0	10	384	48	0												
914	2	H-P	2003	F	A	>24	0		28	10	192	384	0												
914	2	H-P	2004	F	A	>24	0		100	2	96	768	0												
4124	2	H-P	2003	F	A	>24	1		0	10	96	24	0												
4124	2	H-P	2003	F	A	>24	1		28	10	192	384	96												
4124	2	H-P	2004	F	A	>24	1		100	2	192	24	24												
4136	2	H-P	2003	F	A	>24	1		0	10	48	24	0												
4136	2	H-P	2003	F	A	>24	1		28	10	768	48	96												
4136	2	H-P	2004	F	A	>24	1		100	2	384	48	0												
4153	2	H-P	2003	F	A	>24	1		0	10	96	24	0												
4153	2	H-P	2003	F	A	>24	1		28	10	384	0	96												
4153	2	H-P	2004	F	A	>24	1		100	2	96	192	0												
4158	2	H-P	2003	F	A	>24	1		0	10	0	0	0												
4158	2	H-P	2003	F	A	>24	1		28	10	192	96	24												
4158	2	H-P	2004	F	A	>24	1		100	2	24	0	0												
4162	2	H-P	2003	F	A	>24	1		0	10	96	0	48												
4162	2	H-P	2003	F	A	>24	1		28	10	384	1536	0												
4162	2	H-P	2004	F	A	>24	1		100	2	96	24	0												
4187	2	H-P	2003	F	A	>24	1		0	10	48	24	0												
4187	2	H-P	2003	F	A	>24	1		28	10	96	24	0												
4187	2	H-P	2004	F	A	>24	1		100	2	0	24	0												
4191	2	H-P	2003	F	A	>24	0		0	10	48	24	0												
4191	2	H-P	2003	F	A	>24	0		28	10	48	3072	0												
4191	2	H-P	2004	F	A	>24	0		100	2	48	192	48												
4194	2	H-P	2003	F	A	>24	0		0	10	48	24	0												
4194	2	H-P	2003	F	A	>24	0		28	10	48	384	192												
4194	2	H-P	2004	F	A	>24	0		100	2	96	384	24												
4195	2	H-P	2003	F	A	>24	1		0	10	96	24	24												
4195	2	H-P	2003	F	A	>24	1		28	10	192	24	48												
4195	2	H-P	2004	F	A	>24	1		100	2	96	96	96												
4201	2	H-P	2003	F	A	>24	0		0	10	48	96	24												
4201	2	H-P	2003	F	A	>24	0		28	10	96	48	96												
4201	2	H-P	2004	F	A	>24	0		100	2	192	48	0												
4204	2	H-P	2003	F	A	>24	1		0	10	96	24	24												
4204	2	H-P	2003	F	A	>24	1		28	10	96	48	24												
4204	2	H-P	2004	F	A	>24	1		100	2	192	384	0												
4217	2	H-P	2003	F	A	>24	0		0	10	96	24	0												
4217	2	H-P	2003	F	A	>24	0		28	10	192	48	192												
4217	2	H-P	2004	F	A	>24	0		100	2	96	48	48												
4221	2	H-P	2003	F	A	>24	1		0	10	96	0	0												
4221	2	H-P	2003	F	A	>24	1		28	10	384	0	0												
4221	2	H-P	2004	F	A	>24	1		100	2	48	96	0												
4238	2	H-P	2003	F	A	>24	1		0	10	48	48	0												
4238	2	H-P	2003	F	A	>24	1		28	10	384	192	48												
4238	2	H-P	2004	F	A	>24	1		100	2	192	96	0												
4251	2	H-P	2003	F	A	>24	0		0	10	192	24	48												
4251	2	H-P	2003	F	A	>24	0		28	10	768	24	24												
4251	2	H-P	2004	F	A	>24	0		100	2	96	48	0												
6004	2	H-P	2003	F	A	>24	1		0	10	48	192	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
6004	2	H-P	2003	F	A	>24	1		28	10	192	768	192												
6004	2	H-P	2004	F	A	>24	1		100	2	96	48	0												
6012	2	H-P	2003	F	A	>24	0		0	10	96	384	0												
6012	2	H-P	2003	F	A	>24	0		28	10	96	1536	0												
6012	2	H-P	2004	F	A	>24	0		100	2	24	48	0												
6029	2	H-P	2003	F	A	>24	1		0	10	96	192	0												
6029	2	H-P	2003	F	A	>24	1		28	10	768	384	48												
6029	2	H-P	2004	F	A	>24	1		100	2	768	48	0												
6109	2	H-P	2003	F	A	>24	0		0	10	96	96	48												
6109	2	H-P	2003	F	A	>24	0		28	10	96	192	0												
6109	2	H-P	2004	F	A	>24	0		100	2	0	96	0												
6151	2	H-P	2003	F	A	>24	0		0	10	48	48	24												
6151	2	H-P	2003	F	A	>24	0		28	10	48	192	0												
6151	2	H-P	2004	F	A	>24	0		100	2	48	192	0												
6153	2	H-P	2003	F	A	>24	1		0	10	48	384	24												
6153	2	H-P	2003	F	A	>24	1		28	10	384	384	384												
6153	2	H-P	2004	F	A	>24	1		100	2	0	384	0												
6164	2	H-P	2003	F	A	>24	0		0	10	48	48	48												
6164	2	H-P	2003	F	A	>24	0		28	10	96	192	0												
6164	2	H-P	2004	F	A	>24	0		100	2	48	96	0												
6169	2	H-P	2003	F	A	>24	1		0	10	48	192	48												
6169	2	H-P	2003	F	A	>24	1		28	10	192	384	96												
6169	2	H-P	2004	F	A	>24	1		100	2	192	24	48												
6229	2	H-P	2003	F	A	>24	0		0	10	0	48	0												
6229	2	H-P	2003	F	A	>24	0		28	10	0	192	0												
6229	2	H-P	2004	F	A	>24	0		100	2	96	1536	0												
6283	2	H-P	2003	F	A	>24	0		0	10	96	48	0												
6283	2	H-P	2003	F	A	>24	0		28	10	96	192	48												
6283	2	H-P	2004	F	A	>24	0		100	2	192	96	0												
6292	2	H-P	2003	F	A	>24	0		0	10	0	24	0												
6292	2	H-P	2003	F	A	>24	0		28	10	24	96	192												
6292	2	H-P	2004	F	A	>24	0		100	2	192	192	0												
6497	2	H-P	2003	F	A	>24	1		0	10	48	48	0												
6497	2	H-P	2003	F	A	>24	1		28	10	768	384	48												
6497	2	H-P	2004	F	A	>24	1		100	2	96	96	0												
0	4	Neg	2003	F	A	>24	0		0	10	0	24	0												
0	4	Neg	2003	F	A	>24	0		28	10		96	0												
0	4	Neg	2004	F	A	>24	0		100	2	24	96	0												
0	4	Neg	2004	F	A	>24	0		335	9	24	0	0												
0	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
0	4	Neg	2005	F	A	>24	0		448	2	0	24	0												
3	4	Neg	2003	F	A	>24	0		0	10	48	48	24												
3	4	Neg	2003	F	A	>24	0		28	10	0	96	24												
3	4	Neg	2004	F	A	>24	0		100	2	48	48	0												
3	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
3	4	Neg	2004	F	A	>24	0		365	10	96	192	24												
3	4	Neg	2005	F	A	>24	0		448	2	0	0	0												
6	4	Neg	2004	F	A	>24	1		335	9		0	0												
6	4	Neg	2004	F	A	>24	1		365	10	0	0	0												
6	4	Neg	2005	F	A	>24	1		448	2	24	48	24												
7	4	Neg	2003	F	A	>24	0		0	10	0	0	0												
7	4	Neg	2003	F	A	>24	0		28	10	0	0	0												
7	4	Neg	2004	F	A	>24	0		100	2	24	48	48												
7	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
7	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
7	4	Neg	2005	F	A	>24	0		448	2	24	24	0												
17	4	Neg	2003	F	A	>24	0		0	10	48	48	0												
17	4	Neg	2003	F	A	>24	0		28	10	48	24	0												
17	4	Neg	2004	F	A	>24	0		100	2	96	24	48												
17	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
17	4	Neg	2004	F	A	>24	0		365	10	96	192	24												
17	4	Neg	2005	F	A	>24	0		448	2	48	24	24												
21	4	Neg	2003	F	A	>24	1		0	10	48	24													
21	4	Neg	2003	F	A	>24	1		28	10	192		96												
21	4	Neg	2004	F	A	>24	1		100	2	48	24	0												
23	4	Neg	2003	F	A	>24	1		0	10	192	0	0												
23	4	Neg	2003	F	A	>24	1		28	10	192	768	384												
23	4	Neg	2004	F	A	>24	1		100	2	24	48	48												
23	4	Neg	2004	F	A	>24	1		335	9	96	0	0												
23	4	Neg	2004	F	A	>24	1		365	10	192	48	96												
23	4	Neg	2005	F	A	>24	1		448	2	48	24	24												
24	4	Neg	2003	F	A	>24	1		0	10	96	24	0												
24	4	Neg	2003	F	A	>24	1		28	10	192		48												
24	4	Neg	2004	F	A	>24	1		100	2	24	768	0												
24	4	Neg	2004	F	A	>24	1		335	9	48	0	0												
24	4	Neg	2004	F	A	>24	1		365	10	96	384	96												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
24	4	Neg	2005	F	A	>24	1		448	2	24	48	0												
25	4	Neg	2003	F	A	>24	1		0	10	96	0	0												
25	4	Neg	2003	F	A	>24	1		28	10	96	192	0												
25	4	Neg	2004	F	A	>24	1		100	2	384	48	48												
25	4	Neg	2004	F	A	>24	1		335	9	48	0	0												
25	4	Neg	2004	F	A	>24	1		365	10	768	768	384												
25	4	Neg	2005	F	A	>24	1		448	2	48	48	24												
27	4	Neg	2003	F	A	>24	1		0	10	0	24	0												
27	4	Neg	2003	F	A	>24	1		28	10	0	192	96												
27	4	Neg	2004	F	A	>24	1		100	2	24		0												
27	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
27	4	Neg	2004	F	A	>24	1		365	10	0	24	0												
27	4	Neg	2005	F	A	>24	1		448	2	48	48	96												
29	4	Neg	2003	F	A	>24	0		0	10	0	0	0												
29	4	Neg	2003	F	A	>24	0		28	10	0	0	0												
29	4	Neg	2004	F	A	>24	0		100	2															
29	4	Neg	2004	F	A	>24	0		335	9	24	0	0												
29	4	Neg	2004	F	A	>24	0		365	10	0	24	0												
29	4	Neg	2005	F	A	>24	0		448	2	48	48	24												
31	4	Neg	2003	F	A	>24	0		0	10	192	24	0												
31	4	Neg	2003	F	A	>24	0		28	10	384	24	0												
31	4	Neg	2004	F	A	>24	0		100	2	24	24	96												
33	4	Neg	2003	F	A	>24			0	10	24	24	0												
33	4	Neg	2003	F	A	>24			28	10	24	48	48												
33	4	Neg	2004	F	A	>24			100	2															
35	4	Neg	2003	F	A	>24	1		0	10	96	0	0												
35	4	Neg	2003	F	A	>24	1		28	10	96	96	48												
35	4	Neg	2004	F	A	>24	1		100	2		384	48												
35	4	Neg	2004	F	A	>24	1		335	9	24	48	0												
35	4	Neg	2004	F	A	>24	1		365	10	192	96	24												
35	4	Neg	2005	F	A	>24	1		448	2	24	48	0												
36	4	Neg	2005	F	A	>24	1		448	2	0	48	0												
39	4	Neg	2003	F	A	>24	1		0	10	192	48	0												
39	4	Neg	2003	F	A	>24	1		28	10	192	384	48												
39	4	Neg	2004	F	A	>24	1		100	2	24	24	24												
39	4	Neg	2004	F	A	>24	1		335	9	48	24	0												
39	4	Neg	2004	F	A	>24	1		365	10	384	24	24												
39	4	Neg	2005	F	A	>24	1		448	2	48	24	0												
40	4	Neg	2003	F	A	>24	1		0	10	96		0												
40	4	Neg	2003	F	A	>24	1		0	10	24	24	0												
40	4	Neg	2003	F	A	>24	1		28	10	96	192	48												
40	4	Neg	2003	F	A	>24	1		28	10	48	96	0												
40	4	Neg	2004	F	A	>24	1		100	2	48	192	0												
40	4	Neg	2004	F	A	>24	1		100	2															
40	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
40	4	Neg	2004	F	A	>24	1		365	10	0	0	0												
40	4	Neg	2005	F	A	>24	1		448	2	24	48	0												
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44	4	Neg	2003	F	A	>24	0		0	10	48	24	0												
44	4	Neg	2003	F	A	>24	0		28	10	24	0	0												
44	4	Neg	2004	F	A	>24	0		100	2	192	0	24												
44	4	Neg	2004	F	A	>24	0		335	9		0													
44	4	Neg	2004	F	A	>24	0		365	10	24	0	0												
44	4	Neg	2005	F	A	>24	0		448	2	0	24	0												
45	4	Neg	2003	F	A	>24	1		0	10	48	24	0												
45	4	Neg	2003	F	A	>24	1		28	10															
45	4	Neg	2004	F	A	>24	1		100	2	48	48	0												
45	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
45	4	Neg	2004	F	A	>24	1		365	10	0	0	0												
45	4	Neg	2005	F	A	>24	1		448	2	24	24	0												
46	4	Neg	2003	F	A	>24	1		0	10	0	48	0												
46	4	Neg	2003	F	A	>24	1		28	10	24	24	0												
46	4	Neg	2004	F	A	>24	1		100	2			24												
46	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
46	4	Neg	2004	F	A	>24	1		365	10	768	48	384												
46	4	Neg	2005	F	A	>24	1		448	2	48	0	0												
47	4	Neg	2003	F	A	>24	1		0	10	384	48	0												
47	4	Neg	2003	F	A	>24	1		28	10	384	384	192												
47	4	Neg	2004	F	A	>24	1		100	2	192	192	0												
47	4	Neg	2004	F	A	>24	1		335	9	96	48	0												
47	4	Neg	2004	F	A	>24	1		365	10			48												
47	4	Neg	2005	F	A	>24	1		448	2	96	48	48												
48	4	Neg	2003	F	A	>24	1		0	10	0	96	0												
48	4	Neg	2003	F	A	>24	1		28	10	96	768	48												
48	4	Neg	2004	F	A	>24	1		100	2	0	1536	0												
48	4	Neg	2004	F	A	>24	1		335	9	0	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
48	4	Neg	2004	F	A	>24	1		365	10	0	0	0												
48	4	Neg	2005	F	A	>24	1		448	2	48	24	0												
50	4	Neg	2003	F	A	>24	0		0	10	96	24	0												
50	4	Neg	2003	F	A	>24	0		28	10															
50	4	Neg	2004	F	A	>24	0		100	2	96	384	0												
50	4	Neg	2004	F	A	>24	0		335	9	48	0	0												
50	4	Neg	2004	F	A	>24	0		365	10	384	192	192												
50	4	Neg	2005	F	A	>24	0		448	2	48	24	24												
52	4	Neg	2003	F	A	>24	0		0	10	96	24	0												
52	4	Neg	2003	F	A	>24	0		28	10	192	24	0												
52	4	Neg	2004	F	A	>24	0		100	2	96	48	0												
52	4	Neg	2004	F	A	>24	0		335	9	48	0	0												
52	4	Neg	2004	F	A	>24	0		365	10	192	0	0												
52	4	Neg	2005	F	A	>24	0		448	2			24												
54	4	Neg	2003	F	A	>24	1		0	10	0	24	0												
54	4	Neg	2003	F	A	>24	1		28	10	96	384	24												
54	4	Neg	2004	F	A	>24	1		100	2	192	96	0												
54	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
54	4	Neg	2004	F	A	>24	1		365	10	384	768	48												
54	4	Neg	2005	F	A	>24	1		448	2	24	0	0												
68	4	Neg	2003	F	A	>24	0		0	10	0	24	0												
68	4	Neg	2003	F	A	>24	0		28	10	24	48	0												
68	4	Neg	2004	F	A	>24	0		100	2	24	0	48												
68	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
68	4	Neg	2004	F	A	>24	0		365	10	24	48	0												
68	4	Neg	2005	F	A	>24	0		448	2	0	24	0												
101	4	Neg	2003	F	A	>24	0		0	10	96	192	0												
101	4	Neg	2003	F	A	>24	0		28	10		96													
101	4	Neg	2004	F	A	>24	0		100	2	96	192	0												
101	4	Neg	2004	F	A	>24	0		335	9	0	24	0												
101	4	Neg	2004	F	A	>24	0		365	10	48	48	0												
101	4	Neg	2005	F	A	>24	0		448	2	48	0	0												
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102	4	Neg	2004	F	A	>24	0		335	9	48	24	0												
102	4	Neg	2004	F	A	>24	0		365	10	96	24	0												
102	4	Neg	2005	F	A	>24	0		448	2	96	24	0												
105	4	Neg	2003	F	A	>24	0		0	10	0	48	0												
105	4	Neg	2003	F	A	>24	0		28	10	0	48	0												
105	4	Neg	2004	F	A	>24	0		100	2	0	48	0												
105	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
105	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
105	4	Neg	2005	F	A	>24	0		448	2	24	0	0												
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107	4	Neg	2003	F	A	>24	0		0	10	0	24													
107	4	Neg	2003	F	A	>24	0		28	10	0	24	0												
107	4	Neg	2004	F	A	>24	0		100	2	0	48	0												
109	4	Neg	2003	F	A	>24	0		0	10	48	24	0												
109	4	Neg	2003	F	A	>24	0		28	10	192	96	0												
109	4	Neg	2004	F	A	>24	0		100	2	48	24	48												
109	4	Neg	2004	F	A	>24	0		335	9	24	0	0												
109	4	Neg	2004	F	A	>24	0		365	10	384	768	96												
109	4	Neg	2005	F	A	>24	0		448	2	48	24	24												
114	4	Neg	2003	F	A	>24	0		0	10	0	24	0												
114	4	Neg	2003	F	A	>24	0		28	10	0	48	0												
114	4	Neg	2004	F	A	>24	0		100	2	24	48	24												
114	4	Neg	2004	F	A	>24	0		335	9	0	24	0												
114	4	Neg	2004	F	A	>24	0		365	10	192	384	0												
114	4	Neg	2005	F	A	>24	0		448	2	0	0	0												
115	4	Neg	2003	F	A	>24	0		0	10	96	24	0												
115	4	Neg	2003	F	A	>24	0		28	10		0													
115	4	Neg	2004	F	A	>24	0		100	2	0	0	0												
115	4	Neg	2004	F	A	>24	0		335	9	24	0	0												
115	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
115	4	Neg	2005	F	A	>24	0		448	2	48	0	24												
120	4	Neg	2003	F	A	>24	0		0	10															
120	4	Neg	2003	F	A	>24	0		28	10															
120	4	Neg	2004	F	A	>24	0		100	2															
120	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
120	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
120	4	Neg	2005	F	A	>24	0		448	2	24	0	0												
152	4	Neg	2003	F	A	>24	1		0	10	0	0	0												
152	4	Neg	2003	F	A	>24	1		28	10		24													
152	4	Neg	2004	F	A	>24	1		100	2	48	24	24												
152	4	Neg	2004	F	A	>24	1		335	9	24	0	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
152	4	Neg	2004	F	A	>24	1		365	10	48	96	96												
152	4	Neg	2005	F	A	>24	1		448	2															
154	4	Neg	2003	F	A	>24	0		0	10	0	0	0												
154	4	Neg	2003	F	A	>24	0		28	10		0													
154	4	Neg	2004	F	A	>24	0		100	2	0	0	0												
154	4	Neg	2004	F	A	>24	0		335	9			0												
154	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
154	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
154	4	Neg	2004	F	A	>24	1		365	10	0	0	0												
154	4	Neg	2005	F	A	>24	0		448	2	0	24													
156	4	Neg	2004	F	A	>24	1		335	9	0	0	0												
156	4	Neg	2004	F	A	>24	1		365	10	48	384	0												
157	4	Neg	2003	F	A	>24	1		0	10	0	24	0												
157	4	Neg	2003	F	A	>24	1		28	10	96	384	48												
157	4	Neg	2004	F	A	>24	1		100	2	96	96	48												
157	4	Neg	2004	F	A	>24	1		335	9		0	0												
157	4	Neg	2004	F	A	>24	1		365	10	48	96	96												
157	4	Neg	2005	F	A	>24	1		448	2	48	0	96												
163	4	Neg	2003	F	A	>24	0		0	10															
163	4	Neg	2003	F	A	>24	0		28	10	24	96	48												
163	4	Neg	2004	F	A	>24	0		100	2															
167	4	Neg	2003	F	A	>24	1		0	10	24	24	0												
167	4	Neg	2003	F	A	>24	1		28	10															
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167	4	Neg	2004	F	A	>24	1		335	9	0														
167	4	Neg	2004	F	A	>24	1		365	10															
167	4	Neg	2005	F	A	>24	1		448	2	48														
206	4	Neg	2003	F	A	>24	0		0	10	48	24	0												
206	4	Neg	2003	F	A	>24	0		28	10		0													
206	4	Neg	2004	F	A	>24	0		100	2	96	384	48												
206	4	Neg	2004	F	A	>24	0		335	9			0												
206	4	Neg	2004	F	A	>24	0		365	10	0	0	0												
206	4	Neg	2005	F	A	>24	0		448	2	0	0	0												
210	4	Neg	2003	F	A	>24	1		0	10	96	48	0												
210	4	Neg	2003	F	A	>24	1		28	10	768	1536	48												
210	4	Neg	2004	F	A	>24	1		100	2	96	96	48												
210	4	Neg	2004	F	A	>24	1		335	9	48	0	0												
210	4	Neg	2004	F	A	>24	1		365	10	96	0	0												
210	4	Neg	2005	F	A	>24	1		448	2	48	24	24												
212	4	Neg	2003	F	A	>24	0		0	10	96	48	0												
212	4	Neg	2003	F	A	>24	0		28	10	384	48	0												
212	4	Neg	2004	F	A	>24	0		100	2	192	24	0												
212	4	Neg	2004	F	A	>24	0		335	9	24	0	0												
212	4	Neg	2004	F	A	>24	0		365	10	192	0	0												
212	4	Neg	2005	F	A	>24	0		448	2	48	24	0												
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307	4	Neg	2004	F	A	>24	1		100	2	192	48	48												
307	4	Neg	2004	F	A	>24	1		335	9	0	24	0												
307	4	Neg	2004	F	A	>24	1		365	10	192	96	96												
307	4	Neg	2005	F	A	>24	1		448	2	96	24	48												
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318	4	Neg	2004	F	A	>24	1		100	2	96	48	0												
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318	4	Neg	2004	F	A	>24	1		365	10	384	192	0												
318	4	Neg	2005	F	A	>24	1		448	2	48	0	0												
319	4	Neg	2003	F	A	>24	0		0	10															
319	4	Neg	2003	F	A	>24	0		28	10															
319	4	Neg	2004	F	A	>24	0		100	2															
319	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
319	4	Neg	2004	F	A	>24	0		365	10	96	0	0												
319	4	Neg	2005	F	A	>24	0		448	2															
323	4	Neg	2003	F	A	>24	0		0	10															
323	4	Neg	2003	F	A	>24	0		28	10	96	384	0												
323	4	Neg	2004	F	A	>24	0		100	2															
323	4	Neg	2004	F	A	>24	0		335	9	24	192	0												
323	4	Neg	2004	F	A	>24	0		365	10	384	48	0												
323	4	Neg	2005	F	A	>24	0		448	2	48	48	0												
324	4	Neg	2003	F	A	>24	0		0	10															
324	4	Neg	2003	F	A	>24	0		28	10		24													
324	4	Neg	2004	F	A	>24	0		100	2	96		0												
324	4	Neg	2004	F	A	>24	0		335	9	0	0	0												
324	4	Neg	2004	F	A	>24	0		365	10	96	0	0												
324	4	Neg	2005	F	A	>24	0		448	2	24	0	0												
325	4	Neg	2003	F	A	>24	1		0	10	48	96	0												

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
325	4	Neg	2003	F	A	>24	1		28	10	192	768	24											
325	4	Neg	2004	F	A	>24	1		100	2	96	384	24											
325	4	Neg	2004	F	A	>24	1		335	9	0	48	0											
325	4	Neg	2004	F	A	>24	1		365	10	96	384	192											
325	4	Neg	2005	F	A	>24	1		448	2	0	48	0											
338	4	Neg	2003	F	A	>24	0		0	10														
338	4	Neg	2003	F	A	>24	0		28	10	96	384	24											
338	4	Neg	2004	F	A	>24	0		100	2														
353	4	Neg	2003	F	A	>24	1		0	10	192	0	0											
353	4	Neg	2003	F	A	>24	1		28	10	192													
353	4	Neg	2004	F	A	>24	1		100	2	192	24	0											
364	4	Neg	2003	F	A	>24	1		0	10	192	24	0											
364	4	Neg	2003	F	A	>24	1		28	10	192	192	24											
364	4	Neg	2004	F	A	>24	1		100	2														
408	4	Neg	2003	F	A	>24	0		0	10	24	0	0											
408	4	Neg	2003	F	A	>24	0		28	10	24	24	0											
408	4	Neg	2004	F	A	>24	0		100	2	48	24	24											
408	4	Neg	2004	F	A	>24	0		335	9			0											
408	4	Neg	2004	F	A	>24	0		365	10	48	0	0											
408	4	Neg	2005	F	A	>24	0		448	2	0	48	48											
410	4	Neg	2003	F	A	>24			0	10		48												
410	4	Neg	2003	F	A	>24			28	10		192												
410	4	Neg	2004	F	A	>24			100	2														
410	4	Neg	2004	F	A	>24	0		335	9			0											
410	4	Neg	2004	F	A	>24	0		365	10	192	48	0											
410	4	Neg	2005	F	A	>24	0		448	2	24	24	0											
411	4	Neg	2003	F	A	>24	0		0	10	96	0	0											
411	4	Neg	2003	F	A	>24	0		28	10	96	0	24											
411	4	Neg	2004	F	A	>24	0		100	2	96	0	0											
411	4	Neg	2004	F	A	>24	0		335	9	0	0	0											
411	4	Neg	2004	F	A	>24	0		365	10	96	0	0											
411	4	Neg	2005	F	A	>24	0		448	2	24	0	0											
427	4	Neg	2003	F	A	>24	0		0	10		0												
427	4	Neg	2003	F	A	>24	0		28	10	96	384	24											
427	4	Neg	2004	F	A	>24	0		100	2	96	192	24											
427	4	Neg	2004	F	A	>24	0		335	9	0	48	0											
427	4	Neg	2004	F	A	>24	0		365	10	24	48	0											
427	4	Neg	2005	F	A	>24	0		448	2	24	48	24											
437	4	Neg	2003	F	A	>24	1		0	10	96	24	0											
437	4	Neg	2003	F	A	>24	1		28	10	192	48	48											
437	4	Neg	2004	F	A	>24	1		100	2	96	24	24											
437	4	Neg	2004	F	A	>24	1		335	9	48	0	0											
437	4	Neg	2004	F	A	>24	1		365	10	96	384	0											
437	4	Neg	2005	F	A	>24	1		448	2	48	24	24											
455	4	Neg	2003	F	A	>24	0		0	10	96	0	0											
455	4	Neg	2003	F	A	>24	0		28	10	48	48	0											
455	4	Neg	2004	F	A	>24	0		100	2	96	48	96											
455	4	Neg	2004	F	A	>24	0		335	9	0	0												
455	4	Neg	2004	F	A	>24	0		365	10	24	0												
455	4	Neg	2005	F	A	>24	0		448	2	48	0	24											
459	4	Neg	2003	F	A	>24	0		0	10														
459	4	Neg	2003	F	A	>24	0		28	10	96	768	48											
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553	4	Neg	2003	F	A	>24	0		0	10														
553	4	Neg	2003	F	A	>24	0		28	10	192	24	96											
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612	4	Neg	2003	F	A	>24	1		0	10	192	96	96											
612	4	Neg	2003	F	A	>24	1		28	10	384	1536	192											
612	4	Neg	2004	F	A	>24	1		100	2	192	384	96											
612	4	Neg	2004	F	A	>24	1		335	9	48	48	0											
612	4	Neg	2004	F	A	>24	1		365	10	96	24	0											
612	4	Neg	2005	F	A	>24	1		448	2	48	0	24											
616	4	Neg	2003	F	A	>24	0		0	10	0	96	0											
616	4	Neg	2003	F	A	>24	0		28	10	24	96	0											
616	4	Neg	2004	F	A	>24	0		100	2	96	192	0											
616	4	Neg	2004	F	A	>24	0		335	9	0	0	0											
616	4	Neg	2004	F	A	>24	0		365	10	192	768	384											
616	4	Neg	2005	F	A	>24	0		448	2	48	0	48											
618	4	Neg	2003	F	A	>24	1		0	10	192	48	0											
618	4	Neg	2003	F	A	>24	1		28	10	768	384	48											
618	4	Neg	2004	F	A	>24	1		100	2	384	96	48											
618	4	Neg	2004	F	A	>24	1		335	9	0	0	0											
618	4	Neg	2004	F	A	>24	1		365	10	24	0	0											
618	4	Neg	2005	F	A	>24	1		448	2	24	0	0											
619	4	Neg	2003	F	A	>24	1		0	10	24	48	0											
619	4	Neg	2003	F	A	>24	1		28	10	384	48	96											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM			Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur		Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
619	4	Neg	2004	F	A	>24	1		100	2	192	48	48													
619	4	Neg	2004	F	A	>24	1		335	9	24	48	0													
619	4	Neg	2004	F	A	>24	1		365	10	96	768	384													
619	4	Neg	2005	F	A	>24	1		448	2	48	96	96													
621	4	Neg	2003	F	A	>24	1		0	10	192	24	0													
621	4	Neg	2003	F	A	>24	1		28	10	192	384	0													
621	4	Neg	2004	F	A	>24	1		100	2	192	96	0													
621	4	Neg	2004	F	A	>24	1		335	9	48	0														
621	4	Neg	2004	F	A	>24	1		365	10	96	0														
621	4	Neg	2005	F	A	>24	1		448	2	48	0	24													
627	4	Neg	2003	F	A	>24	0		0	10																
627	4	Neg	2003	F	A	>24	0		28	10	384	24	0													
627	4	Neg	2004	F	A	>24	0		100	2	192	48	0													
627	4	Neg	2004	F	A	>24	0		335	9	24	0	0													
627	4	Neg	2004	F	A	>24	0		365	10	48	384	192													
627	4	Neg	2005	F	A	>24	0		448	2	192	0	0													
631	4	Neg	2003	F	A	>24	0		0	10	48	384	0													
631	4	Neg	2003	F	A	>24	0		28	10	48	384	0													
631	4	Neg	2004	F	A	>24	0		100	2	96	192	0													
631	4	Neg	2004	F	A	>24	0		335	9	0	96	0													
631	4	Neg	2004	F	A	>24	0		365	10	0	96	0													
631	4	Neg	2005	F	A	>24	0		448	2	24	0	0													
633	4	Neg	2003	F	A	>24	0		0	10	96	192	0													
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633	4	Neg	2004	F	A	>24	0		335	9	0	48	0													
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633	4	Neg	2005	F	A	>24	0		448	2	24	0	0													
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659	4	Neg	2003	F	A	>24	0		28	10																
659	4	Neg	2004	F	A	>24	0		100	2																
659	4	Neg	2004	F	A	>24	0		335	9		0	0													
659	4	Neg	2004	F	A	>24	0		365	10	0	0	48													
659	4	Neg	2005	F	A	>24	0		448	2																
710	4	Neg	2003	F	A	>24	0		0	10	96	24	0													
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710	4	Neg	2004	F	A	>24	0		365	10	96	0	0													
710	4	Neg	2005	F	A	>24	0		448	2	24	0	0													
711	4	Neg	2003	F	A	>24	1		0	10	96	0	48													
711	4	Neg	2003	F	A	>24	1		28	10	96	48	96													
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711	4	Neg	2004	F	A	>24	1		335	9	0	0	0													
711	4	Neg	2004	F	A	>24	1		365	10	96	0	0													
711	4	Neg	2005	F	A	>24	1		448	2	24	0	0													
720	4	Neg	2003	F	A	>24	1		0	10	48	24	0													
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720	4	Neg	2004	F	A	>24	1		335	9	0	0	0													
720	4	Neg	2004	F	A	>24	1		365	10	48	192	96													
720	4	Neg	2005	F	A	>24	1		448	2	48	0	0													
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722	4	Neg	2003	F	A	>24	0		28	10		48	0													
722	4	Neg	2004	F	A	>24	0		100	2	192	24	0													
722	4	Neg	2004	F	A	>24	0		335	9	0	0	0													
722	4	Neg	2004	F	A	>24	0		365	10	0	0	0													
722	4	Neg	2005	F	A	>24	0		448	2	24	0	0													
730	4	Neg	2003	F	A	>24	0		0	10	192	96	24													
730	4	Neg	2003	F	A	>24	0		28	10	96	48	0													
730	4	Neg	2004	F	A	>24	0		100	2																
730	4	Neg	2004	F	A	>24	0		335	9	48	0	0													
730	4	Neg	2004	F	A	>24	0		365	10	96	192	96													
730	4	Neg	2005	F	A	>24	0		448	2	24	0	24													
732	4	Neg	2003	F	A	>24	1		0	10	192	0	0													
732	4	Neg	2003	F	A	>24	1		28	10	384	96	48													
732	4	Neg	2004	F	A	>24	1		100	2																
732	4	Neg	2004	F	A	>24	1		335	9	48	0	0													
732	4	Neg	2004	F	A	>24	1		365	10	24	0	0													
732	4	Neg	2005	F	A	>24	1		448	2	48	0	0													
834	4	Neg	2003	F	A	>24	0		0	10	0	384	0													
834	4	Neg	2003	F	A	>24	0		28	10	24	1536	0													
834	4	Neg	2004	F	A	>24	0		100	2																
834	4	Neg	2004	F	A	>24	0		335	9	0	96	0													
834	4	Neg	2004	F	A	>24	0		365	10	0	384	0													
834	4	Neg	2005	F	A	>24	0		448	2	48	48	0													

