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# EPIDEMIOLOGY OF CANINE LEPTOSPIROSIS IN NEW ZEALAND

A thesis presented in fulfilment of the requirements for the degree of
Master of Veterinary Science at Massey University, Palmerston North, New Zealand

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2015

## I Abstract:

Leptospirosis is a disease of worldwide significance affecting dogs, livestock, and humans. It can be a severe clinical or subclinical disease, either of which contribute to shedding of leptospires in the environment. This work includes a detailed literature review of leptospirosis, the disease, and its prevention and epidemiology as it pertains to dogs worldwide and in New Zealand where the epidemiology is unique. Original work includes a nationwide sero-prevalence survey quantifying the risk of exposure for serovars Copenhageni, Hardjo and Pomona for New Zealand dogs. An additional survey of South Island farm dogs investigates the prevalence of exposure to and urinary shedding of leptospires in dogs exposed to livestock with a high prevalence of infection. This is the first study to investigate shedding of leptospires in dog urine in New Zealand, and challenges a long held perception that dogs may only serve as a maintenance host for serovar Canicola.

Conclusions:

Urinary shedding of Leptospira spp. was demonstrated in more than 12 (95% C.I: 5-24) % of New Zealand farm dogs. It is speculated that these dogs may be serving as maintenance hosts for a number of serovars in addition to Canicola, and may contribute to the maintenance of this disease in a farm environment, and affected dogs may be a zoonotic risk. Urinary shedding of leptospires can occur in dogs with low or negative MAT titres. Exposure to serovar Copenhageni is common for New Zealand dogs, and farm working dogs appear to be at risk for exposure to serovar Hardjo. Serovar Pomona causes severe clinical disease in dogs in New Zealand. Veterinarians investigating clinical cases suspicious for leptospirosis should consider MAT testing for Pomona and Hardjo in addition to Copenhageni, and concurrent PCR testing on blood and/or urine may expedite a diagnosis. Completion of convalescent serology and culture of urine from suspected cases is strongly encouraged. There is a need for a vaccine to protect dogs at risk of infection with serovar Pomona, and consideration should be given to also including serovar Hardjo in the vaccine. Further work is needed to determine the prevalence and duration of urine shedding by dogs nationwide, with culture confirmation and identification of the serovars isolated.

## II Acknowledgements

I would like to thank my supervisors and mentors from the Massey Veterinary Teaching Hospital and Leptospirosis Research Group: Dr. Nick Cave, Professor Boyd Jones, Dr. Jackie Benschop, Dr. Julie Collins-Emerson, Professor Cord Heuer, and Professor Peter Wilson. You gave endless encouragement and support for me as an early career researcher, and welcomed me as a member of the team.

Thanks to Juan Sanhueza for collaborating with sampling and also to all veterinarians and technical staff involved with sample collection. The contribution to research by commercial veterinary practices is greatly valued, as is the willingness of farmers to allow sampling to take place. I would especially like to acknowledge Energy Vets Inglewood and Waitara, Veterinary Services Hawkes Bay, Taranaki Veterinary Services, the Hunterville Veterinary Club, Tararua Vets, The Vet Centre Marlborough, Vet South, Aorangi Vets, Invermay, Canterbury Vets, Darfield Vet Centre, North Canterbury Vet Clinic, Oxford Vet Clinic, Rangiora Veterinary Centre, Riverside Veterinary Services, Vetlife, and Waimate Vet Services.

Thanks also to my Massey Veterinary Teaching Hospital colleagues for keeping the clinic running when I was absent and for helping me maintain my perspective, and especially to Cathy Dyer for filling in for me in the clinic.

Neil Chesterton, Kelly Garland, Andy David and Oliver Knesl, thank you for believing in me and encouraging me not to let questions go unanswered.

And lastly but by no means least; thank you to my family: Graham, Theodore, Cooper and Azriella for patiently supporting me during the creation of this work, and especially my parents for all the sacrifices you made supporting my education.

The author acknowledges the technical advice and diagnostic testing provided by NZVP, mEpiLab and the Epicentre.

Funding for the study was generously provided by the Wairarapa Vet Association, the Companion Animal Society Companion Animal Council, Zoetis/Pfizer, the Massey University Research Fund, the L.A. Alexander Trust, the Centre for Service and Working Dog Health and the Phyllis Irene Grey Trust.

This work has the approval of the Massey University Animal Ethics Committee # 10/103

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# Chapter 1

# Introduction

# 1 Introduction

Leptospirosis; a bacterial disease of global importance and a significant cause of disease and mortality in animals and humans.

This work contains a comprehensive review of canine leptospirosis, including an overview of the bacteria and its taxonomy as it pertains to understanding epidemiological behaviour and the diagnosis of clinical disease or exposure. The epidemiology of the disease in general terms is also reviewed, along with description of typical and less common clinical presentations and clinical signs, the occurrence of subclinical disease, and treatment both specifically and concurrent supportive care. The review also contains a thorough evaluation of the various diagnostic tests available, including selection and interpretation of the appropriate diagnostic tests along with a discussion on the strengths and weaknesses of each.

The focus is on the disease in dogs, the unique epidemiology of the disease in New Zealand and its impact on the canine population throughout this country. Farm working dogs are a particular focus given their exposure to livestock species that act as maintenance hosts to multiple leptospiral serovars. The role of dogs as a potential maintenance host of serovars other than Canicola is discussed, with consideration to New Zealand's unique leptospiral epidemiology.

Two original investigations described herein characterise canine leptospirosis in New Zealand. The studies include a nationwide prevalence survey of New Zealand dogs with the aim of identifying prevalence and exposure risk in both the North and South Islands of New Zealand. The second study provides further clarification of seroprevalence and exposure risk in South Island farm working dogs, and is the first study to identify and quantify the prevalence of urinary shedding of leptospires by these dogs.

# Chapter 2

# Canine leptospirosis –a review:

## Disease, diagnosis, and epidemiology, and the role of dogs as alternative maintenance hosts for multiple leptospiral serovars.

# 2 Canine leptospirosis – a review

## 2.1 Leptospirosis – an introduction

### *2.1.1 Overview*

Leptospirosis is a bacterial disease caused by pathogenic species of genus *Leptospira* (WHO, 2003) characterised by acute multi-organ system febrile disease affecting man and animals. It is considered to be a significant, re-emerging zoonotic disease throughout the world (Bharti *et al.*, 2003; Langston & Heuter, 2003; WHO, 2003; Stokes & Forrester, 2004; Greene, Sykes, & Brown, 2006).

Leptospires are thin, coiled or spiral shaped organisms with a characteristic terminal hook (P. N. Levett, 2001; Langston & Heuter, 2003). Both pathogenic and saprophytic free living species exist (WHO, 2003), which are morphologically indistinguishable (Faine, 1982). The outer membrane of the organism includes lipopolysaccharide (LPS) and antigenic lipoproteins (LipL21, LipL32, LipL36, LipL41), variation in these outer membrane components allows grouping of leptospires into antigenically distinct serovars and serogroups (Greene, Sykes, & Brown, 2006).

The organism has a worldwide distribution (P. N. Levett, 2001; WHO, 2003; Sykes *et al.*, 2011), prevalent in temperate and tropical regions of developing and industrialised countries both in rural and urban areas (Bharti *et al.*, 2003). The widespread distribution is a reflection of urinary shedding of leptospires into the environment by domestic and wildlife maintenance hosts, and the ability of leptospires to persist in the environment outside of a host (Lau, Smythe, Craig, & Weinstein, 2010; Haake & Levett, 2015). The risk of host exposure to leptospires is dependent on many factors including seasonal variations in climate, density of maintenance hosts, and contact between reservoir and accidental hosts (P. N. Levett, 2001; Bharti *et al.*, 2003).

Infection requires direct contact with infected tissue or urine, or with contaminated soil or water (Faine, 1982; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Adler & de la Peña Moctezuma, 2010; Baer, Turnberg, Yu, & Wohrle, 2010). Pathogenic leptospires can invade damaged skin and intact mucous membranes such as the conjunctiva, spreading rapidly via the bloodstream within minutes (P. N. Levett, 2001; Langston & Heuter, 2003; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Baer, Turnberg, Yu, & Wohrle, 2010). These extracellular pathogens can be highly virulent, adhering to and invading host cells (Fraga, Barbosa, & Isaac, 2011). Vascular endothelial damage is the primary disease mechanism, leading to organ damage including but not limited to hepatocellular ischaemia, renal tubular necrosis, pulmonary haemorrhage, myositis, uveitis and meningitis (P. N. Levett, 2001; Langston & Heuter, 2003; William A Ellis, 2015). The degree of injury and severity of clinical signs may vary between serovars and with differing host susceptibility (Langston & Heuter, 2003). Localisation within the proximal convoluted tubules of the kidney affords some protection from humoral immunity, thereby allowing persistent infection and urinary shedding of the organism (Marshall & Manktelow, 2002; Langston & Heuter, 2003; Stokes & Forrester, 2004; Adler & de la Peña Moctezuma, 2010). Post-infection modification of leptospiral lipoprotein epitopes has been demonstrated, suggesting an additional mechanism of immune system evasion that enables persistent infection (Witchell *et al.*, 2014). The persistence and intensity of shedding varies between individuals, host species and the infecting serovar (William A Ellis, 2015).

## 2.2 The clinician's guide to Taxonomy

Classification of the genus *Leptospira* is currently based on both phenotypic and genotypic characteristics (Faine, Adler, Bolin, & Perolat, 1999; Greene, Sykes, & Brown, 2006), which can be confusing as the two classification systems overlap (Adler & de la Peña Moctezuma, 2010; R.E. Goldstein, 2010).

Phenotypic classification is based on the antigenic similarity of outer membrane LPS antigens (Greene, Sykes, & Brown, 2006; Adler & de la Peña Moctezuma, 2010). According to this classification, *L. interrogans* has more than 200 serovars (P. N. Levett, 2001; Adler & de la Peña Moctezuma, 2010). Those that are antigenically related and have significant antibody cross reactivity are grouped into more than twenty serogroups, many of which can be pathogenic to man and animals (André-Fontaine, 2006; Paul N Levett, 2015). Serovars within the same serogroup are therefore expected to strongly cross react on MAT tests (Sykes *et al.*, 2011) as the MAT detects humoral antibody directed against serovar specific LPS antigen (and thus the MAT is a poor predictor of cell mediated immunity)(Zuerner, 2015). While serogroup classification has no taxonomic status, it has been used for predicting diagnostic and epidemiological behaviour (Faine, Adler, Bolin, & Perolat, 1999; Greene, Sykes, & Brown, 2006; Paul N Levett, 2015) especially where diagnosis of exposure has been made by serological means and further identification of the infecting serovar is not possible. This approach is limited, as while serovars within a serogroup may have similar antigenicity, their genetic composition and pathogenicity may differ significantly (André-Fontaine, 2006; Greene, Sykes, & Brown, 2006). In addition, the MAT test is likely to be less useful for detecting exposure to cell mediated antigens which may be common across serogroups.

For example, *L. interrogans* serovar Copenhageni and *L. interrogans* serovar Icterohaemorrhagiae belong to the Icterohaemorrhagiae serogroup, they will be indistinguishable with each other on MAT testing, and vaccination with serovar Icterohaemorrhagiae will confer immunity against infection with serovar Copenhageni. *L.interrogans* serovar Canicola, however belongs to the Canicola serogroup, and so vaccination with an Icterohaemorrhagiae bacterin is not expected to confer humoral immunity against serovar Canicola and vice versa.

Genotypic classification measures the ratio of DNA similarity using DNA hybridization techniques. There are 18 recognised species of *Leptospira*; such as *L.biflexa, L.borgpetersenii*, and *L.interrogans,* (Brenner, Kaufmann, & Sulzer, 1999; Faine, Adler, Bolin, & Perolat, 1999). Serovars within the same serogroup may belong to different species, for example *L.interrogans* serovar Hardjoprajitno and *L.borgpetersenii* serovar Hardjo both belong to the Sejroe serogroup, and are indistinguishable on agglutination testing (Robinson, Ramadass, Lee, & Marshall, 1982; Brenner, Kaufmann, & Sulzer, 1999). Genotypic classification does not correspond to the system of serogroups, and is less useful for predicting diagnostic test and epidemiological behaviour, as MAT testing relies on phenotypic similarities of surface antigens, pathogenic and non-pathogenic serovars can occur within the same species, and serovars within the same serogroup can belong to multiple different species. Clinical diagnostic laboratories continue to rely on serological classification of leptospires because of the above limitations, and the complexity of species identification (P. N. Levett, 1999).

### *2.2.1 Maintenance and accidental hosts*

Maintenance hosts are those where adaptation of the infecting serovar means clinical disease tends to be mild (Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Adler & de la Peña Moctezuma, 2010; Baer, Turnberg, Yu, & Wohrle, 2010), however urinary colonisation and shedding of leptospires persists, allowing infection to transmit between hosts. Hosts are susceptible to infection with a low infectious dose, and transmission occurs within and between generations of the host thereby becoming endemic (S. C. Hathaway, 1981; Langston & Heuter, 2003; Adler & de la Peña Moctezuma, 2010; Fraga, Barbosa, & Isaac, 2011). Serovars can be maintained by one or more maintenance hosts, for example rats are maintenance hosts of serovars Icterohaemorrhagiae, Copenhageni and Ballum; cattle and sheep are maintenance hosts for serovars Pomona and Hardjo; and dogs are recognised as the only maintenance host for serovar Canicola (P. N. Levett, 2001; André-Fontaine, 2006; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Adler & de la Peña Moctezuma, 2010; William A Ellis, 2015). Knowledge of the serovars and maintenance hosts within a certain geographical region is important for understanding the epidemiology of the disease in that region (P. N. Levett, 2001; Sykes *et al.*, 2011) especially when changes in contact between reservoir and maintenance hosts occur (S. C. Hathaway, 1981).

Infection of accidental hosts tends to be sporadic but is often associated with more severe clinical signs. Urinary shedding by accidental hosts can be transient (S. C. Hathaway, 1981) or persistent (Langston & Heuter, 2003). In addition to the acute or per acute presentation, some authors note that infections of animals can also be subclinical or chronic (Langston & Heuter, 2003; Baer, Turnberg, Yu, & Wohrle, 2010). Humans are not considered maintenance hosts (Fraga, Barbosa, & Isaac, 2011).

The interaction between host species and pathogenic leptospires is dynamic, with altered selection pressure following changes in reservoir and accidental host dynamics, increases in population density and environmental changes (S. C. Hathaway & Blackmore, 1981; William A Ellis, 2015). The distinction between maintenance and accidental hosts is not always clearly defined(Adler & de la Peña Moctezuma, 2010) with leptospires being able to adapt to new host species (WHO, 2003).

### *2.2.2 Environmental risk factors*

Leptospires are dependent on a warm, wet environment with neutral to slightly alkaline stagnant or slow-moving water in order to survive outside a host (P. N. Levett, 2001; Langston & Heuter, 2003; Greene, Sykes, & Brown, 2006). (P. N. Levett, 2001). Given these favourable conditions, leptospires can persist in soil or surface water for up to 6 months (WHO, 2003; André-Fontaine, 2006).

The incidence of disease is higher in developing countries with a tropical climate; a warm, humid environment favours survival of the organism in the environment and establishment of a maintenance host (P. N. Levett, 2001; Sykes *et al.*, 2011), the peak incidence of disease being associated with the rainy season which facilitates survival and transmission of the organism (P. N. Levett, 2001). Many more serovars and a wide range of reservoir hosts are present in tropical environments (P. N. Levett, 2001) and close contact between humans and animals also occurs more frequently in developing countries (P. N. Levett, 2001; Adler & de la Peña Moctezuma, 2010).

In temperate climates, the peak incidence of disease is associated with higher rainfall, the presence of surface water and warmer temperatures resulting in a peak incidence of disease in summer and autumn Large outbreaks of disease are associated with periods of flooding (P. N. Levett, 2001; Langston & Heuter, 2003). Leptospirosis is also an important occupational zoonosis in developed countries of temperate climates (P. N. Levett, 2001) including New Zealand (Marshall & Manktelow, 2002), Denmark (Holk, Nielsen, & Rønne, 2000), Germany (Jansen *et al.*, 2005), and the USA (Campagnolo *et al.*, 2000) where human infection occurs from contact with farmed animals or at meat processing plants.

### *2.2.3 Zoonosis*

Infection of humans by leptospires is primarily acquired from animal sources and is a significant and widespread zoonotic disease (Bharti *et al.*, 2003; Adler & de la Peña Moctezuma, 2010). A wide range of animal species can be a source of zoonotic infection including small mammals such as rodents, and farm or domestic animals, including cattle, sheep, pigs and dogs.

Disease in humans is seen primarily in occupational groups exposed directly or indirectly to animals, such as meat workers, farmers, veterinarians, sewer workers, rice field workers, sugar cane cutters and banana farmers, however it is increasingly being recognised in people involved in recreational activities associated with water (WHO, 2003; K. Brown & Prescott, 2008; Haake & Levett, 2015). These avocational exposures are considered the most important source of infection in tropical regions (Haake & Levett, 2015). Serovars infecting humans include Pomona, Hardjo, Ballum, Canicola, Copenhageni and Icterohaemorrhagiae (Baber & Stuart, 1946; Feigin RD, 1973; Shenberg, Birnbaum, Rodrig, & Torten, 1977; Zaltzman *et al.*, 1981; Venkataraman & Nedunchelliyan, 1992; Trevejo *et al.*, 1998; Pereira *et al.*, 2000; P. N. Levett, 2001; Thornley, Baker, Weinstein, & Maas, 2002; Andre-Fontaine, Branger, Gray, & Klaasen, 2003; Jansen *et al.*, 2005; Greene, Sykes, & Brown, 2006; Gouveia *et al.*, 2008).

Disease in humans may present as the characteristic jaundice, haemorrhage and renal failure known as Weil's disease (WHO, 2003; Adler & de la Peña Moctezuma, 2010). Meningitis and pulmonary haemorrhage may also occur. Late sequelae including chronic fatigue and mood alterations have also been identified (WHO, 2003) although humans rarely become chronic carriers (Adler & de la Peña Moctezuma, 2010). Leptospirosis in humans may also present as a mild, non-specific or flu-like illness that may be confused with other diseases such as Dengue fever, or infection may be asymptomatic, especially in endemic areas (Haake & Levett, 2015). Infection with serovars associated with severe disease in some cases may also present as mild or subclinical disease in other cases (Gouveia *et al.*, 2008). This non-specific presentation, combined with difficulties in diagnosis (discussed later), mean that human leptospirosis is significantly underreported (WHO, 2003).

## 2.3 Leptospirosis in dogs

### *2.3.1 Epidemiology in dogs*

Exposure to leptospires is common in dogs worldwide, and its importance in veterinary practice is likely underestimated (R.E. Goldstein, 2010). While historically thought to be a disease of rural dogs, urban dogs are also at risk (Adin & Cowgill, 2000; Langston & Heuter, 2003; Alton GD, 2009), potentially due to urbanisation allowing increased contact between dogs and wild reservoir hosts (Trevejo *et al.*, 1998).