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM		Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur	Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
836	4	Neg	2003	F	A	>24	0		0	10	0	96	0											
836	4	Neg	2003	F	A	>24	0		28	10	0	192	0											
836	4	Neg	2004	F	A	>24	0		100	2	24	192	96											
836	4	Neg	2004	F	A	>24	0		335	9	0	24	0											
836	4	Neg	2004	F	A	>24	0		365	10	48	384	24											
836	4	Neg	2005	F	A	>24	0		448	2	0	24	0											
838	4	Neg	2003	F	A	>24	1		0	10	24	96	0											
838	4	Neg	2003	F	A	>24	1		28	10														
838	4	Neg	2004	F	A	>24	1		100	2	48	192	0											
838	4	Neg	2004	F	A	>24	1		335	9	0	0	0											
838	4	Neg	2004	F	A	>24	1		365	10	0	24	0											
838	4	Neg	2005	F	A	>24	1		448	2	24	48	24											
840	4	Neg	2003	F	A	>24	1		0	10	24	48	0											
840	4	Neg	2003	F	A	>24	1		28	10	96	768	96											
840	4	Neg	2004	F	A	>24	1		100	2	96	768	192											
840	4	Neg	2004	F	A	>24	1		335	9	48	24	0											
840	4	Neg	2004	F	A	>24	1		365	10	96	768	96											
840	4	Neg	2005	F	A	>24	1		448	2	48	48	24											
845	4	Neg	2003	F	A	>24	1		0	10	24	96	0											
845	4	Neg	2003	F	A	>24	1		28	10	192	768	96											
845	4	Neg	2004	F	A	>24	1		100	2	48	384	24											
845	4	Neg	2004	F	A	>24	1		335	9	48	96	0											
845	4	Neg	2004	F	A	>24	1		365	10	24	384	384											
845	4	Neg	2005	F	A	>24	1		448	2	24	0	0											
846	4	Neg	2003	F	A	>24	0		0	10	0	192	0											
846	4	Neg	2003	F	A	>24	0		28	10	0	96	0											
846	4	Neg	2004	F	A	>24	0		100	2	96	48	0											
846	4	Neg	2004	F	A	>24	0		335	9	0	48	0											
846	4	Neg	2004	F	A	>24	0		365	10	24	96	384											
846	4	Neg	2005	F	A	>24	0		448	2	0	0	0											
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851	4	Neg	2003	F	A	>24	0		28	10	0	48	0											
851	4	Neg	2004	F	A	>24	0		100	2	0	96	0											
851	4	Neg	2004	F	A	>24	0		335	9	0	24	0											
851	4	Neg	2004	F	A	>24	0		365	10	48	768	96											
851	4	Neg	2005	F	A	>24	0		448	2	0	24	0											
853	4	Neg	2003	F	A	>24	1		0	10	0	192	0											
853	4	Neg	2003	F	A	>24	1		28	10		768												
853	4	Neg	2004	F	A	>24	1		100	2	384	768	0											
853	4	Neg	2004	F	A	>24	1		335	9	0	192	0											
853	4	Neg	2004	F	A	>24	1		365	10	0	384	384											
853	4	Neg	2005	F	A	>24	1		448	2	0	48	24											
859	4	Neg	2003	F	A	>24	0		0	10	0	192	48											
859	4	Neg	2003	F	A	>24	0		28	10	24	768	24											
859	4	Neg	2004	F	A	>24	0		100	2	24	384	24											
859	4	Neg	2004	F	A	>24	0		335	9	0	24	0											
859	4	Neg	2004	F	A	>24	0		365	10	0	96	0											
859	4	Neg	2005	F	A	>24	0		448	2	0	0	0											
862	4	Neg	2003	F	A	>24	0		0	10	0	48	0											
862	4	Neg	2003	F	A	>24	0		28	10	0	96	24											
862	4	Neg	2004	F	A	>24	0		100	2	24	96	48											
862	4	Neg	2004	F	A	>24	0		335	9	0	0	0											
862	4	Neg	2004	F	A	>24	0		365	10	48	24	0											
862	4	Neg	2005	F	A	>24	0		448	2	24	24	0											
900	4	Neg	2003	F	A	>24	0		0	10	24	24	0											
900	4	Neg	2003	F	A	>24	0		28	10														
900	4	Neg	2004	F	A	>24	0		100	2	0	24	0											
902	4	Neg	2003	F	A	>24	0		0	10	0	96	0											
902	4	Neg	2003	F	A	>24	0		28	10	0	96	0											
902	4	Neg	2004	F	A	>24	0		100	2														
902	4	Neg	2004	F	A	>24	0		335	9	0	24	0											
902	4	Neg	2004	F	A	>24	0		365	10	0	48	0											
902	4	Neg	2005	F	A	>24	0		448	2		24	0											
905	4	Neg	2003	F	A	>24	0		0	10	0	96	0											
905	4	Neg	2003	F	A	>24	0		28	10	0	96	0											
905	4	Neg	2004	F	A	>24	0		100	2	0	24	0											
905	4	Neg	2004	F	A	>24	0		335	9	0	0	0											
905	4	Neg	2004	F	A	>24	0		365	10	48	192	0											
905	4	Neg	2005	F	A	>24	0		448	2	0	0	0											
908	4	Neg	2003	F	A	>24	0		0	10														
908	4	Neg	2003	F	A	>24	0		28	10	24	24	0											
908	4	Neg	2004	F	A	>24	0		100	2	384	96	0											
908	4	Neg	2004	F	A	>24	0		335	9	0	0	0											
908	4	Neg	2004	F	A	>24	0		365	10	96	96	0											
908	4	Neg	2005	F	A	>24	0		448	2	0	0	0											
914	4	Neg	2003	F	A	>24	1		0	10	96	0	0											

Id	Farm			S	Age		Vaccine		Trial		Serology			Cul.	DFM			Sv	Kidney		Histopathology					
	No	DX	year		Cls	Mt	Dm	Wn	Day	Mt	Hb	Pom	Cop	U-K	LLI	Ur		Tp	HP	GP	FN	DN	Nphs	L-P	GA	Sc
914	4	Neg	2003	F	A	>24	1		28	10	48	0	0													
914	4	Neg	2004	F	A	>24	1		100	2	96															
914	4	Neg	2004	F	A	>24	1		335	9	24	0	0													
914	4	Neg	2004	F	A	>24	1		365	10	0	0	0													
914	4	Neg	2005	F	A	>24	1		448	2	24	24	0													
915	4	Neg	2003	F	A	>24	0		0	10	0	192	0													
915	4	Neg	2003	F	A	>24	0		28	10	0	24	0													
915	4	Neg	2004	F	A	>24	0		100	2	24	24	48													
915	4	Neg	2004	F	A	>24	0		335	9	0	0	0													
915	4	Neg	2004	F	A	>24	0		365	10	0	0	0													
915	4	Neg	2005	F	A	>24	0		448	2	0	0	24													
920	4	Neg	2003	F	A	>24	0		0	10	24	0	0													
920	4	Neg	2003	F	A	>24	0		28	10		0														
920	4	Neg	2004	F	A	>24	0		100	2	48	24	48													
920	4	Neg	2004	F	A	>24	0		335	9	0	0	0													
920	4	Neg	2004	F	A	>24	0		365	10	0	0	0													
920	4	Neg	2005	F	A	>24	0		448	2	24	0	0													
942	4	Neg	2003	F	A	>24	0		0	10	0		0													
942	4	Neg	2003	F	A	>24	0		28	10	24	48	24													
942	4	Neg	2004	F	A	>24	0		100	2	24	48	0													
942	4	Neg	2004	F	A	>24	0		335	9	0	0	0													
942	4	Neg	2004	F	A	>24	0		365	10	0	0	0													
942	4	Neg	2005	F	A	>24	0		448	2	48	0	0													
943	4	Neg	2003	F	A	>24	1		0	10	48	24	0													
943	4	Neg	2003	F	A	>24	1		28	10	96	768	48													
943	4	Neg	2004	F	A	>24	1		100	2	96	192	0													
943	4	Neg	2004	F	A	>24	1		335	9	24	24	0													
943	4	Neg	2004	F	A	>24	1		365	10	0	0	0													
943	4	Neg	2005	F	A	>24	1		448	2	24	0	0													
945	4	Neg	2003	F	A	>24	1		0	10	0	48	0													
945	4	Neg	2003	F	A	>24	1		28	10	96	384	48													
945	4	Neg	2004	F	A	>24	1		100	2	24	96	0													
945	4	Neg	2004	F	A	>24	1		335	9	0	0	0													
945	4	Neg	2004	F	A	>24	1		365	10	192	0	24													
945	4	Neg	2005	F	A	>24	1		448	2	48	0	48													
959	4	Neg	2003	F	A	>24	1		0	10	0	24	0													
959	4	Neg	2003	F	A	>24	1		28	10	0	96	24													
959	4	Neg	2004	F	A	>24	1		100	2	24	24	0													
959	4	Neg	2004	F	A	>24	1		335	9	0	0														
959	4	Neg	2004	F	A	>24	1		365	10																
959	4	Neg	2005	F	A	>24	1		448	2	0	0	0													
40 g	4	Neg	2003	F	A	>24	0		0	10	48	48	0													
40 g	4	Neg	2003	F	A	>24	0		28	10	24	48	0													
40 g	4	Neg	2004	F	A	>24	0		100	2	48															
40 g	4	Neg	2004	F	A	>24	0		335	9	48															
40 g	4	Neg	2004	F	A	>24	0		365	10	48															
40 g	4	Neg	2005	F	A	>24	0		448	2	24	48	0													

Appendix 2b. Weaner weights farm 2, year 2004 by weighting month. Id = Animal Id as used in appendix 2a.

Consecutive.	ID	Farm	year	Month	weight kg.
1	302	2	2004	2	50.2
2	302	2	2004	6	67.4
3	302	2	2004	9	74
4	309	2	2004	2	54.4
5	309	2	2004	6	70.8
6	309	2	2004	9	75
7	316	2	2004	2	49
8	316	2	2004	6	65.4
9	316	2	2004	9	69
10	321	2	2004	2	48.2
11	321	2	2004	6	63
12	321	2	2004	9	70
13	538	2	2004	2	49.4
14	538	2	2004	6	62.8
15	538	2	2004	9	69
16	553	2	2004	2	49.7
17	553	2	2004	6	67.6
18	553	2	2004	9	71
19	700	2	2004	2	49.4
20	700	2	2004	6	68.6
21	700	2	2004	9	73
22	702	2	2004	2	45.4
23	702	2	2004	6	63.8
24	702	2	2004	9	75
25	706	2	2004	2	45.7
26	706	2	2004	6	63
27	706	2	2004	9	71
28	709	2	2004	2	51.2
29	709	2	2004	6	67
30	709	2	2004	9	69
31	714	2	2004	2	47.9
32	714	2	2004	6	60
33	714	2	2004	9	65
34	719	2	2004	2	48.5
35	719	2	2004	6	63
36	719	2	2004	9	68.2
37	723	2	2004	2	48
38	723	2	2004	6	72
39	723	2	2004	9	76
40	730	2	2004	2	58.4
41	730	2	2004	6	75
42	730	2	2004	9	90
43	816	2	2004	2	57.4
44	816	2	2004	6	75
45	816	2	2004	9	91
46	909	2	2004	2	54
47	909	2	2004	6	68
48	909	2	2004	9	81
49	913	2	2004	2	51.2
50	913	2	2004	6	66
51	913	2	2004	9	71
52	919	2	2004	2	56.6
53	919	2	2004	6	70.2
54	919	2	2004	9	76.2
55	920	2	2004	2	51.8
56	920	2	2004	6	70.8
57	920	2	2004	9	81
58	934	2	2004	2	52.6
59	934	2	2004	6	51.2
60	934	2	2004	9	64
61	121	2	2004	2	56.2
62	121	2	2004	6	72.2
63	121	2	2004	9	82
64	311	2	2004	2	55.2
65	311	2	2004	6	77
66	311	2	2004	9	87
67	322	2	2004	2	61
68	322	2	2004	6	76.4
69	322	2	2004	9	92
70	332	2	2004	2	54.4
71	332	2	2004	6	69.8
72	332	2	2004	9	78
73	333	2	2004	2	57
75	333	2	2004	9	87
76	334	2	2004	2	55.2
77	334	2	2004	6	68.8
78	334	2	2004	9	80
79	337	2	2004	2	56
80	337	2	2004	6	76
81	337	2	2004	9	89
82	338	2	2004	2	53.6
83	338	2	2004	6	
84	338	2	2004	9	85
85	339	2	2004	2	60.2
86	339	2	2004	6	75
87	339	2	2004	9	87
88	631	2	2004	2	58.4
89	631	2	2004	6	71.2
90	631	2	2004	9	81.8
91	644	2	2004	2	55.8
92	644	2	2004	6	74.4
93	644	2	2004	9	91
94	735	2	2004	2	61.4
95	735	2	2004	6	78.4
96	735	2	2004	9	88
97	739	2	2004	2	49
98	739	2	2004	6	64.6
99	739	2	2004	9	74.2
100	741	2	2004	2	57
101	741	2	2004	6	71.6
102	741	2	2004	9	85
103	743	2	2004	2	58.6
104	743	2	2004	6	72.8
105	743	2	2004	9	80
106	751	2	2004	2	54
107	751	2	2004	6	78.2
108	751	2	2004	9	89
109	752	2	2004	2	55.8
110	752	2	2004	6	72
111	752	2	2004	9	79
112	845	2	2004	2	49
113	845	2	2004	6	65.4
114	845	2	2004	9	74
115	301	2	2004	2	47.7
116	301	2	2004	6	63.2
117	301	2	2004	9	68
118	304	2	2004	2	41.4
120	304	2	2004	9	59
121	305	2	2004	2	51.4
122	305	2	2004	6	65.8
123	305	2	2004	9	67
124	306	2	2004	2	47.1
125	306	2	2004	6	62.6
126	306	2	2004	9	66.4
127	307	2	2004	2	44.4
128	307	2	2004	6	64.4
129	307	2	2004	9	70.6
130	310	2	2004	2	60.8
131	310	2	2004	6	74
132	310	2	2004	9	81
133	313	2	2004	2	54
134	313	2	2004	6	71.8
135	313	2	2004	9	77
136	314	2	2004	2	53
137	314	2	2004	6	70.2
138	314	2	2004	9	73
139	522	2	2004	2	50.2
140	522	2	2004	6	66.6
141	522	2	2004	9	72
142	701	2	2004	2	47.2
120	304	2	2004	9	59
121	305	2	2004	2	51.4
122	305	2	2004	6	65.8
123	305	2	2004	9	67
124	306	2	2004	2	47.1
125	306	2	2004	6	62.6

Consecutive.	ID	Farm	year	Month	weight kg.
143	701	2	2004	6	61
144	701	2	2004	9	63.8
145	703	2	2004	2	55
146	703	2	2004	6	62
147	703	2	2004	9	68
148	705	2	2004	2	47.6
149	705	2	2004	6	60.6
150	705	2	2004	9	65
151	710	2	2004	2	51.2
152	710	2	2004	6	70.6
153	710	2	2004	9	80
154	712	2	2004	2	48.6
155	712	2	2004	6	65.2
156	712	2	2004	9	68
157	715	2	2004	2	50.8
158	715	2	2004	6	64.2
159	715	2	2004	9	66.8
160	722	2	2004	2	45
161	722	2	2004	6	63.8
162	722	2	2004	9	72
163	729	2	2004	2	47.8
164	729	2	2004	6	62.2
165	729	2	2004	9	69
166	907	2	2004	2	52.4
167	907	2	2004	6	69.2
168	907	2	2004	9	78
169	911	2	2004	2	53.8
170	911	2	2004	6	70.4
171	911	2	2004	9	74
172	17	2	2004	2	67
173	17	2	2004	6	82.6
174	17	2	2004	9	90
175	326	2	2004	2	53.6
176	326	2	2004	6	71.6
177	326	2	2004	9	81
178	329	2	2004	2	60.6
143	701	2	2004	6	61
144	701	2	2004	9	63.8
145	703	2	2004	2	55
146	703	2	2004	6	62
147	703	2	2004	9	68
148	705	2	2004	2	47.6
149	705	2	2004	6	60.6
179	329	2	2004	6	78.4
180	329	2	2004	9	87
181	330	2	2004	2	57.4
182	330	2	2004	6	73.8
183	330	2	2004	9	90
184	335	2	2004	2	51.2
185	335	2	2004	6	68.8
186	335	2	2004	9	82
187	336	2	2004	2	57
188	336	2	2004	6	72.8
189	336	2	2004	9	93
190	542	2	2004	2	54.6
191	542	2	2004	6	71
192	542	2	2004	9	81
193	630	2	2004	2	56.4
194	630	2	2004	6	74.8
195	630	2	2004	9	85
196	636	2	2004	2	54
197	636	2	2004	6	73
198	636	2	2004	9	87
199	731	2	2004	2	54
200	731	2	2004	6	67.8
201	731	2	2004	9	80.8
202	745	2	2004	2	50.6
203	745	2	2004	6	77.4
204	745	2	2004	9	89
205	748	2	2004	2	49
206	748	2	2004	6	71.6
207	748	2	2004	9	80
208	749	2	2004	2	49.2

Consecutive.	ID	Farm	year	Month	weight kg.
209	749	2	2004	6	67.2
210	749	2	2004	9	78
211	757	2	2004	2	55.8
212	757	2	2004	6	82.6
213	757	2	2004	9	96
214	758	2	2004	2	56.2
215	758	2	2004	6	72.6
216	758	2	2004	9	80
217	838	2	2004	2	64
218	838	2	2004	6	86.4
219	838	2	2004	9	104
220	839	2	2004	2	60.4
221	839	2	2004	6	75.4
222	839	2	2004	9	81
223	933	2	2004	2	
224	933	2	2004	6	69.6
225	933	2	2004	9	77.6
226	938	2	2004	2	51.6
227	938	2	2004	6	75
228	938	2	2004	9	86
229	942	2	2004	2	47.7
230	942	2	2004	6	65.2
231	942	2	2004	9	
232	1601	2	2004	2	59
233	1601	2	2004	6	69.4
234	1601	2	2004	9	70
235	1602	2	2004	2	56.5
236	1602	2	2004	6	50
237	1602	2	2004	9	61
238	1603	2	2	2	60
239	1603	2	2	6	69
240	1603	2	2	9	65.4
241	1604	2	2	2	59
242	1604	2	2	6	51
243	1604	2	2	9	62
244	1609	2	2	2	58
245	1609	2	2	6	66
246	1609	2	2	9	67
247	1626	2	2	2	57.5
248	1626	2	2	6	65
249	1626	2	2	9	70
250	1627	2	2	2	50.5
251	1627	2	2	6	59
252	1627	2	2	9	67
253	1630	2	2	2	56.5
254	1630	2	2	6	61
255	1630	2	2	9	69.6
256	S101	2	2	2	40.5
257	S101	2	2	6	
258	S101	2	2	9	66
259	S104	2	2	2	50
260	S104	2	2	6	
261	S104	2	2	9	62.2
262	S105	2	2	2	41.5
263	S105	2	2	6	
264	S105	2	2	9	57.2
265	S106	2	2	2	40.5
266	S106	2	2	6	
267	S106	2	2	9	56
268	S107	2	2	2	44
269	S107	2	2	6	
270	S107	2	2	9	57
271	S134	2	2	2	46
272	S134	2	2	6	57
273	S134	2	2	9	64
274	S135	2	2	2	39.5
275	S135	2	2	6	48.2
276	S135	2	2	9	66
277	S137	2	2	2	
278	S137	2	2	6	52
279	S137	2	2	9	67.4
280	S138	2	2	2	49
281	S138	2	2	6	55

Consecutive.	ID	Farm	year	Month	weight kg.
282	S138	2	2	9	59
283	S139	2	2	2	44
284	S139	2	2	6	52.4
285	S139	2	2	9	59
286	I660	2	2	2	43
287	I660	2	2	6	55
288	I660	2	2	9	65.4
289	I663	2	2	2	47.1
290	I663	2	2	6	61.7
291	I663	2	2	9	74
292	I664	2	2	2	47
293	I664	2	2	6	61.7
294	I664	2	2	9	73
295	I665	2	2	2	46.8
296	I665	2	2	6	61.7
297	I665	2	2	9	69
298	I668	2	2	2	46.4
299	I668	2	2	6	61.7
300	I668	2	2	9	75.4
301	I684	2	2	2	62.5
302	I684	2	2	6	77
303	I684	2	2	9	87
304	I685	2	2	2	43.9
305	I685	2	2	6	61.6
306	I685	2	2	9	71.2
307	I686	2	2	2	43.7
308	I686	2	2	6	61.6
309	I686	2	2	9	74.4
310	I688	2	2	2	59
311	I688	2	2	6	68
312	I688	2	2	9	81
313	I694	2	2	2	63
314	I694	2	2	6	69
315	I694	2	2	9	81
316	I695	2	2	2	65
317	I695	2	2	6	72.4
318	I695	2	2	9	82
319	S158	2	2	2	54.5
320	S158	2	2	6	63
321	S158	2	2	9	65
322	S169	2	2	2	52
323	S169	2	2	6	67
324	S169	2	2	9	71.2
325	S170	2	2	2	54
326	S170	2	2	6	61.2
327	S170	2	2	9	75.4
328	S172	2	2	2	52.5
329	S172	2	2	6	61
330	S172	2	2	9	72
331	S175	2	2	2	46.5
332	S175	2	2	6	59
333	S175	2	2	9	71.4
334	S188	2	2	2	51.5
335	S188	2	2	6	61.6
336	S188	2	2	9	73
337	S189	2	2	2	50.5
338	S189	2	2	6	62
339	S189	2	2	9	74.2
340	S191	2	2	2	52.5
341	S191	2	2	6	66.4
342	S191	2	2	9	72.8
343	S196	2	2	2	47
344	S196	2	2	6	55
345	S196	2	2	9	65.2
346	I605	2	2	2	46.5
347	I605	2	2	6	56.8
348	I605	2	2	9	61.6
349	I607	2	2	2	53.5
350	I607	2	2	6	64.4
351	I607	2	2	9	69.8
352	I610	2	2	2	53
353	I610	2	2	6	61
354	I610	2	2	9	72.6
355	I624	2	2	2	56
356	I624	2	2	6	65
357	I624	2	2	9	69
358	I625	2	2	2	54.5
359	I625	2	2	6	65
360	I625	2	2	9	76
361	I628	2	2	2	46
362	I628	2	2	6	54
363	I628	2	2	9	62
364	I629	2	2	2	50.5
365	I629	2	2	6	61.6
366	I629	2	2	9	72.6
367	I638	2	2	2	58
368	I638	2	2	6	67.2
369	I638	2	2	9	74
370	S100	2	2	2	36
371	S100	2	2	6	
372	S100	2	2	9	53.6
373	S102	2	2	2	46
374	S102	2	2	6	
375	S102	2	2	9	66.4
376	S108	2	2	2	44
377	S108	2	2	6	
378	S108	2	2	9	66
379	S109	2	2	2	38
380	S109	2	2	6	
381	S109	2	2	9	61
382	S111	2	2	2	49.5
383	S111	2	2	6	
384	S111	2	2	9	56
385	S131	2	2	2	47
386	S131	2	2	6	54
387	S131	2	2	9	61
388	S132	2	2	2	44
389	S132	2	2	6	52
390	S132	2	2	9	56
391	S133	2	2	2	52.5
392	S133	2	2	6	62
393	S133	2	2	9	69.2
394	S135	2	2	2	39.5
395	S135	2	2	6	48.2
396	S135	2	2	9	53.6
397	S142	2	2	2	48
398	S142	2	2	6	61
399	S142	2	2	9	67
400	I658	2	2	2	54
401	I658	2	2	6	59
402	I658	2	2	9	65
403	I666	2	2	2	46.7
404	I666	2	2	6	61.7
405	I666	2	2	9	74.2
406	I670	2	2	2	55.5
407	I670	2	2	6	60
408	I670	2	2	9	66.6
409	I671	2	2	2	51.5
410	I671	2	2	6	
411	I671	2	2	9	
412	I683	2	2	2	44.2
413	I683	2	2	6	61.6
414	I683	2	2	9	74.4
415	I687	2	2	2	59
416	I687	2	2	6	70.4
417	I687	2	2	9	81
418	I689	2	2	2	64
419	I689	2	2	6	71
420	I689	2	2	9	81
421	I690	2	2	2	43.1
422	I690	2	2	6	61.6
423	I690	2	2	9	71.2
424	I693	2	2	2	
425	I693	2	2	6	70
426	I693	2	2	9	81.8
427	S166	2	2	2	52

Consecutive.	ID	Farm	year	Month	weight kg.
428	S166	2	2	6	65
429	S166	2	2	9	78
430	S167	2	2	2	47.5
431	S167	2	2	6	63.8
432	S167	2	2	9	75.2
433	S174	2	2	2	55
434	S174	2	2	6	59.5
435	S174	2	2	9	
436	S176	2	2	2	49
437	S176	2	2	6	62
438	S176	2	2	9	73
439	S177	2	2	2	46
440	S177	2	2	6	58.4
441	S177	2	2	9	
442	S192	2	2	2	45.5
443	S192	2	2	6	
444	S192	2	2	9	
445	S194	2	2	2	48.5
446	S194	2	2	6	63.2
447	S194	2	2	9	73
448	S197	2	2	2	44
449	S197	2	2	6	49.4
450	S197	2	2	9	64.2
451	S198	2	2	2	44
452	S198	2	2	6	52.2
453	S198	2	2	9	57.4
454	S199	2	2	2	45.5
455	S199	2	2	6	55.2
456	S199	2	2	9	62.8

Appendix 3 raw data
Chapter 7
(Regional Survey)

Table 3.1.a List of abbreviation and classification used in Appendices 3a, and 3c.

Appendix	Abbreviation and classification			Notes
3a individual animal serology results	Regions		Northland/Auckland	Nothland
			Waikato	Waikato
			Bay of Plenty./ Gisborne	Bo.Plen-Gisborne
			Hawkes Bay	Hawkes Bay
			Taranaki/ Manawatu/ Wanganui/ Wellington	Well-Manawatu
			West Coast/ Nelson/ Malborough	West Coast
			Canterbury	Canterbury
			Otago	Otago
			Southland	Southland
	ID		Animal identification number	Farm number
	No.		Consecutive number of samples.	Laboratory number
3c results questionnaire	Farm No.		Farm Number	Assigned number
	Prevalence	Hb	Hardjobovis	Single seroreactors
		Pom	Pomona	
		Cop	Copenhageni	
		Hb & Pom.	Hardjobovis and Pomona	Dual seroreactors
	Deer demographics	R2♂	2nd year rising male (Stag)	>12 months and < 24 month of age
		R2♀	2nd year rising female2 (Hind)	
		A♂	Adults male (Stag)	>24 months of age
		A♀	Adult female (Hind)	
		weaner		<12 months of age
	Lepto Dx before	Herd leptospira diagnosis in the past		
	Serovar Pom-Hardjo	Hardjobovis, Pomona or Hardjobovis and Pomona		If infected in the past which was the infecting serovar

Appendix 3a. Regional survey individual animal serology results for each serovar, and region and farm number.