In all surveys antibody prevalence is greater than clinical disease suggesting subclinical infections occur commonly. Serovars known to be pathogenic in the dog include Canicola, Icterohaemorrhagiae, Copenhageni, Bratislava, Grippotyphosa, Hardjo, Pomona, Australis, Ballum, and Autumnalis (Wohl, 1996; Adin & Cowgill, 2000; Stokes & Forrester, 2004; R. I. Miller, Ross, Sullivan, & Perkins, 2007; W. A. Ellis, 2010).

Dogs are widely recognised as maintenance hosts for serovar Canicola (H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; André-Fontaine, 2006; Sykes *et al.*, 2011) as they are known to shed this serovar in their urine for an extended period (Langston & Heuter, 2003; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Sykes *et al.*, 2011). Dogs do not meet all of the requirements of a maintenance host as discussed previously; serovar Canicola can cause severe disease in dogs (Wohl, 1996; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003) and reports of dog to dog transmission of leptospirosis are not readily available in the literature, however the declining incidence of infection in dogs since the introduction of vaccines against Canicola still supports this theory, and no other maintenance host for this serovar has been identified (W. A. Ellis, 2010; William A Ellis, 2015). Commonly cited sources of Canicola infection in dogs include raccoons and skunks (Prescott, 2008). Dogs can also act as secondary hosts for other serovars that cause disease (L. Smythe *et al.*, 2002; Stokes & Forrester, 2004) and may also act as maintenance hosts for serovars from the Australis serogroup such as Bratislava (W. A. Ellis, 2010; William A Ellis, 2015). In Europe, disease in dogs is most commonly caused by serovars Icterohaemorrhagiae, Bratislava and Grippotyphosa (W. A. Ellis, 2010), serovars Grippotyphosa and Pomona have increasing significance in the United States (Rentko, Clark, Ross, & Schelling, 1992). Serological evidence of exposure of dogs to Hardjo is not commonly reported worldwide (Rentko, Clark, Ross, & Schelling, 1992; W. A. Ellis, 2010; Gautam, Wu, Guptill, Potter, & Moore, 2010).

In temperate climates, disease incidence peaks in late summer and autumn (Prescott JF, 2002; Ward, Guptill, Prahl, & Wu, 2004; Prescott, 2008; Alton GD, 2009), and is associated with high rainfall (Prescott JF, 2002; Van de Maele, Claus, Haesebrouck, & Daminet, 2008). Disease presentation may be sporadic suggesting direct exposure of individual dogs to maintenance hosts (Adin & Cowgill, 2000). In tropical climates, disease can occur year round (Langston & Heuter, 2003). The distribution of canine cases can be confined to a specific geographic region where the organism is endemic.

The prevalence of disease in the US, Canada and Europe decreased between 1970 and the 1980s likely due to the use of a bivalent vaccine for Canicola and Icterohaemorrhagiae reducing shedding of these strains and reduced maintenance of Canicola within the canine population (Prescott JF, 2002; Andre-Fontaine, Branger, Gray, & Klaasen, 2003; André-Fontaine, 2006). Recent increases in reported prevalence are thought to be a combination of increased contact between dogs and maintenance hosts, urbanization, climate change, increasing awareness by veterinarians or emergence of other pathogenic serovars such as Grippotyphosa, Pomona, Bratislava and Autumnalis (Adin & Cowgill, 2000; Prescott JF, 2002; L. Smythe *et al.*, 2002; Stokes & Forrester, 2004; André-Fontaine, 2006; Sykes *et al.*, 2011).

Some studies suggest an increased risk for male dogs (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Ward, Guptill, Prahl, & Wu, 2004; R. I. Miller, Ross, Sullivan, & Perkins, 2007) and larger breed dogs (Prescott JF, 2002), however other studies were unable to confirm these findings (Alton GD, 2009). Middle aged dogs tend to be at greatest risk of infection compared to younger or older dogs (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Ward, Guptill, Prahl, & Wu, 2004).

### *2.3.2 Clinical disease in dogs*

Leptospiral infection in dogs can be acute or chronic, asymptomatic or subclinical, or it can present as severe, sometimes fatal disease with any combination of fever, anorexia, vomiting, diarrhoea, myalgia, uveitis, CNS dysfunction, jaundice, hepatic failure, renal failure, haemorrhage and pulmonary disease (Prescott JF, 2002; Langston & Heuter, 2003; Stokes & Forrester, 2004; Greene, Sykes, & Brown, 2006; Schuller *et al.*, 2015).

As with humans, an acute non-icteric form of disease is recognised in dogs, as well as the classical severe icteric form known as Weil's disease (William A Ellis, 2015). Dogs may present with hepatic or pulmonary involvement only (Tangeman & Littman, 2013) and per-acute infection and sudden death has been reported (Greene, Sykes, & Brown, 2006). A bi-phasic presentation starting with fever, malaise and septicaemia, followed by an onset of inflammation secondary to antigen-antibody complex mediated inflammation and organ dysfunction is also recognised in dogs as well as humans (Langston & Heuter, 2003).

Disease in dogs is reportedly most frequently caused by serovars Icterohaemorrhagiae, Pomona, Grippotyphosa and Canicola (Adler & de la Peña Moctezuma, 2010; William A Ellis, 2015). Not all serovars exhibit the same pathogenicity for a given host species (Adler & de la Peña Moctezuma, 2010). Some authors suggest clinical presentations can vary with the infecting serovar; Canicola being the cause of an acute renal or gastrointestinal form of the disease, and Icterohaemorrhagiae and Pomona being associated with more hepatic involvement, jaundice or haemorrhagic disease (Langston & Heuter, 2003; Tangeman & Littman, 2013; William A Ellis, 2015). In contrast other authors believe that clinical manifestations may vary depending on the infectious dose and host susceptibility, and that there is no typical syndrome ascribable to a particular serovar (André-Fontaine, 2006; R. E. Goldstein *et al.*, 2006). Signs are reportedly more severe in non-host adapted serovars (Adin & Cowgill, 2000) and for serovar Pomona (R. E. Goldstein *et al.*, 2006) therefore typing of isolates from clinical cases is needed to determine if the disease presentation is associated with the infecting serovar, rather than relying on serological methods (Greene, Sykes, & Brown, 2006).

Clinical signs may also be more severe in non-host adapted serovars (Adin & Cowgill, 2000) and the severity of disease in dogs with Canicola infection is expected to be mild, dogs being recognised as the maintenance host for this serovar (Ellison RS, 1990); however severe disease is also reported for this serovar so in this respect dogs do not meet the definition of a true maintenance host as discussed earlier.

It is recognised that younger dogs (less than 6 months old) tend to have more severe clinical signs (Langston & Heuter, 2003; WHO, 2003; Greene, Sykes, & Brown, 2006). Traditionally larger herding and sporting breed dogs from rural environments have been regarded as being at increased risk of infection over smaller urban, companion dogs (W. A. Ellis, 2010), however in the last decade there has been an increasing incidence of disease reported in smaller dogs and terrier breeds (Lee, Guptill, Johnson, & Moore, 2014)

A 2011 review of canine leptospirosis recommended treatment with doxycycline, ampicillin or penicillin G to clear the leptospiraemic phase, with an extended course of doxycycline prescribed to eliminate renal carriage of the organisms (Sykes *et al.*, 2011). This approach is recommended by other authors (Langston & Heuter, 2003; R. E. Goldstein *et al.*, 2006; Van de Maele, Claus, Haesebrouck, & Daminet, 2008). Leptospires may be intracellular during the acute phase of disease, requiring extended treatment with antibiotics that are effective penetrating into host cells (André-Fontaine, 2006) such as doxycycline.

Other therapy that may be indicated includes intravenous fluid therapy, electrolyte imbalance correction, haemodialysis, anti-emetics, blood transfusions, oxygen and gastroprotectants (Langston & Heuter, 2003; André-Fontaine, 2006; Sykes *et al.*, 2011). Most dogs will often recover if aggressive therapy is initiated early in the course of the disease (André-Fontaine, 2006; R. E. Goldstein *et al.*, 2006) although mortality rates in excess of 50% have also been reported (R. I. Miller, Ross, Sullivan, & Perkins, 2007).

### *2.3.3 Urinary shedding*

Dogs are known to be persistent shedders of serovar Canicola for up to two years (Langston & Heuter, 2003; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Sykes *et al.*, 2011) if not treated with appropriate antimicrobial agents after infection, however the duration of shedding of other serovars has not been determined (Mackintosh, Blackmore, & Marshall, 1980; P. N. Levett, 2001; Andre-Fontaine, Branger, Gray, & Klaasen, 2003; Langston & Heuter, 2003; Wilson *et al.*, 2013). Persistent urinary shedding of serovar Tarrasovi for many months in apparently healthy dogs with negative MAT titres has been documented (Mackintosh, Blackmore, & Marshall, 1980) after experimental inoculation, suggesting persistent shedding of serovars in addition to Canicola does occur. Others have reported isolation of serovars Icterohaemorrhagiae, Pomona and Bratislava from canine urine (Menges, Galton, & Habermann, 1960; Thiermann, 1980; van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Greene, Sykes, & Brown, 2006).

The number of dogs shedding bacteria in their urine following subclinical or chronic disease is unknown (Andre-Fontaine, Branger, Gray, & Klaasen, 2003; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; H.L.B.M. Klaasen, Van Der Veen, Molkenboer, & Sutton, 2013) . Urinary excretion can be continuous or intermittent (Greene, Sykes, & Brown, 2006; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Adler & de la Peña Moctezuma, 2010).

Urinary shedding may occur in apparently healthy dogs (Harkin, Roshto, & Sullivan, 2003; Rojas, 2010) with MAT titres of 100 or less(Mackintosh, Blackmore, & Marshall, 1980; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; Langston & Heuter, 2003) thus serological titres are poor predictors of urinary shedding of leptospires (Harkin,

Roshto, Sullivan, Purvis, & Chengappa, 2003). DNA sequencing or culture identification of leptospires detected in urine of dogs by PCR is not well reported in the literature (Bajani *et al.*, 2003), most studies relying on serology to determine the infecting serovar. For example Harkin *et al* (2003) relied on positive serology titres to Grippotyphosa, Canicola and Icterohaemorrhagiae to identify the infecting serovar in dogs with a positive urine PCR.

### *2.3.4 Zoonosis from dogs*

Human infection by leptospires acquired from infected dogs is described in the literature (Feigin RD, 1973; Wong, Kaplan, Dunkle, Stechenberg, & Feigin, 1977; Trevejo *et al.*, 1998; P. N. Levett, 2001; Andre-Fontaine, Branger, Gray, & Klaasen, 2003; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; K. Brown & Prescott, 2008; Sykes *et al.*, 2011), and suspected in other cases (Jansen *et al.*, 2005; André-Fontaine, 2006; Whitney, Ailes, Myers, Saliki, & Berkelman, 2009) where urinary shedding in the dog has not been confirmed largely due to reliance on the MAT as a screening test for the in-contact animals (Sykes *et al.*, 2011), including human cases of Canicola in New Zealand (Frazer J, 2012). While the reported incidence of leptospiral infection from dogs appears low, zoonotic infection is likely underreported due to the non-specific clinical presentation of disease, low index of suspicion for leptospirosis by medical practitioners, lack of awareness of the role of dogs as a potential source of zoonotic infection and limitations of diagnostic testing (discussed later). Notification of health authorities in many countries is not required(K. Brown & Prescott, 2008). Little is known about the persistence of and therefore zoonotic risk from urinary shedding of leptospires in dogs infected by serovars other than Canicola (P. N. Levett, 2001; Langston & Heuter, 2003), and many authors express concern about zoonotic potential of infection in dogs (Mackintosh, Blackmore, & Marshall, 1980; Wohl, 1996; P. N. Levett, 2001; J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002; Andre-Fontaine, Branger, Gray, & Klaasen, 2003; Sykes *et al.*, 2011) indicating further studies investigating the serovars shed in canine urine and the persistence of such shedding are required.

While the risk of acquiring leptospirosis is well understood for production animal veterinarians, companion animal veterinarians are also at risk of infection in the workplace (Baer, Turnberg, Yu, & Wohrle, 2010). Barrier nursing of suspect canine leptospirosis patients in a veterinary clinic is well documented (Langston & Heuter, 2003; Greene, Sykes, & Brown, 2006).

### *2.3.5 Vaccination*

Vaccination of dogs at risk of infection is the most efficient method of preventing disease in dogs (H.L.B.M. Klaasen, Van Der Veen, Molkenboer, & Sutton, 2013) and controlling leptospirosis in dogs (André-Fontaine, 2006), and is thought to have reduced the incidence of disease from Icterohaemorrhagiae and Canicola in the last decade (Trevejo *et al.*, 1998; André-Fontaine, 2006). Vaccination may not prevent disease if the challenge is high, but should reduce the severity of clinical signs (Andre-Fontaine, Branger, Gray, & Klaasen, 2003). Immunized dogs may still be infected with serovars not present in the vaccine (Adler & de la Peña Moctezuma, 2010) therefore a diagnosis of leptospirosis should not be ruled out in dogs up to date with their annual leptospirosis vaccination (Tangeman & Littman, 2013).

Low (<100) MAT titres after vaccination are reported in the literature, these animals are still protected against challenge, therefore a low or negative MAT should not be used as a measure of vaccine inefficacy (Andre-Fontaine, Branger, Gray, & Klaasen, 2003). The MAT titre in dogs that do show a serological response to vaccination will fall below detectable levels within 6 weeks of vaccination (Hartman, van Houten, Frik, & van der Donk, 1984). Vaccinated animals may also give a weaker serological response to challenge than naïve animals, which must be taken into account when relying on serological tests to diagnose leptospirosis in vaccinated animals (Andre-Fontaine, Branger, Gray, & Klaasen, 2003). Demonstration of seroconversion or a fourfold increase in titre is still required for diagnosis of exposure in a vaccinated animal.

Vaccination can decrease urinary shedding of leptospires, and therefore the potential for zoonotic transmission (Sykes *et al.*, 2011) however it may not completely prevent urinary shedding by infected animals (Feigin RD, 1973; C. Branger *et al.*, 2001; Andre-Fontaine, Branger, Gray, & Klaasen, 2003; H.L.B.M. Klaasen, Van Der Veen, Molkenboer, & Sutton, 2013) especially if the prevalence of infected maintenance hosts and therefore challenge is high (Schmidt, Winn, & Keefe, 1989; Wohl, 1996; Andre-Fontaine, Branger, Gray, & Klaasen, 2003). Variability in the efficacy of different licenced vaccines in preventing urinary shedding is reported (Andre-Fontaine, Branger, Gray, & Klaasen, 2003), Minke *et al* (2009) theorised this may be due to variability in immunogenicity of bacterins used in the vaccine In contrast, other studies report vaccines preventing shedding in more than 90% of dogs challenged in experimental settings (Schreiber *et al.*, 2005; Minke *et al.*, 2009; Wilson *et al.*, 2013). Cell mediated immunity has also been shown to have a role in the prevention of renal carriage and urinary shedding in cattle (R. A. Brown *et al.*, 2003).

Killed whole cell bacterin vaccines are the only licensed vaccines available (Adler, 2015). These vaccines induce a humoral immune response directed against cell membrane lipopolysaccharide (LPS) providing immunity against infection by antigenically related serovars, but provide no cross-protection to other serogroups, therefore several serovars may be incorporated into the vaccines to cover/provide protection against serovars common in a particular geographic region (C. Branger *et al.*, 2001; Adler & de la Peña Moctezuma, 2010; Adler, 2015). Bacterin vaccines are less potent at stimulating an immune response than live vaccines, and confer a short lived rise in IgM and IgG antibody (6-12 months) (Hartman, van Houten, Frik, & van der Donk, 1984; C. Branger *et al.*, 2001; M. J. Day, Horzinek, & Schultz, 2007; Zuerner, 2015) hence annual revaccination is recommended(Adler, 2015). The identity of the antigens involved in protective immunity need further elucidation if vaccines providing heterologous immunity to multiple strains are to be developed (Adler, 2015).

There are no licensed live vaccines available (Adler, 2015). Newer protein vaccines are being developed from specific antigenic epitopes rather than the entire microbial particle, but results to date show lesser protection than that conferred by whole cell bacterin vaccines (Adler & de la Peña Moctezuma, 2010). Incorporation of multiple antigens into a vaccine may be required for protective immunity(Adler & de la Peña Moctezuma, 2010), and further work is required to substantiate the efficacy of these vaccines (Adler, 2015).

### *2.3.6 Immunology*

Immunity to leptospiral infection is primarily humoral – passive transfer of antibody to LPS has shown to be protective against challenge - however cell mediated immunity plays a role in all species (Greene, Sykes, & Brown, 2006; Adler & de la Peña Moctezuma, 2010; Haake & Levett, 2015; Zuerner, 2015) and protection against infection has been reported in vaccinated animals (including dogs) with low or negative MAT titres, suggesting cell mediated immunity is involved in this protection (Greene, Sykes, & Brown, 2006). Consequently a low or negative MAT cannot be used as an indicator of lack of immunity or poor vaccine efficacy (Gueguen S., 2000; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; Minke *et al.*, 2009). A strong cell-mediated immune response to vaccination has been demonstrated in cattle, and it is theorised that cell mediated immunity is required to prevent urinary shedding and abortion due to Hardjo infection in cattle (R. A. Brown *et al.*, 2003). Most vaccine studies focus on humoral protection against acute infection, however less is known about the components of the immune response required to prevent renal colonisation and persistent shedding (Zuerner, 2015). Extrapolation of mechanisms of immunity from laboratory animal trials or between other species is likely to be flawed (Adler, 2015).

Following an initial antigenic exposure (infection or vaccination), the initial humoral response is the production of IgM antibody, a subsequent rise in IgG antibody follows several days later (Michael J Day, 2011). IgG reflects a mature humoral immune response; with subsequent repeat exposure IgG rise is more rapid, and the IgM response is less marked although it may persist at low levels for more than four months (Hartman, van Houten, Frik, & van der Donk, 1984; Greene, Sykes, & Brown, 2006). IgM is an efficient agglutinator with its pentamer structure providing multiple binding sites, whereas IgG has only two binding sites. Because of this agglutination efficiency the Microscopic Agglutination Test (MAT) follows the IgM titre more closely than that of the IgG, and is therefore useful in the early detection of a humoral response to infection. The association between the MAT and IgM also becomes relevant when assessing for immunity after vaccination –protective immunity is maintained in the long term in part by the production of IgG by long-lived plasma cells, consequently IgM and therefore the MAT is not an accurate representation of an animal's protection against infection.

Vaccination or field exposure stimulates ongoing replication of memory B cells, and a marked anamnestic rise in antibody titres may be seen in vaccinated dogs exposed to environmental challenge (André-Fontaine, 2006), a response that may confound interpretation of MAT titres.

## 2.4 Diagnosis

Diagnosis of acute infection requires identification of a significant immune response from the host, or definitive identification of leptospires within fluids or tissues (Sykes *et al.*, 2011; William A Ellis, 2015). Techniques to measure host humoral responses include the Microscopic Agglutination Test (MAT), or Enzyme Linked Immuno-Sorbent Assay (ELISA). Methods of detecting the presence of infectious leptospires include Dark Field Microscopy (DFM), culture, Polymerase Chain Reaction (PCR) and specialised staining of tissues. Each of these tests have their limitations and present challenges with interpretation.

### *2.4.1 Microscopic Agglutination Test*

#### 2.4.1.1 Methods

The Microscopic Agglutination Test (MAT) is the most widely used test for identification of infection in patients with appropriate clinical signs (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002; O'Keefe, 2002; Bajani *et al.*, 2003). It is widely available, relatively inexpensive and there is a large body of information available about its use (Adler & de la Peña Moctezuma, 2010; Sykes *et al.*, 2011). It has a reportedly high sensitivity and specificity, especially when used in the convalescent phase rather than in the acute phase of disease (Bajani *et al.*, 2003; Adler & de la Peña Moctezuma, 2010) and it is considered a gold standard serological test (Nally, Mullen, Callanan, Mischak, & Albalat, 2015).

The technique for performing the MAT is well described in the literature. In summary, serial dilutions of the patient's serum are mixed with cultured *Leptospira* organisms from a panel of serovars representing different serogroups (O'Keefe, 2002; Sykes *et al.*, 2011). Darkfield microscopy is used to assess agglutination of the organism by antibody (largely IgM and to a lesser extent, IgG) (Hartman, van Houten, van der Donk, & Frik, 1984; Greene, Sykes, & Brown, 2006). The titre reported for each serogroup is the greatest dilution of the sera that caused 50% agglutination of the organisms representing that serogroup (reported as a reciprocal of the dilution). Positive titres are regarded as exposure (or vaccination) within 12 months of the test (Sykes *et al.*, 2011). Results should be regarded as serogroup specific, rather than serovar specific as there is significant cross-reactivity between serovars within the same serogroup (Greene, Sykes, & Brown, 2006; Sykes *et al.*, 2011).

There is considerable debate what titres are indicative of acute infection; a single high MAT titre (≥800) to a non-vaccinal serovar and concurrent negative or low (≤400) titres against vaccinal serovars, accompanied by clinical signs of leptospirosis, is highly suggestive of current infection according to some authors (Adin & Cowgill, 2000; Harkin, Roshto, & Sullivan, 2003; Greene, Sykes, & Brown, 2006). Other authors suggest a minimum titre of 200 (Ellison RS, 1990), 400 (Adler & de la Peña Moctezuma, 2010) or even 1600 (Gautam, Wu, Guptill, Potter, & Moore, 2010; M. D. Miller, Annis, Lappin, & Lunn, 2011) is required to confirm infection, especially in vaccinated dogs (Tangeman & Littman, 2013).

A more reliable indicator of acute infection is a fourfold increase in MAT titres. One study reported 45% of leptospiral infections in dogs would have been missed if convalescent serology had not been performed (Tangeman & Littman, 2013). MAT test results are often negative in the first week after infection, especially in younger dogs, therefore a second serum sample should be obtained and MAT tested within two weeks (Adin & Cowgill, 2000; Greene, Sykes, & Brown, 2006; Sykes *et al.*, 2011). Negative initial antibody tests can be explained by the delay before IgM production rises and therefore before MAT agglutination can be detected. MAT titres become positive after about one week, peak around 4 weeks, and remain positive for months after both natural infection or vaccination (Hartman, van Houten, van der Donk, & Frik, 1984; Greene, Sykes, & Brown, 2006). This peak in MAT titres may be blunted following antibiotic treatment (Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Sykes *et al.*, 2011) and a convalescent titre of 100 following initial seronegativity in treated animals is also considered indicative of infection by some authors (Bharti *et al.*, 2003).

A titre of ≥100 can be used as evidence of past exposure for epidemiological serosurveys(Stokes *et al.*, 2007), and for discriminating positive from negative animals, however conclusions about infecting serovars require isolation confirmation as the correlation between the highest MAT and the infecting serovar can be as low as 50% (Faine, 1982; O'Keefe, 2002; P. N. Levett, 2003; Haake & Levett, 2015). In addition, vaccinal titres cannot be differentiated from natural exposure using the MAT; some authors will ascribe different cut-off values for vaccinal and non-vaccinal serovars in order to minimise the confounding effect of vaccination (Stokes *et al.*, 2007).

#### 2.4.1.2 Limitations of the MAT

The problem with interpretation of the MAT or other antibody test results is the high prevalence of subclinical infections and the persistence of antibodies following challenge or vaccination.

Leptospiral vaccines induce antibodies, and the MAT cannot discriminate between vaccine induced and infective titres (R. E. Goldstein *et al.*, 2006; Greene, Sykes, & Brown, 2006; Adler & de la Peña Moctezuma, 2010). Titres of up to 800 or even 3200 have been reported after recent vaccination (Davis *et al.*, 2008; Gautam, Guptill, Wu, Potter,

& Moore, 2010), though typically these are only to the vaccinal serovar (André-Fontaine, 2006; Gautam, Guptill, Wu, Potter, & Moore, 2010) and vaccinal MAT titres rapidly diminish to below 100 over weeks to months (Hartman, van Houten, Frik, & van der Donk, 1984; J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002; Barr, McDonough, Scipioni-Ball, & Starr, 2005). Care must also be taken when interpreting MAT titres in vaccinated animals subjected to natural challenge; the IgM response and therefore MAT titre is expected to be dampened as an anamnestic response in a vaccinated animal is predominantly IgG mediated (Andre-Fontaine, Branger, Gray, & Klaasen, 2003; Greene, Sykes, & Brown, 2006), as opposed to the initial IgM response in a naïve animal subjected to challenge (the MAT titre more closely following the IgM titre as discussed earlier).

While high MAT titres are useful in diagnosing acute infection, they may be less useful when interpreting the degree of exposure within a population (W. A. Ellis, 2010) as low titres may be indicative of prior exposure, cross-reactions to other serogroups, innate IgM mediated immunity or vaccination (Michael J Day, 2011). Sero-prevalence surveys in the literature use titres of from 24 to 100 as an indication of exposure (Mackintosh, Blackmore, & Marshall, 1980; Hilbink, 1989; P. N. Levett, 2001; J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002), and this variability in the minimum titre used makes comparison between different surveys difficult (W. A. Ellis, 2010) and the sero-prevalence reported will vary significantly if a different cut-off titre is used (Ward, Guptill, Prahl, & Wu, 2004). Ideally the MAT cut-off titre should be determined after reviewing serial MAT titres of culture confirmed cases, and comparing these to the titres in the healthy population, but this determination is rarely done (WHO, 2003); The cut off used may vary with the vaccination status of individuals within the study population, a higher cut off being chosen as an indication of exposure in vaccinated animals (Greene, Sykes, & Brown, 2006). The MAT can be used to give an overview of the serogroups present within a population (P. N. Levett, 2001) although isolation and identification techniques are preferred when performing epidemiological studies, selecting serovars to be included in the MAT for a given region, or selecting serogroups to be included in vaccines (Harkin, Roshto, & Sullivan, 2003; Sykes *et al.*, 2011).

Positive titres may be reported for several serogroups. Historically, the highest titre is considered to be that of the infective serogroup (Greene, Sykes, & Brown, 2006; Sykes *et al.*, 2011), which may be true after the antibody response has matured, however paradoxical cross reactions within and across serogroups are frequently reported (Greene, Sykes, & Brown, 2006; R. I. Miller, Ross, Sullivan, & Perkins, 2007; Sykes *et al.*, 2011; Miller MD, 2007). The MAT is generally considered to have poor specificity for identifying the infective serovar but good specificity for identification of the infecting serogroup (P. N. Levett, 2001; R. E. Goldstein *et al.*, 2006; L. D. Smythe *et al.*, 2009). Cross-reactions occur because leptospires have several common antigens. Equal titres to two different serogroups is often ascribed to dual exposure (Harkin, Roshto, & Sullivan, 2003), although isolation or sequencing confirmation of the infecting serovar is infrequently reported. In addition there is considerable inter- and intra-laboratory variability in identifying the infecting serogroup (Adin & Cowgill, 2000; Bharti *et al.*, 2003; Chappel, Goris, Palmer, & Hartskeerl, 2004; Sykes *et al.*, 2011; Fang Fang *et al.*, 2014; Miller MD, 2007).

MAT testing requires the use of a battery of live cultures of each of the serovars known to be present in the local area (Adler & de la Peña Moctezuma, 2010). Even lower specificity could be predicted if only a limited number of serogroups are represented in the panel (Sykes *et al.*, 2011). Maintaining live cultures in a laboratory can lead to contamination of the culture, and the laboratory strain may not be as immunogenic as the wild strains, thereby reducing the accuracy of the MAT further (Christopher, Adler, & Faine, 1982; Faine, 1982; Chappel, Goris, Palmer, & Hartskeerl, 2004; Cerqueira *et al.*, 2010; Fang Fang *et al.*, 2014). Keeping live culture also carries a risk of zoonotic infection of laboratory staff (Sykes *et al.*, 2011).

The MAT is regarded as a poor predictor of urinary shedding of leptospires, although there is some evidence suggesting seropositivity to Hardjo in sheep may be useful for predicting urinary shedding in that species (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; Fang Fang *et al.*, 2014). Chronic infection can be asymptomatic and the MAT has poor sensitivity in detecting such cases; titres may be low or fall below detectable levels while animals are chronically infected (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; WHO, 2003; Van de Maele, Claus, Haesebrouck, & Daminet, 2008; Rojas, 2010).

Protection against challenge is reported in vaccinated animals with a low or negative agglutination titres, presumably because continued immunity is mediated by IgG and potentially cell mediated immunity. Therefore a low or negative MAT cannot be used as an indicator of lack of immunity, or of poor vaccine efficacy after the initial IgM response to vaccination (Hartman, van Houten, van der Donk, & Frik, 1984; Gueguen S., 2000; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; Minke *et al.*, 2009).

#### 2.4.1.3 Summary of the MAT

The MAT is a commonly used diagnostic test for leptospirosis, it is readily available and inexpensive. There are, however, some limitations to its use. It has poor specificity for identifying the infecting serovar. Care must also be taken when interpreting MAT titres on a population basis, and a combination of serology and other tests such as culture and PCR is recommended. Reliance on serological tests such as the MAT alone is likely to result in missed diagnosis of acute leptospirosis in a significant number of cases, especially when convalescent MAT titres are not obtained. The MAT also has poor sensitivity for detecting chronic infection, and is a poor predictor of urinary shedding.

### *2.4.2 Polymerase Chain Reaction*

Polymerase Chain Reaction (PCR) techniques amplify leptospiral DNA, using primers specific to strands of DNA common to all pathogenic leptospires (WHO, 2003). Real time PCR has even greater sensitivity and specificity by use of fluorescent labelled TaqMan probes, and giving a semi-quantitative PCR result (A. Slack *et al.*, 2007).

PCR can detect leptospiral organisms or cell free DNA in serum and urine, and has the potential to expedite diagnosis early in the course of infection before seroconversion or positive culture can be demonstrated (Bharti *et al.*, 2003; Christine Branger *et al.*, 2005; Sykes *et al.*, 2011). PCR may also be useful in diagnosis of leptospirosis in case of chronic renal or hepatic disease (Sykes *et al.*, 2011), and can also aid in identification of the carrier animal that is shedding leptospiral antigen but is culture and microscopy negative (P. N. Levett *et al.*, 2005; Van de Maele, Claus, Haesebrouck, & Daminet, 2008). PCR techniques are also applicable to determining potential for zoonotic infection (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003), and also useful for epidemiological surveys (Christine Branger *et al.*, 2005).

PCR testing can be performed on whole blood (in preference to serum) in the first week after infection (Kositanont *et al.*, 2007; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009), after that organisms are more concentrated in the urine so urine becomes the sample of choice (P. N. Levett, 2001). Simultaneous blood and urine PCR testing is recommended (Greene, Sykes, & Brown, 2006; Sykes *et al.*, 2011; Schuller *et al.*, 2015). PCR can also be performed on tissue such as kidney (Christine Branger *et al.*, 2005; Van de Maele, Claus, Haesebrouck, & Daminet, 2008).

Similarly to the MAT, recent antimicrobial therapy may reduce the sensitivity of the PCR, however the PCR is less susceptible to the effect of antimicrobials as it detects both viable organisms and cell free DNA (Sykes *et al.*, 2011). The lower limit for detection of leptospires reported in one study was 10 organisms per ml in serum and 50 organisms per ml in urine (L. Smythe *et al.*, 2002) although some studies report higher limits of detection (Christine Branger *et al.*, 2005; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009; Rojas, 2010).

Differentiation between pathogenic and saprophytic leptospires is possible via PCR (Christine Branger *et al.*, 2005; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009; Sykes *et al.*, 2011), however current PCR techniques do not differentiate between different serovars or serogroups. At present, identification of the infecting organism requires culture or DNA sequencing, although newer techniques which allow serogroup identification are being developed (Ahmed *et al.*, 2006; Cai *et al.*, 2010; Haake & Levett, 2015).

PCR techniques are reportedly highly sensitive and specific (Christine Branger *et al.*, 2005; P. N. Levett *et al.*, 2005; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009) giving positive results in cases where urine culture was negative (P. N. Levett *et al.*, 2005). In one study, Harkin *et al* reported a PCR sensitivity of 100% for detection of leptospiral DNA , a specificity of 88.3%, a positive predictive value of 33%, and a negative predictive value of 100% when compared to a diagnosis of leptospirosis based on clinical criteria ( a single titre of 400 in a dog with appropriate clinical signs, or a four-fold rise in convalescent antibody titre at two to four weeks) (Harkin, Roshto, & Sullivan, 2003). While the sensitivity of PCR is high, it still has limitations and the true sensitivity is less than 100%. False negative PCR assays may occur with low numbers of leptospires, inefficiency of DNA extraction, or in the presence of PCR inhibitors (Greene, Sykes, & Brown, 2006; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009; Rojas, 2010). Negative PCR results from samples taken after antibiotic administration is documented in the literature (Schuller *et al.*, 2015). Degradation of DNA may occur in stored or frozen urine samples – the PCR sensitivity in such samples may be reduced when compared to fresh samples (Christine Branger *et al.*, 2005). PCR sensitivity may also be reduced if serum is used instead of whole blood as leptospires may be incorporated in the clot (Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009).

PCR may have a low positive predictive value when the MAT (which has poor sensitivity for detecting chronic asymptomatic shedders) is used as a gold standard; the PCR is likely a more sensitive test than serology (Harkin, Roshto, & Sullivan, 2003). In contrast when compared to culture, a real time PCR was found to have a specificity of 99.5% and sensitivity 96.4% (A. Slack *et al.*, 2007), with a positive predictive value of 96.4% and negative predictive value of 99.5%. Positive urine PCR results may occur with concurrent negative serology; asymptomatic infection and urinary shedding of leptospires with low or negative serology is well documented (Harkin, Roshto, & Sullivan, 2003; Christine Branger *et al.*, 2005). Other explanations for a true positive PCR result in the face of negative serology include inadequate time for seroconversion in acute infection, reduced serological sensitivity especially if the infecting serovar is not present in the panel tested, and (rarely) an immunocompromised patient unable to mount an immune response. True false positive PCR results may occur due to non-specific binding, contamination or laboratory error (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; WHO, 2003; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009). Because of the non-specific clinical presentation of leptospirosis, there may also be a risk of misdiagnosis if the positive PCR is an incidental finding to the true presenting complaint in cases where the medical work up of the sick dog is incomplete (Harkin, Roshto, & Sullivan, 2003; Greene, Sykes, & Brown, 2006).

The validity of the PCR results are largely dependent on laboratory quality control – positive and negative controls should be used in each run (WHO, 2003). There is considerable variation in the PCR techniques reported that target different gene sequences or lack robust veterinary clinical validation, leading to recommendations for interpreting PCR results in conjunction with other tests (Greene, Sykes, & Brown, 2006; Stoddard, Gee, Wilkins, McCaustland, & Hoffmaster, 2009; Sykes *et al.*, 2011; Schuller *et al.*, 2015). In contrast, a recent study in New Zealand demonstrated good agreement between PCR results from a veterinary diagnostic laboratory and a research facility (kappa= 0.9), despite the use of different probes, chemistry and DNA extraction techniques (Fang Fang *et al.*, 2014). Quantitative PCR techniques have an increased specificity and lower rate of false positives compared to conventional PCR (P. N. Levett *et al.*, 2005; Palaniappan *et al.*, 2005; Greene, Sykes, & Brown, 2006).

### *2.4.3 Culture*

Culture of *Leptospira* on enriched media is notoriously fickle. The organisms are slow to grow (incubation for 3-6 months may be required before determining a negative culture result) and is prone to overgrowth with contaminants (Bal *et al.*, 1994; P. N. Levett, 2001; WHO, 2003; A. Slack *et al.*, 2007; Adler & de la Peña Moctezuma, 2010). Clean free-catch or cystocentesis urine samples treated with a phosphate buffer prior to transport, blood samples and also fresh tissue can be used for culture, which should be performed by laboratories with expertise in culture and identification (Sykes *et al.*, 2011; Cameron, 2015). Antimicrobial use prior to sampling is known to result in false negatives (Sykes *et al.*, 2011). Tissue, blood or urine should be inoculated into culture medium as soon as the sample is collected; organisms may not survive transport to the laboratory especially in acidic urine or if samples are not chilled (WHO, 2003; Greene, Sykes, & Brown, 2006; Adler & de la Peña Moctezuma, 2010; Sykes *et al.*, 2011). These complexities result in a low sensitivity for culture (Bharti *et al.*, 2003) and explain the numerous reports of positive urine PCR with negative urine culture (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003).

Once the organisms are cultured, serological or molecular techniques are then used to identify the bacteria to a species or serovar level (Haake & Levett, 2015). Despite known difficulties associated with successful culture, many authors encourage culture and subsequent typing of the isolate thereby contributing to a more complete understanding of the epidemiology of the disease (Adler & de la Peña Moctezuma, 2010; Sykes *et al.*, 2011).

### *2.4.4 Darkfield Microscopy*

Leptospires are not visible by standard light microscopy (Sykes *et al.*, 2011) however Darkfield Microscopy (DFM) techniques can be useful for detecting leptospires in blood or urine (O'Keefe, 2002) and is also used for the identification of agglutination in the MAT procedure described earlier. This technique does lack sensitivity and specificity (Adler & de la Peña Moctezuma, 2010); requiring at least 1000 organisms per ml for detection (Marshall & Manktelow, 2002) and debris and other microorganisms may be mistaken for leptospires by inexperienced diagnosticians, skilled and experienced operators are required for accurate identification of the organism (Faine, 1982). Samples must be received by the laboratory in an expedite manner in order to ensure the leptospires are still viable. Leptospires survive poorly in acidic environments, or if they are not chilled (WHO, 2003).