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1	Well-Manawatu	1	92	48	0	0	36	Hawkes bay	2	3045	96	96	0
2	Well-Manawatu	1	96	96	0	0	37	Hawkes bay	2	6189	384	24	0
3	Well-Manawatu	1	99	48	0	0	38	Hawkes bay	2	4119	384	192	24
4	Well-Manawatu	1	101	0	0	0	39	Hawkes bay	2	4332	24	0	0
5	Well-Manawatu	1	103	48	0	0	40	Hawkes bay	2	7326	3072	96	96
6	Well-Manawatu	1	105	48	0	0	41	Hawkes bay	2	227	384	0	24
7	Well-Manawatu	1	116	96	0	0	42	Well-Manawatu	3	2*	24	768	24
8	Well-Manawatu	1	124	192	0	0	43	Well-Manawatu	3	3*	24	768	0
9	Well-Manawatu	1	125	0	0	0	44	Well-Manawatu	3	4*	24	3072	24
10	Well-Manawatu	1	126	96	0	0	45	Well-Manawatu	3	6*	0	3072	0
11	Well-Manawatu	1	127	192	0	0	46	Well-Manawatu	3	7*	48	3072	0
12	Well-Manawatu	1	129	0	0	0	47	Well-Manawatu	3	8*	24	1536	0
13	Well-Manawatu	1	130	384	0	0	48	Well-Manawatu	3	12*	48	384	0
14	Well-Manawatu	1	134	48	0	0	49	Well-Manawatu	3	304	24	1536	192
15	Well-Manawatu	1	135	0	0	0	50	Well-Manawatu	3	2529	48	768	24
16	Well-Manawatu	1	137	192	0	0	51	Well-Manawatu	3	2543	24	3072	24
17	Well-Manawatu	1	140	192	0	0	52	Well-Manawatu	3	2543	24	768	0
18	Well-Manawatu	1	144	192	0	0	53	Well-Manawatu	3	2569	24	768	0
19	Well-Manawatu	1	146	24	0	0	54	Well-Manawatu	3	2571	48	1536	0
20	Well-Manawatu	1	147	192	0	0	55	Well-Manawatu	3	2627	24	1536	0
21	Well-Manawatu	1	148	48	0	0	56	Well-Manawatu	3	27	48	3072	96
22	Hawkes bay	2	876	24	0	0	57	Well-Manawatu	3	27	24	1536	0
23	Hawkes bay	2	177	48	24	0	58	Well-Manawatu	3	2685	96	3072	48
24	Hawkes bay	2	3175	24	24	0	59	Well-Manawatu	3	2706	24	3072	96
25	Hawkes bay	2	855	24	48	0	60	Well-Manawatu	3	2726	24	3072	0
26	Hawkes bay	2	944	24	0	0	61	Well-Manawatu	3	2727	24	3072	192
27	Hawkes bay	2	3123	48	48	24	62	Well-Manawatu	4	81	0	0	0
28	Hawkes bay	2	no id tag	48	24	0	63	Well-Manawatu	4	61	0	0	0
29	Hawkes bay	2	3161	48	0	0	64	Well-Manawatu	4	80	0	0	0
30	Hawkes bay	2	3197	48	96	0	65	Well-Manawatu	4	50E	0	0	0
31	Hawkes bay	2	3113	0	48	24	66	Well-Manawatu	4	6	0	0	0
32	Hawkes bay	2	940	24	24	24	67	Well-Manawatu	4	50	0	0	0
33	Hawkes bay	2	872	24	0	0	68	Well-Manawatu	4	3	0	0	0
34	Hawkes bay	2	176	48	48	0	69	Well-Manawatu	4	30	0	0	0
35	Hawkes bay	2	3110	24	0	0	70	Well-Manawatu	4	79	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
71	Well-Manawatu	4	49	0	0	0	113	Otago	6	231	24	0	0
72	Well-Manawatu	4	36	0	0	0	114	Otago	6	242	768	0	0
73	Well-Manawatu	4	2	0	0	0	115	Otago	6	247	0	0	0
74	Well-Manawatu	4	40	0	0	0	116	Otago	6	257	0	0	0
75	Well-Manawatu	4	33	0	0	0	117	Otago	6	273	48	0	0
76	Well-Manawatu	4	7	0	0	0	118	Otago	6	275	384	0	0
77	Well-Manawatu	4	29	0	0	0	119	Otago	6	295	96	0	0
78	Well-Manawatu	4	11	0	0	0	120	Otago	6	429	192	0	0
79	Well-Manawatu	4	65	0	0	0	121	Northland	7	1	96	24	0
80	Well-Manawatu	4	35	0	0	0	122	Northland	7	2	96	96	0
81	Otago	5	78	0	0	0	123	Northland	7	3	96	24	0
82	Otago	5	116	24	0	0	124	Northland	7	4	192	96	0
83	Otago	5	145	0	0	0	125	Northland	7	5	48	96	0
84	Otago	5	148	0	0	0	126	Northland	7	6	96	0	0
85	Otago	5	149	1536	0	0	127	Northland	7	7	48	0	0
86	Otago	5	181	0	0	0	128	Northland	7	8	48	0	0
87	Otago	5	185	0	0	0	129	Northland	7	9	48	24	0
88	Otago	5	231	768	0	0	130	Northland	7	10	48	48	0
89	Otago	5	238	24	0	0	131	Northland	7	11	192	0	0
90	Otago	5	260	24	0	0	132	Northland	7	12	96	24	0
91	Otago	5	279	0	0	0	133	Northland	7	13	48	0	0
92	Otago	5	281	0	0	0	134	Northland	7	14	96	0	0
93	Otago	5	290	384	0	0	135	Northland	7	15	96	24	0
94	Otago	5	337	192	0	0	136	Northland	7	16	0	0	0
95	Otago	5	339	768	0	0	137	Northland	7	17	96	0	0
96	Otago	5	466	0	0	0	138	Northland	7	18	48	0	0
97	Otago	5	475	48	0	0	139	Northland	7	19	192	0	0
98	Otago	5	619	384	0	0	140	Northland	7	20	0	0	0
99	Otago	5	708	0	0	0	141	Northland	8	1	0	0	0
100	Otago	5	1203	48	0	0	142	Northland	8	2	24	0	0
101	Otago	6	3	1536	0	0	143	Northland	8	3	24	0	0
102	Otago	6	90	24	0	0	144	Northland	8	4	24	0	0
103	Otago	6	91	0	0	0	145	Northland	8	5	24	0	0
104	Otago	6	92	96	0	0	146	Northland	8	6	48	0	0
105	Otago	6	96	384	0	0	147	Northland	8	7	96	0	0
106	Otago	6	101	96	0	0	148	Northland	8	8	96	0	0
107	Otago	6	158	384	0	0	149	Northland	8	9	96	0	0
108	Otago	6	159	384	0	0	150	Northland	8	10	24	0	0
109	Otago	6	175	384	0	0	151	Northland	8	11	96	0	0
110	Otago	6	182	384	0	0	152	Northland	8	12	0	0	0
111	Otago	6	186	96	0	0	153	Northland	8	13	0	0	0
112	Otago	6	190	192	0	0	154	Northland	8	14	48	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
155	Northland	8	15	0	0	0	197	Bo.Plen-Gisborne	10	2264	0	0	24
156	Northland	8	16	0	0	0	198	Bo.Plen-Gisborne	10	2308	48	0	24
157	Northland	8	17	0	0	0	199	Bo.Plen-Gisborne	10	2406	24	0	0
158	Northland	8	18	48	0	0	200	Bo.Plen-Gisborne	10	2407	48	0	24
159	Northland	8	19	0	0	0	201	Canterbury	11	1	24	0	0
160	Northland	8	20	0	0	0	202	Canterbury	11	2	0	0	0
161	Northland	9	2004	96	0	0	203	Canterbury	11	3	0	0	0
162	Northland	9	2005	24	0	0	204	Canterbury	11	4	0	0	0
163	Northland	9	2009	0	0	0	205	Canterbury	11	5	0	0	24
164	Northland	9	2010	384	0	0	206	Canterbury	11	6	0	0	0
165	Northland	9	2017	48	0	0	207	Canterbury	11	7	24	0	0
166	Northland	9	2033	0	0	0	208	Canterbury	11	8	0	0	0
167	Northland	9	1036	192	0	0	209	Canterbury	11	9	0	0	0
168	Northland	9	2042	384	0	24	210	Canterbury	11	10	0	0	0
169	Northland	9	2043	96	0	0	211	Canterbury	11	11	0	0	0
170	Northland	9	2046	24	0	0	212	Canterbury	11	12	0	0	0
171	Northland	9	2054	192	0	0	213	Canterbury	11	13	24	0	0
172	Northland	9	2055	192	0	0	214	Canterbury	11	14	0	0	0
173	Northland	9	2058	384	0	0	215	Canterbury	11	15	0	0	0
174	Northland	9	2061	48	0	0	216	Canterbury	11	16	24	0	0
175	Northland	9	2063	48	0	0	217	Canterbury	11	17	0	0	0
176	Northland	9	2083	48	0	0	218	Canterbury	11	18	24	0	0
177	Northland	9	2086	384	0	0	219	Canterbury	11	19	0	0	0
178	Northland	9	2089	192	0	0	220	Canterbury	11	20	0	0	0
179	Northland	9	2090	96	0	0	221	Otago	13	6	48	0	24
180	Northland	9	2096	48	0	0	222	Otago	13	19	192	0	0
181	Bo.Plen-Gisborne	10	109	0	0	24	223	Otago	13	25	96	0	0
182	Bo.Plen-Gisborne	10	212	0	0	0	224	Otago	13	34	96	0	24
183	Bo.Plen-Gisborne	10	215	0	0	0	225	Otago	13	35	384	0	0
184	Bo.Plen-Gisborne	10	219	0	0	0	226	Otago	13	47	192	0	0
185	Bo.Plen-Gisborne	10	231	0	0	0	227	Otago	13	54	192	0	0
186	Bo.Plen-Gisborne	10	255	24	0	0	228	Otago	13	69	384	0	0
187	Bo.Plen-Gisborne	10	256	24	0	0	229	Otago	13	70	96	0	0
188	Bo.Plen-Gisborne	10	259	0	0	0	230	Otago	13	72	96	0	24
189	Bo.Plen-Gisborne	10	263	0	0	0	231	Otago	13	76	48	0	0
190	Bo.Plen-Gisborne	10	269	24	0	0	232	Otago	13	83	96	0	0
191	Bo.Plen-Gisborne	10	2212	48	0	0	233	Otago	13	96	192	0	0
192	Bo.Plen-Gisborne	10	2232	0	0	0	234	Otago	13	103	192	0	0
193	Bo.Plen-Gisborne	10	2226	48	0	0	235	Otago	13	107	24	0	0
194	Bo.Plen-Gisborne	10	2255	0	0	24	236	Otago	13	113	24	0	0
195	Bo.Plen-Gisborne	10	2257	24	0	0	237	Otago	13	117	192	0	0
196	Bo.Plen-Gisborne	10	2258	48	0	0	238	Otago	13	125	384	0	24

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
239	Otago	13	141	96	0	24	281	West Coast	16	14	0	0	0
240	Otago	14	Nh1	0	0	0	282	West Coast	16	16a	0	0	0
241	Otago	14	Nh2	0	0	0	283	West Coast	16	16b	24	0	0
242	Otago	14	T90	0	0	0	284	West Coast	16	17	0	0	0
243	Otago	14	174	0	0	0	285	West Coast	16	27	0	0	0
244	Otago	14	178	0	0	0	286	West Coast	16	34	0	0	0
245	Otago	14	198	0	0	0	287	West Coast	16	40	0	0	0
246	Otago	14	225	0	0	0	288	West Coast	16	46	0	0	0
247	Otago	14	229	0	0	0	289	West Coast	16	49	24	0	0
248	Otago	14	264	0	0	0	290	West Coast	16	51	0	0	0
249	Otago	14	269	0	0	0	291	West Coast	16	52	0	0	0
250	Otago	14	333	0	0	0	292	West Coast	16	55	0	0	0
251	Otago	14	361	0	0	24	293	West Coast	16	57	0	0	0
252	Otago	14	370	0	0	0	294	West Coast	16		0	0	0
253	Otago	14	409	0	0	0	295	West Coast	16	68	0	0	0
254	Otago	14	414	0	0	24	296	West Coast	16	72	0	0	0
255	Otago	14	416	0	0	0	297	West Coast	16	75	0	0	0
256	Otago	14	424	0	0	0	298	West Coast	16	78	0	0	0
257	Otago	14	460	0	0	0	299	West Coast	16	213	24	0	0
258	Otago	14	490	0	0	0	300	Canterbury	17	1	0	0	0
259	Otago	14	524	0	0	0	301	Canterbury	17	2	0	0	0
260	West Coast	15	1	24	0	0	302	Canterbury	17	3	0	0	0
261	West Coast	15	2	0	0	0	303	Canterbury	17	4	0	0	0
262	West Coast	15	3	24	0	0	304	Canterbury	17	5	0	0	0
263	West Coast	15	4	0	0	0	305	Canterbury	17	6	0	0	0
264	West Coast	15	5	0	0	0	306	Canterbury	17	7	0	0	0
265	West Coast	15	6	48	0	0	307	Canterbury	17	8	0	0	0
266	West Coast	15	7	0	0	0	308	Canterbury	17	9	0	0	0
267	West Coast	15	8	0	0	0	309	Canterbury	17	10	0	0	0
268	West Coast	15	9	48	0	0	310	Canterbury	17	11	0	0	0
269	West Coast	15	10	0	0	0	311	Canterbury	17	12	0	0	0
270	West Coast	15	11	0	0	0	312	Canterbury	17	13	0	0	0
271	West Coast	15	12	0	0	0	313	Canterbury	17	14	0	0	0
272	West Coast	15	13	24	0	0	314	Canterbury	17	15	0	0	0
273	West Coast	15	14	0	0	0	315	Canterbury	17	16	0	0	0
274	West Coast	15	15	0	0	0	316	Canterbury	17	17	0	0	0
275	West Coast	15	16	48	0	0	317	Canterbury	17	18	0	0	0
276	West Coast	15	17	0	0	0	318	Canterbury	17	19	0	0	0
277	West Coast	15	18	48	0	0	319	Canterbury	17	20	0	0	0
278	West Coast	15	19	48	0	0	320	Canterbury	18	1	0	0	0
279	West Coast	15	20	24	0	0	321	Canterbury	18	2	0	0	0
280	West Coast	16	9	0	0	0	322	Canterbury	18	3	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
323	Canterbury	18	4	0	0	0	365	Bo.Plen-Gisborne	20	6	96	0	0
324	Canterbury	18	5	0	0	0	366	Bo.Plen-Gisborne	20	7	48	0	0
325	Canterbury	18	6	0	0	0	367	Bo.Plen-Gisborne	20	8	24	0	0
326	Canterbury	18	7	0	0	0	368	Bo.Plen-Gisborne	20	9	48	0	0
327	Canterbury	18	8	0	0	0	369	Bo.Plen-Gisborne	20	10	48	0	0
328	Canterbury	18	9	0	0	0	370	Bo.Plen-Gisborne	20	11	192	0	0
329	Canterbury	18	10	24	0	0	371	Bo.Plen-Gisborne	20	12	48	0	0
330	Canterbury	18	11	0	0	0	372	Bo.Plen-Gisborne	20	13	48	0	0
331	Canterbury	18	12	24	0	0	373	Bo.Plen-Gisborne	20	14	96	0	0
332	Canterbury	18	13	0	0	0	374	Bo.Plen-Gisborne	20	15	96	0	0
333	Canterbury	18	14	0	0	0	375	Bo.Plen-Gisborne	20	16	192	0	0
334	Canterbury	18	15	0	0	0	376	Bo.Plen-Gisborne	20	17	96	0	0
335	Canterbury	18	16	0	0	0	377	Bo.Plen-Gisborne	20	18	96	0	0
336	Canterbury	18	17	24	0	0	378	Bo.Plen-Gisborne	20	19	48	0	0
337	Canterbury	18	18	0	0	0	379	Bo.Plen-Gisborne	20	20	96	0	0
338	Canterbury	18	19	0	0	0	380	Bo.Plen-Gisborne	21	1	24	0	0
339	Canterbury	18	20	24	0	0	381	Bo.Plen-Gisborne	21	2	0	0	0
340	Bo.Plen-Gisborne	19	7	0	0	0	382	Bo.Plen-Gisborne	21	3	0	0	0
341	Bo.Plen-Gisborne	19	9	0	0	0	383	Bo.Plen-Gisborne	21	4	0	0	0
342	Bo.Plen-Gisborne	19	16	0	0	0	384	Bo.Plen-Gisborne	21	5	0	0	0
343	Bo.Plen-Gisborne	19	28	192	0	0	385	Bo.Plen-Gisborne	21	6	0	0	0
344	Bo.Plen-Gisborne	19	31	48	0	0	386	Bo.Plen-Gisborne	21	7	0	0	0
345	Bo.Plen-Gisborne	19	35	24	0	0	387	Bo.Plen-Gisborne	21	8	0	0	0
346	Bo.Plen-Gisborne	19	36	0	0	0	388	Bo.Plen-Gisborne	21	9	0	0	0
347	Bo.Plen-Gisborne	19	38	0	0	0	389	Bo.Plen-Gisborne	21	10	24	0	0
348	Bo.Plen-Gisborne	19	40	24	0	0	390	Bo.Plen-Gisborne	21	11	24	0	0
349	Bo.Plen-Gisborne	19	42	0	0	0	391	Bo.Plen-Gisborne	21	12	0	0	0
350	Bo.Plen-Gisborne	19	52	0	0	0	392	Bo.Plen-Gisborne	21	13	0	0	0
351	Bo.Plen-Gisborne	19	53	0	0	0	393	Bo.Plen-Gisborne	21	14	0	0	0
352	Bo.Plen-Gisborne	19	56	24	0	0	394	Bo.Plen-Gisborne	21	15	0	0	0
353	Bo.Plen-Gisborne	19	63	24	0	0	395	Bo.Plen-Gisborne	21	16	0	0	0
354	Bo.Plen-Gisborne	19	70	0	0	0	396	Bo.Plen-Gisborne	21	17	24	0	0
355	Bo.Plen-Gisborne	19	73	0	0	0	397	Bo.Plen-Gisborne	21	18	0	0	0
356	Bo.Plen-Gisborne	19	79	0	0	0	398	Bo.Plen-Gisborne	21	19	0	0	0
357	Bo.Plen-Gisborne	19	87	0	0	0	399	Bo.Plen-Gisborne	21	20	24	0	0
358	Bo.Plen-Gisborne	19	88	0	0	0	400	Northland	22	No tag 1	0	0	0
359	Bo.Plen-Gisborne	19	99	0	0	0	401	Northland	22	No tag 2	0	0	0
360	Bo.Plen-Gisborne	20	1	96	0	0	402	Northland	22	No tag 3	0	0	0
361	Bo.Plen-Gisborne	20	2	48	0	0	403	Northland	22	55	0	0	0
362	Bo.Plen-Gisborne	20	3	48	0	0	404	Northland	22	58	0	0	0
363	Bo.Plen-Gisborne	20	4	96	0	0	405	Northland	22	75	0	0	0
364	Bo.Plen-Gisborne	20	5	768	0	0	406	Northland	22	166	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
407	Northland	22	721	0	0	0	449	Well-Manawatu	24	74	0	0	0
408	Northland	22	773	0	0	0	450	Well-Manawatu	24	76	0	0	0
409	Northland	22	728	0	0	0	451	Well-Manawatu	24	77	0	0	0
410	Northland	22	781	0	0	0	452	Well-Manawatu	24	138	0	0	0
411	Northland	22	787	0	0	0	453	Well-Manawatu	24	242	0	0	0
412	Northland	22	793	0	0	0	454	Well-Manawatu	24	274	0	0	0
413	Northland	22	794	0	0	0	455	Well-Manawatu	24	308	0	0	0
414	Northland	22	852	0	0	0	456	Well-Manawatu	24	321	0	0	0
415	Northland	22	859	0	0	0	457	Well-Manawatu	24	333	0	0	0
416	Northland	22	864	0	0	0	458	Well-Manawatu	24	336	0	0	0
417	Northland	22	880	0	0	0	459	Well-Manawatu	24	377	0	0	0
418	Northland	22	883	24	0	0	460	Well-Manawatu	24	394	0	0	0
419	Northland	22	925	24	0	0	461	Otago	25	6	384	0	0
420	Bo.Plen-Gisborne	23	4	24	0	0	462	Otago	25	9	96	0	0
421	Bo.Plen-Gisborne	23	65	0	0	0	463	Otago	25	12	768	0	0
422	Bo.Plen-Gisborne	23	66	0	0	0	464	Otago	25	19	384	0	0
423	Bo.Plen-Gisborne	23	7	0	0	0	465	Otago	25	22	384	0	0
424	Bo.Plen-Gisborne	23	8	0	0	0	466	Otago	25	28	96	0	0
425	Bo.Plen-Gisborne	23	9	0	0	0	467	Otago	25	38	96	0	0
426	Bo.Plen-Gisborne	23	12	24	0	0	468	Otago	25	47	384	0	0
427	Bo.Plen-Gisborne	23	17	0	0	0	469	Otago	25	49	24	0	0
428	Bo.Plen-Gisborne	23	29	24	0	0	470	Otago	25	56	384	0	24
429	Bo.Plen-Gisborne	23	30	24	0	0	471	Otago	25	59	384	0	0
430	Bo.Plen-Gisborne	23	33	0	0	0	472	Otago	25	65	48	0	0
431	Bo.Plen-Gisborne	23	36	0	0	0	473	Otago	25	71	384	0	0
432	Bo.Plen-Gisborne	23	41	0	0	0	474	Otago	25	72	96	0	0
433	Bo.Plen-Gisborne	23	43	0	0	0	475	Otago	25	74	768	0	0
434	Bo.Plen-Gisborne	23	47	0	0	0	476	Otago	25	75	384	0	0
435	Bo.Plen-Gisborne	23	53	0	0	0	477	Otago	25	77	96	0	0
436	Bo.Plen-Gisborne	23	59	0	0	0	478	Northland	26	1	384	0	24
437	Bo.Plen-Gisborne	23	61	0	0	0	479	Northland	26	2	96	0	0
438	Bo.Plen-Gisborne	23	64	24	0	0	480	Northland	26	3	48	0	0
439	Bo.Plen-Gisborne	23	71	0	0	0	481	Northland	26	4	192	0	0
440	Bo.Plen-Gisborne	23	72	0	0	0	482	Northland	26	5	96	0	0
441	Bo.Plen-Gisborne	23	73	0	0	0	483	Northland	26	6	192	0	24
442	Well-Manawatu	24	4	0	0	0	484	Northland	26	7	96	0	0
443	Well-Manawatu	24	17	0	0	0	485	Northland	26	8	96	0	24
444	Well-Manawatu	24	38	0	0	0	486	Northland	26	9	24	0	0
445	Well-Manawatu	24	44	0	0	0	487	Northland	26	10	48	0	24
446	Well-Manawatu	24	45	0	0	0	488	Northland	26	11	192	0	0
447	Well-Manawatu	24	54	0	0	0	489	Northland	26	12	96	0	0
448	Well-Manawatu	24	73	0	0	0	490	Northland	26	13	48	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
491	Northland	26	14	96	0	0	533	Well-Manawatu	28	401	96	0	0
492	Northland	26	15	48	0	0	534	Well-Manawatu	28	404	0	0	24
493	Northland	26	16	96	0	0	535	Well-Manawatu	28	410	0	24	0
494	Northland	26	17	96	0	0	536	Well-Manawatu	28	453	384	0	0
495	Northland	26	18	48	0	0	537	Well-Manawatu	28	466	192	0	0
496	Northland	26	19	192	0	0	538	Hawkes bay	29	22	48	0	0
497	Northland	26	20	192	0	0	539	Hawkes bay	29	28	192	0	0
498	Well-Manawatu	27	2003	0	0	0	540	Hawkes bay	29	63	192	0	0
499	Well-Manawatu	27	2008	0	0	0	541	Hawkes bay	29	99	48	0	0
500	Well-Manawatu	27	2016	0	0	0	542	Hawkes bay	29	133	96	0	0
501	Well-Manawatu	27	2017	0	0	0	543	Hawkes bay	29	164	24	0	0
502	Well-Manawatu	27	2019	0	0	0	544	Hawkes bay	29	204	96	0	0
503	Well-Manawatu	27	2025	0	0	0	545	Hawkes bay	29	221	384	0	0
504	Well-Manawatu	27	2025	0	0	0	546	Hawkes bay	29	234	48	0	0
505	Well-Manawatu	27	2028	0	0	0	547	Hawkes bay	29	248	24	0	0
506	Well-Manawatu	27	2035	0	0	0	548	Hawkes bay	29	257	768	0	0
507	Well-Manawatu	27	2044	0	0	0	549	Hawkes bay	29	354	48	0	0
508	Well-Manawatu	27	2050	0	0	0	550	Hawkes bay	29	354	48	0	0
509	Well-Manawatu	27	2052	0	0	0	551	Hawkes bay	29	360	24	0	0
510	Well-Manawatu	27	2058	0	0	0	552	Hawkes bay	29	366	48	0	0
511	Well-Manawatu	27	2063	0	0	0	553	Hawkes bay	29	379	192	0	0
512	Well-Manawatu	27	2070	0	0	0	554	Hawkes bay	29	407	96	0	0
513	Well-Manawatu	27	2073	0	0	0	555	Hawkes bay	29	456	48	0	0
514	Well-Manawatu	27	2077	0	0	0	556	Hawkes bay	29	476	48	0	0
515	Well-Manawatu	27	2078	0	0	0	557	Hawkes bay	29	504	96	0	0
516	Well-Manawatu	27	2084	0	0	0	558	West Coast	30	110	24	48	48
517	Well-Manawatu	27	2087	0	0	0	559	West Coast	30	111	24	192	24
518	Well-Manawatu	28	15	0	0	0	560	West Coast	30	112	0	48	24
519	Well-Manawatu	28	42	48	0	0	561	West Coast	30	113	24	48	24
520	Well-Manawatu	28	53	0	0	0	562	West Coast	30	114	0	48	24
521	Well-Manawatu	28	55	192	0	0	563	West Coast	30	115	24	24	48
522	Well-Manawatu	28	57	192	0	24	564	West Coast	30	116	24	48	48
523	Well-Manawatu	28	79	192	0	0	565	West Coast	30	117	0	48	24
524	Well-Manawatu	28	90	96	0	0	566	West Coast	30	118	0	24	24
525	Well-Manawatu	28	111	48	0	0	567	West Coast	30	119	24	24	24
526	Well-Manawatu	28	151	96	0	0	568	West Coast	30	120	0	0	24
527	Well-Manawatu	28	164	48	0	0	569	West Coast	30	121	24	0	24
528	Well-Manawatu	28	174	192	0	0	570	West Coast	31	166	48	192	0
529	Well-Manawatu	28	180	96	0	0	571	West Coast	31	1	24	96	24
530	Well-Manawatu	28	192	768	0	0	572	West Coast	31	168	24	192	24
531	Well-Manawatu	28	251	96	0	24	573	West Coast	31	169	96	24	24
532	Well-Manawatu	28	350	48	24	0	574	West Coast	31	170	48	96	24

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
575	West Coast	31	171	192	24	0	617	West Coast	35	51	0	0	0
576	West Coast	31	172	96	384	24	618	West Coast	35	52	0	0	0
577	West Coast	31	173	48	192	0	619	West Coast	35	53	0	0	0
578	West Coast	31	174	48	48	24	620	West Coast	35	54	48	0	0
579	West Coast	31	175	192	768	48	621	West Coast	35	55	48	0	0
580	West Coast	31	176	192	48	0	622	West Coast	35	56	24	0	0
581	West Coast	31	177	192	384	48	623	West Coast	35	57	24	0	0
582	Canterbury	32	201	48	24	24	624	West Coast	35	58	48	24	0
583	Canterbury	32	202	384	0	24	625	West Coast	35	59	48	24	0
584	Canterbury	32	203	768	0	48	626	West Coast	35	60	24	0	0
585	Canterbury	32	204	96	0	24	627	West Coast	35	61	24	0	0
586	Canterbury	32	195	96	48	24	628	West Coast	36	94	48	0	24
587	Canterbury	32	196	96	96	24	629	West Coast	36	95	24	48	0
588	Canterbury	32	197	96	96	48	630	West Coast	36	96	192	0	0
589	Canterbury	32	198	96	48	24	631	West Coast	36	97	48	0	0
590	Canterbury	32	199	96	48	24	632	West Coast	36	98	96	0	0
591	Canterbury	32	200	24	48	48	633	West Coast	36	99	192	0	0
592	West Coast	33	14	24	48	0	634	West Coast	36	100	24	0	0
593	West Coast	33	15	24	48	0	635	West Coast	36	101	48	0	0
594	West Coast	33	16	0	48	0	636	West Coast	36	102	384	0	48
595	West Coast	33	17	24	0	0	637	West Coast	36	103	48	24	0
596	West Coast	33	18	24	0	0	638	West Coast	36	104	24	24	24
597	West Coast	33	19	24	0	0	639	West Coast	36	105	24	0	0
598	West Coast	33	20	24	0	0	640	West Coast	36	106	48	48	0
599	West Coast	33	21	24	24	0	641	West Coast	36	107	384	0	0
600	West Coast	33	22	0	24	0	642	West Coast	37	108	192	0	24
601	West Coast	33	23	0	0	0	643	West Coast	37	109	24	0	0
602	West Coast	33	24	24	24	24	644	West Coast	37	110	24	24	0
603	West Coast	33	25	24	0	0	645	West Coast	37	111	24	0	0
604	West Coast	34	26	24	0	24	646	West Coast	37	112	48	24	0
605	West Coast	34	27	24	0	0	647	West Coast	37	113	24	0	0
606	West Coast	34	28	24	0	0	648	West Coast	37	114	24	0	0
607	West Coast	34	29	96	0	0	649	West Coast	37	115	24	24	0
608	West Coast	34	30	24	0	0	650	West Coast	37	116	48	24	0
609	West Coast	34	31	24	24	0	651	West Coast	37	117	24	24	0
610	West Coast	34	32	48	24	0	652	West Coast	38	118	768	24	0
611	West Coast	34	33	24	0	24	653	West Coast	38	119	96	24	0
612	West Coast	34	34	0	48	0	654	West Coast	38	120	96	0	0
613	West Coast	34	35	24	24	0	655	West Coast	38	121	96	0	0
614	West Coast	34	36	48	0	0	656	West Coast	38	122	192	0	0
615	West Coast	34	37	24	24	0	657	West Coast	38	123	384	0	0
616	West Coast	35	50	24	0	24	658	West Coast	38	124	24	96	24

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
659	West Coast	38	125	24	96	24	701	West Coast	42	193	24	0	0
660	West Coast	38	126	0	48	24	702	West Coast	42	194	0	0	0
661	West Coast	38	127	24	48	0	703	West Coast	42	195	0	0	24
662	West Coast	38	128	24	48	24	704	West Coast	42	196	24	0	24
663	West Coast	38	129	24	48	24	705	West Coast	42	197	0	0	0
664	West Coast	39	39	96	768	0	706	West Coast	42	198	24	24	0
665	West Coast	39	40	48	1536	48	707	West Coast	42	199	24	0	0
666	West Coast	39	41	192	768	24	708	West Coast	42	200	0	0	24
667	West Coast	39	42	96	48	24	709	West Coast	42	201	24	0	24
668	West Coast	39	43	24	768	24	710	West Coast	42	202	24	0	24
669	West Coast	39	44	48	192	96	711	West Coast	42	203	0	0	24
670	West Coast	39	45	48	192	24	712	West Coast	43	204	0	0	24
671	West Coast	39	46	48	24	24	713	West Coast	43	205	0	0	24
672	West Coast	39	47	24	48	24	714	West Coast	43	206	0	0	24
673	West Coast	39	48	48	384	48	715	West Coast	43	207	24	24	0
674	West Coast	39	49	24	192	24	716	West Coast	43	208	24	24	0
675	West Coast	39	50	24	384	0	717	West Coast	44	31	24	0	0
676	West Coast	40	159	24	24	0	718	West Coast	44	32	24	24	24
677	West Coast	40	160	0	24	0	719	West Coast	44	33	24	0	24
678	West Coast	40	161	24	0	0	720	West Coast	44	34	24	24	24
679	West Coast	40	162	0	24	0	721	West Coast	44	35	24	24	24
680	West Coast	40	163	24	24	0	722	West Coast	44	36	48	0	24
681	West Coast	40	164	24	24	0	723	West Coast	44	37	24	0	24
682	West Coast	40	165	24	0	24	724	West Coast	44	38	24	0	0
683	West Coast	40	166	24	0	24	725	West Coast	44	39	0	0	24
684	West Coast	40	1	0	0	0	726	West Coast	44	40	0	0	24
685	West Coast	40	168	0	0	24	727	West Coast	44	41	0	0	24
686	West Coast	40	169	0	0	24	728	West Coast	44	42	0	0	24
687	West Coast	40	170	24	0	24	729	West Coast	45	90	192	24	24
688	West Coast	41	171	0	0	24	730	West Coast	45	91	96	24	0
689	West Coast	41	172	0	0	0	731	West Coast	45	92	24	48	24
690	West Coast	41	173	0	0	24	732	West Coast	45	93	96	48	24
691	West Coast	41	174	48	0	0	733	West Coast	45	94	24	48	0
692	West Coast	41	175	192	0	0	734	West Coast	45	95	96	48	48
693	West Coast	41	176	48	0	0	735	West Coast	45	96	192	48	24
694	West Coast	41	177	24	0	0	736	West Coast	45	97	48	48	24
695	West Coast	41	178	24	0	0	737	West Coast	45	98	96	0	24
696	West Coast	41	179	24	0	0	738	West Coast	45	99	384	24	48
697	West Coast	41	180	0	0	0	739	West Coast	45	100	24	0	0
698	West Coast	41	181	24	0	24	740	West Coast	45	101	24	24	24
699	West Coast	41	182	0	0	0	741	Canterbury	46	131	48	0	24
700	West Coast	42	192	24	0	24	742	Canterbury	46	132	48	0	24