### *2.4.5 Enzyme Linked Immunosorbent Assay*

An Enzyme Linked Immunosorbent Assay (ELISA) is designed to detect very low concentrations of antibodies or proteins using the principle of antibody binding and specificity. An antigen or antibody is bound to a fixed surface (e.g. a 96-well plate) and a sample is added to the surface in a liquid form. In an indirect ELISA, protein antigen adheres to the plastic plate, and a solution that contains antibody specific to the antigen (e.g. serum) is added. Any antibody in the added sample that binds to the fixed antigen is then detected using an enzyme-linked anti-Ig antibody which produces a coloured reaction product. A substrate is then added, the action of enzyme and substrate leads to a colour change, the result being reported as the greatest dilution to cause a 50% colour density compared to the positive control (Terpstra, Ligthart, & Schoone, 1985), or as an optical density which is then compared to a standard curve reference. The intensity of the enzyme-induced colour change is thus proportional to the number of antibody molecules bound to the fixed antigen, and can be described in terms of an antibody titre.

Indirect ELISAs have been used to detect and quantify serum antibodies to specific leptospiral serovars. In these assays, the plates are coated with heat extracted or sonicated antigen prepared from cultured leptospires, and incubated with serial dilutions of test serum, and then further incubated with the antibody conjugate, which is specific to either IgG or IgM in the test serum (Terpstra, Ligthart, & Schoone, 1985). Thus, an ELISA produces an objective, continuous variable (optical density) that can easily be compared between test sera. This objective interpretation of results has an advantage over the MAT, which requires subjective assessment to identify 50% agglutination (Ribotta, Higgins, Gottschalk, & Lallier, 2000). As with the MAT a suitable cut off titre for the diagnosis of acute disease should be selected based on the prevalence of leptospirosis in the region of interest, a higher cut-off being used for areas where disease is endemic and many healthy dogs have low titres from previous exposure (WHO, 2003). In non-endemic areas, low titres are supportive of recent exposure or current infection.

ELISA is usually genus specific (Terpstra, Ligthart, & Schoone, 1985; H.L.B.M. Klaasen, Van Der Veen, Molkenboer, & Sutton, 2013), as the leptospiral protein antigens used cross react between serogroups, unlike the lipopolysaccharide antigens used in the MAT which are serogroup or serovar specific (Trueba, Bolin, & Thoen, 1990; Ribotta, Higgins, Gottschalk, & Lallier, 2000). Work is being done to develop serogroup and possibly even serovar specific ELISA if serogroup or serovar specific antigen fractions can be developed (Terpstra, Ligthart, & Schoone, 1985; Trueba, Bolin, & Thoen, 1990; Ribotta, Higgins, Gottschalk, & Lallier, 2000).

These techniques can be used to specifically detect IgG or IgM antibody, the IgM specific assay being particularly useful for detection in acute infection even earlier than the MAT (Hartman, van Houten, Frik, & van der Donk, 1984; Weekes, Everard, & Levett, 1997; Cumberland, Everard, Wheeler, & Levett, 2001; WHO, 2003). This increased sensitivity of an IgM ELISA over the MAT early in the acute phase of illness is likely due to the MAT assay requiring the presence of a greater amount of antibody for a positive result compared to the ELISA (Terpstra, Ligthart, & Schoone, 1985; Haake & Levett, 2015). The ability of the ELISA to differentiate IgM from IgG may aid in

differentiation of low titres due to infection or vaccination, and an IgG assay may be a better indicator of immunity post vaccination than agglutination tests (Hartman, van Houten, Frik, & van der Donk, 1984).

The ELISA does not require maintenance of live culture by the laboratory, and as such is a recommended screening test to be used where laboratories do not have the facilities to perform a MAT. Unlike MAT which requires live culture, the ELISA is able to be standardised, using a reproducible antigenic preparation and objective interpretation of results and thereby giving repeatable results (Ribotta, Higgins, Gottschalk, & Lallier, 2000; WHO, 2003). The sensitivity of the ELISA, as with the MAT, improves when testing convalescent rather than acute phase samples, but the specificity is reportedly good both early and later in the course of disease (Cumberland, Everard, & Levett, 1999; Signorini, Lottersberger, Tarabla, & Vanasco, 2013). Cumberland reports the sensitivity of ELISA of 52% at admission and 93% on convalescent samples, compared to culture. In the same study, the MAT sensitivity at admission was 30% and on convalescent samples 76%, compared to culture. Cross reactions with non-specific non-leptospiral antigens have been reported (Hartman, van Houten, van der Donk, & Frik, 1984). It is the opinion of some authors that the ELISA tests require further validation, and that the ELISA should not be used as the sole means of diagnosis (Adler & de la Peña Moctezuma, 2010; Haake & Levett, 2015; Schuller *et al.*, 2015).

Given the increased sensitivity of the ELISA in the early stages of infection in comparison to the MAT, it is likely to perform better than the MAT as a screening test, enabling rapid diagnosis of acute disease, and the sensitivity of the test is not limited by selection of appropriate serovars as it is for the MAT. In a meta-analysis of ELISA assays, Signorini regarded the IgM ELISA as the preferred diagnostic test irrespective of the stage of disease (Signorini, Lottersberger, Tarabla, & Vanasco, 2013). Confirmation of the diagnosis and infecting serogroup by MAT is still recommended (Ribotta, Higgins, Gottschalk, & Lallier, 2000; Signorini, Lottersberger, Tarabla, & Vanasco, 2013).

### *2.4.6 Other tests*

Staining of tissues with special stains such as Warthin-Starry silver stains or Immunofluorescent stains can be used to demonstrate the presence of leptospires in tissues, however these techniques have similarly poor sensitivity and specificity as DFM (WHO, 2003). Tissue PCR and DNA hybridisation techniques are also reported (Sykes *et al.*, 2011). Alternative serological tests are described in the literature, often available as commercial test kits. They include Indirect Haemagglutination Assays that have variable performance and are not as sensitive early in the course of disease, and IgM dipstick assays with reported high sensitivity in the acute phase of the disease which may even outperform the MAT and ELISA at that stage of the disease (Bajani *et al.*, 2003). Identification of specific polypeptide sequences in urine using capillary-electrophoresis mass spectrometry in experimental settings shows promise as a highly sensitive method of identifying chronically infected hosts (Nally, Mullen, Callanan, Mischak, & Albalat, 2015). Gene sequencing or DNA-DNA hybridisation of cultured organisms or of isolated and amplified DNA can used to identify the infecting organism (Ahmed *et al.*, 2006; A. T. Slack, Symonds, Dohnt, & Smythe, 2006).

## 2.5 Epidemiology of leptospirosis in New Zealand

New Zealand has the highest rate of leptospirosis in humans for developed countries with a temperate climate (Thornley, Baker, Weinstein, & Maas, 2002), and leptospirosis is the most important zoonotic disease in this country (Heuer *et al.*, 2010). As these human infections are often occupationally acquired through farming and meat processing activities, much work has been done to define the epidemiology of Leptospirosis in this country, thereby enabling implementation of control measures such as livestock vaccination and personal protection and hygiene strategies (S. C. Hathaway, 1981; Marshall & Manktelow, 2002; Thornley, Baker, Weinstein, & Maas, 2002; Dorjee *et al.*, 2008; Haake & Levett, 2015). Historically most human infections in New Zealand have been ascribed to serovars Hardjo and Pomona, however there are increasing reports of human infection with serovar Ballum, and less frequently Tarrasovi and Copenhageni (Crump, Murdoch, & Baker, 2001; Thornley, Baker, Weinstein, & Maas, 2002; Keenan, 2007; F Fang *et al.*, 2014), and in addition to occupational exposure, these infections may also be acquired through contact with leptospire contaminated soil or water during recreational activities (Haake & Levett, 2015). Only six pathogenic serovars are known to be endemic in New Zealand, and these belong to five serogroups and only two species (Table 1).

**Table 1 Leptospiral serovars endemic in New Zealand and their maintenance hosts (S. C. Hathaway, 1981; T. D. Day, O'Connor, Waas, Pearson, & Matthews, 1998; P. N. Levett, 2001; Marshall & Manktelow, 2002; Ayanegui-Alcerreca *et al.*, 2007; Dorjee *et al.*, 2008; William A Ellis, 2015)**

| Species | Serovar | Serogroup | Maintenance Hosts |
|---|---|---|---|
| *L. borgpetersenii* | Balcanica | Sejroe | Brushtail Possum |
| *L. borgpetersenii* | Ballum | Ballum | Black Rat, Mouse, Hedgehog |
| *L. borgpetersenii* | Hardjo | Sejroe | Cattle, Deer, Sheep |
| *L. borgpetersenii* | Tarrasovi | Tarrasovi | Pig |
| *L. interrogans* | Copenhageni | Icterohaemorrhagiae | Brown Rat, Mouse, Hedgehog |
| *L. interrogans* | Pomona[1] | Pomona | Pig, Cattle, Sheep |

Serovars Canicola and Australis have been isolated in human patients in NZ, but cannot be ascribed endemic status in New Zealand (Crump, Murdoch, & Baker, 2001; Marshall & Manktelow, 2002). Transmission of Balcanica from possums to livestock appears uncommon (Crump, Murdoch, & Baker, 2001). There is some serological evidence for the presence of serovar Arborea (serogroup Ballum) in New Zealand deer, however this finding is yet to be confirmed with culture isolation of the organism (Subharat *et al.*, 2011).

As the epidemiology of leptospirosis is influenced by the distribution of reservoir hosts, contact between maintenance and accidental hosts and environmental factors, the epidemiology of leptospirosis in New Zealand is unique because of its geographic isolation and the limited number of serovars and serogroups endemic in a small number of maintenance hosts (S. C. Hathaway, 1981; Crump, Murdoch, & Baker, 2001). The wetter west coast of New Zealand is likely more favourable for leptospire survival outside the host, but microclimates able to facilitate leptospire survival are likely to occur throughout the country (Brockie, 1977).

As discussed previously, the role of maintenance and accidental hosts is not always distinct, there is the potential for adaptation of leptospires to new reservoir hosts due to selection pressure and host dynamics in this unique environment. Being a country with a large dairy industry where 80% of dairy herds show evidence of exposure to Hardjo (S. C. Hathaway, 1981), this serovar is likely to have a more significant role than in other ecosystems. In addition, recent studies have indicated that shedding of leptospires by vaccinated dairy cattle is still prevalent despite widespread vaccination (Parramore, 2014).

The presence of few serovars with little serogroup overlap is likely to increase the specificity of the MAT and predictive value of diagnostic tests compared to countries with a greater number of serovars and serogroups represented (P. N. Levett, 2001; Sykes *et al.*, 2011). All serovars present in New Zealand are found in different serogroups aside from serovars Balcanica and Hardjo, which are from the Sejroe serogroup, and as such are indistinguishable on serologic testing as discussed earlier.

[1] Recent studies suggest the New Zealand Pomona isolates may in fact be the closely related *L.interrogans* Kennewicki, also from the Pomona serogroup; this finding has potential implications with regard to vaccine efficacy and disease presentation (Fang, 2014).

## 2.6 Canine leptospirosis in New Zealand

Three sero-prevalence surveys of the New Zealand dog population have been reported, along with a collection of individual case reports from veterinarians and diagnostic laboratories. Infection of dogs with serovar Copenhageni is reported most commonly in New Zealand, other serovars that have been determined to infect dogs in New Zealand include Tarrasovi, Pomona, Hardjo and Ballum (Mackintosh et al 1980; Ellison and Hilbink 1990; Hilbink et al 1992; Hill 1999).

### *2.6.1 Sero-prevalence surveys of New Zealand dogs*

In 1980 Mackintosh *et al* reported a survey of Manawatu dairy farm dogs where titres of 24 or greater were found to serovars Hardjo, Ballum, Copenhageni and Tarrasovi. They also reported isolation of serovar Pomona from farm working dogs from a property experiencing bovine abortion due to Pomona. The conclusion drawn from this work was that leptospirosis may be more common in dogs than previously thought, that these dogs may be a source of zoonotic infection, and further investigation into the importance of dogs as short term or maintenance carriers was required (Mackintosh, Blackmore, & Marshall, 1980).

Hilbink *et al* conducted a nationwide survey of New Zealand dogs between 1990 and 1991 to assess the sero-prevalence of antibodies to serovars Copenhageni, Ballum and Canicola. MAT titres of 200 or greater to Copenhageni were found in 5% of dogs from the urban Auckland area, 1.5% from the Northland/Auckland region, and 1.7% from Waikato. Titres of 100 or greater to Copenhageni occurred in 0.9% of dogs nationwide, and 2.7% of dogs from the Auckland/Northland region, and 1.7% from Waikato. Titres of 100 or greater to Ballum were identified in 0.7% of dogs nationwide, scattered throughout the country. Only one titre of 100 to Canicola was identified, and it was concluded this result was likely from a cross- or non- specific reaction. From this study it was concluded that canine infection with Copenhageni occurs frequently in the Auckland and Waikato regions with sporadic disease occurring elsewhere, a finding consistent with disease reported by the veterinary diagnostic laboratories at that time (Ellison RS, 1990). This study also identified low numbers of positive titres to Copenhageni and Ballum in Taranaki, Manawatu, and in the South Island. Serovar Ballum was thought to be a cause of sporadic infection, and should still be considered a potential cause of clinical disease. Following this study a vaccine against Copenhageni was made available for dogs in New Zealand and vaccination of dogs in the northern North Island was recommended (Hilbink, Penrose, & McSporran, 1992).

A further survey of dogs from the lower half of the North Island of New Zealand was reported by O'Keefe *et al* in 2002. This survey was conducted in response to an increasing incidence of infection in dogs in this region being reported by veterinary diagnostic laboratories (Hill, 1999). Serovars included in the MAT panel included *L.borgpetersenii* serovar Hardjo, *L.interrogans* serovars Icterohaemorrhagiae, Pomona and Canicola, and *L.kirschneri* serovar Grippotyphosa.
Titres of 100 or greater were reported in 9.5% of dogs to the following serovars:

Hardjo: 3.5% of dogs surveyed, 5% of dogs on dairy farms, 4.5% of dogs on dry stock farms and 0.7% of urban dogs.

Pomona: 1.3% of dogs surveyed, 0% of dairy farm dogs, 2.6% of dry stock farm dogs, 1.4% of urban dogs.

Copenhageni: 7.9% of dairy farm dogs, 8.8% of dry stock farm dogs, and 11.9% of urban dogs.

Three dogs from Taranaki had positive titres to Canicola, but further investigation determined these titres were likely cross-reactions and the true infecting serovar was Ballum, which was known to be carried by rats in that area. No sera were positive for Grippotyphosa. Rural dogs were at significantly more likely to be seropositive to Hardjo than urban dogs, consistent with intermittent transmission of this serovar to dogs from the cattle maintenance host. O'Keefe theorised that titres following challenge with Hardjo may be lower than those generated by other serovars (no reasoning behind this theory was given), but the cut off for a positive MAT remained 100 rather than 50 in order to minimise false positives from vaccine-induced titres. This MAT cut off of 100 was similar to that used by Hilbink *et al,* allowing comparison to be made between these two studies.

O'Keefe concluded that infection of dogs with leptospires is common in New Zealand, in particular with serovar Copenhageni. Protection of at risk dogs with a vaccine against Copenhageni, Pomona and Hardjo should be considered; a canine vaccine containing these serovars was reportedly in use at the time of the survey. Concern was also raised at the possibility of zoonotic infection because of the prevalence of Copenhageni exposure in the dog population (J. S. O'Keefe, J. A. Jenner, N. C. Sandifer, A. Antony, & N. B. Williamson, 2002).

From these surveys we can conclude that the prevalence of exposure to *Leptospira* in New Zealand dogs has increased between 1992 and 2002. Exposure to serovar Copenhageni is now common in dogs throughout the North Island, exposure to Hardjo is associated with rural or farm dogs, and sporadic infection of dogs with serovars Ballum and Pomona does occur in New Zealand. Serovars Canicola and Grippotyphosa are exotic to New Zealand. No surveys have confirmed infection with culture or PCR, nor correlated the reported titres to clinical disease.

### *2.6.2 Disease reports in New Zealand dogs*

Isolation of leptospires from dogs in New Zealand was first reported in 1953, serovar Pomona being isolated from the kidneys of a farm dog on a Waikato property experiencing an outbreak of bovine abortion due to the same serovar (Te Punga & Bishop, 1953). All three farm dogs had positive agglutination titres to Pomona.

Mackintosh *et al* reported the first New Zealand case of canine infection with serovar Tarrasovi in 1980 in a pack of hounds in the Auckland region (Mackintosh, Blackmore, & Marshall, 1980). There had been an outbreak of acute leptospirosis within the pack with positive titres to serogroup Icterohaemorrhagiae recorded in the affected dogs, titres to serogroup Tarassovi were negative. At the same time serovar Tarassovi (serogroup Tarassovi) was cultured from the urine of three apparently healthy hounds. Titres to Tarassovi developed after two months, titres to serogroup Icterohaemorrhagiae also persisted, so a dual infection was proposed even though serogroup Icterohaemorrhagiae was not cultured. Several dogs were identified as persistent urinary shedders of serovar Tarrasovi with MAT titres to Tarrasovi not exceeding 48. The source of infections may have been from exposure to rodents (serovar Copenhageni) and the feeding of raw bovine and equine kidneys (serovar Tarrasovi). Experimental inoculation of five naïve dogs with the Tarrasovi isolate resulted in minimal clinical signs but a persistent leptospiruria lasting more than 7 months in all dogs. MAT titres to Tarrasovi in these experimentally challenged dogs ranged between 96 and 192 two months after inoculation, and after 5 months ranged between less than 24 and 96. Mackintosh concluded that canine leptospiruria may persist even when MAT titres drop to low or negative levels.

In 1990, Ellison reported that cases of clinical leptospirosis in dogs due to Copenhageni infection occurred commonly in the Auckland/Waikato regions but not in other areas of New Zealand. The Ruakura Animal Health Laboratory confirmed three cases of canine Copenhageni infection in 1986, case numbers rose each year to reach twenty cases in 1989. A MAT titre of greater than 200 was considered diagnostic for the disease caused by that serovar when accompanied by appropriate clinical signs and clinical pathology. The disease presented as acute non-specific vomiting, diarrhoea, lethargy and anorexia followed by jaundice within 24 hours and this presentation was considered typical for canine leptospirosis. A mortality rate of 40% was reported. Clinical pathology indicated severe renal and/or hepatic dysfunction. Disease was most frequently seen in dogs less than one year of age and older than four years of age, cattle and sheep dog breeds were most frequently represented in the case reports. Positive titres to Canicola were infrequently reported, and were ascribed to being cross-reactions in all cases; the author also concluded that if Canicola was endemic in New Zealand it should cause only mild disease but with a high rate of infection, in contrast to the sporadic nature of Copenhageni infection with subsequent severe disease. Infection with Pomona was considered uncommon in dogs, and this serovar was considered to be less pathogenic in dogs.

Cave *et al* reported clinical disease consistent with leptospirosis in more than 12 working farm dogs from the Tararua region, with positive agglutination to serovar Pomona in 2012. These authors also describe an increase in the number of serological requests for canine leptospirosis submitted to a Veterinary Diagnostic Laboratory between 2011 and 2012, and an apparent increase in the number of positive titres to serovar Pomona compared to Copenhageni (Cave, Harland, & Allott, 2013). Surveillance quarterly reports from New Zealand Veterinary Diagnostic Laboratories provide a representative summary of recently diagnosed cases of canine leptospirosis (Table 2).