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
743	Canterbury	46	133	24	0	24	785	Otago	49	76	96	0	0
744	Canterbury	46	134	24	0	24	786	Otago	49	77	24	0	0
745	Canterbury	46	135	48	0	24	787	Otago	49	78	48	0	0
746	Canterbury	46	136	48	0	24	788	Otago	49	79	24	0	0
747	Canterbury	46	137	384	0	24	789	Otago	49	80	48	0	0
748	Canterbury	46	138	96	0	24	790	Otago	50	10	96	0	0
749	Canterbury	46	139	24	24	0	791	Otago	50	26	384	0	0
750	Canterbury	46	140	48	0	0	792	Otago	50	98	192	0	0
751	Canterbury	46	141	96	0	24	793	Otago	50	113	96	0	0
752	Canterbury	46	142	192	0	48	794	Otago	50	288	96	48	0
753	Southland	48	1	0	0	0	795	Otago	50	427	96	0	0
754	Southland	48	2	96	0	0	796	Otago	50	439	24	0	0
755	Southland	48	3	0	0	0	797	Otago	50	449	48	0	0
756	Southland	48	4	96	0	0	798	Otago	50	522	48	0	0
757	Southland	48	5	96	0	0	799	Otago	50	613	24	0	0
758	Southland	48	6	48	0	0	800	Otago	50	626	48	0	0
759	Southland	48	7	192	0	0	801	Otago	50	700	48	0	0
760	Southland	48	8	96	0	0	802	Otago	50	953	24	0	0
761	Southland	48	9	192	0	0	803	Otago	50	955	96	0	0
762	Southland	48	10	48	0	0	804	Otago	50	956	192	0	0
763	Southland	48	11	48	0	0	805	Otago	50	957	384	0	0
764	Southland	48	12	96	0	0	806	Otago	50	987	96	0	0
765	Southland	48	13	48	0	0	807	Otago	50	1335	384	0	0
766	Southland	48	14	0	0	0	808	Otago	50	1348	384	0	0
767	Southland	48	15	24	0	0	809	Otago	50	02-955	192	0	0
768	Southland	48	16	48	0	0	810	Canterbury	51	433	96	0	0
769	Southland	48	17	0	0	0	811	Canterbury	51	434	48	0	0
770	Otago	49	61	48	0	0	812	Canterbury	51	485	48	0	0
771	Otago	49	62	48	0	0	813	Canterbury	51	1587	96	0	0
772	Otago	49	63	96	0	0	814	Canterbury	51	642	0	0	0
773	Otago	49	64	48	0	0	815	Canterbury	51	650	96	0	0
774	Otago	49	65	48	0	0	816	Canterbury	51	618	24	0	0
775	Otago	49	66	48	0	0	817	Canterbury	51	824	24	0	0
776	Otago	49	No tag	768	0	0	818	Canterbury	51	825	24	0	0
777	Otago	49	68	192	0	0	819	Canterbury	51	826	768	0	0
778	Otago	49	69	24	0	0	820	Canterbury	51	827	384	0	0
779	Otago	49	70	24	0	0	821	Canterbury	51	828	24	0	0
780	Otago	49	71	24	0	0	822	Canterbury	51	1579	0	0	0
781	Otago	49	72	48	0	0	823	Canterbury	51	1563	0	0	0
782	Otago	49	73	48	0	0	824	Canterbury	51	1743	0	0	0
783	Otago	49	74	48	0	0	825	Canterbury	51	1755	24	0	0
784	Otago	49	75	96	0	0	826	Canterbury	51	1757	24	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
827	Canterbury	51	1759	24	0	0	869	Canterbury	53	y9886	48	0	0
828	Canterbury	51	1769	24	0	0	870	Canterbury	54	95	24	0	0
829	Canterbury	51	1779	48	0	0	871	Canterbury	54	130	192	0	0
830	Waikato	52	22	0	0	0	872	Canterbury	54	140	0	0	0
831	Waikato	52	42	0	0	0	873	Canterbury	54	141	0	0	0
832	Waikato	52	1196	0	0	0	874	Canterbury	54	143	48	0	0
833	Waikato	52	4927	0	0	0	875	Canterbury	54	146	96	0	0
834	Waikato	52	9587	0	0	0	876	Canterbury	54	147	0	0	0
835	Waikato	52	9641	0	0	0	877	Canterbury	54	149	192	0	0
836	Waikato	52	9650	0	0	0	878	Canterbury	54	156	48	0	0
837	Waikato	52	9711	0	0	0	879	Canterbury	54	161	24	0	0
838	Waikato	52	9733	0	0	0	880	Canterbury	54	163	48	0	0
839	Waikato	52	9741	0	0	0	881	Canterbury	54	182	0	0	0
840	Waikato	52	9746	24	0	0	882	Canterbury	54	183	48	0	0
841	Waikato	52	9802	0	0	0	883	Canterbury	54	187	192	0	0
842	Waikato	52	9812	0	0	0	884	Canterbury	54	188	96	0	0
843	Waikato	52	9835	0	0	0	885	Canterbury	54	189	192	0	0
844	Waikato	52	9837	0	0	0	886	Canterbury	54	190	96	0	0
845	Waikato	52	9837	0	0	0	887	Canterbury	54	194	24	0	0
846	Waikato	52	9905	0	0	0	888	Canterbury	54	195	96	0	0
847	Waikato	52	9918	0	0	0	889	Canterbury	54	196	48	0	0
848	Waikato	52	9919	0	0	0	890	Canterbury	55	54	48	0	0
849	Waikato	52	9939	0	0	0	891	Canterbury	55		48	0	0
850	Canterbury	53	20	96	0	0	892	Canterbury	55	147	96	0	0
851	Canterbury	53	50	24	0	0	893	Canterbury	55	149	384	0	0
852	Canterbury	53	140	96	0	0	894	Canterbury	55	193	48	0	0
853	Canterbury	53	614	96	0	0	895	Canterbury	55	212	768	0	0
854	Canterbury	53	088h4	96	0	0	896	Canterbury	55	213	96	0	0
855	Canterbury	53	b2	24	0	0	897	Canterbury	55	224	96	0	0
856	Canterbury	53	bw8	48	0	24	898	Canterbury	55	245	96	0	0
857	Canterbury	53	g7	48	0	0	899	Canterbury	55	246	96	0	0
858	Canterbury	53	o1989	48	0	0	900	Canterbury	55	371	96	0	0
859	Canterbury	53	r131	768	0	24	901	Canterbury	55	446	192	0	0
860	Canterbury	53	r59	192	0	0	902	Canterbury	55	447	48	0	0
861	Canterbury	53	w4	96	0	0	903	Canterbury	55	455	96	0	0
862	Canterbury	53	y119	24	0	0	904	Canterbury	55	465	192	0	0
863	Canterbury	53	y147	24	0	0	905	Canterbury	55	580	48	0	0
864	Canterbury	53	y154	48	0	0	906	Canterbury	55	10066	48	0	0
865	Canterbury	53	y161	96	0	0	907	Canterbury	55	230a	48	0	0
866	Canterbury	53	y188	48	0	0	908	Canterbury	55	h6	96	0	0
867	Canterbury	53	y47	96	0	0	909	Canterbury	55	r77	96	0	0
868	Canterbury	53	y57	1536	0	0	910	Canterbury	56	97	96	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
911	Canterbury	56	131	48	0	0	953	Northland	58	164	192	0	0
912	Canterbury	56	892	384	0	0	954	Northland	58	1	192	0	0
913	Canterbury	56	1329	192	0	0	955	Northland	58	168	384	0	0
914	Canterbury	56	1373	48	0	0	956	Northland	58	169	96	0	0
915	Canterbury	56	8125	96	0	0	957	Northland	58	170	96	0	0
916	Canterbury	56	8318	48	0	0	958	Northland	58	171	48	0	0
917	Canterbury	56	8325	192	0	0	959	Northland	58	187	48	0	0
918	Canterbury	56	8329	192	0	0	960	Northland	58	188	96	0	0
919	Canterbury	56	9136	48	0	0	961	Northland	58	189	96	0	0
920	Canterbury	56	9143	96	0	0	962	Northland	58	200	48	0	0
921	Canterbury	56	9149	24	0	24	963	Northland	58	202	96	0	0
922	Canterbury	56	9155	48	0	0	964	Northland	58	203	96	0	0
923	Canterbury	56	92	48	0	0	965	Northland	58	204	384	0	0
924	Canterbury	56	9279	192	0	0	966	Northland	58	209	384	0	0
925	Canterbury	56	9283	24	0	0	967	Northland	58	210	96	0	0
926	Canterbury	56	9284	48	0	0	968	Northland	58	260	48	0	0
927	Canterbury	56	9334	96	0	0	969	Northland	58	164a	48	0	0
928	Canterbury	56	a6	48	0	0	970	Otago	59	1	48	0	0
929	Canterbury	56	or/115	48	0	24	971	Otago	59	2	24	0	0
930	Northland	57	18	96	0	0	972	Otago	59	3	0	0	0
931	Northland	57	20	192	0	0	973	Otago	59	4	0	0	0
932	Northland	57	21	96	0	0	974	Otago	59	5	0	0	0
933	Northland	57	28	48	0	0	975	Otago	59	6	0	0	0
934	Northland	57	29	48	0	0	976	Otago	59	7	0	0	0
935	Northland	57	33	96	0	0	977	Otago	59	8	0	0	0
936	Northland	57	62	96	0	0	978	Otago	59	9	48	0	0
937	Northland	57	82	96	0	0	979	Otago	59	10	0	0	0
938	Northland	57	84	384	0	0	980	Otago	59	11	0	0	0
939	Northland	57	122	192	0	0	981	Otago	59	12	24	0	0
940	Northland	57	144	768	0	0	982	Otago	59	13	24	0	0
941	Northland	57	157	192	0	0	983	Otago	59	14	48	0	0
942	Northland	57	165	96	0	0	984	Otago	59	15	24	0	0
943	Northland	57	213	96	0	0	985	Otago	59	16	24	0	0
944	Northland	57	226	96	0	0	986	Otago	59	17	48	0	0
945	Northland	57	231	96	0	0	987	Otago	59	18	24	0	0
946	Northland	57	232	384	0	0	988	Otago	59	19	0	0	0
947	Northland	57	236	192	0	0	989	Otago	59	20	24	0	0
948	Northland	57	237	48	0	0	990	West Coast	60	33	48	0	0
949	Northland	57	343	96	0	0	991	West Coast	60	36	48	0	0
950	Northland	58	104	96	0	0	992	West Coast	60	38	48	0	0
951	Northland	58	155	192	0	0	993	West Coast	60	1377	48	0	0
952	Northland	58	158	192	0	0	994	West Coast	60	3055	96	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
995	West Coast	60	3059	48	0	0	1037	Southland	62	8	48	0	0
996	West Coast	60	3070	48	0	0	1038	Southland	62	9	96	0	0
997	West Coast	60	3075	24	0	0	1039	Southland	62	10	192	0	0
998	West Coast	60	3077	192	0	0	1040	Southland	62	11	0	0	0
999	West Coast	60	3079	48	0	0	1041	Southland	62	12	384	0	0
1000	West Coast	60	3900	48	0	0	1042	Sou hland	62	13	48	0	0
1001	West Coast	60	4056	24	0	0	1043	Southland	62	14	0	0	0
1002	West Coast	60	6117	48	0	0	1044	Southland	62	15	48	0	0
1003	West Coast	60	9297	24	0	0	1045	Southland	62	16	48	0	0
1004	West Coast	60	1 F	24	0	0	1046	Sou hland	62	17	96	0	0
1005	West Coast	60	14a	192	0	0	1047	Sou hland	62	18	0	0	0
1006	West Coast	60	14b	24	0	0	1048	Southland	62	19	48	0	0
1007	West Coast	60	25 Purpule	0	0	0	1049	Sou hland	62	20	0	0	0
1008	West Coast	60	Nt Big	192	0	0	1050	Bo.Plen-Gisborne	63	287	192	0	0
1009	West Coast	60	Nt Big	24	0	0	1051	Bo.Plen-Gisborne	63	210	96	0	0
1010	Southland	61	1	0	0	0	1052	Bo.Plen-Gisborne	63	238	48	0	0
1011	Southland	61	2	0	0	0	1053	Bo.Plen-Gisborne	63	22	384	0	0
1012	Southland	61	3	0	0	0	1054	Bo.Plen-Gisborne	63	29	48	0	0
1013	Southland	61	4	0	0	0	1055	Bo.Plen-Gisborne	63	145	192	0	0
1014	Southland	61	5	0	0	0	1056	Bo.Plen-Gisborne	63	240	0	0	0
1015	Southland	61	6	0	0	0	1057	Bo.Plen-Gisborne	63	176	384	0	0
1016	Southland	61	7	0	0	0	1058	Bo.Plen-Gisborne	63	232	48	0	0
1017	Southland	61	8	0	0	0	1059	Bo.Plen-Gisborne	63	215	192	0	0
1018	Southland	61	9	0	0	0	1060	Bo.Plen-Gisborne	63	239	24	0	0
1019	Southland	61	10	0	0	0	1061	Bo.Plen-Gisborne	63	151	48	0	0
1020	Southland	61	11	0	0	0	1062	Bo.Plen-Gisborne	63	124	192	0	0
1021	Southland	61	12	0	0	0	1063	Bo.Plen-Gisborne	63	2	192	0	0
1022	Southland	61	13	0	0	0	1064	Bo.Plen-Gisborne	63	77	96	0	0
1023	Southland	61	14	0	0	0	1065	Bo.Plen-Gisborne	63	10	192	0	0
1024	Sou hland	61	15	0	0	0	1066	Bo.Plen-Gisborne	63	150	384	0	0
1025	Southland	61	16	0	0	0	1067	Bo.Plen-Gisborne	63	55	0	0	0
1026	Southland	61	17	0	0	0	1068	Bo.Plen-Gisborne	63	211	48	0	0
1027	Southland	61	18	0	0	0	1069	Bo.Plen-Gisborne	63	168	384	0	0
1028	Southland	61	19	0	0	0	1070	West Coast	64	1	0	0	0
1029	Southland	61	20	0	0	0	1071	West Coast	64	2	0	0	0
1030	Southland	62	1	96	0	0	1072	West Coast	64	3	24	0	0
1031	Southland	62	2	0	0	0	1073	West Coast	64	4	24	0	0
1032	Southland	62	3	48	0	0	1074	West Coast	64	5	24	0	0
1033	Southland	62	4	0	0	0	1075	West Coast	64	6	0	0	0
1034	Southland	62	5	96	0	0	1076	West Coast	64	7	48	0	0
1035	Southland	62	6	48	0	0	1077	West Coast	64	8	24	0	0
1036	Southland	62	7	96	0	0	1078	West Coast	64	9	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1079	West Coast	64	10	0	0	0	1121	Canterbury	66	552	48	768	24
1080	West Coast	64	11	24	0	0	1122	Canterbury	66	766	48	384	24
1081	West Coast	64	12	48	0	0	1123	Canterbury	66	542	48	768	24
1082	West Coast	64	13	24	0	0	1124	Canterbury	66	752	384	192	24
1083	West Coast	64	14	24	0	0	1125	Canterbury	66	531	192	384	0
1084	West Coast	64	15	0	0	0	1126	Canterbury	66	723	24	192	0
1085	West Coast	64	16	24	0	0	1127	Canterbury	66	1027	768	96	0
1086	West Coast	64	17	24	0	0	1128	Canterbury	66	653	24	0	0
1087	West Coast	64	18	24	0	0	1129	Canterbury	66	525	384	48	0
1088	West Coast	64	19	0	0	0	1130	Canterbury	66	522	384	48	0
1089	West Coast	64	20	0	0	0	1131	Bo.Plen-Gisborne	67	7	0	0	0
1090	Otago	65	288	48	0	0	1132	Bo.Plen-Gisborne	67	834	0	24	0
1091	Otago	65	280	24	0	0	1133	Bo.Plen-Gisborne	67	690	0	0	0
1092	Otago	65	279	192	0	0	1134	Bo.Plen-Gisborne	67	700	0	0	0
1093	Otago	65	278	48	0	0	1135	Bo.Plen-Gisborne	67	844	0	0	0
1094	Otago	65	276	768	0	0	1136	Bo.Plen-Gisborne	67	698	0	0	0
1095	Otago	65	230	192	0	0	1137	Bo.Plen-Gisborne	67	695	0	0	0
1096	Otago	65	273	768	0	0	1138	Bo.Plen-Gisborne	67	689	0	0	0
1097	Otago	65	271	768	0	0	1139	Bo.Plen-Gisborne	67	723	0	0	0
1098	Otago	65	264	384	0	0	1140	Bo.Plen-Gisborne	67	841	0	0	0
1099	Otago	65	262	384	0	0	1141	Bo.Plen-Gisborne	67	821	0	0	0
1100	Otago	65	292	192	0	0	1142	Bo.Plen-Gisborne	67	837	0	0	0
1101	Otago	65	257	96	0	0	1143	Bo.Plen-Gisborne	67	790	0	0	0
1102	Otago	65	251	96	0	0	1144	Bo.Plen-Gisborne	67	694	0	0	0
1103	Otago	65	240	192	0	0	1145	Bo.Plen-Gisborne	67	692	0	0	0
1104	Otago	65	239	0	0	0	1146	Bo.Plen-Gisborne	67	726	0	0	0
1105	Otago	65	277	192	0	0	1147	Bo.Plen-Gisborne	67	831	0	0	0
1106	Otago	65	235	24	0	0	1148	Bo.Plen-Gisborne	67	721	0	0	0
1107	Otago	65	226	384	0	0	1149	Bo.Plen-Gisborne	67	842	0	0	0
1108	Otago	65	225	0	0	0	1150	Bo.Plen-Gisborne	67	703	0	0	0
1109	Otago	65	223	96	0	0	1151	Canterbury	68	1	0	0	0
1110	Otago	65	215	48	0	0	1152	Canterbury	68	2	0	0	0
1111	Canterbury	66	606	192	0	0	1153	Canterbury	68	3	0	0	0
1112	Canterbury	66	1005	384	1536	24	1154	Canterbury	68	4	0	0	0
1113	Canterbury	66	588	192	384	0	1155	Canterbury	68	5	0	0	0
1114	Canterbury	66	848	0	0	48	1156	Canterbury	68	6	0	0	0
1115	Canterbury	66	583	0	192	0	1157	Canterbury	68	7	0	0	0
1116	Canterbury	66	795	48	0	24	1158	Canterbury	68	8	0	0	0
1117	Canterbury	66	558	24	384	24	1159	Canterbury	68	9	0	0	0
1118	Canterbury	66	782	192	0	0	1160	Canterbury	68	10	0	0	0
1119	Canterbury	66	557	48	768	0	1161	Canterbury	68	11	24	0	0
1120	Canterbury	66	776	24	768	0	1162	Canterbury	68	12	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1163	Canterbury	68	13	24	0	0	1205	Otago	70	240	0	0	0
1164	Canterbury	68	14	0	0	0	1206	Otago	70	241	0	0	0
1165	Canterbury	68	15	0	0	0	1207	Otago	70	247	0	0	0
1166	Canterbury	68	16	0	0	0	1208	Otago	70	278	0	0	0
1167	Canterbury	68	17	0	0	0	1209	Otago	70	314	24	0	0
1168	Canterbury	68	18	0	0	0	1210	Otago	70	396	24	0	0
1169	Canterbury	68	19	0	0	0	1211	West Coast	71	y48	0	0	0
1170	Canterbury	68	20	0	0	0	1212	West Coast	71	or68	48	0	0
1171	Canterbury	69	1	24	0	0	1213	West Coast	71	y520	96	384	0
1172	Canterbury	69	2	0	0	0	1214	West Coast	71	y197	0	0	0
1173	Canterbury	69	3	0	0	0	1215	West Coast	71	y374	48	0	0
1174	Canterbury	69	4	0	0	0	1216	West Coast	71	or249	0	0	0
1175	Canterbury	69	5	0	0	0	1217	West Coast	71	y359	24	0	0
1176	Canterbury	69	6	0	0	0	1218	West Coast	71	or88	96	0	0
1177	Canterbury	69	7	0	0	0	1219	West Coast	71	y26	0	0	0
1178	Canterbury	69	8	0	0	0	1220	West Coast	71	y861	24	0	0
1179	Canterbury	69	9	0	0	0	1221	West Coast	71	y225	0	0	0
1180	Canterbury	69	10	0	0	0	1222	West Coast	71	o835	96	0	0
1181	Canterbury	69	11	0	0	0	1223	West Coast	71	y341	96	0	0
1182	Canterbury	69	12	0	0	0	1224	West Coast	71	y56	48	0	0
1183	Canterbury	69	13	0	0	0	1225	West Coast	71	y326	48	0	0
1184	Canterbury	69	14	0	0	0	1226	West Coast	71	y172	48	0	0
1185	Canterbury	69	15	0	0	0	1227	West Coast	71	y180	48	0	0
1186	Canterbury	69	16	0	0	0	1228	West Coast	71	b82	96	0	0
1187	Canterbury	69	17	0	0	0	1229	West Coast	71	o151	48	0	0
1188	Canterbury	69	18	0	0	0	1230	West Coast	71	b81	0	0	0
1189	Canterbury	69	19	0	0	0	1231	West Coast	71	85353	0	0	0
1190	Canterbury	69	20	0	0	0	1232	West Coast	71	219	24	0	0
1191	Otago	70	27	0	0	0	1233	Bo.Plen-Gisborne	72	1	24	0	0
1192	Otago	70	34	0	0	0	1234	Bo.Plen-Gisborne	72	4	0	0	0
1193	Otago	70	37	0	0	0	1235	Bo.Plen-Gisborne	72	17	24	0	0
1194	Otago	70	45	0	0	0	1236	Bo.Plen-Gisborne	72	200	0	0	0
1195	Otago	70	47	0	0	0	1237	Bo.Plen-Gisborne	72	204	0	0	0
1196	Otago	70	50	0	0	0	1238	Bo.Plen-Gisborne	72	207	0	0	0
1197	Otago	70	92	0	0	0	1239	Bo.Plen-Gisborne	72	210	0	0	0
1198	Otago	70	104	0	0	0	1240	Bo.Plen-Gisborne	72	211	24	0	0
1199	Otago	70	106	0	0	0	1241	Bo.Plen-Gisborne	72	212	24	0	0
1200	Otago	70	136	0	0	0	1242	Bo.Plen-Gisborne	72	215	24	0	0
1201	Otago	70	139	0	0	0	1243	Bo.Plen-Gisborne	72	216	0	0	0
1202	Otago	70	164	0	0	0	1244	Bo.Plen-Gisborne	72	217	0	0	0
1203	Otago	70	196	0	0	0	1245	Bo.Plen-Gisborne	72	221	0	0	0
1204	Otago	70	232	0	0	0	1246	Bo.Plen-Gisborne	72	222	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1247	Bo.Plen-Gisborne	72	225	0	0	0	1289	Southland	74	16	24	0	0
1248	Bo.Plen-Gisborne	72	503	0	0	0	1290	Southland	74	17	48	0	0
1249	Bo.Plen-Gisborne	72	521	0	0	0	1291	Southland	74	18	24	0	0
1250	Bo.Plen-Gisborne	72	658	0	0	0	1292	Southland	74	19	96	0	0
1251	Bo.Plen-Gisborne	72	846	0	0	0	1293	Southland	74	20	96	0	0
1252	Bo.Plen-Gisborne	72	b15	0	0	0	1294	Bo.Plen-Gisborne	75	Ntag 1	384	0	0
1253	Bo.Plen-Gisborne	72	nt3	0	0	0	1295	Bo.Plen-Gisborne	75	Ntag 2	192	0	0
1254	Southland	73	1	0	0	0	1296	Bo.Plen-Gisborne	75	Ntag 3	96	0	0
1255	Southland	73	2	0	0	0	1297	Bo.Plen-Gisborne	75	Ntag 4	384	0	0
1256	Southland	73	3	24	0	0	1298	Bo.Plen-Gisborne	75	Ntag 5	96	0	0
1257	Southland	73	4	96	0	0	1299	Bo.Plen-Gisborne	75	Ntag 6	96	0	0
1258	Southland	73	5	96	0	0	1300	Bo.Plen-Gisborne	75	Ntag 7	192	0	0
1259	Southland	73	6	48	0	0	1301	Bo.Plen-Gisborne	75	Ntag 8	384	0	0
1260	Southland	73	7	96	0	0	1302	Bo.Plen-Gisborne	75	Ntag 9	768	0	0
1261	Southland	73	8	192	0	0	1303	Bo.Plen-Gisborne	75	Ntag 10	96	0	0
1262	Southland	73	9	48	0	0	1304	Bo.Plen-Gisborne	75	Ntag 11	96	0	0
1263	Southland	73	10	192	0	0	1305	Bo.Plen-Gisborne	75	Ntag 12	384	0	0
1264	Southland	73	11	384	0	0	1306	Bo.Plen-Gisborne	75	Ntag 13	768	0	0
1265	Southland	73	12	384	0	0	1307	Bo.Plen-Gisborne	75	g137	24	0	0
1266	Southland	73	13	24	0	0	1308	Bo.Plen-Gisborne	75	t876	24	0	0
1267	Southland	73	14	48	0	0	1309	Bo.Plen-Gisborne	75	y884	48	0	0
1268	Southland	73	15	96	0	0	1310	Bo.Plen-Gisborne	75	y887	48	0	0
1269	Southland	73	16	192	0	0	1311	Bo.Plen-Gisborne	75	y888	96	0	0
1270	Southland	73	17	96	0	0	1312	Bo.Plen-Gisborne	75	y917	48	0	0
1271	Southland	73	18	96	0	0	1313	Bo.Plen-Gisborne	75	y919	96	0	0
1272	Southland	73	19	384	0	0	1314	Bo.Plen-Gisborne	75	y925	96	0	0
1273	Southland	73	20	48	0	0	1315	Bo.Plen-Gisborne	75	y928	96	0	0
1274	Southland	74	1	96	0	0	1316	Bo.Plen-Gisborne	75	y945	96	0	0
1275	Southland	74	2	24	0	0	1317	Bo.Plen-Gisborne	75	y954	192	0	0
1276	Southland	74	3	192	0	0	1318	Otago	76	40	384	0	0
1277	Southland	74	4	96	0	0	1319	Otago	76	121	96	0	0
1278	Southland	74	5	0	0	0	1320	Otago	76	125	96	0	0
1279	Southland	74	6	24	0	0	1321	Otago	76	127	384	0	0
1280	Southland	74	7	192	0	0	1322	Otago	76	136	96	0	0
1281	Southland	74	8	0	0	0	1323	Otago	76	161	24	0	0
1282	Southland	74	9	48	0	0	1324	Otago	76	168	96	0	0
1283	Southland	74	10	24	0	0	1325	Otago	76	179	192	0	0
1284	Southland	74	11	48	0	0	1326	Otago	76	180	192	0	0
1285	Southland	74	12	96	0	0	1327	Otago	76	211	24	0	0
1286	Southland	74	13	24	0	0	1328	Otago	76	223	192	0	0
1287	Southland	74	14	48	0	0	1329	Otago	76	259	96	0	0
1288	Southland	74	15	0	0	0	1330	Otago	76	263	48	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1331	Otago	76	295	96	0	0	1373	Bay of Plenty-Gisborne	78	68	24	0	0
1332	Otago	76	296	48	0	0	1374	Bay of Plenty-Gisborne	78	74	48	0	0
1333	Otago	76	298	48	0	0	1375	Bay of Plenty-Gisborne	78	95	24	0	0
1334	Otago	76	299	96	0	0	1376	Bay of Plenty-Gisborne	78	97	24	0	0
1335	Otago	76	356	24	0	0	1377	Bay of Plenty-Gisborne	78	99	0	0	0
1336	Otago	76	407	384	0	0	1378	Bay of Plenty-Gisborne	78	104	0	0	0
1337	Otago	76	432	96	0	0	1379	Bay of Plenty-Gisborne	78	106	48	0	0
1338	Otago	76	603	48	0	0	1380	Bay of Plenty-Gisborne	78	107	48	0	0
1339	Southland	77	1	384	0	0	1381	Bay of Plenty-Gisborne	78	108	96	0	0
1340	Southland	77	2	96	0	0	1382	Bay of Plenty-Gisborne	78	109	24	0	0
1341	Southland	77	3	192	0	0	1383	Bay of Plenty-Gisborne	78	110	48	0	0
1342	Southland	77	4	192	0	0	1384	Bay of Plenty-Gisborne	78	112	24	0	0
1343	Southland	77	5	96	0	0	1385	Bay of Plenty-Gisborne	78	113	24	0	0
1344	Southland	77	6	48	0	0	1386	Bay of Plenty-Gisborne	78	115	192	0	0
1345	Southland	77	7	192	0	0	1387	Bay of Plenty-Gisborne	78	117	24	0	0
1346	Southland	77	8	384	0	0	1388	Bay of Plenty-Gisborne	78	121	24	0	0
1347	Southland	77	9	384	0	0	1389	Bay of Plenty-Gisborne	78	128	1536	0	0
1348	Southland	77	10	192	0	0	1390	Bay of Plenty-Gisborne	78	129	24	0	0
1349	Southland	77	11	96	0	0	1391	Bay of Plenty-Gisborne	78	132	24	0	0
1350	Southland	77	12	48	0	0	1392	Bay of Plenty-Gisborne	78	138	0	0	0
1351	Southland	77	13	192	0	0	1393	Bay of Plenty-Gisborne	78	139	96	0	0
1352	Southland	77	14	48	0	0	1394	Bay of Plenty-	78	140	192	0	0

								Gisborne					
1353	Southland	77	15	96	0	0	1395	Bay of Plenty-Gisborne	78	146	192	0	0
1354	Southland	77	16	48	0	0	1396	Bay of Plenty-Gisborne	78	156	0	0	0
1355	Southland	77	17	96	0	0	1397	Bay of Plenty-Gisborne	78	157	0	0	0
1356	Southland	77	18	48	0	0	1398	Bay of Plenty-Gisborne	78	159	24	0	0
1357	Southland	77	19	96	0	0	1399	Bay of Plenty-Gisborne	78	162	96	0	0
1358	Southland	77	20	192	0	0	1400	Bay of Plenty-Gisborne	78	164	96	0	0
1359	Bo.Plen-Gisborne	78	10	24	0	0	1401	Bay of Plenty-Gisborne	78	168	24	0	0
1360	Bo.Plen-Gisborne	78	11	24	0	0	1402	Bay of Plenty-Gisborne	78	169	24	0	0
1361	Bo.Plen-Gisborne	78	20	0	0	0	1403	Bay of Plenty-Gisborne	78	171	0	0	0
1362	Bo.Plen-Gisborne	78	21	0	0	0	1404	Bay of Plenty-Gisborne	78	175	0	0	0
1363	Bo.Plen-Gisborne	78	24	192	0	0	1405	Bay of Plenty-Gisborne	78	182	0	0	0
1364	Bo.Plen-Gisborne	78	32	96	0	0	1406	Southland	79	1	24	0	0
1365	Bo.Plen-Gisborne	78	34	24	0	0	1407	Southland	79	2	48	0	0
1366	Bo.Plen-Gisborne	78	49	48	0	0	1408	Southland	79	3	96	0	0
1367	Bo.Plen-Gisborne	78	55	48	0	0	1409	Southland	79	4	96	0	0
1368	Bo.Plen-Gisborne	78	58	48	0	0	1410	Southland	79	5	192	0	0
1369	Bo.Plen-Gisborne	78	59	192	0	0	1411	Southland	79	6	192	0	0
1370	Bo.Plen-Gisborne	78	62	24	0	0	1412	Southland	79	7	48	0	0
1371	Bo.Plen-Gisborne	78	63	48	0	0	1413	Southland	79	8	768	0	0
1372	Bo.Plen-Gisborne	78		24	0	0	1414	Southland	79	9	192	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1415	Southland	79	10	192	0	0	1457	Waikato	82	159	384	0	0
1416	Southland	79	11	192	0	0	1458	Waikato	82	346	48	0	0
1417	Southland	79	12	192	0	0	1459	Waikato	82	396	24	0	0
1418	Southland	79	13	96	0	0	1460	Waikato	82	399	192	0	0
1419	Southland	79	14	96	0	0	1461	Waikato	82	428	0	0	0
1420	Southland	79	15	192	0	0	1462	Waikato	82	474	24	0	0
1421	Southland	79	16	192	0	0	1463	Waikato	82	476	96	0	0
1422	Southland	79	17	48	0	0	1464	Waikato	82	517	192	0	0
1423	Southland	79	18	0	0	0	1465	Waikato	82	522	96	3072	0
1424	Southland	79	19	48	0	0	1466	Waikato	82	532	96	0	0
1425	Southland	79	20	96	0	0	1467	Waikato	82	543	24	0	0
1426	Southland	80	11	24	0	0	1468	Waikato	82	596	96	0	0