**Table 2 Summary of clinical cases of canine leptospirosis reported by New Zealand Veterinary Diagnostic Laboratories in Surveillance reports 2011-2014, including age, breed, region, MAT titres, PCR results and summarised renal and hepatic clinical pathology where available.**

| Date | Region | Age | Breed | Azotaemia | Hepatic dysfunction / Jaundice | MAT Copenhageni | MAT Pomona | MAT Hardjo | PCR |
|---|---|---|---|---|---|---|---|---|---|
| July-Sept 2011 | Hawkes Bay | 9 | Huntaway | + | + | Not available | 400 | Not available | Not performed |
| | Hawkes Bay | 1 | Huntaway | + | + | Not available | >1600 | Not available | Not performed |
| Jan-March 2012 | Auckland | 6 | Schnauzer | + | + | Not performed | Not performed | Not performed | + (urine and EDTA blood) |
| | Taihape | 7 | Heading dog | + | + | Not available | 1600 | Negative at 1:25 | Not performed |
| | Taranaki | Not available | Heading dog | + | Not available | Not performed | Not performed | Not performed | Suspicious |
| | Rangitikei | 0.5 | Beardie | Not performed | + | >1600 | >1600 | 200 | + (urine) |
| | Tararua | 7 | Heading dog | + | + | Not available | >1600 | Not available | Not performed |
| | Wanganui | 7 | Huntaway | + | Not available | >1600 | >1600 | Not available | - (urine) |
| | Wellington | 4 | Rottweiler | + | + | 800 | Not available | Not available | Not performed |
| April-June 2012 | Wanganui | 7 | Huntaway | + | Not available | Negative at 1:25 | 800 | Not available | Not performed |
| Oct-Dec 2012 | Wanganui | 3 | Labrador | Not available | + | 50 | >1600* | Not available | Not performed |
| July-Sept 2013 | Hawkes Bay | 5 | Heading dog | + | Normal liver parameters | Not available | >1600** | Not available | Not performed |
| Oct-Dec 2013 | Auckland | 5 | American Bulldog | + | + | 50 | Negative at 1:25 | Negative at 1:25 | + ( blood) |
| Jan-March 2014 | Wairarapa | 8 | Heading dog | + | + | Not available | >1600 | Not available | Not performed |
| April-June 2014 | Wairarapa | 8 | Huntaway | + | + | Negative at 1:25 | 800 | Negative at 1:25 | Not performed |

*End point titrated to >25,600

**End point titrated to 819,200

From this summary there is an apparent association between farm dog breeds and infection with serovar Pomona, which occurs throughout the southern half of the North Island. In addition, positive urine PCR was recorded in a dog with a low MAT titre (50). These dogs usually had a history of lethargy and anorexia, and variable combinations of fever, abdominal pain, vomiting, jaundice and polyuria/polydipsia, which was fatal in some cases (Anonymous, 2011; Anonymous, 2012a; Anonymous, 2012b; Anonymous, 2013a; Anonymous, 2013b; Anonymous, 2014a; Anonymous, 2014c; Anonymous, 2014b). Two dogs had dual titres of >1600 to Copenhageni and Pomona; continued dilutions may differentiate one from another, however culture and typing or sequencing would be required to identify the infecting serovar(s). Interestingly these two serovars are from different serogroups yet appear to have a very strong cross-reaction. The vaccination history was unknown for these dogs.

A review article from one Veterinary Diagnostic Laboratory also reported an apparent increase in the number of canine leptospirosis cases since 2011, although the author cautions that an increased awareness of this disease by veterinarians may be partly responsible for the increase in reported cases (Thompson, 2012). Interestingly, canine leptospirosis MAT testing in New Zealand has traditionally been for serovar Copenhageni alone, but with increased awareness of disease, practitioners are now being

advised to request testing for serovars Pomona and Hardjo (Thompson, 2012). The protocol of testing for Copenhageni alone may mean a diagnosis of leptospirosis was missed in cases where testing for additional serovars was not requested.

Serovars Pomona and Copenhageni are associated with severe acute leptospirosis in New Zealand dogs. Exposure to Hardjo is also reported. It does appear that sero-prevalence of antibodies are much higher than reports of clinical disease and this observation suggests exposure and subclinical or mild disease is common (Greene, Sykes, & Brown, 2006; William A Ellis, 2015), or that disease is being underdiagnosed due to the non-specific clinical presentation or from the complexities of the available diagnostic tests.

Farm dogs appear to be at increased risk of infection with serovar Pomona, which is highly pathogenic, in contrast to Ellison's 2002 conclusion. Frequent exposure of farm dogs to serovar Hardjo is evident, but clinical disease is reported rarely – either Hardjo is less pathogenic, or it does not stimulate an elevation in MAT titres that fit the serological diagnostic criteria such that it is overlooked as a cause of disease. Hardjo is reported as a cause of canine leptospirosis in the literature (Adin & Cowgill, 2000; Harkin, Roshto, & Sullivan, 2003; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; Ward, Guptill, Prahl, & Wu, 2004; R. I. Miller, Ross, Sullivan, & Perkins, 2007) although these reports are largely based on serological evidence alone. Harkin did suggest that low titres to Hardjo may still be clinically significant, given the presence of dogs with Hardjo titres of 50 and concurrent positive urine PCR in that study (Harkin, Roshto, & Sullivan, 2003).

These sero-prevalence surveys and disease reports rarely include confirmation of infection with culture or typing of the infectious organism, therefore the epidemiological data is incomplete and indicates that infection is likely underdiagnosed (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003).

#### 2.6.2.1 Canine leptospirosis vaccines in New Zealand

Prior to 2001 a multivalent canine vaccine was available containing serovars Copenhageni, Pomona and Hardjo, however at the time of writing the only available licensed canine leptospirosis vaccine in New Zealand is a monovalent Icterohaemorrhagiae vaccine, which confers protection against Copenhageni, being within the same serogroup (Hartman, van Houten, Frik, & van der Donk, 1984; Schoone *et al.*, 1989; Cave, Harland, & Allott, 2013).

Following the cluster of clinical cases caused by Pomona in farm dogs in 2012, an off-label trial of a trivalent livestock vaccine was conducted to assess its potential for providing protection against an extended panel of serovars for farm working dogs. The vaccine induced increased MAT titres to Hardjo and Pomona, with a lesser response to Copenhageni, suggesting the vaccine may confer immunity to these serovars (Cave, Harland, & Allott, 2013). No challenge trials have been conducted to date and the vaccine remains off-label; veterinarians should therefore ensure its use in dogs is justified by demonstrating significant risk of exposure in the dogs to be vaccinated.

### *2.6.3 Urinary shedding and zoonotic potential of New Zealand dogs*

While positive urine PCR has been demonstrated in dogs with acute leptospirosis, there are no reported investigations into the prevalence and persistence of urinary shedding of leptospires by New Zealand dogs either following clinical disease or in the general dog population. While dogs do not meet all of the criteria for a maintenance host for the serovars present in New Zealand, it has been theorised that atypical maintenance host populations may occur (S.C. Hathaway, 1978). Given the absence of Canicola in the New Zealand ecosystem, there may be a role for adaptation of other leptospiral serovars to maintenance by dogs. This lack of data raises the question of the role of dogs as transient or persistent shedders of leptospires, potentially contributing to the epidemiology of livestock leptospirosis and zoonotic infection of humans.

### 2.7 Summary and conclusions

Leptospirosis in dogs is a significant concern in the North Island of New Zealand. While infection is frequently mild or subclinical, severe or even fatal disease occurs, and the true incidence of acute disease in this country is underreported. The epidemiology of leptospirosis in New Zealand is unique, and the infecting serovars within a population or region vary with changes in reservoir populations and environmental conditions. Conclusions based on studies in other countries are not necessarily applicable in this country. Significant alteration in the epidemiology of leptospirosis in the South Island is expected with increased dairy farming and herd density with movement of livestock from the North to the South Island, yet since O'Keefe's 2002 survey, there have been no further studies to determine if the prevalence in New Zealand dogs has continued to increase, or to determine if the risk of exposure or disease occurrence in South Island dogs has changed.

It is clear that updated prevalence data for dogs are needed, both in the North Island where significant changes in the regional prevalence and distribution of a number of leptospiral serovars is likely to have occurred since the last prevalence study was published in 2002, but also in the South Island of New Zealand where no current data are available. Increasing reports of clinical disease by serovars other than Copenhageni suggest a shift in the epidemiology of leptospirosis in dogs, which is not unexpected given the potential for changes in the serovars within the maintenance rodent population and the significant movement of livestock within New Zealand. Confirmation of increased risk of exposure and disease in dogs (especially in farm working dogs) will enable veterinarians to better advise on vaccination requirements in their regions. The current licensed canine vaccine in New Zealand protects against serovar Copenhageni and is not expected to provide protection against Pomona or Hardjo. Demonstration of significant risk of infection with Hardjo and Pomona is needed to prompt a review of the serovars contained in commercial vaccines for dogs. Veterinarians also need to know the likely prevalence of individual serovars on a regional basis for the purpose of diagnosis; leptospirosis is easily overlooked if the veterinarian has a low index of suspicion or if the diagnostic test selection is incomplete because knowledge of serovars in a given region is not current. Accurate diagnosis of acute disease and therefore improved case reporting rates would be facilitated by the use of ELISA tests early in the course of disease, along with concurrent PCR and convalescent serology.

No data are available on the rate of urinary shedding of leptospires in New Zealand dogs, or on the serovars implicated in such shedding. Because of the high rate of human infection in New Zealand, much work has been done to control leptospirosis in this country, particularly on farms and in slaughterhouses, yet little is known of the role of dogs contributing to the epidemiology of leptospirosis on farms and persistence of leptospires in the environment, or in domestic and wildlife maintenance hosts and incidental hosts. While dogs may not meet the strict definition of a maintenance host for serovars present in New Zealand, dogs may persistently shed leptospires into their urine thereby contributing to the cycle of persistent infection in an ecosystem; therefore research into the prevalence and persistence of urinary shedding of leptospires by dogs in New Zealand is required to further define the role of dogs in the epidemiology of leptospirosis in this country, and to quantify the potential for zoonotic infection Vaccination of farm dogs against an increased number of serovars may be indicated to assist in the management of leptospirosis in a farm environment as part of a farm wide vaccination and control strategy. Demonstration of urinary shedding of leptospires by dogs will indicate potential zoonotic risk, either indirectly through facilitating persistence of leptospires on farms, or directly in a veterinary clinical setting when treating suspect clinical cases, or in a domestic pet setting which while supposedly a smaller risk nonetheless it is one that should not be overlooked especially when the rate of human infection with serovars other than those associated with livestock has been increasing. Culture and typing of isolated serovars is required to confirm vaccination and control recommendations.

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# Chapter 3
# A serological study of leptospiral antibodies in New Zealand dogs

## 3 A serological study of leptospiral antibodies in New Zealand dogs

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New Zealand Veterinary Journal 2013, 61:2, 98-106, DOI: 10.1080/00480169.2012.719212

### 3.1 Abstract

AIM: To investigate the prevalence of titres to four endemic leptospiral serovars in dog sera from the lower half of the North Island, and the South Island of New Zealand submitted to diagnostic laboratories, and to explore the association between the prevalence of seropositive samples to leptospirosis and breed group, age group and sex.

METHODS: Serum samples from 655 dogs residing in the central and lower North Island and from the South Island of New Zealand were sourced from the Massey University Veterinary Teaching Hospital and from submissions to New Zealand Veterinary Pathology in 2005. They were screened by the Microscopic Agglutination Test (MAT) against *Leptospira interrogans* serovars Copenhageni and Pomona and *L. borgpetersenii* serovars Hardjo and Ballum. Titres greater or equal to 96 were considered positive. Variables investigated for their association with the prevalence of seropositive samples to leptospirosis included serovar, breed, North vs. South Island, age and sex.

RESULTS: Positive MAT titres to Leptospira interrogans serovar Copenhageni were found in 10.3 % of dogs (95% CI 8.1–12.9), and were more common than positive titres to other leptospiral serovars. Small breeds did not have a lower prevalence of Copenhageni titres than other breeds. Positive titres to Leptospira borgpetersenii serovar Hardjo were associated with breeds of dogs used as farm working dogs.

There was no significant difference in the prevalence of positive leptospiral titres between dogs from the North or South Islands. Dogs greater than 12 years of age were less likely to have positive titres to Leptospira than younger dogs. No association was found between positive titres and sex.

CONCLUSIONS: Breeds of dogs used as farm working were at greater risk of exposure to *Leptospira borgpetersenii* serovar Hardjo. Small breeds did not have a lower risk of seropositivity to Copenhageni than farm working breeds. Further study should be undertaken to confirm the prevalence of positive titres to leptospirosis in farm dogs and dogs resident in the South Island.

CLINICAL RELEVANCE: The risk of dogs being exposed to *Leptospira interrogans* serovar Copenhageni, and requirement for vaccination against serovar Copenhageni, cannot be determined by geographical location or breed group. Vaccination against *Leptospira borgpetersenii* serovar Hardjo is likely to be beneficial in working dogs.

KEY WORDS: Ballum, canine, Copenhageni, dog, Hardjo, Hardjobovis, Leptospira, Leptospirosis, Microscopic Agglutination Test, MAT, New Zealand, Pomona, sero-prevalence, serosurvey, survey

## 3.2 Introduction

Leptospirosis is a disease of worldwide significance, both for its effect on companion animals, farm animals and wildlife, and for its zoonotic potential. Clinical signs of disease in animals are those of an acute, multi-systemic febrile illness. Common features include fever, anorexia, depression, hepatitis, jaundice, and renal failure. Serological diagnosis is usually based on the Microscopic Agglutination Test (MAT) detecting leptospiral antibodies in serum by microscopic identification of agglutination with live or formalised cultures of leptospires at serial dilutions (L. D. Smythe *et al.*, 2009).

Worldwide, leptospiral serovars commonly associated with disease in dogs include Copenhageni, Icterohaemorrhagiae, Pomona, Bratislava, Autumnalis, Grippotyphosa, Saxkoebing and Canicola (Birnbaum *et al.*, 1998; André-Fontaine, 2006; Geisen *et al.*, 2007; R. I. Miller, Ross, Sullivan, & Perkins, 2007). Leptospirosis has the potential to cause severe or even fatal disease depending on host susceptibility and the infecting serovar (Wohl, 1996). Dogs have been identified as potential maintenance hosts of pathogenic leptospiral serovars (Rojas, 2010), with colonisation of renal tubules resulting in intermittent but persistent shedding of organisms in the urine. These dogs may be asymptomatic, yet be a source of infection for humans and other animals. Leptospirosis is endemic in the livestock, rodent, and possum populations of New Zealand (S. C. Hathaway, 1981; Marshall & Manktelow, 2002). The six leptospiral serovars known to be endemic in New Zealand are *L. interrogans* serovars Pomona and Copenhageni, and *L. borgpetersenii* serovars Balcanica, Hardjo, Ballum and Tarassovi (S. C. Hathaway, 1981)

Exposure to Leptospira organisms is common in dogs in New Zealand (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). Serovar Copenhageni has been identified in dogs in New Zealand (S. C. Hathaway, 1981; Hill, 1999; J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002) and antibodies to other serovars including Hardjo, Pomona and Ballum, have also been identified in this country. These four serovars are considered most likely to cause disease in dogs in New Zealand. Although serovar Canicola is commonly found in dogs overseas, there is no strong evidence for it being present in New Zealand and it is considered an exotic disease (Hilbink, 1989).

Serovar Copenhageni is established in the rat population in the north of the North Island (Brockie, 1977; S. C. Hathaway, 1981). Infections of dogs with this serovar have been reported more commonly in this region (Hilbink, 1989), thus leptospirosis vaccination of dogs in this area of the country became commonplace (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). However, there is evidence that antibodies to Copenhageni occur in dogs resident further south in the North Island (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). It is possible that this finding is due to displacement of serovar Ballum in the maintenance host rodent population by a better host adapted serovar such as Copenhageni (S. C. Hathaway, 1981), or due to movements of the canine population (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). Farm dogs have a higher prevalence of titres to the serovars Hardjo than urban dogs (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002).

Currently available leptospiral vaccines for dogs in New Zealand contain inactivated Leptospira interrogans serovar Icterohaemorrhagiae (Anonymous, 2011) which is antigenically similar to serovar Copenhageni, being from the same serogroup; Icterohaemorrhagiae (Bharti *et al.*, 2003) and will stimulate active immunity to both serovars (Schoone *et al.*, 1989). Vaccines for cattle, sheep, deer, goats and pigs contain serovars Pomona, Hardjo and in some vaccines serovar Copenhageni (Anonymous, 2011).

New Zealand has one of the highest rates of human leptospirosis compared with developed countries of similar climate (Thornley, Baker, Weinstein, & Maas, 2002). If changes occurred in the epidemiology of leptospirosis in ruminant or wildlife populations in New Zealand, the exposure of dogs to different serovars could change and a rise in prevalence might be seen. Dogs could then be an increasingly relevant source of infection for the human population. In Ireland, 37/525 (7.05%) of canine urine samples from the University College Veterinary Hospital, Dublin, and from animal shelters in Ireland, that were tested by PCR were positive for leptospiral DNA (Rojas, 2010). There are no data available on the rate of urinary shedding of leptospires in New Zealand dogs, or the prevalence of positive titres to leptospirosis in South Island dogs.

A serological prevalence study was conducted to provide further information on the epidemiology of canine leptospirosis infection in New Zealand. The aim of the survey was to investigate the prevalence of titres to four endemic leptospiral serovars in dogs, identifying patterns of risk, and generating further hypotheses for investigation of canine leptospirosis in New Zealand.

## 3.3 Materials and methods

### 3.3.1 *Data collection and handling*

The study population was a convenience sample of 655 canine serum samples submitted consecutively to New Zealand Veterinary Pathology (NZVP) laboratories and the Massey University Veterinary Clinic, Palmerston North between January 2005 and May 2005. Blood was collected into plain vacutainer tubes by veterinarians in practice, and submitted to NZVP for diagnostic purposes unrelated to this study. The samples were from dogs resident in 25 different regions; 606 samples were from the lower half of the North Island and 49 from the South Island (Table 3). The number of dogs sampled from each area was compared to the estimated dog population for that region (Anonymous., 2003; Anonymous., 2004; Anonymous., 2006) to quantify any regional bias of the sampling strategy (Table 3).

Information provided with the sera included breed, sex, age and the region the animal resided when the blood was collected. No information that could identify the owner of the dog was made available, therefore confidentiality was protected. Samples were excluded from the study that had incomplete records for quality control checks, or that were duplicate samples from a single dog (n=85).

### 3.3.2 *Serological testing*

Sera were tested against four serovars most likely to cause disease in dogs in New Zealand; *L. interrogans* serovars Copenhageni and Pomona, and *L. borgpetersenii* serovars Hardjo and Ballum.