1427	Southland	80	12	0	0	0	1469	Waikato	82	601	48	48	0
1428	Southland	80	13	192	0	0	1470	Waikato	82	621	24	0	0
1429	Southland	80	14	24	0	0	1471	Waikato	82	669	48	48	0
1430	Southland	80	15	48	0	0	1472	Waikato	82	727	96	0	0
1431	Southland	80	16	48	0	0	1473	Waikato	82	881	96	0	0
1432	Southland	80	17	192	0	0	1474	Waikato	82	1510	48	0	0
1433	Southland	80	18	24	0	0	1475	Otago	83	301	24	0	0
1434	Southland	80	19	96	0	0	1476	Otago	83	302	0	0	0
1435	Southland	81	1	24	0	0	1477	Otago	83	303	24	0	0
1436	Southland	81	2	48	0	0	1478	Otago	83	304	0	0	0
1437	Southland	81	3	24	0	0	1479	Otago	83	305	0	0	0
1438	Southland	81	4	24	0	0	1480	Otago	83	306	0	0	0
1439	Southland	81	5	0	0	0	1481	Otago	83	307	0	0	0
1440	Southland	81	6	24	0	0	1482	Otago	83	308	0	0	0
1441	Southland	81	7	96	0	0	1483	Otago	83	309	0	0	0
1442	Southland	81	8	0	0	0	1484	Otago	83	310	0	0	0
1443	Southland	81	9	48	0	0	1485	Otago	83	311	0	0	0
1444	Southland	81	10	0	0	0	1486	Otago	83	312	0	0	0
1445	Southland	81	11	96	0	0	1487	Otago	83	313	0	0	0
1446	Southland	81	12	384	0	0	1488	Otago	83	314	0	0	0
1447	Southland	81	13	48	0	0	1489	Otago	83	315	0	0	0
1448	Southland	81	14	96	0	0	1490	Otago	83	316	0	0	0
1449	Southland	81	15	24	0	0	1491	Otago	83	317	0	0	0
1450	Southland	81	16	384	0	0	1492	Otago	83	318	0	0	0
1451	Southland	81	17	96	0	0	1493	Otago	83	319	0	0	0
1452	Southland	81	18	96	0	0	1494	Otago	83	320	0	0	0
1453	Southland	81	19	96	0	0	1495	Southland	84	1	192	0	0
1454	Southland	81	20	96	0	0	1496	Southland	84	2	96	0	0
1455	Waikato	82	5	24	0	0	1497	Southland	84	3	48	0	0
1456	Waikato	82	94	192	192	0	1498	Southland	84	4	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1499	Southland	84	5	24	0	0	1541	Southland	86	7	48	0	0
1500	Southland	84	6	0	0	0	1542	Southland	86	8	0	0	0
1501	Southland	84	7	48	0	0	1543	Southland	86	9	384	0	0
1502	Southland	84	8	192	0	0	1544	Southland	86	10	192	0	0
1503	Southland	84	9	48	0	0	1545	Southland	86	11	24	0	0
1504	Southland	84	10	96	0	0	1546	Southland	86	12	24	0	0
1505	Southland	84	11	192	0	0	1547	Southland	86	13	0	0	0
1506	Southland	84	12	48	0	0	1548	Southland	86	14	0	0	0
1507	Southland	84	13	24	0	0	1549	Southland	86	15	96	0	0
1508	Southland	84	14	24	0	0	1550	Southland	86	16	48	0	0
1509	Southland	84	15	48	0	0	1551	Southland	86	17	24	0	0
1510	Southland	84	16	48	0	0	1552	Southland	86	18	0	0	0
1511	Southland	84	17	48	0	0	1553	Southland	86	19	0	0	0
1512	Southland	84	18	48	0	0	1554	Southland	86	20	0	0	0
1513	Southland	84	19	24	0	0	1555	Southland	87	1	0	0	0
1514	Southland	84	20	96	0	0	1556	Southland	87	2	0	0	0
1515	Southland	85	1	384	96	0	1557	Southland	87	3	24	0	0
1516	Southland	85	2	48	0	0	1558	Southland	87	4	0	0	0
1517	Southland	85	3	48	192	0	1559	Southland	87	5	24	0	0
1518	Southland	85	4	24	192	0	1560	Southland	87	6	0	0	0
1519	Southland	85	5	48	24	0	1561	Southland	87	7	24	0	0
1520	Southland	85	6	24	384	0	1562	Southland	87	8	0	0	0
1521	Southland	85	7	48	24	0	1563	Southland	87	9	48	0	0
1522	Southland	85	8	24	96	0	1564	Southland	87	10	24	0	0
1523	Southland	85	9	96	768	0	1565	Southland	87	11	48	0	0
1524	Southland	85	10	96	0	0	1566	Southland	87	12	24	0	0
1525	Southland	85	11	48	768	0	1567	Southland	87	13	24	0	0
1526	Southland	85	12	96	0	0	1568	Southland	87	14	0	0	0
1527	Southland	85	13	192	384	0	1569	Southland	87	15	24	0	0
1528	Southland	85	14	0	384	0	1570	Southland	87	16	48	0	0
1529	Southland	85	15	48	48	0	1571	Southland	87	17	24	0	0
1530	Southland	85	16	48	24	0	1572	Southland	87	18	48	0	0
1531	Southland	85	17	96	96	0	1573	Southland	87	19	0	0	0
1532	Southland	85	18	48	24	0	1574	Southland	87	20	0	0	0
1533	Southland	85	19	384	24	0	1575	Southland	88	1	24	0	0
1534	Southland	85	20	24	0	0	1576	Southland	88	2	24	0	0
1535	Southland	86	1	192	0	0	1577	Southland	88	3	24	0	0
1536	Southland	86	2	24	0	0	1578	Southland	88	4	0	0	0
1537	Southland	86	3	0	0	0	1579	Southland	88	5	48	0	0
1538	Southland	86	4	0	0	0	1580	Southland	88	6	0	0	0
1539	Southland	86	5	96	0	0	1581	Southland	88	7	24	0	0
1540	Southland	86	6	48	0	0	1582	Southland	88	8	24	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1583	Southland	88	9	48	0	0	1625	Otago	90	3368	0	0	0
1584	Southland	88	10	24	0	0	1626	Otago	90	3374	0	0	0
1585	Southland	88	11	48	0	0	1627	Otago	90	3376	0	0	0
1586	Southland	88	12	48	0	0	1628	Otago	90	3387	0	0	0
1587	Southland	88	13	48	0	0	1629	Otago	90	3402	0	0	0
1588	Southland	88	14	96	0	0	1630	Otago	90	3405	24	0	0
1589	Southland	88	15	96	0	0	1631	Otago	90	3407	0	0	0
1590	Southland	88	16	48	0	0	1632	Otago	90	3408	0	0	0
1591	Southland	88	17	192	0	0	1633	Southland	91	1	192	0	0
1592	Southland	88	18	96	0	0	1634	Southland	91	2	96	0	0
1593	Southland	88	19	96	0	0	1635	Southland	91	3	0	0	0
1594	Waikato	89	2001	24	0	0	1636	Southland	91	4	24	0	0
1595	Waikato	89	2001	24	0	0	1637	Southland	91	5	48	0	0
1596	Waikato	89	2006	24	0	0	1638	Southland	91	6	24	0	0
1597	Waikato	89	2012	48	0	0	1639	Southland	91	7	0	0	0
1598	Waikato	89	2010	0	0	0	1640	Southland	91	8	96	0	0
1599	Waikato	89	2021	0	0	0	1641	Southland	91	9	24	0	0
1600	Waikato	89	2022	24	0	0	1642	Southland	91	10	0	0	0
1601	Waikato	89	2023	48	0	0	1643	Southland	91	11	384	0	0
1602	Waikato	89	2036	24	0	0	1644	Southland	91	12	48	0	0
1603	Waikato	89	2035	24	0	0	1645	Southland	91	13	48	0	0
1604	Waikato	89	2043	24	0	0	1646	Southland	91	14	0	0	0
1605	Waikato	89	2045	48	0	0	1647	Southland	91	15	48	0	0
1606	Waikato	89	2046	96	0	0	1648	Southland	91	16	48	0	0
1607	Waikato	89	2048	24	0	0	1649	Southland	91	17	0	0	0
1608	Waikato	89	2051	24	0	0	1650	Southland	91	18	192	0	0
1609	Waikato	89	2058	48	0	0	1651	Southland	91	19	0	0	0
1610	Waikato	89	2059	0	0	0	1652	Southland	91	20	0	0	0
1611	Waikato	89	2065	0	0	0	1653	Canterbury	92	NT 1	0	0	0
1612	Waikato	89	2100	0	0	0	1654	Canterbury	92	5	0	0	0
1613	Otago	90	3335	0	0	0	1655	Canterbury	92	598	0	0	0
1614	Otago	90	3337	0	0	0	1656	Canterbury	92	621	0	0	0
1615	Otago	90	3339	0	0	0	1657	Canterbury	92	634	0	0	0
1616	Otago	90	3340	0	0	0	1658	Canterbury	92	681	0	0	0
1617	Otago	90	3341	0	0	0	1659	Canterbury	92	682	0	0	0
1618	Otago	90	3347	0	0	0	1660	Canterbury	92	689	0	0	0
1619	Otago	90	3349	0	0	0	1661	Canterbury	92	694	0	0	0
1620	Otago	90	3357	0	0	0	1662	Canterbury	92	696	0	0	0
1621	Otago	90	3358	0	0	0	1663	Canterbury	92	722	0	0	0
1622	Otago	90	3360	0	0	0	1664	Canterbury	92	723	0	0	0
1623	Otago	90	3365	0	0	0	1665	Canterbury	92	749	0	0	0
1624	Otago	90	33	0	0	0	1666	Canterbury	92	776	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1667	Canterbury	92	790	0	0	0	1709	Canterbury	94	wh 1.45	0	0	0
1668	Canterbury	92	920	0	0	0	1710	Canterbury	94	y 1.	48	0	0
1669	Canterbury	92	1106	0	0	0	1711	Canterbury	94	Y9.515	0	0	0
1670	Canterbury	92	1448	0	0	0	1712	Canterbury	94	YO 0.63	0	0	0
1671	Canterbury	92	1469	0	0	0	1713	Canterbury	95	10	0	0	0
1672	Canterbury	92	1522	0	0	0	1714	Canterbury	95	12	24	0	0
1673	Canterbury	93	5	0	0	0	1715	Canterbury	95	157	0	0	0
1674	Canterbury	93	6	0	0	0	1716	Canterbury	95	170	192	0	0
1675	Canterbury	93	8	0	0	0	1717	Canterbury	95	183	0	0	0
1676	Canterbury	93	28	0	0	0	1718	Canterbury	95	200	96	0	0
1677	Canterbury	93	29	0	0	0	1719	Canterbury	95	210	0	0	0
1678	Canterbury	93	30	0	0	0	1720	Canterbury	95	211	24	0	0
1679	Canterbury	93	34	0	0	0	1721	Canterbury	95	266	48	0	0
1680	Canterbury	93	39	0	0	0	1722	Canterbury	95	325	96	0	0
1681	Canterbury	93	47	0	0	0	1723	Canterbury	95	329	0	0	0
1682	Canterbury	93	52	0	0	0	1724	Canterbury	95	483	0	0	0
1683	Canterbury	93	39 A	0	0	0	1725	Canterbury	95	500	48	0	0
1684	Canterbury	93	NT 1	0	0	0	1726	Canterbury	95	522	192	0	0
1685	Canterbury	93	NT 11	0	0	0	1727	Canterbury	95	527	0	0	0
1686	Canterbury	93	NT 2	0	0	0	1728	Canterbury	95	616	48	0	0
1687	Canterbury	93	NT 3	0	0	0	1729	Canterbury	95	626	0	0	0
1688	Canterbury	93	NT 5	0	0	0	1730	Canterbury	95	629	0	0	0
1689	Canterbury	93	NT 6	0	0	0	1731	Canterbury	95	703	0	0	0
1690	Canterbury	93	NT 7	0	0	0	1732	Canterbury	95	805	0	0	0
1691	Canterbury	93	NT 8	0	0	0	1733	Canterbury	96	1	0	0	0
1692	Canterbury	93	NT 9	0	0	0	1734	Canterbury	96	16	0	0	0
1693	Canterbury	94	2.23	24	0	0	1735	Canterbury	96	20	0	0	0
1694	Canterbury	94	g 3.61	0	0	0	1736	Canterbury	96	207	0	0	0
1695	Canterbury	94	g 4.74	0	0	0	1737	Canterbury	96	223	0	0	0
1696	Canterbury	94	G 6.11	24	0	0	1738	Canterbury	96	259	0	0	0
1697	Canterbury	94	g 6.59	0	0	0	1739	Canterbury	96	2	0	0	0
1698	Canterbury	94	G.7.	0	0	0	1740	Canterbury	96	275	0	0	0
1699	Canterbury	94	Gr 5.5	96	0	0	1741	Canterbury	96	00 45	0	0	0
1700	Canterbury	94	lg	48	0	0	1742	Canterbury	96	gr 30	0	0	0
1701	Canterbury	94	lg 685	0	0	0	1743	Canterbury	96	nt 2	0	0	0
1702	Canterbury	94	Lg 7.52	48	0	0	1744	Canterbury	96	nt 5	0	0	0
1703	Canterbury	94	Lg 8.2	96	0	0	1745	Canterbury	96	O 270	0	0	0
1704	Canterbury	94	Lg 8.65	96	0	0	1746	Canterbury	96	or 199	0	0	0
1705	Canterbury	94	lg 8.	24	0	0	1747	Canterbury	96	or 254	0	0	0
1706	Canterbury	94	Lu 2.41	96	0	0	1748	Canterbury	96	y 14	0	0	0
1707	Canterbury	94	Or 6.63	0	0	0	1749	Canterbury	96	y 203	0	0	0
1708	Canterbury	94	Or 618	48	0	0	1750	Canterbury	96	y 216	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1751	Canterbury	96	y 266	0	0	0	1793	Well-Manawatu	99	2B	24	48	24
1752	Canterbury	96	y5	0	0	0	1794	Well-Manawatu	99	6B	24	48	24
1753	Canterbury	97	113 A	96	0	0	1795	Well-Manawatu	99	8B	24	48	24
1754	Canterbury	97	13 B	48	0	0	1796	Well-Manawatu	99	9B	24	48	24
1755	Canterbury	97	45	24	0	0	1797	Well-Manawatu	99	1B	48	48	24
1756	Canterbury	97	99	0	0	0	1798	Well-Manawatu	99	7B	48	48	24
1757	Canterbury	97	112	192	0	0	1799	Well-Manawatu	99	3B	96	48	24
1758	Canterbury	97	59	0	0	0	1800	Well-Manawatu	99	4B	96	48	24
1759	Canterbury	97	89	48	0	0	1801	Well-Manawatu	99	5B	96	48	24
1760	Canterbury	97	663	0	0	0	1802	Well-Manawatu	100	6C	0	24	0
1761	Canterbury	97	139	0	0	0	1803	Well-Manawatu	100	7C	0	24	0
1762	Canterbury	97	83	0	0	0	1804	Well-Manawatu	100	1C	0	48	0
1763	Canterbury	97	755	0	0	0	1805	Well-Manawatu	100	2C	0	48	0
1764	Canterbury	97	132	96	0	0	1806	Well-Manawatu	100	3C	0	48	0
1765	Canterbury	97	8	24	0	0	1807	Well-Manawatu	100	5C	0	48	0
1766	Canterbury	97	120	48	0	0	1808	Well-Manawatu	100	8C	0	48	0
1767	Canterbury	97	142	24	0	0	1809	Well-Manawatu	100	4C	0	96	0
1768	Canterbury	97	120 B	0	0	0	1810	Well-Manawatu	100	9C	0	96	0
1769	Canterbury	97	644	0	0	0	1811	Well-Manawatu	101	11D	0	0	0
1770	Canterbury	97	46	48	0	0	1812	Well-Manawatu	101	1D	0	0	0
1771	Canterbury	97	115	96	0	0	1813	Well-Manawatu	101	2D	0	0	0
1772	Canterbury	97	832	24	0	0	1814	Well-Manawatu	101	12D	24	0	0
1773	Otago	98	y59	0	0	0	1815	Well-Manawatu	101	13D	24	0	0
1774	Otago	98	y83	0	0	0	1816	Well-Manawatu	101	8D	24	0	0
1775	Otago	98	y129	0	0	0	1817	Well-Manawatu	101	10D	24	24	0
1776	Otago	98	y23	0	0	0	1818	Well-Manawatu	101	3D	48	0	0
1777	Otago	98	659	0	0	0	1819	Well-Manawatu	101	4D	48	0	0
1778	Otago	98	y190	0	0	0	1820	Well-Manawatu	101	6D	48	0	0
1779	Otago	98	y266	0	0	0	1821	Well-Manawatu	101	5D	192	0	0
1780	Otago	98	y83/98	0	0	0	1822	Well-Manawatu	101	7D	192	0	0
1781	Otago	98	red 118	0	0	0	1823	Well-Manawatu	101	9D	768	24	0
1782	Otago	98	red 112	0	0	0	1824	Well-Manawatu	102	1E	0	0	0
1783	Otago	98	216	24	0	0	1825	Well-Manawatu	102	8E	24	0	0
1784	Otago	98	372	0	0	0	1826	Well-Manawatu	102	11E	0	0	0
1785	Otago	98	y41	24	0	0	1827	Well-Manawatu	102	4E	0	24	0
1786	Otago	98	red 402	0	0	0	1828	Well-Manawatu	102	5E	48	0	0
1787	Otago	98	y189	0	0	0	1829	Well-Manawatu	102	10E	48	0	0
1788	Otago	98	y120	0	0	0	1830	Well-Manawatu	102	6E	48	0	0
1789	Otago	98	y156	0	0	0	1831	Well-Manawatu	102	13E	48	0	0
1790	Otago	98	y46	0	0	0	1832	Well-Manawatu	102	12E	384	0	0
1791	Otago	98	171	0	0	0	1833	Well-Manawatu	102	9E	768	0	0
1792	Otago	98	y150	0	0	0	1834	Well-Manawatu	102	7E	48	24	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1835	Well-Manawatu	102	2E	1536	0	24	1877	Well-Manawatu	105	15H	96	0	24
1836	Well-Manawatu	102	3E	384	0	48	1878	Well-Manawatu	105	4H	96	24	24
1837	Well-Manawatu	103	8F	0	0	0	1879	Well-Manawatu	105	5H	192	24	24
1838	Well-Manawatu	103	10F	0	0	0	1880	Well-Manawatu	105	10H	96	48	0
1839	Well-Manawatu	103	13F	0	0	0	1881	Well-Manawatu	105	14H	48	48	24
1840	Well-Manawatu	103	1F	24	0	0	1882	Well-Manawatu	107	8J	0	0	0
1841	Well-Manawatu	103	3F	24	0	0	1883	Well-Manawatu	107	7J	0	0	0
1842	Well-Manawatu	103	5F	24	0	0	1884	Well-Manawatu	107	1J	0	0	0
1843	Well-Manawatu	103	6F	24	0	0	1885	Well-Manawatu	107	2J	0	0	0
1844	Well-Manawatu	103	7F	24	0	0	1886	Well-Manawatu	107	3J	0	0	0
1845	Well-Manawatu	103	9F	0	24	0	1887	Well-Manawatu	107	4J	0	0	0
1846	Well-Manawatu	103	11F	0	0	24	1888	Well-Manawatu	107	5J	0	0	0
1847	Well-Manawatu	103	12F	0	0	24	1889	Well-Manawatu	107	6J	0	0	0
1848	Well-Manawatu	103	4F	24	0	24	1890	Well-Manawatu	107	10J	0	0	0
1849	Well-Manawatu	103	14F	24		0	1891	Well-Manawatu	107	11J	0	0	0
1850	Well-Manawatu	103	15F	24		24	1892	Well-Manawatu	107	13J	0	24	0
1851	Well-Manawatu	103	2F	96	0	0	1893	Well-Manawatu	107	14J	0	24	0
1852	Well-Manawatu	104	3G	0	0	0	1894	Well-Manawatu	107	15J	0	24	0
1853	Well-Manawatu	104	6G	0	0	0	1895	Well-Manawatu	107	12J	0	24	0
1854	Well-Manawatu	104	11G	0	0	0	1896	Well-Manawatu	107	9J	24	48	0
1855	Well-Manawatu	104	14G	0	0	0	1897	Hawkes Bay	108	1	0	0	0
1856	Well-Manawatu	104	8G	24	0	0	1898	Hawkes Bay	108	2	0	0	0
1857	Well-Manawatu	104	15G	48	0	0	1899	Hawkes Bay	108	3	0	0	0
1858	Well-Manawatu	104	5G	48	0	0	1900	Hawkes Bay	108	4	0	0	0
1859	Well-Manawatu	104	7G	48	0	0	1901	Hawkes Bay	108	5	0	0	0
1860	Well-Manawatu	104	10G	48	0	0	1902	Hawkes Bay	108	6	0	0	0
1861	Well-Manawatu	104	9G	192	0	0	1903	Hawkes Bay	108	7	24	0	0
1862	Well-Manawatu	104	12G	768	0	0	1904	Hawkes Bay	108	8	0	0	0
1863	Well-Manawatu	104	13G	1536	0	0	1905	Hawkes Bay	108	9	0	0	0
1864	Well-Manawatu	104	4G	48	0	24	1906	Hawkes Bay	108	10	0	0	0
1865	Well-Manawatu	104	1G	96	96	0	1907	Hawkes Bay	108	11	0	0	0
1866	Well-Manawatu	104	2G	96	48	24	1908	Hawkes Bay	108	12	0	0	0
1867	Well-Manawatu	105	2H	24	0	0	1909	Hawkes Bay	108	13	0	0	0
1868	Well-Manawatu	105	6H	0	24	0	1910	Hawkes Bay	108	14	0	0	0
1869	Well-Manawatu	105	9H	96	0	0	1911	Hawkes Bay	108	15	0	0	0
1870	Well-Manawatu	105	11H	96	0	0	1912	Hawkes Bay	108	16	0	0	0
1871	Well-Manawatu	105	12H	96	0	0	1913	Hawkes Bay	108	17	0	0	0
1872	Well-Manawatu	105	3H	192	0	0	1914	Hawkes Bay	108	18	0	0	0
1873	Well-Manawatu	105	1H	192	0	0	1915	Hawkes Bay	108	19	0	0	0
1874	Well-Manawatu	105	7H	192	0	0	1916	Hawkes Bay	108	20	0	0	0
1875	Well-Manawatu	105	8H	48	24	0	1917	Well-Manawatu	109	2	0	192	0
1876	Well-Manawatu	105	13H	96	24	0	1918	Well-Manawatu	109	16	24	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni	No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
1919	Well-Manawatu	109	18	0	96	0	1961	Hawkes Bay	111	126	0	0	0
1920	Well-Manawatu	109	24	0	48	0	1962	Hawkes Bay	111	9069	0	0	0
1921	Well-Manawatu	109	25-a	0	24	0	1963	Hawkes Bay	111	6005	0	0	0
1922	Well-Manawatu	109	25-b	0	96	0	1964	Hawkes Bay	111	30	0	0	0
1923	Well-Manawatu	109	29	24	1532	0	1965	Hawkes Bay	111	62	0	0	0
1924	Well-Manawatu	109	86	48	0	0	1966	Hawkes Bay	111	84	0	0	0
1925	Well-Manawatu	109	102	48	96	24	1967	Hawkes Bay	111	5125	0	0	0
1926	Well-Manawatu	109	113	96	0	0	1968	Hawkes Bay	111	26	0	0	0
1927	Well-Manawatu	109	120	0	96	24	1969	Hawkes Bay	111	75	0	0	0
1928	Well-Manawatu	109	168	0	0	0	1970	Hawkes Bay	111	6	0	0	0
1929	Well-Manawatu	109	173	24	0	0	1971	Hawkes Bay	111	91	0	0	0
1930	Well-Manawatu	109	175	0	96	0	1972	Hawkes Bay	111	74	0	0	0
1931	Well-Manawatu	109	179	24	0	0	1973	Hawkes Bay	111	13	0	0	0
1932	Well-Manawatu	109	182	0	192	0	1974	Hawkes Bay	111	105	0	0	0
1933	Well-Manawatu	109	183	24	384	0	1975	Hawkes Bay	111	3084	0	0	0
1934	Well-Manawatu	109	185	24	96	0	1976	Hawkes Bay	112	5	0	192	0
1935	Well-Manawatu	109	186	0	96	0	1977	Hawkes Bay	112	37	0	96	0
1936	Hawkes Bay	110	1	96	96	0	1978	Hawkes Bay	112	97	0	24	0
1937	Hawkes Bay	110	2	38	0	0	1979	Hawkes Bay	112	106	24	96	24
1938	Hawkes Bay	110	3	24	0	0	1980	Hawkes Bay	112	62	0	96	0
1939	Hawkes Bay	110	4	384	48	0	1981	Hawkes Bay	112	36	0	48	0
1940	Hawkes Bay	110	5	768	48	0	1982	Hawkes Bay	112	81	0	0	0
1941	Hawkes Bay	110	6	384	0	0	1983	Hawkes Bay	112	15	0	0	0
1942	Hawkes Bay	110	7	768	0	0	1984	Hawkes Bay	112	205	24	0	0
1943	Hawkes Bay	110	8	96	96	0	1985	Hawkes Bay	112	57	0	48	0
1944	Hawkes Bay	110	9	48	96	0	1986	Hawkes Bay	112	37	0	96	0
1945	Hawkes Bay	110	10	96	0	0	1987	Hawkes Bay	112	6	0	0	0
1946	Hawkes Bay	110	11	192	48	0	1988	Hawkes Bay	112	43	24	0	0
1947	Hawkes Bay	110	12	48	96	0	1989	Hawkes Bay	112	25	0	24	0
1948	Hawkes Bay	110	13	0	0	0	1990	Hawkes Bay	112	53	0	192	24
1949	Hawkes Bay	110	14	48	192	0	1991	Hawkes Bay	112	38	0	0	0
1950	Hawkes Bay	110	15	192	0	0	1992	Hawkes Bay	112	35	0	24	24
1951	Hawkes Bay	110	16	48	384	0	1993	Hawkes Bay	112	89	0	96	0
1952	Hawkes Bay	110	17	48	0	24	1994	Hawkes Bay	112	48	0	768	24
1953	Hawkes Bay	110	18	96	0	0	1995	Hawkes Bay	112	34	0	24	0
1954	Hawkes Bay	110	19	96	48	0	1996	Hawkes Bay	112	47	0	96	24
1955	Hawkes Bay	110	20	96	0	24	1997	Hawkes Bay	113	1179	0	48	0
1956	Hawkes Bay	111	7141	0	0	0	1998	Otago	114	00-369	0	0	0
1957	Hawkes Bay	111	7031	0	0	0	1999	Otago	114	00-382	0	0	0
1958	Hawkes Bay	111	9017	0	0	0	2000	Otago	114	00-413	0	0	0
1959	Hawkes Bay	111	6	0	0	0	2001	Otago	114	00-42	0	0	0
1960	Hawkes Bay	111	4002	0	0	0	2002	Otago	114	00-481	0	0	0

No.	Region	Farm	ID	Hardjobovis	Pomona	Copenhageni
2003	Otago	114	00-49	192	0	0
2004	Otago	114	00-491	0	0	0
2005	Otago	114	00-63	24	0	0
2006	Otago	114	00-8	0	0	0
2007	Otago	114	00-97	24?	0	0
2008	Otago	114	99-005	96	0	0
2009	Otago	114	99-016	48	0	0
2010	Otago	114	99-064	96	0	0
2011	Otago	114	99-088	0	0	0
2012	Otago	114	99-097	0	0	0
2013	Otago	114	99-101	48	0	0
2014	Otago	114	99-110	96	0	0
2015	Otago	114	99-306	48	0	0
2016	Otago	114	99-454	96	0	0
2017	Otago	114	b315	96	0	0
2018	Hawkes Bay	113	39/533	24	48	0
2019	Hawkes Bay	113	Jason/25	0	1536	0
2020	Hawkes Bay	113	o6	0	96	0
2021	Hawkes Bay	113	O66	0	48	0
2022	Hawkes Bay	113	carl/205	192	48	0
2023	Hawkes Bay	113	1165	0	24	0
2024	Hawkes Bay	113	1051	24	0	0
2025	Hawkes Bay	113	471	1536	48	0
2026	Hawkes Bay	113	452	192	0	24
2027	Hawkes Bay	113	00/458	48	48	24
2028	Hawkes Bay	113	99/473	192	24	0
2029	Hawkes Bay	113	484	48	0	0
2030	Hawkes Bay	113	675	48	0	0
2031	Hawkes Bay	113	O76	0	0	24
2032	Hawkes Bay	113	660	48	0	0
2033	Hawkes Bay	113	00/264	96	0	0
2034	Hawkes Bay	113	1203	192	0	192
2035	Hawkes Bay	113	00/225	0	0	0
2036	Hawkes Bay	113	162	384	48	0
2037	Hawkes Bay	113	84	0	0	192

Appendix 3 b. Next Page. Leptospirosis Farm questionnaire

Massey University

Deer Farm Leptospirosis Survey.

Please note: All the information contained within this survey will be totally confidential. Each farmer will be given individual results and an overall summary of the survey but no individual farmer or property will be identified.

Surveyer to complete: **Note: answers should be filling in the white boxes, thank you.**

Name of sample collector:				Sampling Date:	
Herd No.		Farm Sample.		Slaughter Sample.	

Farm Name.			
Contact person: Owner/Manager.			
Farm Address.			
Telephones/Fax	Home:	Work:	Cell:
@-mail address.			
Veterinarian.	Name:		Telephone:

Tick (more than one if appropriate):

Farm	Venison		Velvet		Breeding		Finishing		Other		
Deer species	Red deer		Wapiti/Elk		Mix red-Wapiti				Other		
		Weaners.		Rising 2 year old			Adults.				
		Total		Male		Female		Male		Female	
Numbers: Deer											
		Cattle (dairy)		Cattle (beef)		Sheep		Goats		Pigs	
Numbers: Other stock (all ages)											
Have you brought deer in to your herd in the past 2 years?							Yes		No		
Do cattle, sheep, goat, pig, graze deer pastures?							Yes		No		
If yes: (tick)	Cattle(Dairy)		Cattle(Beef)		Sheep		Goat		Pig		
Deer fenced area Ha.?											

General Health:

1.-Vaccines given in 2004 to deer on the farm? Tick											
5 in 1		7 in 1		Yersiniavax				Leptospira			
2.- Would you be interested in participate in a future survey for Johne's disease in deer?							Yes		No		
3.-Has leptospirosis ever been diagnosed by a Vet. in your deer?							Yes		No		
4. - If yes, what were the signs? (Death, red water, abortions, others?	Observations:										
5. - In which class(es) of deer?											
6. - In what years? Date.											
7. -What serovars were confirmed? If you do not know the answer (VETERINARIAN QUESTION) (Tick).											
Serovars	Hardjobovis		Pomona		Copenhageni		Other/which				
8.-Has leptospirosis been diagnosed by a Vet. in other stock in your property?							Yes		No		
If yes: (tick)	Cattle(Dairy)		Cattle(Beef)		Sheep		Goat		Pig		
9. - If Leptospirosis has been diagnosed in other stock in your property, What serovars were confirmed? If you do not know the answer (VETERINARIAN QUESTION) (Tick).											
Serovars (tick)	Hardjobovis		Pomona		Copenhageni		Other/which				
10. - Do deer have access to the following water sources? Tick *your option(s).*											
Troughs		Dams		Streams		Irrigation ditches			Other		
11.-Have you or your workers had had leptospirosis?							Yes		No		
12.-If yes, in what year?		Were you working with deer?		Yes		No		Other stock			

I agree to serum samples being retained for a serum bank to be used for possible future purposes. The source of serum will remain confidential. Agreed by the under signed.

Farmer signature_____. Date_____.

Appendix 3 C. Questionnaire results (farms, herd prevalence for each serovar and data in the order of the questions in the).
0= No, 1= Yes.

Farm No.	Lepto.	Prevalence				Type of production					Deer type			Deer demographics					
		Hb.	Pom.	Cop.	Hb & Pom.	Venison	Velvet	Breeding	Finishing	Other	Red deer	Wapiti/ Elk	Red X Wapiti	Weaners	R2-♂	R2-♀	A-♂	A-♀	Total
1	1	80.95	0.00	0.00	0.00	1	0	1	0	0	0	0	1	144	11	27	10	167	359
2	1	95.00	40.00	0.00	35.00	1	1	1	0	0	1	0	0	536	42	100	8	700	1386
3	1	95.00	100.00	25.00	95.00	1	0	1	1	0	0	0	1	750	0	0	10	150	910
4	0	0.00	0.00	0.00	0.00	1	0	0	0	1	1	0	1	72	6	14	11	84	187
5	1	55.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	2500	0	570	130	2600	5800
6	1	85.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	2800	0	206	0	1000	4006
7	1	90.00	20.00	0.00	20.00	1	1	1	1	0	1	0	1	600	150	150	600	450	1950
8	1	60.00	0.00	0.00	0.00	0	1	1	0	0	1	0	0	120	70	0	280	120	590
9	1	90.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	100	50	43	5	120	318
10	1	50.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	380	110	54	80	400	1024
11	1	25.00	0.00	0.00	0.00	1	1	0	0	0	0	0	1	260	10	40	47	260	617
13	1	100.00	0.00	0.00	0.00	1	0	1	1	0	1	0	0	560	0	140	15	590	1305
14	0	0.00	0.00	0.00	0.00	1	1	1	1	0	0	0	1	600	50	130	50	620	1450
15	1	45.00	0.00	0.00	0.00	1	1	0	1	0	0	0	1	400	40	80	25	360	905
16	1	15.00	0.00	0.00	0.00	1	1	1	1	0	0	0	1	550	400	40	70	220	1280
17	0	0.00	0.00	0.00	0.00	1	0	1	1	0	0	0	1	700	0	176	7	180	1063
18	1	20.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	115	0	21	0	99	235
19	1	30.00	0.00	0.00	0.00	0	0	1	0	0	1	0	1	100	50	50	10	140	350
20	1	100.00	0.00	0.00	0.00	0	1	1	0	0	1	0	0	200	75	45	133	165	618
21	1	25.00	0.00	0.00	0.00	1	1	1	0	0	1	1	1	62	0	0	0	0	62
22	0	0.00	0.00	0.00	0.00	1	0	1	1	0	1	0	0	120	0	40	5	220	385
23	1	22.73	0.00	0.00	0.00	0	1	0	0	1	1	0	0	300	54	23	400	600	1377
24	0	0.00	0.00	0.00	0.00	1	0	1	0	0	1	0	0	300	20	40	10	350	720
25	1	100.00	0.00	0.00	0.00	1	0	1	0	0	0	0	1	850	5	90	20	370	1335
26	1	100.00	0.00	0.00	0.00	1	0	1	1	0	1	0	0	130	40	50	4	150	374
28	1	80.00	0.00	0.00	0.00	0	0	1	0	0	1	0	0	0	0	0	0	200	200
29	1	100.00	0.00	0.00	0.00	1	1	1	0	0	1	0	0	400	60	40	12	500	1012
31	1	100.00	83.33	16.67	83.33	1	0	1	0	0	1	0	0	2169	0	199	28	902	3298
33	1	75.00	25.00	0.00	16.67	1	0	1	0	0	0	0	1	97	4	19	3	92	215
34	1	91.67	8.33	0.00	0.00	1	0	1	0	0	1	0	0	200	100	70	180	250	800
40	1	58.33	0.00	0.00	0.00	0	0	1	0	1	1	0	0	600	150	144	380	580	1854
48	1	76.47	0.00	0.00	0.00	1	1	1	1	0	1	1	0	312	11	104	16	300	743
49	1	100.00	0.00	0.00	0.00	1	0	0	1	0	1	0	0	186	0	42	6	194	428
51	1	80.00	0.00	0.00	0.00	1	1	1	0	0	1	0	1	410	100	146	250	200	1106
52	0	0.00	0.00	0.00	0.00	1	1	1	1	0	1	0	1	550	0	55	319	1140	2064

Farm No.	Lepto.	Prevalence				Type of production					Deer type			Deer demographics					
		Hb.	Pom.	Cop.	Hb & Pom.	Venison	Velvet	Breeding	Finishing	Other	Red deer	Wapiti/ Elk	Red X Wapiti	Weaners	R2-♂	R2-♀	A-♂	A-♀	Total
53	1	100.00	0.00	0.00	0.00	1	1	1	0	0	1	1	0	704	55	65	65	855	1744
55	1	100.00	0.00	0.00	0.00	0	1	1	0	0	0	1	1	0	420	0	12	510	942
56	1	100.00	0.00	0.00	0.00	1	1	1	0	0	1	0	0	372	105	125	180	450	1232
57	1	100.00	0.00	0.00	0.00	1	1	1	1	0	1	0	1	500	220	240	17	600	1577
58	1	100.00	0.00	0.00	0.00	0	1	1	0	0	1	0	0	260	120	50	240	320	990
59	1	55.00	0.00	0.00	0.00	1	1	1	1	0	1	0	1	280	80	80	140	350	930
60	1	95.00	0.00	0.00	0.00	1	1	0	0	0	1	0	0	0	6	6	29	54	95
50	1	100.00	0.00	0.00	0.00	1	0	0	1	0	0	0	0	1740	180	0	60	1500	3480
61	0	0.00	0.00	0.00	0.00	0	0	1	0	0	0	0	1	80	0	46	8	118	252
62	1	70.00	0.00	0.00	0.00	0	0	1	1	0	0	0	1	166	0	15	25	200	406
63	1	90.00	0.00	0.00	0.00	1	0	1	0	0	0	0	0	2000	0	0	0	0	2000
64	1	60.00	0.00	0.00	0.00	0	0	1	1	0	1	0	0	437	100	70	4	263	874
65	1	90.48	0.00	0.00	0.00	1	1	1	1	0	1	0	1	430	25	80	100	500	1135
66	1	90.00	75.00	0.00	70.00	1	1	1	1	0	1	0	0	1100	200	275	800	1200	3575
67	0	0.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	220	100	80	150	180	730
68	0	0.00	0.00	0.00	0.00	1	1	1	1	0	1	0	1	50	15	125	10	320	520
69	0	0.00	0.00	0.00	0.00	1	1	1	0	0	1	1	0	1000	0	180	27	900	2107
70	0	0.00	0.00	0.00	0.00	1	1	1	1	0	1	0	1	290	110	83	550	896	1929
71	1	68.18	4.55	0.00	4.55	1	1	0	1	0	1	0	0	100	22	55	58	90	325
73	1	90.00	0.00	0.00	0.00	0	0	1	0	0	0	0	1	15	0	15	3	88	121
74	1	85.00	0.00	0.00	0.00	1	0	1	1	0	0	1	0	250	0	83	2	130	465
75	1	100.00	0.00	0.00	0.00	0	0	0	0	0	1	0	0	0	0	0	0	1250	1250
76	1	100.00	0.00	0.00	0.00	1	1	0	1	0	0	0	0	500	300	350	27	400	1577
77	1	100.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	60	1	12	8	58	139
77	1	78.72	0.00	0.00	0.00	0	0	1	0	0	1	0	0	18	1	1	2	40	62
79	1	95.00	0.00	0.00	0.00	1	1	1	1	0	1	0	1	290	10	40	30	280	650
80	1	88.89	0.00	0.00	0.00	1	1	1	1	0	1	0	0	300	150	150	150	150	900
81	1	85.00	0.00	0.00	0.00	0	0	1	0	0	0	0	1	0	0	0	4	150	154
82	1	95.00	20.00	0.00	20.00	1	1	1	1	0	1	0	0	0	1	210	78	1800	2089
83	0	0.00	0.00	0.00	0.00	0	0	1	1	0	0	0	0	75	23	20	10	112	240
84	1	90.00	0.00	0.00	0.00	0	0	1	0	0	0	0	1	6	0	0	6	110	122
85	1	95.00	55.00	0.00	50.00	0	0	1	1	0	1	0	0	0	0	50	5	150	205
86	1	60.00	0.00	0.00	0.00	1	0	1	0	0	1	0	0	32	12	12	1	36	93
87	1	60.00	0.00	0.00	0.00	1	1	1	1	0	1	1	0	250	3	3	25	600	881
88	1	89.47	0.00	0.00	0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
89	1	73.68	0.00	0.00	0.00	1	1	0	0	0	1	0	0	81	6	29	12	89	217
90	0	0.00	0.00	0.00	0.00	0	0	0	0	1	1	0	0	450	85	150	40	500	1225
91	1	65.00	0.00	0.00	0.00	0	1	1	0	0	1	0	1	400	45	45	400	400	1290
92	0	0.00	0.00	0.00	0.00	1	0	1	0	0	1	0	0	140	10	58	6	165	379
107	1	0.00	0.00	0.00	6.67	0	0	1	1	0	1	1	0	300	0	0	2	300	602
108	0	0.00	0.00	0.00	0.00	1	1	1	1	0	1	0	0	250	85	120	100	270	825
109	1	47.37	63.16	0.00	21.05	1	1	1	0	0	1	0	0	27	60	12	24	27	150
110	1	95.00	50.00	0.00	55.00	1	0	0	1	0	0	0	1	600	150	500	0	50	1300

111	0	0.00	0.00	0.00	0.00	0	1	1	0	0	1	0	0	168	28	24	25	161	406
112	1	14.29	52.38	0.00	4.76	1	0	0	0	0	1	0	0	0	85	130	0	0	215
114	0	0.00	0.00	0.00	0.00	0	0	1	0	1	1	0	0	600	150	160	380	580	1870

Appendix 3 C. Continuation.

Farm No	Other species					Management and practices			Vaccines			
	Dairy	Beef	Sheep	Goats	Pigs	Deer fenced area	Brought in deer	Graze in deer pastures	5 in 1	7 in 1	Yersiniavax	Leptospira
1	0	0	80	0	0	40	0	1	1	0	1	0
2	0	150	300	0	3	396	0	1	1	0	1	0
3	200	80	200	0	0	190	1	1	1	0	0	0
4	0	0	0	0	0	22	1	0	1	0	1	0
5	0	200	0	0	0	1800	1	0	0	0	0	0
6	0	550	5000	0	0	600	1	1	0	0	1	0
7	0	60	60	0	0	257	1	1	1	0	1	0
8	0	0	30	0	0	68	0	0	1	0	0	0
9	0	0	0	0	0	38	1	0	0	0	0	0
10	0	0	0	0	0	0	1	1	0	0	0	0
11	0	0	200	0	0	128	0	1	0	0	0	0
13	0	180	4000	0	0	200	1	1	0	0	1	0
14	0	120	4000	0	0	230	1	1	0	0	0	0
15	0	0	20	0	1	90	1	1	0	0	0	0
16	0	10	260	0	2	160	1	1	1	0	1	0
17	0	200	100	0	0	160	1	1	0	0	0	0
18	0	21	2350	0	0	50	1	1	1	0	1	0
19	100	80	120	15	0	65	1	1	0	0	0	0
20	0	57	240	105	0	150	1	1	0	0	0	0
21	0	60	1300	20	0	150	0	1	0	0	1	0
22	0	0	0	0	0	50	1	1	0	0	0	0
23	0	0	64	0	0	0	1	1	0	0	0	0
24	0	0	0	0	0	70	0	0	0	0	0	0
25	0	40	1500	0	0	100	1	1	0	0	0	0
26	0	600	350	50	0	63	0	1	0	0	0	0
28	0	30	0	0	0	40	0	1	0	0	0	0
29	0	450	300	0	0	300	1	1	0	0	0	0
31	30	434	1040	0	0	850	1	1	0	0	0	0

Farm No	Other species					Management and practices			Vaccines			
	Dairy	Beef	Sheep	Goats	Pigs	Deer fenced area	Brought in deer	Graze in deer pastures	5 in 1	7 in 1	Yersiniavax	Leptospira
33	0	7	0	0	0	20	0	0	0	0	0	0
34	0	0	0	0	0	150	1	0	0	0	0	0
40	0	0	0	0	0	160	0	1	1	0	1	0
48	0	0	120	20	0	96	1	1	1	0	1	0
49	0	5	0	0	0	44	1	1	1	0	0	0
51	0	100	180	0	0	300	1	1	0	0	1	0
52	0	0	350	0	0	679	1	1	0	0	0	0
53	0	0	0	0	0	166	1	0	0	0	1	0
55	0	150	0	0	0	92	1	0	0	0	1	0
56	0	11	15	0	0	200	1	1	0	0	0	0
57	0	26	2	0	3	0	1	1	1	0	0	0
58	0	0	100	0	0	80	1	1	0	0	1	0
59	0	0	0	0	2	230	1	0	0	0	0	0
60	0	11	0	0	0	30	0	1	0	0	0	0
50	0	400	9000	0	0	600	1	1	0	0	0	0
61	0	26	800	0	0	40	1	1	0	0	0	0
62	0	0	0	0	0	0	0	0	1	0	0	0
63	0	0	0	0	0	0	0	0	0	0	0	0
64	4	62	0	43	0	80	1	0	0	0	1	0
65	0	0	1400	0	0	0	1	0	0	0	1	0
66	0	250	400	0	0	485	1	1	1	0	1	1
67	0	7	0	25	0	85	1	1	0	0	0	0
68	300	200	50	0	75	50	1	0	0	0	0	0
69	0	77	35	0	8	0	0	0	0	0	1	0
70	0	230	8000	0	0	375	1	0	0	0	1	0
71	0	0	14	0	0	40	0	1	1	0	1	1
73	0	2	50	0	0	0	1	1	0	0	0	0
74	0	8	0	0	0	80	0	0	0	0	0	0
75	0	230	1700	0	0	300	0	1	0	0	0	0
76	0	150	0	0	0	247	1	0	0	0	0	0
77	95	4	23	0	0	18	0	1	0	0	1	0
77	5	0	0	0	0	10	0	0	0	0	0	0
79	0	0	600	0	0	150	0	0	0	0	0	0
80	20	100	0	0	0	0	1	1	0	0	0	0
81	0	20	1000	0	0	30	1	1	0	0	0	0
82	0	850	4500	0	0	550	1	1	0	0	1	0
83	0	5	130	8	0	0	0	1	0	0	0	0
84	70	0	0	0	0	0	0	1	0	0	0	0

Farm No	Other species					Management and practices			Vaccines			
	Dairy	Beef	Sheep	Goats	Pigs	Deer fenced area	Brought in deer	Graze in deer pastures	5 in 1	7 in 1	Yersiniavax	Leptospira
85	0	0	0	1200	0	80	0	1	0	0	1	0
86	0	0	0	0	0	12	0	1	0	0	0	0
87	0	58	0	0	0	100	1	1	0	0	1	0
88	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	1	1	0	20	1	1	1	0	1	0
90	0	0	0	0	0	180	0	0	1	0	1	0
91	0	200	650	0	0	400	1	1	0	0	1	0
92	0	0	0	0	0	57	0	0	0	0	0	0
107	0	50	200	0	0	250	1	1	0	0	0	0
108	0	278	2100	0	0	110	1	1	0	0	0	0
109	0	0	0	0	0	22	0	1	1	0	1	0
110	0	0	0	0	0	185	1	1	0	0	1	1
111	0	0	0	0	0	35	0	0	0	0	1	0
112	0	60	200	0	0	65	1	1	1	0	1	0
114	0	0	0	0	0	160	0	1	1	0	1	0

Appendix 3 C. Continuation.

Farm No	Lepto. DX. before	Signs				Affected age group			Date	Serovars Confirmed	Serovars	Water sources					Leptospirosis in Humans			
		Death	Red water	Abortion	Other	Weaners	Yearlings	Adults				Troughs	Dams	Streams	Irrigation Ditches	Other	Farm workers	Year	Working with	
																			Deer	Other stock
1	1	0	0	0	0	1	1	1	93	1	Hardjobovis	1	0	1	0	0	0		0	
2	0	1	0	0	0	1	0	0	02	0	Pom-Hardjo	1	1	1	0	0	0		0	
3	1	1	0	0	0	1	0	0	03	1	Pom-Hardjo	1	1	0	0	0	0		0	
4	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
5	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
6	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
7	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
8	0	0	0	0	0	0	0	0		0		1	0	0	0	1	0		0	
9	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0		0	
10	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
11	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
13	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	

Farm No	Lepto. DX. before	Signs				Affected age group			Date	Serovars Confirmed	Serovars	Water sources					Leptospirosis in Humans			
		Death	Red water	Abortion	Other	Weaners	Yearlings	Adults				Troughs	Dams	Streams	Irrigation Ditches	Other	Farm workers	Year	Working with Deer	Working with Other stock
14	1	0	0	0	0	0	0	0	95	0		1	0	1	0	0	0		0	
15	1	0	0	1	0	0	0	1	95-96	0		1	1	1	0	0	0		0	
16	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
17	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
18	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
19	0	0	0	0	0	0	0	0		0		1	0	0	0	1	0		0	
20	1	1	1	0	0	1	1	1	98	1	Pomona	1	0	1	0	0	0		0	
21	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
22	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
23	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
24	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
25	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
26	0	0	0	0	0	0	0	0		0		1	0	0	0	1	0		0	
28	0	0	0	0	0	0	0	0	02	1	Hardjobovis	1	1	0	0	0	0		0	
29	1	1	0	0	1	1	1	1	92	1	Pom-Hardjo	1	1	1	0	0	0		0	
31	0	0	0	0	0	0	0	0		0		1	1	1	0	1	0		0	
33	0	0	0	0	0	0	0	0		0		1	0	0	1	0	0		0	
34	0	0	0	0	0	0	0	0		0		1	0	0	1	0	0		0	
40	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0		0	
48	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
49	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
51	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
52	0	0	0	0	0	0	0	0		0		1	1	0	0	0	0		0	
53	0	0	0	0	0	0	0	0		0		1	1	0	0	0	0		0	
55	0	0	0	0	0	0	0	0		0		1	1	0	0	0	0		0	
56	0	0	0	0	0	0	0	0		0		1	1	0	0	0	0		0	
57	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
58	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
59	0	0	0	0	0	0	0	0		0		1	0	0	0	1	0		0	
60	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
50	0	0	0	0	0	0	0	0		0		0	1	1	0	0	0		0	
61	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
62	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
63	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0		0	
64	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
65	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
66	1	1	1	0	0	1	0	0	04	0		1	1	1	1	0	0		0	
67	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
68	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
69	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
70	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	

Farm No	Lepto. DX. before	Signs				Affected age group			Date	Serovars Confirmed	Serovars	Water sources					Leptospirosis in Humans			
		Death	Red water	Abortion	Other	Weaners	Yearlings	Adults				Troughs	Dams	Streams	Irrigation Ditches	Other	Farm workers	Year	Working with Deer	Working with Other stock
71	0	0	0	0	0	0	0	0		0		1	0	0	0	1	0		0	
73	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
74	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
75	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0		0	
76	0	0	0	0	0	0	0	0		0		1	1	0	0	0	0		0	
77	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
77	0	0	0	0	0	0	0	0		0		0	0	1	0	0	0		0	
79	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
80	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
81	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
82	0	0	0	0	0	0	0	0		0		1	1	1	0	0	1	2002	1	Beef
83	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
84	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
85	0	0	0	0	0	0	0	0		0		0	0	1	0	0	0		0	
86	0	0	0	0	0	0	0	0		0		1	1	0	0	1	0		0	
87	0	0	0	0	0	0	0	0		0		1	0	0	0	0	0		0	
88	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0		0	
89	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
90	0	0	0	0	0	0	0	0		0		1	0	0	0	1	0		0	
91	0	0	0	0	0	0	0	0		0		0	0	1	0	0	0		0	
92	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
107	0	0	0	0	0	0	0	0		0		1	0	1	0	0	0		0	
108	0	0	0	0	0	0	0	0		0		1	1	1	0	0	0		0	
109	1	0	0	0	0	0	0	0		0	Hardjobovis	1	0	0	0	0	0		0	
110	1	1	1	0	0	1	0	0	04	0		1	1	1	0	0	0		0	
111	1	0	0	0	0	1	1	1	92	1	Hardjobovis	0	1	0	0	0	0		0	
112	1	0	0	0	0	1	1	1	92	1	Hardjobovis	1	0	1	0	0	0		0	
114	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0		0	

Appendix 4 raw data

CHAPTER 8.

(Deer Slaughter Premises)

Appendix 4.1 Complete raw data for Serum and kidney samples collected at the Deer Slaughter premises Sample number (Sm.No.), year (1992=1992-3, 2004=2003-4), animal identification (An. ID), Farm, age (years.), sex (F=female, M=male), Vaccine (Vacc.) (0= negative, 1= positives), MAT titre result expressed as the highest positive dilution, Hb.= Hardjobovis, Pom.= Pomona, Cop= Copenhageni, Histopathology (HP.) (0= negative, 1= positives) kidney macroscopic lesions (Ws. = White spots, Rs. = Red spots (0= negative, 1= positives) and culture results (0= negative, 1= positive). Postmortem samples (pm).

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
1	1992	72	31 a	2	M	0	0	96	0		0	0	
2	1992	8	31a	2	M	0	0	96	0		0	0	
3	1992	9	31 a	2	M	0	0	0	0		0	0	
4	1992	10	31a	2	M	0	0	48	0		0	0	
5	1992	11	31 a	2	M	0	0	192	0		0	0	
6	1992	12	31a	2	M	0	0	192	0		0	0	
7	1992	13	31 a	2	M	0	0	0	0		0	0	
8	1992	14	31a	2	M	0	0	0	0		0	0	
9	1992	15	31 a	2	M	0	0	0	0		0	0	
10	1992	16	31a	2	M	0	0	0	0		0	0	
11	1992	17	32	>2	M	0	192	48	1536		0	0	
12	1992	18	32	>2	M	0	192	0	1536		0	0	
13	1992	19	32	>2	M	0	24	0	192		0	0	
14	1992	20	32	>2	M	0	24	0	96		0	0	
15	1992	21	32	>2	M	0	24	0	192		0	0	
16	1992	22	32	>2	M	0	0	0	24		0	0	
17	1992	23	32	>2	M	0	48	0	96		0	0	
18	1992	24	32	>2	M	0	0	0	96		0	0	
19	1992	25	32	>2	M	0	24	0	96		0	0	
20	1992	26	32	>2	M	0	48	0	192		0	0	
21	1992	27	32	>2	M	0	48	24	768		0	0	
22	1992	28	32	>2	M	0	0	1536	1536		0	0	
23	1992	42	33	2	M	0	48	0	48		0	0	
24	1992	43	33	2	M	0	48	0	24		0	0	
25	1992	44	33	2	M	0	0	0	24		0	0	
26	1992	45	33	2	M	0	24	0	24		0	0	
27	1992	46	33	2	M	0	24	0	48		0	0	
28	1992	47	33	2	M	0	0	0	96		0	0	
29	1992	48	33	2	M	0	0	0	24		0	0	
30	1992	49	33	2	M	0	192	0	24		0	0	
31	1992	50	33	2	M	0	48	0	24		0	0	
32	1992	51	33	2	M	0	24	0	96		0	0	
33	1992	52	33	2	M	0	0	0	96		0	0	
34	1992	53	33	2	M	0	0	48	1536		0	0	
35	1992	60	34	1	M	0	24	0	48		0	0	
36	1992	61	34	1	M	0	48	0	0		0	0	
37	1992	62	34	1	M	0	24	0	48		0	0	
38	1992	63	34	1	M	0	384	0	96		0	0	
39	1992	64	34	1	M	0	48	0	48		0	0	
40	1992	65	34	1	M	0	24	0	48		0	0	
41	1992	66	34	1	M	0	48	0	48		0	0	
42	1992	67	34	1	M	0	384	0	96		0	0	
43	1992	68	34	1	M	0	48	0	24		0	0	
44	1992	69	34	1	M	0	96	0	0		0	0	
45	1992	70	34	1	M	0	0	0	48		0	0	
46	1992	71	34	1	M	0	48	0	96		0	0	
47	1992	86	35	2	M	0	192	0	0		0	0	
48	1992	87	35	2	M	0	48	0	0		0	0	
49	1992	88	35	2	M	0	24	0	0		0	0	
50	1992	89	35	2	M	0	0	0	0		0	0	
51	1992	90	35	2	M	0	48	0	0		0	0	
52	1992	91	35	2	M	0	0	0	0		0	0	
53	1992	92	35	2	M	0	24	0	0		0	0	
54	1992	93	35	2	M	0	24	0	0		0	0	
55	1992	94	35	2	M	0	48	0	0		0	0	