The MAT testing was performed by experienced technicians at the Hopkirk Institute, Massey University, modified from the technique described in the World Health Organisation Guidelines for the Control of Leptospirosis (Faine, 1982). Doubling dilutions of serum ranging from 1:24 to 1:3072 were incubated with live culture at room temperature for 2–4 h, and then examined by dark-field microscopy. The end point of the reaction was determined to be the titre at which half of the culture had been agglutinated. Agglutination at a 1:96 dilution or greater was considered positive, and titres reported as a reciprocal of the last dilution causing agglutination. If serum agglutinated for more than one culture, we considered it positive for the antigen it reacted to at the highest dilution. If the result included more than one serovar at equal greatest dilution, the sample was considered positive for all serovars at that dilution. Within test, between test and between operator variability were monitored as part of routine quality control procedures in the laboratory.

### 3.3.3 *Statistical analysis*

The continuous variable age was categorised into quartiles (5-6; 6–9; 10–11 and 12≤ years of age). The 160 breeds were classified into four broad categories; small breeds such as Chihuahua, Shi Tzu, Sheltie; terrier types and their crosses likely to be ratters; farm working breeds such as Heading dogs, Huntaways, Sheepdogs, pig dogs; and then all other breeds and cross-breeds.

To investigate the association between the prevalence of positive leptospiral titres (to any serovar, and then stratified by individual serovars) and putative risk factors (breed group, island, age and sex), each risk factor was tested individually for significance in a logistic regression model using the software package R version 2.12.0 (R Development Core team, 2010, R Foundation for Statistical Computing, Vienna, Austria). Factors found significant at the $p \le 0.20$ level were used to build a multivariable model by a stepwise selection process retaining variables significant at $p \le 0.05$ level.

## 3.4 Results

### *3.4.1 Descriptive results*

The study population included 622 dogs with data available on age, and 33 dogs were of unknown age (Appendix 2). The age range of sampled dogs was skewed towards older dogs (Figure 1). There were 284 male and neutered male dogs, 330 female and neutered female dogs, and 41 of undetermined sex within the sample population. The sample population included 149 small breed dogs, 78 terrier or terrier cross breeds, 29 working breed dogs, and 399 dogs of any other or unknown breed. Samples obtained included dogs resident in the North Island (n=604) and the South Island (n=51) of New Zealand (Table 3).

The count and prevalence of positive titres to any one of the four serovars nationwide was 98/655 (15 (95% CI 12.3–18.0)%), and for individual serovars; Copenhageni 67/648 (10.3 (95% CI 8.1–12.9)%), Hardjo 23/649 (3.5 (95% CI 2.3–5.3)%), Pomona 7/651 (1.1 (95% CI 0.4–2.2)%) and Ballum 5/654 (0.8 (95% CI 0.2–1.8)%) (Figures 2 and 3) (Appendix 3). The count and prevalence stratified according to putative risk factors is presented in Table 4, and stratified by island presented in Table 5. Complete results for some samples could not be obtained because of equivocal MAT results or insufficient volume of serum available for analysis (n=1 for serovar Ballum, n=7 for serovar Copenhageni, n=4 for serovar Pomona, n=6 for serovar Hardjo). The frequency of positive and negative MAT titres is given in Appendix 2.

**Figure 1 Histogram of the age of dogs sampled in 2005 for a serological survey of canine leptospirosis in New Zealand.**

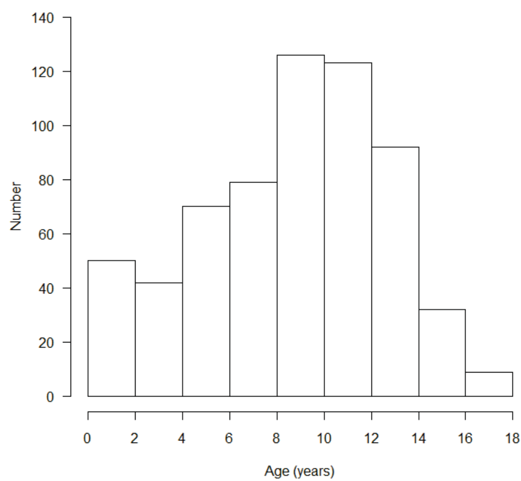


Figure 2 Histograms of frequency of Microscopic Agglutination Test (MAT) titres to serovars (a) Copenhageni, (b) Hardjo, (c) Pomona and (d) Ballum in New Zealand dogs sampled in 2005; the vertical dotted line indicating the cut off titre of 96 or greater for titres to be regarded as positive.

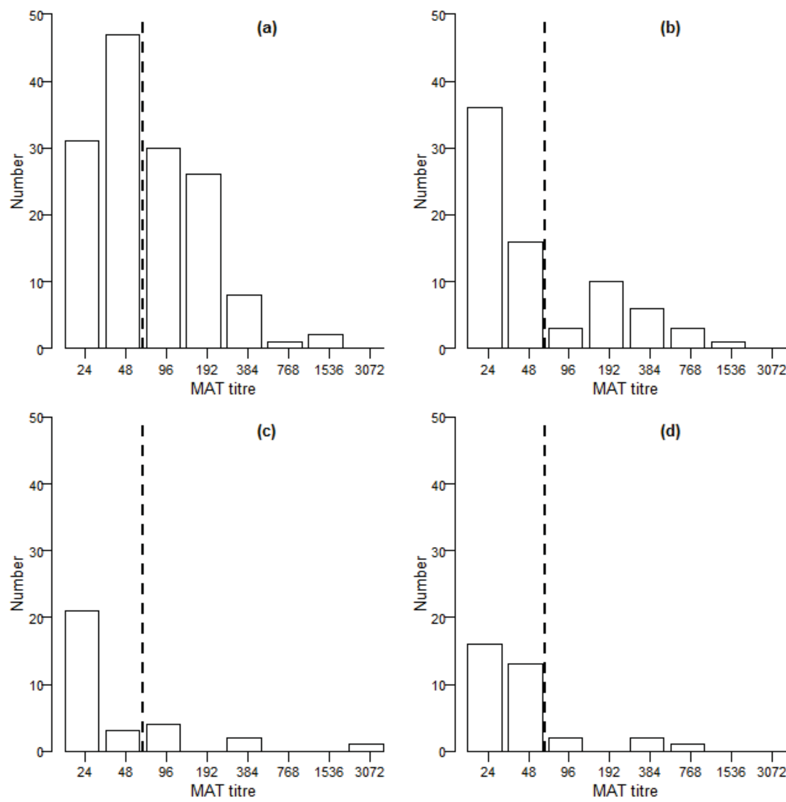



**Figure 3 Point estimates and 95% confidence intervals of the prevalence of microscopic agglutination test titres >96 to serovars Copenhageni, Hardjo, Pomona and Ballum, or to any one of these serovars in the population of New Zealand dogs sampled in 2005.**

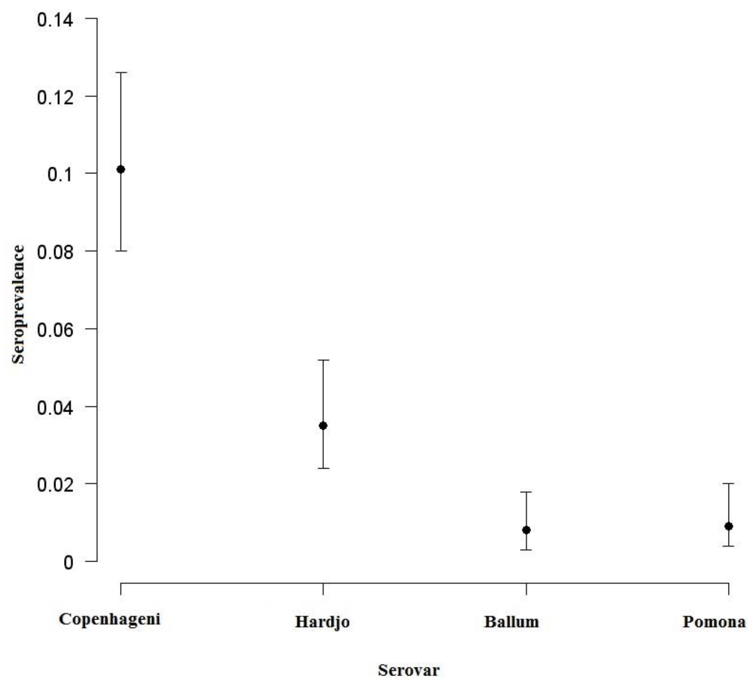


**Table 3 Number of dogs sampled, estimate of population at risk and number of dogs sampled per 10,000 dogs in the at risk population for each region of New Zealand where dogs were sampled for a survey of Microscopic Agglutination Test titres to leptospires in 2005 (Population at risk data obtained from Regional Council Dog Control survey 2003, National Dog Database survey 2004, and Department of Internal Affairs survey 2006).**

| Region | Number Sampled | Estimated population at risk | Number sampled per 10,000 in population |
|---|---|---|---|
| **North Island** | | | |
| Wellington | 220 | 6852 | 321.1 |
| Palmerston North | 108 | 7006 | 154.2 |
| Kapiti | 63 | 6005 | 104.9 |
| Manawatu | 68 | 7234 | 94.0 |
| Lower Hutt | 52 | 8800 | 59.1 |
| Masterton | 22 | 5548 | 39.7 |
| Stratford | 8 | 2256 | 35.5 |
| New Plymouth | 25 | 9300 | 26.9 |
| Central Hawkes Bay | 14 | 5772 | 24.3 |
| South Taranaki | 8 | 5080 | 15.7 |
| Tararua | 5 | 7004 | 7.1 |
| Wanganui | 4 | 7534 | 5.3 |
| Rangitikei | 2 | 5145 | 3.9 |
| Horowhenua | 2 | 5543 | 3.6 |
| South Wairarapa | 1 | 2787 | 3.6 |
| Hastings | 4 | 11914 | 3.4 |
| TOTAL | 606 | 105510 | 57.4 |
| | | | |
| **South Island** | | | |
| Nelson | 10 | 4342 | 23.0 |
| Dunedin City | 9 | 13079 | 6.9 |
| Central Otago | 3 | 5821 | 5.2 |
| Christchurch City | 12 | 29665 | 4.1 |
| Gore | 3 | 7341 | 4.1 |
| Southland | 5 | 12989 | 3.9 |
| Timaru | 3 | 8224 | 3.7 |
| Marlborough | 3 | 9863 | 3.0 |
| Canterbury | 1 | 49900 | 0.2 |
| TOTAL | 49 | 141224 | 3.5 |

**Table 4 Number and percentage of dogs sampled in 2005 for each variable of interest, and number and percentage of each variable with a Microscopic Agglutination Test (MAT) titre of ≥96 for any one of Leptospira serovars Copenhageni, Hardjo, Pomona or Ballum.**

| Variable | Level | Count (%of study population) | Positive MAT – any serovar (%of level) |
|---|---|---|---|
| Age | <6 years | 113 (17.3) | 24 (21.2) |
| | 6-9 years | 186 (28.4) | 28 (15.1) |
| | 10-11 years | 142 (21.7) | 21 (14.8) |
| | ≥12 years | 182 (27.8) | 18 (9.9) |
| Sex | Male | 284 (43.4) | 2 (7.0) |
| | Female | 330 (50.4) | 50 (15.2) |
| | Unknown | 41 (6.3) | 2 (4.9) |
| | Entire | 422 (64.4) | 67 (15.9) |
| | Desexed | 192 (29.3) | 29 (15.1) |
| Breed | Small breeds | 149 (22.7) | 26 (17.5) |
| | Terriers | 78 (11.9) | 10 (12.8) |
| | Working dog breeds | 29( 4.4) | 6 (20.7) |
| | All other breeds | 399 (60.9) | 56 (14.0) |
| Island | North | 606 (92.5) | 92 (15.2) |
| | South | 49 (7.5) | 6 (12.2) |

**Table 5 Count and prevalence of Microscopic Agglutination Test titres ≥ 96 to individual serovars Copenhageni, Hardjo, Ballum and Pomona, and to any one of these serovars in New Zealand dogs sampled in 2005[2].**

| | **North Island** | | **South Island** | | **Nationwide** | |
|---|---|---|---|---|---|---|
| ***Serovar*** | ***Count*** | ***Prevalence(95%CI)*** | ***Count*** | ***Prevalence(95%CI)*** | ***Count*** | ***Prevalence (95%CI)*** |
| **Copenhageni** | 64 | 10.7 (8.3-13.5) | 3 | 6 (1.3-16.5) | 67 | 10.3 (8.1-12.9) |
| **Hardjo** | 21 | 3.5(2.2-5.3) | 2 | 4.1 (0.5-14.0) | 23 | 3.5 (2.3-5.3) |
| **Ballum** | 5 | 0.8(0.3-1.9) | 0 | 0 (0-10.3) | 5 | 0.8 (0.2-1.8) |
| **Pomona** | 6 | 1(0.4-2.2) | 1 | 2 (0-10.4) | 7 | 1.1 (0.4-2.2) |
| **Any one serovar** | 92 | 15.2(12.5-18.3) | 6 | 11.8 (4.4-23.9) | 98 | 15 (12.3-17.9) |

[2] A MAT result was not obtained for all serovars from every serum sample obtained due insufficient sample size or equivocal agglutination.

### *3.4.2 Association between prevalence of positive leptospiral titres and putative risk factors*

#### 3.4.2.1 All serovars

There was a significant association between age quartile and the prevalence of positive MAT tests to any one of the four serovars tested (Table 6). Dogs aged 12 years and older were less likely to have positive leptospiral titres when compared with the reference group (5-6 years) (OR 0.41, 95% CI 0.21–0.79). There was no significant association between the prevalence of positive titres to any one of the serovars tested and breed group, island, or sex (Table 6) One hundred dogs had positive titres to at least one of the tested serovars of 96 or greater; of these, only four were positive to more than one serovar with both titres greater or equal to 96. All four included a positive titre to Hardjo, the second positive titres were all equal or less than the Hardjo titre, and included serovars Copenhageni, Pomona and Ballum.

#### 3.4.2.2 Serovar Copenhageni

In the univariate analysis there was a significant association between the prevalence of positive titres to serovar Copenhageni and breed group as well as age quartile (Table 6). Multivariable logistic regression for serovar Copenhageni (age and breed variables) showed dogs 12 years old and older were less likely to have positive Copenhageni titres than younger dogs (OR 0.37, 95% CI=0.17–0.86) adjusted for the effect of breed (Table 7). There was evidence of an age-related trend (though not statistically significant), with a declining odds ratio of positive titres to Copenhageni with increasing age. Compared with dogs aged less than 6 years, those aged 6–9 years, 10–12 years and $\geq$12 years had 0.88 (95% CI 0.45– 1.75), 0.57 (95% CI 0.26–1.23) and 0.37 (95% CI 0.12–0.82) times the adjusted odds of being positive for Copenhageni, respectively. Terrier breeds were less likely to have positive Copenhageni titres than other breed groups (OR 0.34, 95% CI 0.10–0.95) corrected for the effect of age (Table 6) There was no significant association between the prevalence of positive titres to serovar Copenhageni and island or sex (Table 6).

#### 3.4.2.3 Serovar Hardjo

There was a significant association between the prevalence of positive titres to serovar Hardjo and breed group (Table 6). Breeds of dog typically used as farm working dogs were at significantly increased risk for positive Hardjo titres than other breed groups (OR 10.14, 95% CI 2.34–52.09). There was no significant association between the prevalence of positive titres to serovar Hardjo and age quartile, island, or sex (Table 6).

#### 3.4.2.4 Serovars Pomona and Ballum

There was no significant association between the prevalence of positive titres to serovars Pomona or Ballum and breed group, age quartile, sex, or island.

Table 6 Univariable analysis of association between age, breed group, island or sex with positive MAT to serovars Copenhageni, Hardjo, Pomona and Ballum, or to any one of these serovars in New Zealand dogs sampled in 2005.

| Risk Factor | Level | Copenhageni | | Hardjo | | Pomona | | Ballum | | Any one serovar | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Estimate** | ***p*** | **Estimate** | ***p*** | **Estimate** | ***p*** | **Estimate** | ***p*** | **Estimate** | ***p*** |
| **Age (years)** | **<6** | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | |
| | 6-9 | -0.125 | 0.713 | -0.379 | 0.506 | -1.193 | 0.332 | -18.550 | 0.996 | -0.223 | 0.456 |
| | 10-11 | -0.556 | 0.157 | -0.959 | 0.182 | 0.188 | 0.839 | 0.181 | 0.845 | -0.441 | 0.182 |
| | ≥12 | -0.989 | 0.015 | -0.505 | 0.392 | -1.177 | 0.339 | -18.550 | 0.996 | -0.899 | 0.008 |
| **Breed Group** | **Small breed** | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | |
| | Terriers | -1.181 | 0.036 | 1.183 | 0.112 | 16.930 | 0.991 | 0.647 | 0.649 | -0.363 | 0.366 |
| | Working | -1.595 | 0.126 | 2.296 | 0.003 | 0.000 | 1.000 | -14.576 | 0.994 | 0.210 | 0.678 |
| | All other | -0.443 | 0.120 | 0.211 | 0.751 | 16.210 | 0.991 | 0.108 | 0.926 | -0.258 | 0.320 |
| **Island** | **South Island** | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***ref*** | |
| | North Island | 0.630 | 0.302 | -0.160 | 0.832 | -0.681 | 0.532 | 14.780 | 0.992 | 0.298 | 0.506 |
| **Sex** | **Female** | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | |
| | Male | 0.219 | 0.401 | -0.430 | 0.341 | 0.489 | 0.329 | -1.248 | 0.266 | 0.079 | 0.722 |
| | Unknown | -0.423 | 0.167 | -0.585 | 0.577 | 1.456 | 0.239 | 15.169 | 0.993 | -1.248 | 0.092 |
| | Entire | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | | ***Ref*** | |
| | Desexed | 0.024 | 0.933 | -0.215 | 0.659 | 0.797 | 0.332 | -0.598 | 0.594 | -0.059 | 0.807 |
| | Unknown | -1.523 | 0.138 | -0.470 | 0.653 | 1.297 | 0.266 | -14.917 | 0.993 | -1.303 | 0.077 |

(*Ref=reference value*)

Table 7 Multivariable analysis of the association between age group and breed group with positive Microscopic Agglutination Tests to serovar Copenhageni in New Zealand dogs sampled in 2005.

| **Risk Factor** | **Level** | **Estimate** | ***p*** | **OR (95% CI)** |
|---|---|---|---|---|
| Age (years) | <6 | *Ref* | *Ref* | *Ref* |
| | 6-9 | -0.126 | 0.713 | 0.88 (0.45-1.79) |
| | 10-11 | -0.565 | 0.152 | 0.57 (0.26-1.23) |
| | ≥12 | -0.988 | 0.016 | 0.37 (0.16-0.82) |
| Breed group | Small breeds | *Ref* | *Ref* | *Ref* |
| | Terriers | -1.071 | 0.059 | 0.34 (0.10-10.95) |
| | Working | -1.616 | 0.124 | 0.20 (0.01-1.03) |
| | Other | -0.427 | 0.140 | 0.65 (0.37-1.17) |

*(Ref=reference value)*

### 3.5 Discussion

This study confirmed that Leptospira interrogans serovar Copenhageni was the most common leptospiral serovar that this population of dogs had positive titres to (Figure 3). In addition, the prevalence of titres to Leptospira interrogans serovar Copenhageni in the population of North Island dogs sampled (10.7 (95%CI 8.3–13.5)%) was similar to the prevalence of 9.5% reported in 2002 by O'Keefe (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). In comparison, a 1992 survey by Hilbink reported a prevalence of positive titres to Copenhageni of 0.9% nationwide, but 5% in the Auckland area (Hilbink, 1989). There was little change in the prevalence of Pomona (1.0 (95% CI 0.4–2.2)%)) and Hardjo (3.5 (95%CI 2.2–5.3)%) titres in the North Island when compared with the 2002 study by O'Keefe et al., where the reported prevalence for Pomona was 1.3% and Hardjo was 3.5%.