Snl.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
56	1992	95	35	2	M	0	24	768	0		0	0	
57	1992	96	35	2	M	0	0	0	0		0	0	
58	1992	97	35	2	M	0	0	0	0		0	0	
59	1992	2	36	1	M	0	48	0	0		0	0	
60	1992	3	36	1	M	0	48	0	0		0	0	
61	1992	4	36	1	M	0	192	0	0		0	0	
62	1992	5	36	1	M	0	0	0	0		0	0	
63	1992	6	36	1	M	0	0	0	0		0	0	
64	1992	7	36	1	M	0	48	0	0		0	0	
65	1992	8	36	1	M	0	192	0	0		1	0	
66	1992	9	36	1	M	0	0	0	0		0	0	
67	1992	10	36	1	M	0	0	0	0		0	0	
68	1992	11	36	1	M	0	48	0	0		0	0	
69	1992	23	37	1	M	0	384	1536	96		0	0	
70	1992	24	37	1	M	0	96	1536	24		1	0	
71	1992	25	37	1	M	0	24	1536	0		0	0	
72	1992	26	37	1	M	0	0	768	0		0	0	
73	1992	27	37	1	M	0	192	1536	0		1	1	
74	1992	28	37	1	M	0	96	1536	0		1	1	
75	1992	29	37	1	M	0	96	1536	0		0	0	
76	1992	30	37	1	M	0	24	384	0		0	0	
77	1992	31	37	1	M	0	48	192	0		0	0	
78	1992	32	37	1	M	0	24	1536	24		0	0	
79	1992	33	37	1	M	0	48	768	0		1	1	
80	1992	34	37	1	M	0	384	1536	0		0	0	
81	1992	60	38	1	M	0	0	0	0		0	0	
82	1992	61	38	1	M	0	0	0	0		0	0	
83	1992	62	38	1	M	0	0	0	0		0	0	
84	1992	63	38	1	M	0	0	0	0		0	0	
85	1992	64	38	1	M	0	0	0	0		0	0	
86	1992	65	38	1	M	0	0	0	0		0	0	
87	1992	66	38	1	M	0	0	0	0		0	0	
88	1992	67	38	1	M	0	0	0	0		0	0	
89	1992	68	38	1	M	0	0	24	0		0	0	
90	1992	69	38	1	M	0	0	0	0		0	0	
91	1992	70	38	1	M	0	0	0	0		0	0	
92	1992	71	38	1	M	0	0	0	0		0	0	
93	1992	108	39	1	M	0	0	0	24		0	0	
94	1992	110	39	1	M	0	0	0	24		0	0	
95	1992	111	39	1	M	0	0	0	0		0	0	
96	1992	112	39	1	M	0	0	0	0		0	0	
97	1992	113	39	1	M	0	0	0	0		0	0	
98	1992	114	39	1	M	0	0	0	0		0	0	
99	1992	115	39	1	M	0	0	0	0		0	0	
100	1992	116	39	1	M	0	0	0	24		1	0	
101	1992	117	39	1	M	0	0	0	0		0	0	
102	1992	118	39	1	M	0	0	0	0		1	0	
103	1992	119	39	1	M	0	0	0	0		0	0	
104	1992	120	39	1	M	0	0	0	96		0	0	
105	1992	7	40	1	M	0	0	0	0		0	0	
106	1992	8	40	1	M	0	0	0	0		1	0	
107	1992	9	40	1	M	0	0	0	0		0	0	
108	1992	10	40	1	M	0	0	0	0		0	0	
109	1992	11	40	1	M	0	0	0	0		0	0	
110	1992	12	40	1	M	0	0	0	0		0	0	
111	1992	13	40	1	M	0	0	0	0		0	0	
112	1992	14	40	1	M	0	0	96	0		0	0	
113	1992	15	40	1	M	0	0	0	0		0	0	
114	1992	16	40	1	M	0	0	0	0		0	0	
115	1992	17	40	1	M	0	48	0	0		0	0	
116	1992	18	40	1	M	0	0	0	0		0	0	
117	1992	44	41	>2	F	0	0	0	48		0	0	
118	1992	45	41	>2	F	0	0	0	0		0	0	
119	1992	46	41	>2	F	0	384	0	0		0	0	
120	1992	47	41	>2	F	0	96	0	0		0	0	
121	1992	48	41	>2	F	0	0	0	24		0	0	
122	1992	49	41	>2	F	0	0	0	0		0	0	
123	1992	50	41	2	M	0	1536	192	24		0	0	
124	1992	51	41	2	M	0	96	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
125	1992	52	41	2	M	0	96	0	24		0	0	
126	1992	53	41	2	M	0	48	0	0		0	0	
127	1992	54	42	2	M	0	0	1536	0		0	0	
128	1992	55	42	2	M	0	96	384	0		0	0	
129	1992	56	42	2	M	0	0	384	0		0	1	
130	1992	57	42	2	M	0	192	0	0		1	0	
131	1992	58	42	2	M	0	0	0	0		0	0	
132	1992	59	42	2	M	0	48	24	0		0	0	
133	1992	60	42	2	M	0	24	0	0		0	0	
134	1992	61	42	2	M	0	384	0	0		1	0	
135	1992	62	42	2	M	0	48	0	0		0	0	
136	1992	63	42	2	M	0	96	0	0		0	0	
137	1992	64	42	2	M	0	96	0	0		1	0	
138	1992	65	42	2	M	0	0	0	0		0	0	
139	1992	120	43	1	M	0	0	0	0		0	0	
140	1992	121	43	1	M	0	0	0	0		0	0	
141	1992	122	43	1	M	0	0	0	0		0	0	
142	1992	123	43	1	M	0	0	0	0		0	0	
143	1992	124	43	1	M	0	0	0	0		1	0	
144	1992	125	43	1	M	0	0	0	0		0	0	
145	1992	126	43	1	M	0	0	0	0		0	0	
146	1992	127	43	1	M	0	0	192	0		0	0	
147	1992	128	43	1	M	0	0	0	24		0	0	
148	1992	129	43	1	M	0	0	0	0		1	0	
149	1992	130	43	1	M	0	0	0	0		1	0	
150	1992	131	43	1	M	0	0	0	0		0	0	
151	1992	8	44	1	M	0	0	0	0		0	0	
152	1992	10	44	1	M	0	0	0	0		0	0	
153	1992	11	44	1	M	0	0	0	0		0	0	
154	1992	12	44	1	M	0	0	0	0		0	0	
155	1992	13	44	1	M	0	0	0	0		0	0	
156	1992	14	44	1	M	0	0	0	0		0	0	
157	1992	15	44	1	M	0	0	0	0		0	0	
158	1992	16	44	1	M	0	0	0	0		0	1	
159	1992	17	44	1	M	0	0	0	0		0	0	
160	1992	18	44	1	M	0	0	0	0		0	0	
161	1992	20	44	1	M	0	0	0	0		0	0	
162	1992	21	44	1	M	0	0	0	0		0	0	
163	1992	30	45	1	M	0	0	0	0		0	1	
164	1992	31	45	1	M	0	0	0	0		0	0	
165	1992	32	45	1	M	0	0	0	0		0	0	
166	1992	33	45	1	M	0	0	0	0		0	0	
167	1992	34	45	1	M	0	0	0	0		0	0	
168	1992	35	45	1	M	0	0	0	0		0	0	
169	1992	36	45	1	M	0	0	0	0		0	0	
170	1992	37	45	1	M	0	0	0	0		0	1	
171	1992	38	45	1	M	0	0	0	0		0	0	
172	1992	39	45	1	M	0	0	0	0		0	0	
173	1992	40	45	1	M	0	0	0	0		0	0	
174	1992	41	45	1	M	0	0	0	0		0	0	
175	1992	50	46	1	M	0	0	0	0		0	1	
176	1992	51	46	1	M	0	0	0	0		0	1	
177	1992	52	46	1	M	0	0	0	0		0	0	
178	1992	53	46	1	M	0	0	0	0		0	0	
179	1992	54	46	1	M	0	0	0	0		1	0	
180	1992	55	46	1	M	0	0	0	0		0	0	
181	1992	56	46	1	M	0	0	0	0		0	0	
182	1992	57	46	1	M	0	0	0	0		0	0	
183	1992	58	46	1	M	0	0	0	0		0	0	
184	1992	59	46	1	M	0	0	0	0		0	0	
185	1992	60	46	1	M	0	0	0	0		0	0	
186	1992	61	46	1	M	0	0	0	0		0	0	
187	1992	70	47	1	M	0	192	0	0		0	0	
188	1992	71	47	1	M	0	48	0	0		0	0	
189	1992	72	47	1	M	0	96	0	0		0	0	
190	1992	73	47	1	M	0	48	0	0		0	0	
191	1992	74	47	1	M	0	48	0	0		0	0	
192	1992	75	47	1	M	0	192	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
193	1992	76	47	1	M	0	96	0	0		1	0	
194	1992	77	47	1	M	0	96	0	0		0	0	
195	1992	78	47	1	M	0	48	0	0		0	0	
196	1992	79	47	1	M	0	48	0	0		0	0	
197	1992	80	47	1	M	0	96	0	0		0	0	
198	1992	81	47	1	M	0	96	0	0		0	0	
199	1992	90	48	2	M	0	192	0	0		0	0	
200	1992	91	48	2	M	0	768	0	0		0	0	
201	1992	92	48	1	M	0	48	0	0		0	0	
202	1992	93	48	2	M	0	192	0	0		0	0	
203	1992	94	48	1	M	0	192	0	0		0	0	
204	1992	95	48	2	M	0	192	0	0		0	0	
205	1992	96	48	1	M	0	24	0	0		0	0	
206	1992	97	48	2	M	0	192	0	0		0	0	
207	1992	98	48	1	M	0	0	0	0		1	0	
208	1992	99	48	1	M	0	192	0	0		1	0	
209	1992	100	48	2	M	0	48	0	0		1	0	
210	1992	101	48	1	M	0	96	0	0		0	0	
211	1992	127	49	2	M	0	96	0	0		1	0	
212	1992	128	49	2	M	0	96	0	0		0	0	
213	1992	129	49	2	M	0	192	0	0		1	0	
214	1992	130	49	2	M	0	768	0	0		1	0	
215	1992	131	49	2	M	0	48	0	0		1	0	
216	1992	132	49	2	M	0	48	48	0		1	0	
217	1992	133	49	2	M	0	768	0	0		0	0	
218	1992	134	49	2	M	0	96	0	0		1	0	
219	1992	135	49	2	M	0	96	0	0		1	0	
220	1992	136	49	2	M	0	1536	24	0		0	0	
221	1992	137	49	2	M	0	96	0	0		0	0	
222	1992	138	49	2	M	0	48	0	0		1	0	
223	1992	58	50	2	M	0	1536	1536	0		0	0	
224	1992	59	50	>2	M	0	48	48	0		0	0	
225	1992	60	50	>2	M	0	24	384	0		0	0	
226	1992	61	50	2	M	0	96	1536	0		0	0	
227	1992	62	50	2	M	0	192	0	0		0	0	
228	1992	63	50	>2	M	0	48	0	0		1	0	
229	1992	64	50	>2	M	0	0	768	0		0	0	
230	1992	65	50	>2	M	0	48	0	0		0	0	
231	1992	66	50	>2	M	0	96	0	0		1	0	
232	1992	67	50	>2	M	0	48	0	0		1	0	
233	1992	68	50	>2	M	0	96	1536	0		0	0	
234	1992	69	50	2	M	0	384	0	0		0	0	
235	1992	75	51	1	M	0	0	0	0		0	0	
236	1992	76	51	1	M	0	0	0	0		1	0	
237	1992	77	51	1	M	0	0	0	0		0	0	
238	1992	78	51	1	M	0	0	0	0		0	0	
239	1992	79	51	1	M	0	0	0	0		0	0	
240	1992	80	51	1	M	0	0	0	0		0	0	
241	1992	81	51	1	M	0	0	0	0		0	0	
242	1992	82	51	1	M	0	0	0	0		0	0	
243	1992	83	51	1	M	0	0	0	0		0	0	
244	1992	84	51	1	M	0	0	0	0		1	0	
245	1992	85	51	1	M	0	0	0	0		0	0	
246	1992	86	51	1	M	0	0	0	0		0	0	
247	1992	95	52	>2	M	0	192	0	0		0	0	
248	1992	96	52	>2	M	0	24	0	0		0	0	
249	1992	97	52	>2	M	0	192	0	0		0	0	
250	1992	98	52	>2	M	0	96	0	0		0	0	
251	1992	99	52	>2	F	0	48	0	0		0	0	
252	1992	100	52	>2	F	0	192	0	0		0	0	
253	1992	101	52	>2	F	0	96	0	0		1	0	
254	1992	102	52	>2	M	0	192	0	0		0	0	
255	1992	103	52	>2	M	0	48	192	0		0	0	
256	1992	104	52	>2	M	0	768	192	0		0	0	
257	1992	105	53	1	M	0	0	0	0		0	0	
258	1992	106	53	1	M	0	0	0	0		0	0	
259	1992	107	53	1	M	0	0	0	0		0	0	
260	1992	108	53	1	M	0	0	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
261	1992	109	53	1	M	0	0	0	0		0	0	
262	1992	110	53	1	M	0	0	0	0		0	0	
263	1992	111	53	1	M	0	0	0	0		0	0	
264	1992	112	53	1	M	0	0	0	0		0	0	
265	1992	113	53	1	M	0	0	0	0		0	0	
266	1992	114	53	1	M	0	0	0	0		0	0	
267	1992	115	53	1	M	0	0	0	0		0	0	
268	1992	116	53	1	M	0	0	0	0		0	0	
269	1992	117	54	1	M	0	48	0	0		0	0	
270	1992	118	54	1	M	0	0	0	0		0	0	
271	1992	119	54	1	M	0	0	0	0		0	0	
272	1992	120	54	1	M	0	0	0	0		0	0	
273	1992	121	54	1	M	0	0	0	0		0	0	
274	1992	122	54	1	M	0	0	0	0		0	0	
275	1992	123	54	1	M	0	0	0	0		1	0	
276	1992	124	54	1	M	0	48	0	0		0	0	
277	1992	125	54	1	M	0	0	96	0		0	0	
278	1992	126	54	1	M	0	0	0	0		0	0	
279	1992	127	54	1	M	0	0	0	0		0	0	
280	1992	128	54	1	M	0	0	0	0		0	0	
281	1992	143	55	2	M	0	768	1536	0		0	1	
282	1992	144	55	2	M	0	768	1536	0		0	0	
283	1992	145	55	2	M	0	384	192	0		1	0	
284	1992	146	55	2	M	0	192	1536	0		1	0	
285	1992	147	55	2	M	0	384	0	0		1	0	
286	1992	148	55	2	M	0	96	1536	0		1	0	
287	1992	149	55	2	M	0	384	1536	0		1	0	
288	1992	150	55	2	M	0	96	1536	0		0	0	
289	1992	151	55	2	M	0	192	1536	0		1	0	
290	1992	152	55	2	M	0	48	768	0		0	0	
291	1992	153	55	2	M	0	48	1536	0		1	0	
292	1992	154	55	2	M	0	768	1536	0		0	0	
293	1992	5	56	2	M	0	96	24	48		0	0	
294	1992	6	56	2	M	0	96	0	24		1	0	
295	1992	7	56	2	M	0	24	0	0		0	1	
296	1992	8	56	2	M	0	24	0	0		0	0	
297	1992	9	56	2	M	0	0	0	0		0	0	
298	1992	10	56	2	M	0	0	0	0		1	0	
299	1992	11	56	2	M	0	48	0	0		0	0	
300	1992	12	56	2	M	0	24	0	0		1	0	
301	1992	13	56	>2	M	0	96	0	24		0	0	
302	1992	14	56	>2	M	0	192	0	0		0	0	
303	1992	15	56	2	M	0	0	0	24		0	0	
304	1992	16	56	2	M	0	96	0	48		0	0	
305	1992	20	57	1	M	0	96	0	24		0	1	
306	1992	21	57	1	M	0	192	0	0		0	0	
307	1992	22	57	1	M	0	192	0	0		0	0	
308	1992	23	57	1	M	0	96	0	0		0	0	
309	1992	24	57	1	M	0	384	0	0		1	0	
310	1992	25	57	1	M	0	1536	0	0		0	0	
311	1992	26	57	1	M	0	192	0	0		0	0	
312	1992	27	57	1	M	0	384	0	0		1	0	
313	1992	28	57	1	M	0	192	0	0		1	0	
314	1992	29	57	1	M	0	768	0	0		1	0	
315	1992	30	57	1	M	0	384	0	0		0	0	
316	1992	31	57	1	M	0	768	0	0		1	0	
317	1992	43	58	>2	M	0	48	48	0		0	0	
318	1992	44	58	2	M	0	96	0	0		1	0	
319	1992	45	58	2	M	0	192	0	24		1	0	
320	1992	46	58	>2	M	0	192	0	0		0	0	
321	1992	47	58	2	M	0	768	0	0		1	0	
322	1992	48	58	>2	M	0	768	0	0		0	0	
323	1992	49	58	2	M	0	768	24	0		0	1	
324	1992	50	58	>2	M	0	192	24	0		0	0	
325	1992	51	58	2	M	0	96	0	0		0	0	
326	1992	52	58	2	M	0	96	1536	0		0	0	
327	1992	53	58	2	M	0	96	192	0		0	0	
328	1992	54	58	>2	M	0	384	96	24		0	0	
329	1992	112	59	>2	M	0	48	24	0		1	0	
330	1992	113	59	>2	M	0	48	48	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
331	1992	114	59	>2	M	0	0	0	0		1	0	
332	1992	115	59	>2	M	0	384	24	0		0	0	
333	1992	116	59	>2	M	0	96	24	24		0	0	
334	1992	117	59	>2	M	0	96	96	24		1	0	
335	1992	118	59	>2	M	0	96	0	0		1	0	
336	1992	119	59	>2	M	0	0	96	0		1	0	
337	1992	120	59	>2	M	0	24	192	0		0	0	
338	1992	121	59	>2	M	0	1536	48	0		0	0	
339	1992	122	59	>2	M	0	48	48	0		1	0	
340	1992	123	59	>2	M	0	96	48	0		0	0	
341	1992	139	60	2	M	0	1536	0	0		1	0	
342	1992	140	60	>2	M	0	96	0	0		0	0	
343	1992	141	60	2	M	0	384	0	0		0	0	
344	1992	142	60	2	M	0	96	0	0		0	0	
345	1992	143	60	2	M	0	384	0	0		0	0	
346	1992	144	60	2	M	0	768	0	0		0	0	
347	1992	34	60	2	M	0	384	0	24		1	0	
348	1992	35	60	>2	M	0	384	0	0		0	1	
349	1992	36	60	2	M	0	1536	24	0		0	0	
350	1992	37	60	2	M	0	192	0	0		0	1	
351	1992	38	60	>2	M	0	192	0	0		1	0	
352	1992	39	60	>2	M	0	1536	0	0		0	0	
353	1992	1	61	>2	M	0	96	0	0		0	1	
354	1992	2	61	>2	M	0	48	0	0		0	0	
355	1992	3	61	>2	M	0	192	0	0		0	0	
356	1992	4	61	>2	M	0	192	0	0		0	0	
357	1992	5	61	>2	M	0	384	0	0		0	1	
358	1992	6	61	>2	M	0	1536	0	0		0	0	
359	1992	7	61	>2	M	0	1536	0	0		0	0	
360	1992	8	61	>2	M	0	384	0	0		0	0	
361	1992	9	61	>2	M	0	48	0	0		0	0	
362	1992	10	61	>2	M	0	384	0	0		0	0	
363	1992	11	61	>2	M	0	384	0	0		0	0	
364	1992	12	61	>2	M	0	192	0	0		0	1	
365	1992	50	62	>2	M	0	48	0	0		0	0	
366	1992	51	62	>2	M	0	1536	0	0		0	0	
367	1992	52	62	>2	M	0	768	0	0		1	0	
368	1992	53	62	>2	M	0	192	0	0		0	0	
369	1992	54	62	>2	M	0	768	0	0		0	0	
370	1992	55	62	>2	M	0	1536	0	0		1	0	
371	1992	56	62	>2	M	0	96	0	0		0	0	
372	1992	57	62	>2	M	0	768	0	0		1	0	
373	1992	58	62	>2	M	0	96	0	0		0	0	
374	1992	59	62	>2	M	0	192	0	0		0	0	
375	1992	60	62	>2	M	0	768	0	0		0	0	
376	1992	61	62	>2	M	0	768	0	0		0	0	
377	1992	66	63	1	M	0	0	0	0		0	0	
378	1992	67	63	1	M	0	0	0	0		0	0	
379	1992	68	63	1	M	0	0	0	0		0	1	
380	1992	69	63	1	M	0	0	0	0		1	0	
381	1992	70	63	1	M	0	0	0	0		0	0	
382	1992	71	63	1	M	0	0	0	0		0	0	
383	1992	72	63	1	M	0	0	0	0		1	0	
384	1992	73	63	1	M	0	0	0	0		0	0	
385	1992	74	63	1	M	0	0	0	0		1	0	
386	1992	75	63	1	M	0	48	0	0		0	0	
387	1992	76	63	1	M	0	0	0	0		0	0	
388	1992	77	63	1	M	0	0	0	0		0	0	
389	1992	80	64	2	M	0	384	0	0		0	0	
390	1992	81	64	2	M	0	1536	0	0		0	0	
391	1992	82	64	2	M	0	768	0	0		0	0	
392	1992	83	64	2	M	0	96	0	0		0	0	
393	1992	84	64	2	M	0	768	0	0		0	0	
394	1992	85	64	2	M	0	1536	0	0		0	0	
395	1992	86	64	2	M	0	768	0	0		1	1	
396	1992	87	64	2	M	0	48	0	0		0	0	
397	1992	88	64	2	M	0	192	0	0		0	0	
398	1992	89	64	2	M	0	48	0	0		0	1	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
399	1992	90	64	2	M	0	768	0	0		0	0	
400	1992	91	64	2	M	0	192	0	0		0	0	
401	1992	109	65	>2	F	0	96	0	0		0	0	
402	1992	110	65	>2	F	0	96	0	0		1	0	
403	1992	111	65	>2	F	0	96	0	0		0	0	
404	1992	112	65	>2	F	0	192	0	0		0	0	
405	1992	113	65	>2	F	0	192	0	0		0	0	
406	1992	114	65	>2	F	0	1536	0	0		0	0	
407	1992	115	65	>2	F	0	96	0	0		0	0	
408	1992	116	65	>2	F	0	96	24	0		0	0	
409	1992	117	65	>2	F	0	1536	0	0		0	0	
410	1992	118	65	>2	F	0	384	0	0		0	0	
411	1992	119	65	>2	F	0	24	0	0		0	0	
412	1992	120	65	>2	F	0	48	0	0		0	0	
413	1992	18	66	>2	M	0	24	96	0		0	0	
414	1992	19	66	>2	M	0	96	96	0		0	0	
415	1992	20	66	>2	M	0	0	0	0		0	1	
416	1992	21	66	>2	M	0	24	0	0		0	0	
417	1992	22	66	>2	M	0	24	48	24		0	0	
418	1992	23	66	>2	M	0	0	0	0		0	1	
419	1992	24	66	>2	M	0	0	96	0		0	0	
420	1992	25	66	>2	M	0	24	96	0		0	0	
421	1992	26	66	>2	M	0	96	48	24		0	0	
422	1992	27	66	>2	M	0	384	0	24		0	0	
423	1992	28	66	>2	M	0	24	48	24		0	0	
424	1992	29	66	>2	M	0	24	192	96		0	0	
425	1992	63	67	>2	M	0	192	96	0		1	0	
426	1992	64	67	>2	M	0	48	24	0		0	0	
427	1992	65	67	>2	M	0	384	48	0		0	0	
428	1992	66	67	>2	M	0	96	0	0		1	0	
429	1992	67	67	>2	M	0	1536	0	0		0	0	
430	1992	68	67	>2	M	0	96	48	0		1	0	
431	1992	69	67	>2	M	0	768	0	0		0	0	
432	1992	70	67	>2	M	0	48	48	0		1	0	
433	1992	71	67	>2	M	0	192	48	0		0	0	
434	1992	72	67	>2	M	0	96	24	0		1	0	
435	1992	73	67	>2	M	0	192	96	0		0	0	
436	1992	74	67	>2	M	0	96	24	48		1	0	
437	1992	86	68	2	M	0	96	48	0		1	1	
438	1992	87	68	2	M	0	96	0	0		0	0	
439	1992	88	68	2	M	0	48	0	0		0	0	
440	1992	89	68	2	M	0	48	0	0		0	0	
441	1992	90	68	2	M	0	96	0	0		1	1	
442	1992	91	68	2	M	0	0	0	0		0	0	
443	1992	92	68	2	M	0	0	48	0		0	0	
444	1992	93	68	2	M	0	0	48	0		1	0	
445	1992	94	68	2	M	0	0	384	24		1	0	
446	1992	95	68	2	M	0	192	0	0		0	0	
447	1992	96	68	2	M	0	24	1536	24		1	1	
448	1992	97	68	2	M	0	24	768	24		1	0	
449	1992	108	69	>2	M	0	0	0	0		0	0	
450	1992	109	69	>2	M	0	48	48	0		0	0	
451	1992	110	69	>2	M	0	0	48	0		0	0	
452	1992	111	69	>2	M	0	24	96	0		0	0	
453	1992	112	69	>2	M	0	24	48	0		0	0	
454	1992	113	69	>2	M	0	24	192	0		0	0	
455	1992	114	69	>2	M	0	24	0	0		0	0	
456	1992	115	69	>2	M	0	0	384	0		0	0	
457	1992	116	69	>2	M	0	0	48	0		0	0	
458	1992	117	69	>2	M	0	48	48	0		0	0	
459	1992	118	69	>2	M	0	0	96	24		0	0	
460	1992	119	69	>2	M	0	0	192	0		0	0	
461	1992	15	70	1	M	0	1536	0	0		1	0	
462	1992	16	70	1	M	0	96	0	0		1	0	
463	1992	17	70	1	M	0	192	0	0		1	0	
464	1992	18	70	1	M	0	96	0	0		0	0	
465	1992	19	70	1	M	0	96	0	0		0	0	
466	1992	20	70	1	M	0	384	0	0		1	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
467	1992	21	70	1	M	0	192	0	0		0	0	
468	1992	22	70	1	M	0	96	0	0		0	0	
469	1992	23	70	1	M	0	96	0	0		0	0	
470	1992	24	70	1	M	0	384	0	0		1	0	
471	1992	25	70	1	M	0	192	0	0		1	0	
472	1992	26	70	1	M	0	768	0	0		0	0	
473	1992	39	71	2	M	0	192	0	0		0	0	
474	1992	40	71	2	M	0	96	0	0		0	0	
475	1992	41	71	2	M	0	384	48	0		0	0	
476	1992	42	71	2	M	0	768	0	0		0	0	
477	1992	43	71	2	M	0	192	0	0		0	0	
478	1992	44	71	2	M	0	192	0	0		0	0	
479	1992	45	71	2	M	0	1536	24	0		1	0	
480	1992	46	71	2	M	0	192	24	24		0	0	
481	1992	47	71	2	M	0	192	24	0		1	0	
482	1992	48	71	2	M	0	48	24	0		0	0	
483	1992	49	71	2	M	0	192	96	0		0	0	
484	1992	50	71	2	M	0	96	96	24		0	0	
485	1992	91	72	1	M	0	768	96	0		1	0	
486	1992	92	72	1	M	0	768	24	0		1	0	
487	1992	93	72	1	M	0	192	48	0		1	0	
488	1992	94	72	1	M	0	768	48	0		1	0	
489	1992	95	72	1	M	0	768	24	0		1	0	
490	1992	96	72	1	M	0	1536	48	0		1	1	
491	1992	97	72	1	M	0	48	0	0		0	0	
492	1992	98	72	1	M	0	384	0	0		1	0	
493	1992	99	72	1	M	0	768	48	0		1	0	
494	1992	100	72	1	M	0	96	96	0		1	0	
495	1992	101	72	1	M	0	1536	192	0		0	0	
496	1992	102	72	1	M	0	384	96	0		1	0	
497	1992	20	73	2	M	0	24	0	48		0	0	
498	1992	21	73	>2	M	0	48	0	0		1	0	
499	1992	22	73	2	M	0	96	0	0		0	0	
500	1992	23	73	2	M	0	48	0	0		0	1	
501	1992	24	73	2	M	0	24	0	0		0	0	
502	1992	25	73	2	M	0	96	0	0		0	1	
503	1992	26	73	2	M	0	768	0	0		0	1	
504	1992	27	73	2	M	0	192	0	0		0	0	
505	1992	28	73	>2	M	0	192	48	0		1	1	
506	1992	29	73	2	M	0	384	0	0		1	0	
507	1992	30	73	>2	M	0	384	24	0		0	0	
508	1992	31	73	>2	M	0	96	0	0		1	1	
509	1992	61	74	2	M	0	384	0	24		0	0	
510	1992	62	74	>2	M	0	192	48	24		1	0	
511	1992	63	74	2	M	0	96	192	24		1	0	
512	1992	64	74	2	M	0	1536	192	0		0	0	
513	1992	65	74	2	M	0	96	24	0		0	0	
514	1992	66	74	>2	M	0	96	0	0		0	0	
515	1992	67	74	2	M	0	384	0	0		0	0	
516	1992	68	74	2	M	0	384	24	0		1	0	
517	1992	69	74	2	M	0	768	24	0		1	0	
518	1992	70	74	2	M	0	192	48	0		0	0	
519	1992	10	75	>2	M	0	384	0	24		0	0	
520	1992	11	75	>2	M	0	1536	0	0		0	0	
521	1992	12	75	>2	M	0	384	0	0		0	0	
522	1992	13	75	>2	M	0	192	0	0		0	0	
523	1992	14	75	>2	M	0	96	0	0		0	0	
524	1992	15	75	>2	M	0	48	0	0		0	0	
525	1992	16	75	2	M	0	768	0	0		1	0	
526	1992	17	75	2	M	0	768	0	0		0	0	
527	1992	18	75	>2	M	0	1536	0	0		1	0	
528	1992	19	75	2	M	0	1536	0	0		0	0	
529	1992	20	75	>2	M	0	1536	0	0		0	0	
530	1992	21	75	2	M	0	384	0	0		0	0	
531	1992	34	76	1	M	0	192	0	0		0	0	
532	1992	35	76	1	M	0	48	0	0		0	0	
533	1992	36	76	1	M	0	192	0	0		0	1	
534	1992	37	76	1	M	0	768	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
535	1992	38	76	1	M	0	384	0	0		1	0	
536	1992	39	76	1	M	0	384	0	0		1	0	
537	1992	40	76	1	M	0	768	0	0		0	0	
538	1992	41	76	1	M	0	768	0	0		0	1	
539	1992	42	76	1	M	0	96	0	0		1	0	
540	1992	43	76	1	M	0	0	0	0		0	1	
541	1992	44	76	1	M	0	0	0	0		0	0	
542	1992	45	76	1	M	0	96	0	0		0	0	
543	1992	48	77	>2	M	0	96	0	0		0	0	
544	1992	49	77	>2	M	0	96	0	0		0	0	
545	1992	50	77	>2	M	0	0	0	0		1	1	
546	1992	51	77	>2	M	0	192	0	0		0	0	
547	1992	52	77	>2	M	0	384	0	0		0	0	
548	1992	53	77	>2	M	0	96	0	0		0	0	
549	1992	54	77	>2	M	0	1536	0	24		0	1	
550	1992	55	77	>2	M	0	48	0	0		0	0	
551	1992	56	77	>2	M	0	192	0	0		0	0	
552	1992	57	77	>2	M	0	24	0	0		0	1	
553	1992	118	78	>2	M	0	0	24	24		1	1	
554	1992	119	78	>2	M	0	0	96	24		1	0	
555	1992	120	78	>2	M	0	0	0	0		1	0	
556	1992	121	78	>2	M	0	0	0	0		1	0	
557	1992	122	78	>2	M	0	768	0	0		0	0	
558	1992	123	78	>2	M	0	0	0	0		1	0	
559	1992	124	78	>2	M	0	0	0	0		1	0	
560	1992	125	78	2	M	0	0	0	24		1	1	
561	1992	126	78	>2	M	0	0	0	0		1	0	
562	1992	127	78	>2	M	0	0	1536	24		0	0	
563	1992	128	78	>2	M	0	384	24	0		0	1	
564	1992	129	78	2	M	0	0	192	48		0	0	
565	1992	1	79	1	M	0	0	0	0		0	0	
566	1992	2	79	1	M	0	0	0	0		0	0	
567	1992	3	79	1	M	0	0	0	0		0	0	
568	1992	4	79	1	M	0	0	0	0		0	1	
569	1992	5	79	1	M	0	0	0	0		0	1	
570	1992	6	79	1	M	0	0	0	0		0	0	
571	1992	7	79	1	M	0	0	0	0		0	1	
572	1992	8	79	1	M	0	0	0	0		0	0	
573	1992	9	79	1	M	0	0	0	0		1	0	
574	1992	10	79	1	M	0	0	0	0		0	0	
575	1992	24	80	1	M	0	96	0	0		0	0	
576	1992	25	80	2	M	0	768	0	0		0	1	
577	1992	26	80	1	M	0	768	0	0		0	0	
578	1992	27	80	2	M	0	96	0	0		0	0	
579	1992	28	80	1	M	0	96	0	0		1	0	
580	1992	29	80	1	M	0	192	0	0		0	0	
581	1992	30	80	1	M	0	768	0	0		0	0	
582	1992	31	80	1	M	0	768	0	0		0	0	
583	1992	32	80	1	M	0	384	0	0		0	0	
584	1992	33	80	1	M	0	96	0	0		1	0	
585	1992	34	80	2	M	0	0	0	0		0	0	
586	1992	35	80	1	M	0	96	0	0		1	0	
587	1992	39	81	>2	M	0	0	0	0		0	0	
588	1992	40	81	>2	M	0	384	0	0		0	0	
589	1992	41	81	>2	M	0	0	0	0		1	0	
590	1992	42	81	>2	M	0	0	0	0		0	0	
591	1992	43	81	>2	M	0	24	0	0		1	0	
592	1992	44	81	>2	M	0	48	0	0		1	0	
593	1992	45	81	>2	M	0	48	0	0		1	0	
594	1992	46	81	>2	M	0	0	0	0		0	0	
595	1992	47	81	>2	M	0	0	0	0		0	0	
596	1992	48	81	>2	M	0	48	0	0		0	0	
597	1992	49	81	>2	M	0	0	0	0		0	1	
598	1992	50	81	>2	M	0	24	0	0		0	0	
599	1992	68	82	1	M	0	0	0	0		1	0	
600	1992	69	82	1	M	0	0	0	0		1	0	
601	1992	70	82	1	M	0	0	0	0		0	0	
602	1992	71	82	1	M	0	0	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
603	1992	72	82	1	M	0	0	0	0		0	0	
604	1992	73	82	1	M	0	0	0	0		1	1	
605	1992	74	82	1	M	0	0	0	0		0	0	
606	1992	75	82	1	M	0	0	0	0		1	0	
607	1992	76	82	1	M	0	0	0	0		1	0	
608	1992	77	82	1	M	0	0	0	0		0	0	
609	1992	78	82	1	M	0	0	0	0		0	0	
610	1992	79	82	1	M	0	96	0	0		0	0	
611	1992	88	83	1	M	0	1536	0	0		0	0	
612	1992	89	83	1	M	0	192	0	0		1	0	
613	1992	90	83	1	M	0	384	0	0		0	0	
614	1992	91	83	1	M	0	768	0	0		1	0	
615	1992	92	83	1	M	0	192	0	0		1	0	
616	1992	93	83	1	M	0	768	0	0		1	0	
617	1992	94	83	1	M	0	384	0	0		1	0	
618	1992	95	83	1	M	0	48	24	0		1	0	
619	1992	96	83	1	M	0	1536	0	0		1	0	
620	1992	97	83	1	M	0	1536	0	0		1	0	
621	1992	98	83	1	M	0	0	24	0		0	0	
622	1992	99	83	1	M	0	96	0	0		0	0	
623	2004	177	2	1	M	0	0	48	0	1	1	0	1
624	2004	180	2	>4	F	0	24	0	0	0	1	0	0
625	2004	85	2	1	M	0	0	0	0	0	0	0	0
626	2004	190	2	1	M	0	24	48	0	1	0	0	1
627	2004	28	2	1	M	0	0	0	0	1	0	0	0
628	2004	35	2	1	M	0	24	0	0	0	0	1	0
629	2004	40	2	1	M	0	192	24	0	1	0	0	0
630	2004	770	2	1	M	0	0	24	0	1	1	1	0
631	2004	769	2	1	M	0	48	48	0	1	1	0	0
632	2004	176	2	>4	F	0	48	48	0	1	1	0	0
633	2004	177	2	>4	F	0	48	24	0	0	1	1	0
634	2004	188	2	1	M	0	48	0	0	0	0	0	1
635	2004	189	2	1	M	0	0	0	0	1	0	0	1
636	2004	191	2	1	M	0	0	24	0	0	0	0	0
637	2004	196	2	1	M	0	24	192	0	0	1	0	0
638	2004	212	2	>4	F	0	48	192	0	0	0	0	0
639	2004	227	2	>4	F	0	384	0	24	1	0	0	0
640	2004	270	2	1	M	0	96	0	0	1	0	0	0
641	2004	531	2	1	M	0	0	0	0	1	0	0	1
642	2004	615	2	>4	F	0	96	48	48	0	0	0	0
643	2004	616	2	>4	F	0	768	0	24	0	1	0	1
644	2004	959	2	1	M	O	0	96	0	1	0	0	0
645	2004	970	2	1	M	O	24	192	0	1	1	0	0
646	2004	966	2	1	M	O	96	96	0	1	1	0	1
647	2004	912	2	1	M	O	24	0	0	1	0	0	1
648	2004	977	2	1	M	O	24	384	0	1	0	0	0
649	2004	669	2	1	M	O	48	96	0	1	1	0	0
650	2004	682	2	1	M	O	24	0	0	1	0	0	0
651	2004	873	2	1	M	O	0	0	0	0	0	0	0
652	2004	874	2	1	M	O	24	0	0	0	0	0	1
653	2004	875	2	1	M	O	24	0	0	1	0	0	1
654	2004	855	2	>4	F	0	24	48	0	0	0	0	0
655	2004	872	2	>4	F	0	24	0	0	0	0	0	0
656	2004	876	2	>4	F	0	24	0	0	0	1	0	0
657	2004	884	2	>4	F	0	48	0	0	0	0	0	0
658	2004	916	2	>4	F	0	768	0	0	1	0	0	0
659	2004	940	2	>4	F	0	24	24	24	0	1	0	0
660	2004	944	2	>4	F	0	24	0	0	0	0	0	0
661	2004	845	2	1	M	0	24	0	0	1	0	0	0
662	2004	946	2	>4	F	0	0	0	0	0	0	0	0
663	2004	3019	2	>4	F	0	24	24	24	1	0	0	0
664	2004	3025	2	>4	F	0	384	0	0	0	0	0	0
665	2004	3028	2	>4	F	0	48	96	24	0	0	0	0
666	2004	3029	2	>4	F	0	96	24	0	0	0	0	0
667	2004	3034	2	>4	F	0	48	24	0	0	0	0	0
668	2004	3036	2	>4	F	0	48	0	0	0	0	0	0
669	2004	3040	2	>4	F	0	24	48	0	0	0	0	0
670	2004	3045	2	>4	F	0	96	96	0	0	1	0	0

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
671	2004	3091	2	>4	F	0	48	384	0	0	0	0	0
672	2004	3110	2	>4	F	0	24	0	0	0	0	0	0
673	2004	3113	2	>4	F	0	0	48	24	1	1	0	0
674	2004	3118	2	>4	F	0	0	48	0	0	0	0	0
675	2004	3123	2	>4	F	0	48	48	24	0	0	0	0
676	2004	3125	2	>4	F	0	192	96	0	0	0	0	0
677	2004	3133	2	>4	F	0	3072	24	48	0	1	0	0
678	2004	3139	2	>4	F	0	96	24	0	1	0	0	0
679	2004	3148	2	>4	F	0	24	48	0	0	1	0	0
680	2004	3156	2	>4	F	0	48	0	0	0	0	0	0
682	2004	3161	2	>4	F	0	48	0	0	0	1	0	0
683	2004	3164	2	>4	F	0	48	0	0	0	0	0	0
684	2004	3175	2	>4	F	0	24	24	0	0	0	0	0
685	2004	3176	2	>4	F	0	48	96	0	0	0	1	0
686	2004	3195	2	>4	F	0	48	24	48	0	0	0	0
687	2004	3196	2	>4	F	0	0	0	0	0	0	0	1
688	2004	3197	2	>4	F	0	48	96	0	0	0	0	0
689	2004	4119	2	>4	F	0	384	192	24	1	1	0	1
690	2004	4332	2	>4	F	0	24	0	0	0	0	1	0
691	2004	5020	2	>4	F	0	384	192	48	1	1	0	0
692	2004	5087	2	>4	F	0	48	0	0	1	0	0	0
693	2004	5185	2	>4	F	0	0	48	0	1	0	0	0
694	2004	5293	2	>4	F	0	48	48	48	0	0	0	0
695	2004	6177	2	>4	F	0	0	0	0	1	0	1	0
696	2004	6189	2	>4	F	0	384	24	0	0	0	0	0
697	2004	6378	2	>4	F	0	48	0	0	0	0	0	0
698	2004	7326	2	>4	F	0	3072	96	96	1	1	0	1
699	2004	7338	2	>4	F	0	0	24	24	0	0	0	0
700	2004	7471	2	>4	F	0	24	24	0	1	0	0	0
701	2004	7529	2	>4	F	0	24	48	48	0	0	0	1
702	2004	515*	2	>4	F	0	96	0	0	1	1	0	0
703	2004	523*	2	>4	F	0	48	24	0	1	0	0	0
704	2004	0 id tag	2	>4	F	0	48	24	0	0	0	0	0
705	2004	0 id. tag	2	>4	F	0	24	0	0	0	0	0	0
706	2004	90	1	2	M	0	0	0	0	0	0	0	0
707	2004	91	1	2	M	0	48	0	0	0	1	0	0
708	2004	92	1	2	M	0	0	0	24	1	0	0	0
709	2004	93	1	2	M	0	0	0	0	0	0	0	0
710	2004	94	1	2	M	0	24	0	24	1	0	1	0
711	2004	97	1	2	M	0	24	0	0	0	0	0	0
712	2004	99	1	2	M	0	24	0	0	1	0	1	0
713	2004	100	1	2	M	0	24	0	0	0	0	0	0
714	2004	101	1	2	M	0	0	0	0	1	0	0	0
715	2004	102	1	2	M	0	24	0	0	0	0	0	0
716	2004	15	1	2	M	0	24	0	0	1	0	0	0
717	2004	42	1	2	M	0	48	0	0	1	0	0	0
718	2004	53	1	2	M	0	24	0	24	0	0	1	1
719	2004	55	1	2	M	0	24	0	0	0	0	0	0
720	2004	57	1	2	M	0	24	0	0	1	0	0	0
721	2004	79	1	2	M	0	0	0	0	0	0	0	0
722	2004	90	1	2	M	0	0	0	0	0	0	0	0
723	2004	111	1	2	M	0	24	0	24	0	0	0	0
724	2004	151	1	2	M	0	24	0	0	0	0	0	0
725	2004	164	1	2	M	0	24	0	0	0	0	0	0
726	2004	174	1	2	M	0	0	0	0	0	0	0	0
727	2004	180	1	2	M	0	24	0	24	1	0	0	0
728	2004	192	1	2	M	0	0	0	24	1	0	0	0
729	2004	251	1	2	M	0	0	0	0	0	0	0	0
730	2004	350	1	2	M	0	48	0	0	1	0	0	0
731	2004	401	1	2	M	0	24	0	0	1	0	0	0
732	2004	404	1	2	M	0	24	0	0	0	0	0	0
733	2004	410	1	2	M	0	48	0	0	0	0	1	0
734	2004	453	1	2	M	0	0	0	0	0	0	0	0
735	2004	466	1	2	M	0	0	0	0	0	0	0	0
736	2004	127	1	2	M	0	24	0	0	0	0	0	0
737	2004	132	1	2	M	0	48	0	0	0	0	0	0
738	2004	134	1	2	M	0	24	0	0	0	0	0	0
739	2004	140	1	2	M	0	0	0	0	0	0	0	0