The original data set contained many dog breeds (n=108) and dogs of unknown breed (n=75), making analysis of breed associations challenging. When grouped into broad breed categories, breeds of dog typically used for farm working dogs were at increased risk of having a positive titre to serovar Hardjo, confirming the previous finding of O'Keefe. (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). The confidence intervals reported for the prevalence of Hardjo titres in farm working dogs are wide, which reflects the small sample population.

For the purposes of this study, all positive Hardjo titres were presumed to be due to exposure to, or vaccination with, *L. borgpetersenii* Hardjo strain Hardjobovis. Other serovars within the Sejroe serogroup such as L. interrogans Hardjo strain Hardjoprajitno and L. borgpetersenii Balcanica (Brenner et al. 1999) are indistinguishable from each other and the strain Hardjobovis on MAT (Robinson, Ramadass, Lee, & Marshall, 1982). However, there is no firm evidence for Hardjoprajitno being present in New Zealand, and transmission of serovar Balcanica among the maintenance hosts, Brushtail possums (Trichosuris vulpecula), is associated with sexual behaviour (T. D. Day, O'Connor, Waas, Pearson, & Matthews, 1998). Therefore it seems reasonable to assume that accidental infection of dogs with serovar Balcanica is uncommon. Canine vaccines containing Hardjo were withdrawn from the market in New Zealand prior to 2001. Given the short duration of vaccine induced titres (Hartman, van Houten, Frik, & van der Donk, 1984), the Hardjo titres in this study are unlikely to be a result of administration of previously available licensed vaccines. If dogs were developing antibodies against Hardjo after off-label cattle vaccine administration by farmers we would expect titres to Pomona, and possibly Copenhageni, in the same dog as these serovars are also present in cattle leptospirosis vaccines. Therefore the prevalence of titres to Hardjo was most likely to reflect natural exposure to L. borgpetersenii Hardjo strain Hardjobovis.

Terrier breeds did not have a higher prevalence of titres to Copenhageni than other breeds; thus the hypothesis that increased contact with rats and therefore increased risk of having positive leptospiral titres by this breed group was not supported by this study. The sample size in this breed group (n=78) was low, and may not have been sufficient to detect a difference in prevalence of positive leptospiral titres by breed.

There is an anecdotal perception among veterinarians that urban dogs are at lesser risk of exposure to leptospires than other dogs. In this study, however, small breeds presumed to live in an urban environment did not have a lower incidence of titres to *Leptospira interrogans* serovar Copenhageni than other breeds. This finding may be due to titres from vaccination; however vaccine induced titres rarely result in titres greater than 300, and these titres only persist for 3 to 12 weeks after vaccination, falling below MAT titres of 1:100 (Hartman, van Houten, Frik, & van der Donk, 1984; J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). Therefore the prevalence of Copenhageni titres in these dogs most likely reflects natural exposure in naive or vaccinated dogs. There is no available data on the rate of leptospiral vaccine use in dogs in New Zealand.

The breed classification for this study was based on data supplied on the laboratory submission form; the potential for misclassification combined with the large numbers of dogs of unknown breed will contribute to bias in this study. Further studies should include information on dog activity and exposure to risk factors in addition to breed.

Vaccine induced titres against serovar Icterohaemorrhagiae makes interpretation of multiple positive titres and Copenhageni titres more difficult. Previous studies have shown cross-reactions from vaccine induced titres are unlikely to raise the MAT titre in excess of the serovars contained in the vaccine (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991), and MAT titres remain low after vaccine administration (H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003), suggesting that elevated MAT titres to leptospires reflects natural exposure. Harkin et al. (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003) suggested that differentiating vaccine induced responses and natural exposure based on titres is flawed, as vaccinated dogs will have an increased antibody response when exposed to natural infection. The higher prevalence of titres to serovar Copenhageni in this study may reflect a population of vaccinated dogs responding to natural challenge, rather than increased titres after natural infection unrelated to vaccine administration (Harkin, Roshto, & Sullivan, 2003). If data on the number of dogs vaccinated against leptospirosis in New Zealand were available further inferences could be made about the significance of positive titres to serovar Copenhageni.

There was no statistically significant difference in prevalence of positive leptospiral titres between the North and South Islands. This finding refutes the traditionally held belief that exposure of dogs to leptospires is largely confined to areas north of Taupo. Many authors outside of New Zealand report changing epidemiology of canine leptospirosis (Birnbaum *et al.*, 1998; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; Hartskeerl, Collares-Pereira, & Ellis, 2011). These changes include increased incidence or recognition of clinical disease caused by serovars not currently included in commercially available canine vaccines, and may also be due to changing contact with wildlife and livestock reservoir hosts. In other parts of the world, increased use of vaccines against leptospirosis in dogs may be contributing to the changing epidemiology but this seems an unlikely cause in the South Island of New Zealand where, presumably, relatively few dogs are vaccinated against leptospirosis. While there is no available information on changing epidemiology of leptospirosis in the South Island, it is anticipated that the increasing dairy herd density in the South Island is likely to

contribute to changes in leptospiral epidemiology in this part of New Zealand. The presence of positive leptospiral titres in the low number of samples obtained from South Island indicates further investigation is required to clarify the prevalence and distribution of positive leptospiral titres in dogs residing in the South Island of New Zealand.

Dogs aged 12 years or older had a significantly reduced prevalence of positive titres to Leptospira, in particular to serovar Copenhageni when compared to dogs less than 6 years of age. This may be due to reduced exposure in older animals less likely to roam or be involved in activities that would lead to exposure to natural infection. If there was a larger number of dogs less than 12 years old included in the sample population, observed trends towards increasing prevalence in younger dogs may have achieved statistical significance.

There was no positive association that could be made with either sex (male or female) or reproductive status (entire or neutered) and the presence of a leptospiral MAT titre of ≥96 (Table 6). This finding is in contrast to other studies which showed significantly higher sero-prevalence in male dogs which were thought to be more likely to roam and therefore be exposed to infection (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Birnbaum *et al.*, 1998).

O'Keefe considered titres of 100 or greater as positive (J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002). For this study, we regarded titres of 96 or greater as positive, to enable comparison with the O'Keefe paper. There is no consensus on what minimum titre constitutes a positive result, and there is variability in titres reported by different laboratories testing identical samples (M. D. Miller, Annis, Lappin, & Lunn, 2011)). Excluding positive titres less than 96 is expected to decrease the sensitivity of the assays used in this study, and therefore reduce the sero-prevalence estimates given, however this should increase the specificity of the positive results thus making conclusions regarding factors associated with the prevalence of positive leptospiral titres more compelling

For the purpose of this study, we determined that the highest titre identified the infecting serovar and equal highest titres resulted from more than one infecting serovar. Cross-reactivity between different serovars belonging to different serogroups is less common than cross reactivity between serovars in the same serogroup (Levett 2003). With the exception of L. borgpetersenii Hardjo strain Hardjo-bovis and L. borgpetersenii Balcanica, serovars present in New Zealand are from different serogroups, therefore different serovars can be differentiated on the basis of serological results (S. C. Hathaway, 1981). Titres for an infecting serovar also predominate over cross-reacting serovars after the first few weeks of acute infection (Scanziani *et al.*, 1994) and titres from natural exposure persist for 2 to 5 months (Scanziani *et al.*, 1994; Birnbaum *et al.*, 1998) supporting our premise that the infecting serovar produced the greatest titre.

The presentation and severity of acute leptospirosis can vary with the infecting serovar (Faine, 1982; Bolin & Alt, 2001) When testing for canine leptospirosis is indicated, consideration should be given to testing for multiple serovars known to be circulating in the local canine population, especially for the diagnosis of acute disease (Sykes *et al.*, 2011). Interestingly, Levett showed MAT testing had low specificity for predicting the infecting serovar in individual cases of leptospirosis in humans in Barbados (P. N. Levett, 2003). Serological identification of serovars present is therefore more useful on a population basis to identify serogroups of interest (P. N. Levett, 2003), whereas the diagnosis of acute disease only requires confirmation of leptospiral infection; treatment of acute disease does not vary with infecting serovar. Where a rapid diagnosis is required, PCR testing of blood or urine can expedite the diagnosis of leptospiral infection. Real-time PCR is a highly sensitive technique for identification of leptospiral DNA in blood or urine and can provide rapid, semi-quantitative results. PCR techniques may preclude the requirement for culture or for paired MAT tests. However, differentiation of the serovars involved still relies on MAT or culture (L. Smythe *et al.*, 2002) multilocus sequence typing (Ahmed *et al.*, 2006), or 16srRNA gene sequencing (Morey *et al.*, 2006).

No data on the presenting problem were available for us to draw any conclusions regarding health status of the animals being blood sampled, however the findings in this study were consistent with other sero-prevalence surveys in countries with a similar climate to New Zealand, showing a much higher sero-prevalence than the incidence of clinical signs of leptospirosis (W. A. Ellis, 2010; Rojas, 2010; Sykes *et al.*, 2011) This low incidence of clinical signs of leptospirosis compared to the sero-prevalence could have two reasons: subclinical infections are commonplace, or veterinarians are failing to diagnose mild cases of leptospirosis. The non-specific clinical signs of infection and difficulties with diagnosis also mean that the true prevalence of leptospiral infections in dogs is likely to be higher than reported (Hartskeerl, Collares-Pereira, & Ellis, 2011).

Chronic infection, leptospiruria and persistent asymptomatic shedding of leptospires are possible sequelae to acute infection (Rojas, 2010). The same authors showed a prevalence of urinary shedding of leptospires of 7.05% in dogs surveyed from the University College Veterinary Hospital, Dublin and dogs from shelters in Ireland, which is similar to the percentage identified in studies in the USA (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003). Harkin proposed that dogs may be a maintenance host for zoonotic infection; a concern that was also raised by O'Keefe et al. (J. S. O'Keefe, J. A. Jenner, N. C. Sandifer, A. Antony, & N. B. Williamson, 2002; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003). Transmission of leptospires from dogs to humans has been reported (Weekes, Everard, & Levett, 1997). MAT testing provides information on exposure of dogs, however studies have shown healthy seronegative dogs may still actively shed leptospires in their urine (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Anonymous., 2003; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003). Compared with PCR testing of canine urine, serological testing is a poor predictor of urinary shedding and zoonotic potential (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003). No information is currently available on the prevalence of urinary shedding of leptospires by dogs in New Zealand; thus further investigation is required in order to quantify the zoonotic risk posed by dogs.

Historically, vaccination against leptospirosis has been recommended for dogs living north of Taupo because of the prevalence of serovar Copenhageni in the rat population in the north of the North Island (Hilbink et al. 1992). No nationwide surveys on canine leptospirosis or maintenance hosts have been conducted since then. This study supports the conclusion that exposure to serovar Copenhageni is common for dogs resident in the south of the North Island. While the incidence of clinical leptospirosis is low, acute leptospirosis can be severe and life threatening.

Vaccination with Icterohaemorrhagiae provides immunity against infection by serovar Copenhageni, being from the same serogroup (Icterohaemorrhagiae) (Schoone *et al.*, 1989; Sykes *et al.*, 2011), and should therefore be considered as a component of vaccines used in dogs in all regions of the North Island where Copenhageni is known to be prevalent. Inclusion of serovar Hardjo as part of canine leptospirosis vaccines should be considered for dogs at increased risk of exposure to this serovar, such as working and on-farm dogs. However, we are unable to accurately define the risk of exposure to any serovar, and until this risk is known, we cannot make firm recommendations regarding vaccination of dogs against Leptospira in New Zealand. Vaccination should be considered on a case by case basis, considering the available information of risk, along with any potential side effects of and expenses incurred with vaccination.

Convenience-based sampling of sera submitted to NZVP for other diagnostic purposes means that results are not representative of the total canine population. This introduces a selection bias into our study; older dogs are more likely to be blood sampled because of geriatric disease conditions or geriatric screening, and young and healthy dogs are more likely to be excluded from sampling. We assume that the age distribution of this study population is skewed towards older dogs, although there is no general population data to test that assumption. In this study, older dogs were less likely to be seropositive than younger dogs, thus the seropositive rate may be less than that in the whole population. In contrast, healthy dogs are presumably less likely to be seropositive than diseased dogs, thus the study population may have a higher seropositive rate than the true population. In addition, veterinarians from different regions or practice types may have preference for using other veterinary pathology laboratories, which may lead to reduced numbers of samples from rural dogs being submitted to NZVP. This sampling strategy means the sample population is not representative of the total canine population in New Zealand, and therefore these results may not be able to be generalised to all New Zealand dogs, and further emphasises the need for a prospective study with an unbiased sampling strategy. Grouping of breeds into four broad categories based on presumed activity is likely subject to non-directional misclassification bias; breed data were missing for some dogs, and assumptions were made on activity, rural vs. urban residence and therefore risk of exposure based on breed may be incorrect. Dogs of a breed typically used as a farm working dog may be resident in an urban environment, while some dogs of small breed that were presumed urban for the purposed of this study may be resident in a rural environment. This sampling strategy is likely to have resulted in dogs resident in rural environments being underrepresented in the study population, this may mean significant associations between positive leptospiral titres and risk factors did not achieve statistical significance in this study. Our presumption that the greatest titre reflects the infecting serovar, and that there is only one infecting serovar is also another potential source of misclassification bias. Also, only useable serological results were included in data analysis, meaning different numbers of sera were tested for each serovar; no analysis was conducted to investigate the frequency of equivocal results for each leptospiral serovar tested. The estimates on the population at risk were obtained from records on numbers of registered dogs from the Regional Council Dog Control Survey, the National Dog Database Survey 2004, and the 2006 Department of Internal Affairs Survey. These estimates are based on the number of registered dogs, this combined with the multiple sources of data mean estimates are likely to be inaccurate, and an underestimate of the true population at risk. While having limitations, this is the best information available on dog population estimates. These limitations will mean estimates of the proportion of the population at risk sampled for this study is likely to be less than stated in this survey. The samples included in this study were collected over a 5-month period during summer and autumn. Seasonal rainfall variations affecting survivability and transmission of leptospires, in combination with a short duration of titres after exposure may have confounded these results. However, the summer and autumn months in New Zealand typically have very different rainfalls, and the sampling period could be considered to cover the lowest risk period, and highest risk periods of warm, wet weather. Future studies could more closely examine the seasonal variations in exposure.

This study provides prevalence estimates on four endemic leptospiral serovars in this population of dogs in New Zealand in 2005. The study has confirmed positive titres to serovar Copenhageni do occur, though it remains unclear whether the positive titres are related to natural exposure or, in some cases, from vaccination. Small breeds of dogs presumed to live in urban environments were no less at risk for positive titres to serovar Copenhageni than other breeds. The study also suggests that dogs in the South Island are exposed to Leptospira organisms, and that dogs of breeds typically used on farm are at increased risk for exposure to serovar Hardjo. Further studies in New Zealand are required to confirm the prevalence of leptospiral exposure in South Island and working dogs, and to determine the species and serovar of leptospires shed in canine urine in order to further assess the zoonotic risk and aid our understanding of the epidemiology of leptospirosis.

### 3.6 Acknowledgements

This study was funded by Pfizer Animal Health (NZ) Ltd. The authors acknowledge the support and contribution to this study from colleagues at the Veterinary Teaching Hospital, Institute of Veterinary and Biomedical Sciences, and the EpiCentre, Massey University.

# Chapter 4

# A leptospirosis survey of farm working dogs in the South Island of New Zealand.

# 4 A leptospiral survey of farm working dogs in the South Island of New Zealand

## 4.1 Abstract:

Antibodies to *Leptospira* serovars Copenhageni, Pomona, Hardjo and Ballum have been identified in New Zealand dogs and these serovars have also been reported in notified human cases. Prior to 2012 infections of dogs with serovar Copenhageni have been reported most commonly in the North Island of New Zealand, however a serological study published in 2012 (Chapter 3) suggested that dogs from the North and South Islands are at similar risk of exposure. In addition, previous studies report that antibody titres to Hardjo are associated with farm working dog breeds, but recent reports of clinical disease in working dogs attribute infection to serovar Pomona. Farm dogs are exposed to livestock with a high prevalence of antibody to Hardjo and Pomona. No suitable data are available on urinary shedding of leptospires by dogs in New Zealand, or on the association of seropositivity in dogs and their urinary shedding with leptospiral serovars in livestock.

The aims of this cross-sectional pilot study were to determine the prevalence of positive antibody titres to serovars Copenhageni, Pomona, and Hardjo, and to determine the prevalence of urinary shedding of leptospires, in healthy unvaccinated South Island farm dogs. A further aim was to evaluate the association of positive serological titres or urine PCR with putative dog and farm level risk factors including the age and sex of the dog, and the serological prevalence in livestock on these farms.

The prevalence of MAT titres ≥100 to any one of the three serovars tested in South Island farm working dogs was lower (1/129 dogs or 1(95% C.I: 0-2)%) than that reported in a nationwide study in 2012 (15 (95% C.I: 12.3–18.0)%). The serovar with the highest prevalence (titres ≥25) in the study population was Hardjo (23/129 dogs or (18 (95% C.I: 10-30) %). Titres to Copenhageni were associated with a high livestock prevalence together with the presence of horses on the same farm.

Urinary shedding of leptospires was identified in more than 12 (95% C.I: 5-24) % of South Island farm dogs. While positive MAT titres were associated with a strong positive urine PCR (OR 3.06 (95% C.I:1.28-7.30)), this study also demonstrated the presence of urinary shedding in apparently healthy dogs with negative MAT titres. Identification of the serovar (s) shed in canine urine in New Zealand is required, as is establishment of the prevalence and duration of urinary shedding in dogs nationwide. Further work is needed to confirm any association between a high livestock prevalence and the serological status of dogs on the same property or in contact with livestock.

The study was designed to test the following principle hypotheses: that the prevalence of positive MAT titres ≥100 to serovars Hardjo, Copenhageni and Pomona in South Island dogs is similar to the prevalence of positive MAT titres ≥100 in New Zealand dogs reported in 2012; for Copenhageni 10.3 (95% C.I: 8.1-12.9)%, Hardjo 3.5 (95% CI: 2.3-5.3)% and Pomona 1.1 (95% C.I: 0.4-2.2)%, that the most prevalent serovar farm working dogs are exposed to is Hardjo, that the prevalence of positive urine PCR tests will be ≥ 5%, and that positive urine PCR can occur with negative serology.