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
740	2004	147	1	2	M	0	0	0	0	0	0	0	0
741	2004	150	1	2	M	0	0	0	0	0	0	0	0
742	2004	153	1	2	M	0	0	0	0	0	0	0	0
743	2004	154	1	2	M	0	24	0	0	0	0	1	0
744	2004	156	1	2	M	0	24	0	0	0	0	0	0
745	2004	110	31	1	F	0	24	48	48		0	1	
746	2004	111	31	1	F	0	24	192	24		0	1	
747	2004	112	31	1	F	0	0	48	24		1	0	
748	2004	113	31	1	F	0	24	48	24		0	1	
749	2004	114	31	1	F	0	0	48	24		0	1	
750	2004	115	31	1	F	0	24	24	48		0	1	
751	2004	116	31	1	F	0	24	48	48		0	1	
752	2004	117	31	1	F	0	0	48	24		1	1	
753	2004	118	31	1	F	0	0	24	24		1	1	
754	2004	119	31	1	F	0	24	24	24		1	1	
755	2004	120	31	1	F	0	0	0	24		1	1	
756	2004	121	31	1	F	0	24	0	24		0	1	
757	2004	166	4a	1	M	0	48	192	0		1	1	
758	2004	167	4a	1	M	0	24	96	24		1	1	
759	2004	168	4a	1	M	0	24	192	24		1	1	
760	2004	169	4a	1	M	0	96	24	24		1	1	
761	2004	170	4a	1	M	0	48	96	24		1	1	
762	2004	171	4a	1	M	0	192	24	0		1	1	
763	2004	172	4a	1	M	0	96	384	24		1	1	
764	2004	173	4a	1	M	0	48	192	0		1	1	
765	2004	174	4a	1	M	0	48	48	24		1	1	
766	2004	175	4a	1	M	0	192	768	48		1	1	
767	2004	176	4a	1	M	0	192	48	0		1	1	
768	2004	177	4a	1	M	0	192	384	48		1	1	
769	2004	195	5	1	M	0	96	48	24		1	1	
770	2004	196	5	1	M	0	96	96	24		0	1	
771	2004	197	5	1	M	0	96	96	48		0	1	
772	2004	198	5	1	M	0	96	48	24		0	1	
773	2004	199	5	1	M	0	96	48	24		0	1	
774	2004	200	5	1	M	0	24	48	48		0	1	
775	2004	201	5	1	F	0	48	24	24		1	1	
776	2004	202	5	1	F	0	384	0	24		1	1	
777	2004	203	5	1	F	0	768	0	48		0	1	
778	2004	204	5	1	F	0	96	0	24		0	1	
779	2004	205	5	1	F	0	384	0	24		1	1	
780	2004	206	5	1	M	0	96	96	24		0	1	
781	2004	14	6	1	M	0	24	48	0		0	1	
782	2004	15	6	1	M	0	24	48	0		0	1	
783	2004	16	6	1	M	0	0	48	0		0	1	
784	2004	17	6	1	M	0	24	0	0		0	1	
785	2004	18	6	1	M	0	24	0	0		0	1	
786	2004	19	6	1	M	0	24	0	0		0	1	
787	2004	20	6	1	M	0	24	0	0		0	1	
788	2004	21	6	1	F	0	24	24	0		1	1	
789	2004	22	6	1	F	0	0	24	0		1	1	
790	2004	23	6	1	M	0	0	0	0		1	1	
791	2004	24	6	1	M	0	24	24	24		0	1	
792	2004	25	6	1	F	0	24	0	0		0	1	
793	2004	26	7	1	F	0	24	0	24		0	1	
794	2004	27	7	1	M	0	24	0	0		0	1	
795	2004	28	7	1	M	0	24	0	0		0	1	
796	2004	29	7	1	F	0	96	0	0		0	1	
797	2004	30	7	1	M	0	24	0	0		1	1	
798	2004	31	7	1	M	0	24	24	0		1	1	
799	2004	32	7	1	F	0	48	24	0		0	1	
800	2004	33	7	1	F	0	24	0	24		1	1	
801	2004	34	7	1	M	0	0	48	0		1	1	
802	2004	35	7	1	M	0	24	24	0		0	1	
803	2004	36	7	1	F	0	48	0	0		0	1	
804	2004	37	7	1	F	0	24	24	0		1	1	
805	2004	50	8	1	F	0	24	0	24		0	1	
806	2004	51	8	1	M	0	0	0	0		0	1	
807	2004	52	8	1	F	0	0	0	0		1	1	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
808	2004	53	8	1	F	0	0	0	0		0	1	
809	2004	54	8	1	F	0	48	0	0		1	1	
810	2004	55	8	1	M	0	48	0	0		1	1	
811	2004	56	8	1	M	0	24	0	0		0	1	
812	2004	57	8	1	M	0	24	0	0		1	1	
813	2004	58	8	1	M	0	48	24	0		0	1	
814	2004	59	8	1	M	0	48	24	0		1	1	
815	2004	60	8	1	M	0	24	0	0		0	1	
816	2004	61	8	1	M	0	24	0	0		1	1	
817	2004	94	9	1	M	0	48	0	24		0	1	
818	2004	95	9	1	M	0	24	48	0		0	1	
819	2004	96	9	1	M	0	192	0	0		1	1	
820	2004	97	9	1	M	0	48	0	0		0	1	
821	2004	98	9	1	M	0	96	0	0		0	1	
822	2004	99	9	1	M	0	192	0	0		0	1	
823	2004	100	9	2	M	0	24	0	0		0	1	
824	2004	101	9	2	M	0	48	0	0		1	1	
825	2004	102	9	2	M	0	384	0	48		0	1	
826	2004	103	9	2	M	0	48	24	0		0	1	
827	2004	104	9	2	M	0	24	24	24		0	1	
828	2004	105	9	2	M	0	24	0	0		0	1	
829	2004	106	9	2	M	0	48	48	0		0	1	
830	2004	107	9	2	M	0	384	0	0		0	1	
831	2004	108	10	2	M	0	192	0	24		0	1	
832	2004	109	10	2	M	0	24	0	0		0	1	
833	2004	110	10	2	M	0	24	24	0		0	1	
834	2004	111	10	2	M	0	24	0	0		0	1	
835	2004	112	10	1	M	0	48	24	0		0	1	
836	2004	113	10	1	M	0	24	0	0		0	1	
837	2004	114	10	1	M	0	24	0	0		0	1	
838	2004	115	10	1	M	0	24	24	0		0	1	
839	2004	116	10	1	M	0	48	24	0		1	1	
840	2004	117	10	1	M	0	24	0	0		0	1	
841	2004	118	10	1	M	0	24	24	0		0	1	
842	2004	119	10	1	M	0	24	0	0		0	1	
843	2004	120	10	1	M	0	48	24	0		0	1	
844	2004	117	10	1	M	0	24	24	0		0	1	
845	2004	118	11	1	M	0	768	24	0		0	1	
846	2004	119	11	1	M	0	96	24	0		0	1	
847	2004	120	11	1	M	0	96	0	0		0	1	
848	2004	121	11	1	M	0	96	0	0		0	1	
849	2004	122	11	1	M	0	192	0	0		0	1	
850	2004	123	11	1	M	0	384	0	0		0	1	
851	2004	124	11	1	M	0	24	96	24		1	1	
852	2004	125	11	1	M	0	24	96	24		0	1	
853	2004	126	11	1	M	0	0	48	24		1	1	
854	2004	127	11	1	M	0	24	48	0		0	1	
855	2004	128	11	1	M	0	24	48	24		0	1	
856	2004	129	11	1	M	0	24	48	24		0	1	
857	2004	27	12	1	M	0	0	0	0		1	1	
858	2004	28	12	1	M	0	0	0	0		0	1	
859	2004	29	12	1	M	0	0	0	0		0	1	
860	2004	30	12	1	M	0	0	0	0		1	1	
861	2004	31	12	1	M	0	0	0	0		1	1	
862	2004	32	12	1	M	0	0	0	0		1	1	
863	2004	33	12	1	M	0	0	0	0		0	1	
864	2004	34	12	1	M	0	0	0	0		0	1	
865	2004	35	12	1	M	0	0	0	0		1	1	
866	2004	36	12	1	M	0	0	0	0		0	1	
867	2004	37	12	1	M	0	0	0	0		1	1	
868	2004	38	12	1	M	0	0	0	0		0	1	
869	2004	39	13	1	F	0	96	768	0		0	1	
870	2004	40	13	1	F	0	48	1536	48		1	1	
871	2004	41	13	1	F	0	192	768	24		1	1	
872	2004	42	13	1	F	0	96	48	24		1	1	
873	2004	43	13	1	F	0	24	768	24		0	1	
874	2004	44	13	1	F	0	48	192	96		1	1	
875	2004	45	13	1	F	0	48	192	24		1	1	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
876	2004	46	13	1	F	0	48	24	24		1	1	
877	2004	47	13	1	F	0	24	48	24		0	1	
878	2004	48	13	1	F	0	48	384	48		1	1	
879	2004	49	13	1	F	0	24	192	24		0	1	
880	2004	50	13	1	F	0	24	384	0		1	0	
881	2004	159	14	1	F	0	24	24	0		0	1	
882	2004	160	14	1	F	0	0	24	0		0	0	
883	2004	161	14	1	M	0	24	0	0		0	1	
884	2004	162	14	1	M	0	0	24	0		0	1	
885	2004	163	14	1	F	0	24	24	0		0	1	
886	2004	164	14	1	M	0	24	24	0		1	1	
887	2004	165	14	1	M	0	24	0	24		0	1	
888	2004	166	14	1	F	0	24	0	24		0	1	
889	2004	167	14	1	M	0	0	0	0		1	1	
890	2004	168	14	1	M	0	0	0	24		0	1	
891	2004	169	14	1	F	0	0	0	24		1	1	
892	2004	170	14	1	F	0	24	0	24		0	1	
893	2004	171	15	1	F	0	0	0	24		0	1	
894	2004	172	15	1	F	0	0	0	0		0	1	
895	2004	173	15	1	F	0	0	0	24		0	1	
896	2004	174	15	1	F	0	48	0	0		0	1	
897	2004	175	15	1	M	0	192	0	0		1	1	
898	2004	176	15	1	F	0	48	0	0		0	1	
899	2004	177	15	1	F	0	24	0	0		0	1	
900	2004	178	15	1	F	0	24	0	0		0	1	
901	2004	179	15	1	F	0	24	0	0		0	1	
902	2004	180	15	1	F	0	0	0	0		0	1	
903	2004	181	15	1	F	0	24	0	24		0	1	
904	2004	182	15	1	F	0	0	0	0		1	1	
905	2004	192	16	1	M	0	24	0	24		0	1	
906	2004	193	16	1	F	0	24	0	0		0	1	
907	2004	194	16	1	F	0	0	0	0		0	1	
908	2004	195	16	1	M	0	0	0	24		0	1	
909	2004	196	16	1	F	0	24	0	24		0	1	
910	2004	197	16	1	M	0	0	0	0		0	1	
911	2004	198	16	1	F	0	24	24	0		0	1	
912	2004	199	16	1	M	0	24	0	0		0	1	
913	2004	200	16	1	M	0	0	0	24		0	1	
914	2004	201	16	1	F	0	24	0	24		0	1	
915	2004	202	16	1	F	0	24	0	24		0	1	
916	2004	203	16	1	M	0	0	0	24		1	1	
917	2004	204	17	1	F	0	0	0	24		0	1	
918	2004	205	17	1	M	0	0	0	24		1	1	
919	2004	206	17	2	F	0	0	0	24		0	1	
920	2004	207	17	2	F	0	24	24	0		0	1	
921	2004	208	17	2	F	0	24	24	0		1	1	
922	2004	209	17	1	M	0	96	48	0		0	1	
923	2004	210	17	1	M	0	192	48	0		0	1	
924	2004	211	17	2	M	0	96	96	0		1	1	
925	2004	212	17	2	M	0	96	384	0		0	1	
926	2004	213	17	2	M	0	384	768	0		1	1	
927	2004	214	17	2	M	0	192	1536	0		1	1	
928	2004	215	17	2	M	0	384	1536	0		1	1	
929	2004	31	18	2	M	0	24	0	0		1	1	
930	2004	32	18	2	M	0	24	24	24		1	1	
931	2004	33	18	2	M	0	24	0	24		0	1	
932	2004	34	18	2	M	0	24	24	24		0	1	
933	2004	35	18	2	M	0	24	24	24		0	1	
934	2004	36	18	2	M	0	48	0	24		1	1	
935	2004	37	18	2	M	0	24	0	24		0	1	
936	2004	38	18	2	M	0	24	0	0		1	1	
937	2004	39	18	2	M	0	0	0	24		0	1	
938	2004	40	18	2	M	0	0	0	24		1	1	
939	2004	41	18	2	M	0	0	0	24		1	1	
940	2004	42	18	2	M	0	0	0	24		0	1	
941	2004	90	19	2	M	0	192	24	24		1	1	
942	2004	91	19	2	M	0	96	24	0		1	1	
943	2004	92	19	2	M	0	24	48	24		1	1	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
944	2004	93	19	2	M	0	96	48	24		0	1	
945	2004	94	19	2	M	0	24	48	0		0	1	
946	2004	95	19	2	M	0	96	48	48		0	1	
947	2004	96	19	2	M	0	192	48	24		0	1	
948	2004	97	19	2	M	0	48	48	24		0	1	
949	2004	98	19	2	M	0	96	0	24		0	1	
950	2004	99	19	2	M	0	384	24	48		1	1	
951	2004	100	19	2	M	0	24	0	0		0	1	
952	2004	101	19	2	M	0	24	24	24		0	1	
953	2004	131	20	2	F	0	48	0	24		0	1	
954	2004	132	20	2	F	0	48	0	24		0	1	
955	2004	133	20	1	F	0	24	0	24		0	1	
956	2004	134	20	2	F	0	24	0	24		0	1	
957	2004	135	20	2	F	0	48	0	24		0	1	
958	2004	136	20	2	F	0	48	0	24		0	1	
959	2004	137	20	2	F	0	384	0	24		0	1	
960	2004	138	20	2	F	0	96	0	24		0	1	
961	2004	139	20	2	F	0	24	24	0		0	1	
962	2004	140	20	2	F	0	48	0	0		1	1	
963	2004	141	20	2	F	0	96	0	24		0	1	
964	2004	142	20	2	F	0	192	0	48		0	1	
965	2004	1	22	2	M	0	48	48	24		0	0	
966	2004	2	22	2	M	0	24	48	24		0	0	
967	2004	3	22	2	M	0	96	48	24		0	0	
968	2004	4	22	2	M	0	96	48	24		0	0	
969	2004	5	22	2	M	0	96	48	24		0	0	
970	2004	6	22	2	M	0	24	48	24		0	0	
971	2004	7	22	2	M	0	48	48	24		0	0	
972	2004	8	22	2	M	0	24	48	24		0	0	
973	2004	9	22	2	M	0	24	48	24		0	0	
974	2004	10	22	2	M	0	24	24	24		1	0	
975	2004	11	22	2	M	0	24	24	24		0	0	
976	2004	12	22	2	M	0	48	48	24		1	0	
977	2004	1	23	1	M	0	0	48	0		0	0	
978	2004	2	23	1	M	0	0	48	0		0	0	
979	2004	3	23	1	M	0	0	48	0		0	0	
980	2004	4	23	1	M	0	0	96	0		0	0	
981	2004	5	23	1	M	0	0	48	0		0	0	
982	2004	6	23	1	M	0	0	24	0		0	0	
983	2004	7	23	1	M	0	0	24	0		0	0	
984	2004	8	23	1	M	0	0	48	0		0	0	
985	2004	9	23	1	M	0	0	96	0		0	0	
986	2004	10	23	1	M	0	0	24	0		0	0	
987	2004	11	23	1	M	0	0	48	0		0	0	
988	2004	12	23	1	M	0	0	96	0		0	0	
989	2004	1	24	1	M	0	0	0	0		0	0	
990	2004	2	24	1	M	0	0	0	0		0	0	
991	2004	3	24	1	M	0	48	0	0		1	0	
992	2004	4	24	1	M	0	48	0	0		1	0	
993	2004	5	24	1	M	0	192	0	0		1	0	
994	2004	6	24	1	M	0	48	0	0		1	0	
995	2004	7	24	1	M	0	192	0	0		1	0	
996	2004	8	24	1	M	0	24	0	0		1	0	
997	2004	9	24	1	M	0	768	24	0		0	0	
998	2004	10	24	1	M	0	24	24	0		1	0	
999	2004	11	24	1	M	0	0	0	0		1	0	
1000	2004	12	24	1	M	0	24	0	0		1	0	
1001	2004	13	24	1	M	0	24	0	0		1	0	
1002	2004	1	25	1	M	0	0	0	0		1	0	
1003	2004	2	25	1	M	0	1536	0	24		1	0	
1004	2004	3	25	1	M	0	384	0	48		1	0	
1005	2004	4	25	1	M	0	384	24	0		1	0	
1006	2004	5	25	1	M	0	48	0	0		1	0	
1007	2004	6	25	1	M	0	48	0	0		1	0	
1008	2004	7	25	1	M	0	48	24	0		1	0	
1009	2004	8	25	1	M	0	24	0	0		1	0	
1010	2004	9	25	1	M	0	768	0	0		1	0	
1011	2004	10	25	1	M	0	48	0	0		1	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
1012	2004	11	25	1	M	0	48	0	0		0	0	
1013	2004	12	25	1	M	0	384	0	0		0	0	
1014	2004	13	25	1	M	0	48	0	0		0	0	
1015	2004	1	26	1	M	0	24	0	0		0	0	
1016	2004	2	26	1	M	0	96	0	0		1	0	
1017	2004	3	26	1	M	0	24	0	0		0	0	
1018	2004	4	26	1	M	0	24	0	24		0	0	
1019	2004	5	26	1	M	0	24	0	0		0	0	
1020	2004	6	26	1	M	0	24	0	0		0	0	
1021	2004	7	26	1	M	0	24	0	0		0	0	
1022	2004	8	26	1	M	0	0	0	0		0	0	
1023	2004	9	26	1	M	0	0	24	0		0	0	
1024	2004	10	26	1	M	0	0	0	0		0	0	
1025	2004	11	26	1	M	0	0	0	24		0	0	
1026	2004	12	26	1	M	0	0	0	24		0	0	
1027	2004	13	26	1	M	0	0	0	0		0	0	
1028	2004	14	26	1	M	0	24	0	0		0	0	
1029	2004	15	26	1	M	0	24	0	24		0	0	
1030	2004	1	27	1	M	0	96	96	0		0	0	
1031	2004	2	27	1	M	0	96	48	24		0	0	
1032	2004	3	27	1	M	0	0	0	0		0	0	
1033	2004	4	27	1	M	0	48	0	24		1	0	
1034	2004	5	27	1	M	0	48	0	0		0	0	
1035	2004	6	27	1	M	0	0	0	0		0	0	
1036	2004	7	27	1	M	0	48	0	0		0	0	
1037	2004	8	27	1	M	0	24	0	0		0	0	
1038	2004	9	27	1	M	0	192	0	0		1	0	
1039	2004	10	27	1	M	0	48	0	0		0	0	
1040	2004	11	27	1	M	0	0	0	0		0	0	
1041	2004	12	27	1	M	0	768	0	0		0	0	
1042	2004	13	27	1	M	0	1536	0	0		0	0	
1043	2004	14	27	1	M	0	0	0	0		0	0	
1044	2004	15	27	1	M	0	48	0	0		1	0	
1045	2004	1	28	1	M	0	192	0	0		0	0	
1046	2004	2	28	1	M	0	24	0	0		0	0	
1047	2004	3	28	1	M	0	192	0	0		1	0	
1048	2004	4	28	1	M	0	96	24	24		0	0	
1049	2004	5	28	1	M	0	192	24	24		0	0	
1050	2004	6	28	1	M	0	0	24	0		0	0	
1051	2004	7	28	1	M	0	192	0	0		0	0	
1052	2004	8	28	1	M	0	48	24	0		0	0	
1053	2004	9	28	1	M	0	96	0	0		0	0	
1054	2004	10	28	1	M	0	96	48	0		0	0	
1055	2004	11	28	1	M	0	96	0	0		0	0	
1056	2004	12	28	1	M	0	96	0	0		0	0	
1057	2004	13	28	1	M	0	96	24	0		0	0	
1058	2004	14	28	1	M	0	48	48	24		0	0	
1059	2004	15	28	1	M	0	96	0	24		0	0	
1060	2004	1	29	1	M	0	48	192	0		0	0	
1061	2004	2	29	1	M	0	24	192	0		0	0	
1062	2004	3	29	1	M	0	0	192	0		0	0	
1063	2004	4	29	1	M	0	0	384	0		0	0	
1064	2004	5	29	1	M	0	48	384	0		0	0	
1065	2004	6	29	1	M	0	24	384	0		0	0	
1066	2004	7	29	1	M	0	24	24	0		0	0	
1067	2004	8	29	1	M	0	24	0	0		0	0	
1068	2004	9	29	1	M	0	48	192	0		1	0	
1069	2004	10	29	1	M	0	0	384	0		0	0	
1070	2004	11	29	1	M	0	48	24	24		0	0	
1071	2004	12	29	1	M	0	0	192	24		0	0	
1072	2004	13	29	1	M	0	0	24	0		1	0	
1073	2004	14	29	1	M	0	48	48	24		0	0	
1074	2004	15	29	1	M	0	24	96	0		1	0	
1075	2004	1	30	1	M	0	0	0	0		0	0	
1076	2004	2	30	1	M	0	0	0	0		0	0	
1077	2004	3	30	1	M	0	0	0	0		0	0	
1078	2004	4	30	1	M	0	0	0	0		0	0	
1079	2004	5	30	1	M	0	0	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
1080	2004	6	30	1	M	0	0	0	0		0	0	
1081	2004	7	30	1	M	0	0	0	0		1	0	
1082	2004	8	30	1	M	0	0	0	0		1	0	
1083	2004	9	30	1	M	0	24	48	0		0	0	
1084	2004	10	30	1	M	0	0	0	0		0	0	
1085	2004	11	30	1	M	0	0	0	0		0	0	
1086	2004	12	30	1	M	0	0	24	0		0	0	
1087	2004	13	30	1	M	0	0	24	0		1	0	
1088	2004	14	30	1	M	0	0	24	0		1	0	
1089	2004	15	30	1	M	0	0	24	0		1	0	
1090	2004	2638	3	1	M	0	0	384	0		1	1	
1091	2004	2500	3	1	M	0	0	96	48		1	0	
1092	2004	77	3	1	M	0	192	384	0		0	0	
1093	2004	76	3	1	M	0	24	1536	0		1	0	
1094	2004	1262	3	1	M	0	0	96	48		0	0	
1095	2004	2571	3	1	M	0	96	384	0		1	0	
1096	2004	2588	3	1	M	0	24	96	0		1	1	
1097	2004	1460	3	1	M	0	24	192	0		0	0	
1098	2004	1306	3	1	M	0	24	384	24		0	0	
1099	2004	1300	3	1	M	0	0	96	0		1	0	
1100	2004	1485	3	1	M	0	24	192	0		1	0	
1032	2004	3	27	1	M	0	0	0	0		0	0	
1032	2004	3	27	1	M	0	0	0	0		0	0	
1033	2004	4	27	1	M	0	48	0	24		1	0	
1034	2004	5	27	1	M	0	48	0	0		0	0	
1035	2004	6	27	1	M	0	0	0	0		0	0	
1036	2004	7	27	1	M	0	48	0	0		0	0	
1037	2004	8	27	1	M	0	24	0	0		0	0	
1038	2004	9	27	1	M	0	192	0	0		1	0	
1039	2004	10	27	1	M	0	48	0	0		0	0	
1040	2004	11	27	1	M	0	0	0	0		0	0	
1041	2004	12	27	1	M	0	768	0	0		0	0	
1042	2004	13	27	1	M	0	1536	0	0		0	0	
1043	2004	14	27	1	M	0	0	0	0		0	0	
1044	2004	15	27	1	M	0	48	0	0		1	0	
1045	2004	1	28	1	M	0	192	0	0		0	0	
1046	2004	2	28	1	M	0	24	0	0		0	0	
1047	2004	3	28	1	M	0	192	0	0		1	0	
1048	2004	4	28	1	M	0	96	24	24		0	0	
1049	2004	5	28	1	M	0	192	24	24		0	0	
1050	2004	6	28	1	M	0	0	24	0		0	0	
1051	2004	7	28	1	M	0	192	0	0		0	0	
1052	2004	8	28	1	M	0	48	24	0		0	0	
1053	2004	9	28	1	M	0	96	0	0		0	0	
1054	2004	10	28	1	M	0	96	48	0		0	0	
1055	2004	11	28	1	M	0	96	0	0		0	0	
1056	2004	12	28	1	M	0	96	0	0		0	0	
1057	2004	13	28	1	M	0	96	24	0		0	0	
1058	2004	14	28	1	M	0	48	48	24		0	0	
1059	2004	15	28	1	M	0	96	0	24		0	0	
1060	2004	1	29	1	M	0	48	192	0		0	0	
1061	2004	2	29	1	M	0	24	192	0		0	0	
1062	2004	3	29	1	M	0	0	192	0		0	0	
1063	2004	4	29	1	M	0	0	384	0		0	0	
1064	2004	5	29	1	M	0	48	384	0		0	0	
1065	2004	6	29	1	M	0	24	384	0		0	0	
1066	2004	7	29	1	M	0	24	24	0		0	0	
1067	2004	8	29	1	M	0	24	0	0		0	0	
1068	2004	9	29	1	M	0	48	192	0		1	0	
1069	2004	10	29	1	M	0	0	384	0		0	0	
1070	2004	11	29	1	M	0	48	24	24		0	0	
1071	2004	12	29	1	M	0	0	192	24		0	0	
1072	2004	13	29	1	M	0	0	24	0		1	0	
1073	2004	14	29	1	M	0	48	48	24		0	0	
1074	2004	15	29	1	M	0	24	96	0		1	0	
1075	2004	1	30	1	M	0	0	0	0		0	0	
1076	2004	2	30	1	M	0	0	0	0		0	0	
1077	2004	3	30	1	M	0	0	0	0		0	0	

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
1078	2004	4	30	1	M	0	0	0	0		0	0	
1079	2004	5	30	1	M	0	0	0	0		0	0	
1080	2004	6	30	1	M	0	0	0	0		0	0	
1081	2004	7	30	1	M	0	0	0	0		1	0	
1082	2004	8	30	1	M	0	0	0	0		1	0	
1083	2004	9	30	1	M	0	24	48	0		0	0	
1084	2004	10	30	1	M	0	0	0	0		0	0	
1085	2004	11	30	1	M	0	0	0	0		0	0	
1086	2004	12	30	1	M	0	0	24	0		0	0	
1087	2004	13	30	1	M	0	0	24	0		1	0	
1088	2004	14	30	1	M	0	0	24	0		1	0	
1089	2004	15	30	1	M	0	0	24	0		1	0	
1090	2004	1262	3	1	M	0	0	384	0	1	1	1	0
1091	2004	1288	3	1	M	0	0	96	48	1	1	0	0
1092	2004	1275	3	1	M	0	192	384	0	1	0	0	1
1093	2004	1299	3	1	M	0	24	1536	0	1	1	0	1
1094	2004	1306	3	1	M	0	0	96	48	0	0	0	1
1095	2004	1422	3	1	M	0	96	384	0	1	1	0	0
1096	2004	1435	3	1	M	0	24	96	0	1	1	1	0
1097	2004	1444	3	1	M	0	24	192	0	1	0	0	0
1098	2004	1460	3	1	M	0	24	384	24	0	0	0	0
1099	2004	1465	3	1	M	0	0	96	0	1	1	0	1
1100	2004	1463	3	1	M	0	24	192	0	0	1	0	0
1101	2004	1467	3	1	M	0	0	384	48	1	1	0	1
1102	2004	1470	3	1	M	0	0	24	0	1	1	0	0
1103	2004	1473	3	1	M	0	48	24	0	0	0	0	0
1104	2004	1485	3	1	M	0	0	96	0	1	1	0	1
1105	2004	1475	3	1	F	0	24	96	24	1	1	0	1
1106	2004	1477	3	1	F	0	0	24	24	1	1	0	1
1107	2004	2528	3	1	F	0	0	192	0	1	1	0	0
1108	2004	2529	3	1	F	0	192	192	24	1	1	0	1
1109	2004	2543	3	1	F	0	96	96	0	1	0	0	0
1110	2004	1479	3	1	F	0	0	24	24	1	0	0	0
1111	2004	1482	3	1	F	0	0	384	0	0	1	0	1
1112	2004	2571	3	1	F	0	192	96	0	1	1	0	0
1113	2004	2573	3	1	F	0	0	96	0	1	1	0	0
1114	2004	1486	3	1	F	0	0	96	24	1	1	0	0
1115	2004	1488	3	1	F	0	0	96	24	1	0	0	0
1116	2004	2597	3	1	F	0	0	96	24	1	0	0	0
1117	2004	2433	3	1	F	0	24	96	0	1	0	0	1
1118	2004	2446	3	1	M	0	0	24	0	1	1	1	0
1119	2004	2448	3	1	F	0	0	0	96	1	1	0	0
1120	2004	2500	3	1	F	0	24	96	0	1	1	0	0
1121	2004	2672	3	1	F	0	96	24	0	1	0	0	0
1122	2004	2648	3	1	M	0	24	1536	24	1	1	0	1
1123	2004	2685	3	1	F	0	192	768	0	1	0	0	0
1124	2004	2577	3	1	M	0	24	24	24	1	1	1	0
1125	2004	2584	3	1	F	0	0	1536	24	1	1	0	0
1126	2004	2709	3	1	M	0	24	0	96	1	1	0	0
1127	2004	2588	3	1	F	0	0	96	48	0	1	0	0
1128	2004	2727	3	1	F	0	24	192	0	1	1	1	0
1129	2004	2597	3	1	F	0	24	192	0	1	1	1	0
1130	2004	2741	3	1	F	0	24	96	0	1	1	1	1
1131	2004	2600	3	1	F	0	0	0	0	1	1	0	0
1132	2004	2627	3	1	F	0	24	384	0	1	1	0	1
1133	2004	2630	3	1	F	0	0	192	24	1	1	0	0
1134	2004	2636	3	1	M	0	0	0	0	1	1	0	0
1135	2004	2637	3	1	F	0	0	384	96	1	1	0	1
1136	2004	2638	3	1	F	0	24	0	0	1	1	0	1
1137	2004	2800	3	1	F	0	0	384	0	1	1	0	0
1138	2004	2804	3	1	F	0	0	192	0	1	1	0	0
1139	2004	2839	3	1	F	0	24	48	48	1	1	1	1
1140	2004	2832	3	1	F	0	0	96	0	0	0	0	0
1141	2004	2656	3	1	F	0	24	96	24	1	0	0	1
1142	2004	2668	3	1	F	0	24	24	24	1	1	0	0
1143	2004	2677	3	1	M	0	48	768	0	1	1	0	0
1144	2004	2902	3	1	F	0	96	192	24	1	1	0	0
1145	2004	2707	3	1	F	0	24	24	24	1	1	0	1

Sm.No.	Year	An.ID	Farm	Age	Sex	Vacc.	Hb.	Pom.	Cop.	Hp.	Ws.	RS.	Cult.
1146	2004	2913	3	1	F	0	192	96	0	1	1	0	0
1147	2004	2926	3	1	F	0	96	384	0	1	1	0	1
1148	2004	2726	3	1	F	0	24	384	96	1	1	0	1
1149	2004	2756	3	1	F	0	0	0	0	0	1	0	0
1150	2004	2973	3	1	F	0	0	96	96	1	1	1	1
1151	2004	2977	3	1	F	0	192	96	24	1	1	0	0
1152	2004	2981	3	1	F	0	24	48	0	1	0	0	0
1153	2004	2985	3	1	M	0	0	384	0	1	1	0	0
1154	2004	2764	3	1	F	0	24	192	48	1	1	0	0
1155	2004	2767	3	1	M	0	48	96	48	1	0	0	0
1156	2004	3003	3	1	F	0	192	192	24	1	1	0	0
1157	2004	3009	3	1	F	0	48	96	24	1	1	0	0
1158	2004	2768	3	1	F	0	24	96	0	1	1	0	1
1159	2004	3019	3	1	F	0	192	96	0	1	1	0	0
1160	2004	3035	3	1	F	0	192	96	0	1	1	0	0
1161	2004	2834	3	1	F	0	0	0	0	1	1	0	0
1162	2004	3037	3	1	F	0	96	96	0	1	1	0	0
1163	2004	3049	3	1	F	0	96	96	0	1	0	0	0
1164	2004	2871	3	1	M	0	24	96	24	1	1	1	0
Postmortem (pm) samples from farms 2 and 3													
1	2004	54	2-pm	1	M	0	0	0	0	ND	1	0	1
2	2004	56	2-pm	1	M	0	24	48	0	ND	0	0	0
3	2004	123	2-pm	1	M	0	24	24	48	ND	0	0	1
4	2004	190	2-pm	1	M	0	24	0	0	ND	0	1	0
5	2004	278	2-pm	1	M	0	96	0	0	ND	0	0	0
6	2004	900	2-pm	1	F	0	0	0	0	ND	0	0	1
7	2004	a-19	2-pm	1	M	0	0	96	0	ND	0	0	1
8	2004	98	2-pm	1	M	0	24	192	0	ND	1	0	0
9	2004	456	3-pm	1	M	0	96	96	0	ND	1	0	1
10	2004	987	3-pm	1	M	0	24	0	0	ND	0	0	0
11	2004	345	3-pm	1	M	0	48	96	0	ND	1	0	0
12	2004	654	3-pm	1	M	0	24	0	0	ND	0	0	0
13	2004	321	3-pm	1	M	0	0	0	0	ND	0	0	0
14	2004	938	3-pm	1	M	0	24	0	0	ND	0	0	1
15	2004	900	3-pm	1	M	0	24	0	0	ND	0	0	1
16	2004	594	3-pm	1	M	0	24	0	0	ND	0	0	0
Vaccinated herd not included in stastical analysis													
17	2003	10	21	1	M	1	384	1536	24		1	0	
18	2003	11	21	1	M	1	96	384	24		0	0	
19	2003	12	21	1	M	1	192	1536	48		0	1	
20	2003	1	21	1	M	1	96	48	24		0	0	
21	2003	2	21	1	M	1	384	3072	24		1	0	
22	2003	3	21	1	M	1	384	768	24		0	1	
23	2003	4	21	1	M	1	192	3072	24		0	1	
24	2003	5	21	1	M	1	192	48	24		0	0	
25	2003	6	21	1	M	1	96	3072	24		1	0	
26	2003	7	21	1	M	1	1536	1536	48		1	0	
27	2003	8	21	1	M	1	96	96	24		1	0	
28	2003	9	21	1	M	1	192	1536	24		1	0	