<u>Conclusions:</u>

The prevalence of MAT titres of ≥100 in South Island dogs to serovar Hardjo was 1 (95% C.I: 0-2)%, lower than that previously reported for North Island dogs. No titres ≥100 were identified to serovars Copenhageni or Pomona. The most prevalent serovar identified on serology (titres ≥25) was serovar Hardjo with a prevalence of 18 (95% C.I: 10-30)%, however the prevalence of Copenhageni titres was only slightly lower at 14 (95% C.I: 8-24)%. The prevalence of strong positive urine PCR tests was 12 (95% C.I: 5-24) % and dogs with positive urine PCR and negative serology were identified.

### 4.2 Introduction

Leptospiral infection in dogs is reported around the world, and is recognised as a common infection, which can cause severe clinical disease, or subclinical infection (Greene, Sykes, & Brown, 2006) Common clinical signs include depression, anorexia, vomiting, diarrhoea, and icterus. Dogs frequently present with an acute onset illness, and a primary enteric disease is often initially suspected, however affected dogs often develop a marked jaundice (Langston & Heuter, 2003). Infection causes both severe hepatic and renal disease, along with pulmonary haemorrhage, vasculitis, coagulopathy, myositis and uveitis (Prescott JF, 2002; Langston & Heuter, 2003; R. E. Goldstein *et al.*, 2006; Greene, Sykes, & Brown, 2006; Geisen *et al.*, 2007; Sykes *et al.*, 2011). Mortality rates of 50% have been reported (R. I. Miller, Ross, Sullivan, & Perkins, 2007).

Antibodies to *Leptospira* serovars Copenhageni, Pomona, Hardjo, Tarrasovi and Ballum have been identified in dogs in New Zealand (Mackintosh, Blackmore, & Marshall, 1980; Ellison RS, 1990; Hill, 1999). Antibodies to these serovars have also been identified in humans in New Zealand (Crump, Murdoch, & Baker, 2001; Thornley, Baker, Weinstein, & Maas, 2002). Antibodies to serovar Copenhageni in dogs are identified more commonly in the North Island, (Hilbink, Penrose, & McSporran, 1992; J. O'Keefe, J. Jenner, N. Sandifer, A. Antony, & N. Williamson, 2002; Harland *et al.*, 2012) and in more than 14% of sampled dogs (Harland *et al.*, 2012).

A recent sero-prevalence survey (Chapter 3) (Harland *et al.*, 2012) reported that positive agglutination titres to Hardjo were associated with breeds of dogs commonly used as farm working dogs, and that dogs from the North and South Islands were at similar risk of exposure (Harland *et al.*, 2012). Recent observations by veterinary practitioners and veterinary diagnostic laboratory reports have attributed severe clinical disease in dogs to serovar Pomona (Anonymous, 2012a; Anonymous, 2012b; Anonymous, 2013a; Anonymous, 2013b; Cave, Harland, & Allott, 2013; Anonymous, 2014a; Anonymous, 2014c; Anonymous, 2014b). These observations are of concern when considering the exposure of farm dogs to livestock with a high prevalence of antibodies to Hardjo and Pomona, where the only vaccine licensed for use in dogs provides protection against serovar Copenhageni alone (Cave, Harland, & Allott, 2013). Being from a different serogroup, this vaccine is not expected to confer immunity against Hardjo or Pomona (Hartman, van Houten, Frik, & van der Donk, 1984; Cave, Harland, & Allott, 2013).

Dogs are recognised as maintenance hosts of the leptospiral serovar Canicola, with urinary shedding of leptospires identified in 7.05% of dogs sampled in Ireland (Rojas, 2010), however the prevalence and duration of urinary shedding of leptospires by dogs infected with other serovars is not known (H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; Langston & Heuter, 2003; André-Fontaine, 2006; H.L.B.M. Klaasen, Van Der Veen, Molkenboer, & Sutton, 2013). Persistent renal colonisation and urinary shedding in dogs has been documented for serovars Tarrasovi, Pomona, Bratislava and Icterohaemorrhagiae (Menges, Galton, & Habermann, 1960; Mackintosh, Blackmore, & Marshall, 1980; Thiermann, 1980; van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Greene, Sykes, & Brown, 2006). Dogs may also act as maintenance hosts for serovars from the Australis serogroup (William A Ellis, 2015), serovars in this serogroup include Australis and Bratislava (P. N. Levett, 2001). Adaptation of leptospires to different maintenance hosts has been thought to occur with changes in selection pressure on the organism - the delineation of accidental and maintenance hosts is not always clear (WHO, 2003). The development of atypical maintenance hosts, persistent shedders or propagation of epidemics in the presence of alternative maintenance hosts given favourable environmental and population dynamics has already been proposed (S.C. Hathaway, 1978; William A Ellis, 2015). Little is known about the role of dogs in the epidemiology of leptospirosis in New Zealand, in particular the prevalence of urinary shedding of leptospiral organisms by dogs and the contribution dogs make to the maintenance of serovars in the environment or to exposure of wildlife and livestock maintenance hosts. Given that the epidemiology of canine leptospirosis in New Zealand is unique - serovar Canicola being exotic to this country, and only a limited number of endemic serovars and maintenance hosts being present in the country; it is therefore possible that dogs may act as persistent shedders or even maintenance hosts of serovars other than Canicola.

Humans are accidental hosts for *Leptospira*, and zoonotic infection from dogs has been reported in the literature (Feigin RD, 1973; Wong, Kaplan, Dunkle, Stechenberg, & Feigin, 1977; Trevejo *et al.*, 1998; P. N. Levett, 2001; H.L.B.M. Klaasen, Molkenboer, Vrijenhoek, & Kaashoek, 2003; André-Fontaine, 2006; K. Brown & Prescott, 2008; Sykes *et al.*, 2011). New Zealand has one of the highest rates of notified human leptospirosis of developed countries with a temperate climate (Thornley, Baker, Weinstein, & Maas, 2002), and there is an increasing rate of human infections with serovars that are not occupationally acquired such as serovar Ballum (Crump, Murdoch, & Baker, 2001; Thornley, Baker, Weinstein, & Maas, 2002; Keenan, 2007; F Fang *et al.*, 2014). If zoonotic serovars were found in the urine of dogs, it would indicate clear risk for human infection.

There are no data available on urinary shedding of leptospires by dogs in NZ, or on the association between positive Leptospiral serology in dogs with Leptospiral serovars in livestock or humans. Given this absence of comprehensive data on urinary shedding by New Zealand dogs when seropositivity has been shown to be common in this country, investigation into the prevalence of urinary shedding by dogs is necessary. In addition, dogs of breeds commonly used as farm dogs have been shown to be at increased risk of seropositivity to serovar Hardjo (Harland *et al.*, 2012). This same study found that dogs from the South Island of New Zealand were at similar risk of seropositivity as North Island dogs. The limitations of the 2012 study included a small sample size from the South Island (n=51), many of the South Island dogs were resident in urban areas ( Christchurch City, Nelson, Dunedin City), and the assumption that breeds commonly used as working dogs were resident on a farm. Given these limitations, further investigation into the prevalence of seropositivity to leptospiral serovars in South Island dogs and working farm dogs is required.

The aims of this study were to determine the prevalence of positive MAT titres to leptospiral serovars Hardjo, Pomona and Copenhageni, and the prevalence of urinary shedding of leptospires in farm working dogs from the South Island of New Zealand. The association between positive antibody titres or positive urine PCR in dogs and putative risk factors for exposure to leptospires are also investigated.

## 4.3 Materials and Methods

### *4.3.1 Sample Collection*

The study population was thirty South Island farms where livestock had previously been tested for exposure to Hardjobovis and Pomona (Dreyfus, 2013), and the farmers had agreed to participate in further research projects. The current cross sectional survey was conducted between March and September 2013 by contracting local veterinary clinics to obtain blood and urine samples and interview farmers who were regular clients. Up to eight dogs were sampled on each farm, and those less than 3 months of age were excluded to avoid confounding by maternally derived antibody. The veterinarian taking the samples recorded the vaccination history, health status, age and exposure to livestock (sheep, cattle, pigs and deer), vermin, horses and free standing water on a standard survey form (Appendix 4). Serum was collected by venepuncture and urine by free catch, new needles, syringes, urine collection cups and pipettes were used for each dog. Serum was collected into a glass blood collection tube (Vacutainer, BD), urine was collected into a plastic cup and then decanted into a sterile specimen tube containing a buffer solution (Assay Assure, Thermo Scientific). Serum samples were obtained by venepuncture of tail or jugular vein from twenty sentinel livestock species (either cattle, sheep or deer) were obtained at the same farm visit, the species of livestock sampled was convenience based, with the first 20 animals being sampled.

### *4.3.2 Canine Serology*

Canine serum was tested for antibodies to Leptospiral serovars Copenhageni, Pomona and Hardjo by the Microscopic Agglutination Test (MAT), performed by a commercial veterinary diagnostic laboratory, New Zealand Veterinary Pathology (NZVP, Tennent Drive, Palmerston North). The MAT was performed using standard laboratory methods (Faine, 1982); serial dilutions of serum being incubated with culture of each serovar and observed for agglutination, the final reported titre being the reciprocal of the greatest dilution causing 50% agglutination.

### *4.3.3 Canine PCR*

Canine urine was analysed by NZVP for the presence of Leptospiral DNA using a commercially available real time, quantitative, genus specific PCR kit - Patholept TaqVet - that targets the 16s gene (Laboratoire Service International, Lissieu, France). This test has been validated by the manufacturer for use in all animal species using tissue, urine or whole (EDTA) blood. The extraction and TaqMan qPCR were performed according to the manufacturer's instructions. The thermal cycling and annealing sequence comprised one hold at 94°C for ten minutes, followed by 45 cycles of 20 seconds denaturation at 94°C and extension for one minute at 60°C, using a Corbett Rotor Gene 6000. Fluorescence was captured after each cycle at the end of the extension. An internal negative control was used with each batch. Samples with a threshold cycle (Ct) of less than 37 were classified as positive, and samples with a Ct from 37 to 45 were classified as weak or suspect positives.

### *4.3.4 Livestock Serology*

Livestock sera were tested for antibodies to serovars Hardjo and Pomona by a research laboratory (mEpiLab, Hopkirk Research Institute, Tennent Drive, Palmerston North) using the MAT as described above. Livestock prevalence was arbitrarily categorised as "high" if more than 1 animal had a MAT titre of $\geq$100 to Pomona or more than 6 animals had a MAT titre of $\geq$100 to Hardjo. Otherwise the prevalence was classified as "low".

### *4.3.5 Statistical Analysis*

The prevalence of positive MAT tests and positive urine PCR in dogs was calculated using Generalized Estimating Equations (GEE) to adjust for the effect of clustering of dogs within farms, where dogs resident on the same property are more likely to have similar PCR and MAT test results than dogs resident on different properties (Liang & Zeger, 1986).

Associations between putative explanatory variables (farm level variables included the sero-prevalence in livestock, the presence of vermin, domestic pigs, deer, sheep, cattle, horses and natural waterways, and individual level variables included the age and sex of the dog) and the outcomes of interest (positive serology and positive urine PCR) were explored using univariable logistic regression. Variables were allowed to enter a multivariable model if the Likelihood Ratio Test (LRT) was statistically significant at $p \leq 0.2$. The model was built in a backwards stepwise fashion, removing least significant variables one at a time, and retaining those variables that were significant at $p \leq 0.05$. Where more than one variable was significant at $p \leq 0.05$, the odds ratio and confidence intervals reported were adjusted for the presence of the other significant variables. Variables not significant at $p \leq 0.05$ were still retained where their presence changed an exposure coefficient by more than 15%, indicating the presence of confounding. Two way interaction terms were tested for significance, and significant interaction terms were included, even if the individual variables were not significant on their own. Results were adjusted for the effect of clustering on farms using GEE.

The association between positive urine PCR and positive MAT tests in dogs was tested using logistic regression, and then adjusted for the effect of clustering of dogs on farms using GEE.Statistical analysis was performed using the software package R, version 3.1.1 (R Development Core team, 2014, R Foundation for Statistical Computing, Vienna, Austria).

This study was approved by the Massey University Animal Ethics Committee, protocol 10/103.

## 4.4 Results:

### *4.4.1 Descriptive results:*

One hundred and twenty nine dogs from thirty farms were included in this study (Appendix 5). All dogs were apparently healthy at the time of sample collection, and none of the dogs had received a leptospirosis vaccine at any time. The median number of dogs sampled per farm was 4 (min:1 IQR:3 max:8) 128 serum samples and 94 urine samples were obtained. There were 59 male dogs, 60 female dogs, and 10 dogs of unknown sex. The dogs ranged in age from nine months to fourteen years of age, the age of ten dogs was unknown (Figure 4.) The prevalence of positive MAT titres in livestock was classified as "High" on 11 farms, and "Low" on 18 farms (livestock were not sampled on one farm). The number of dogs and farms associated with each factor is shown in Table 8. Thirty dogs (23%) were seropositive at any titre $\geq$25 to any of the three serovars tested (Hardjo, Pomona and Copenhageni) (Figure 5). The titre distribution by serovar is shown in Figure 2. Titres were predominantly 25, only one dog had a MAT titre greater than 50 (a titre of 200 to Hardjo), this dog was from a high livestock prevalence farm, and no urine was collected from this dog. The frequency of positive MAT and PCR results is shown in Table 10.

**Figure 4 Age distribution of farm dogs from the South Island of New Zealand that had urine and serum samples collected for leptospiral serology and PCR.**

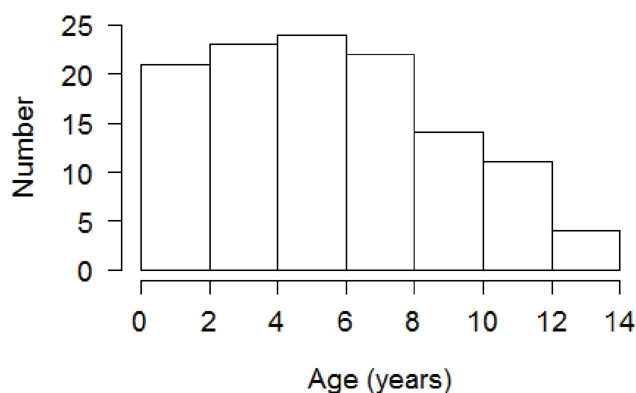
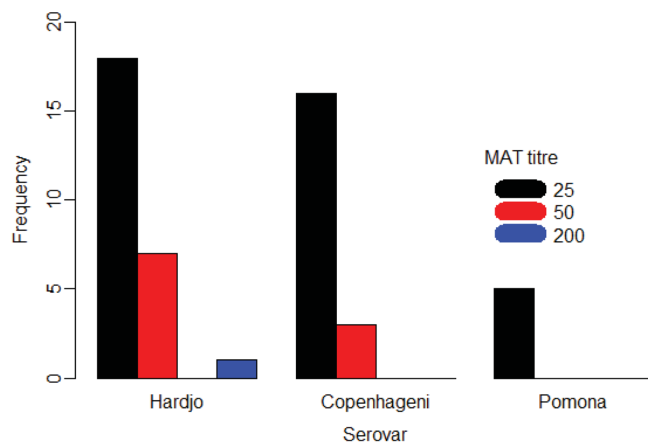



**Table 8 Frequency (percentage) of presence and exposure to putative environmental risk factors for exposure to Leptospira in 129 farm dogs from 30 farms in the South Island of New Zealand.**

| **Risk factor** | **Number of dogs exposed (%)** | **Number of farms with risk factor present (%)** |
|---|---|---|
| Sheep | 121 (93.7) | 28 (93) |
| Cattle | 123 (95.3) | 29 (97) |
| Deer | 114 (88.4) | 27 (90) |
| Pigs | 42 (32.6) | 10 (33) |
| Waterways | 126 (97.7) | 29 (97) |
| Vermin | 121 (93.8) | 29 (97) |
| Horse | 26 (20.2) | 9 (30) |
| High Livestock Prevalence | 22 (17.1) | 6 (20) |

**Figure 5 Frequency of MAT titres to leptospiral serovars Copenhageni, Pomona and Hardjo in farm dogs from the South Island of New Zealand**




### *4.4.2 Prevalence Results:*

The prevalence of positive MAT titres for all serovars tested and each serovar individually is given in Table 9. Ten dogs had dual MAT titres:

Pomona 25 Hardjo 25 1 dog

Hardjo 25 Copenhageni 50 2 dogs

Hardjo 25 Copenhageni 25 2 dogs

Hardjo 50 Copenhageni 25 5 dogs[3]

There were 16 dogs with positive urine PCR tests (11 dogs with strong positive urine PCR and 5 dogs with weak positive urine PCR). These were from 11 farms (three farms had two dogs with positive urine PCR, one farm had four dogs with positive urine PCR). The prevalence of positive urine PCR in dogs was 17 (95% C.I: 9-28) %, adjusted for the effect of clustering of dogs on farms. The prevalence of strong positive urine PCR in dogs was 12 (95% C.I: 5-24) %, adjusted for clustering.

**Table 9 Prevalence and confidence intervals of positive MAT titres to serovars Hardjo, Pomona and Copenhageni in farm dogs from the South Island of New Zealand, adjusted for the effect of clustering of dogs on farms.**

| MAT titre | Prevalence (95% C.I:)% |
|---|---|
| Any serovar ≥25 | 26 (15-41) |
| Any serovar ≥100 | 1 (0-2) |
| Hardjo ≥25 | 18 (10-30) |
| Hardjo ≥100 | 1 (0-2) |
| Copenhageni ≥25 | 14 (8-24) |
| Copenhageni ≥100 | 0 (n/a)[4] |
| Pomona ≥25 | 3 (2-6) |
| Pomona ≥100 | 0 (n/a) |

[3] These 5 dogs were all from same farm, with high livestock seroprevalence and were PCR negative.

[4] No confidence interval was calculated where the prevalence was 0%

Association between Serology and PCR results:

**Table 10 Frequency of urine PCR results for leptospires and MAT titres to any of the three leptospiral serovars tested (Hardjo, Copenhageni, and Pomona) in South Island farm dogs.**

| | MAT negative *(no reactivity at 1:25 dilution)* | MAT ≥25 |
|---|---|---|
| Negative urine PCR | 58 | 19 |
| Weak Positive Urine PCR | 4 | 1 |
| Strong Positive urine PCR | 5 | 6 |

On simple logistic regression, the associations between positive (both weak and strong positive) urine PCR and serology to all serovars, and to each serovar individually, was not statistically significant.

With only the strong positive PCR results, there was a statistically significant association between a strong positive urine PCR and a positive MAT titre of ≥25 to any serovar (p=0.05), and to serovar Copenhageni individually (p=0.05). The odds of having a strong positive urine PCR result in dogs with a MAT titre of ≥25 to any serovar was 3.06 (95% C.I: 1.28-7.30) times the odds of having a strong positive urine PCR in dogs with negative serology to all serovars tested, adjusted for the effect of clustering of dogs on farms. The odds of having a strong positive urine PCR result in dogs with a MAT titre of ≥25 to serovar Copenhageni was 3.79 (95% C.I: 1.06-13.54) times the odds of having a strong positive urine PCR results in dogs with a negative MAT titre to serovar Copenhageni, adjusted for the effect of clustering.

### *4.4.3 Association between Serology and putative risk factors:*

Significant associations between canine serology and putative risk factors are shown in Tables 11, 12 and 13 (univariable models) and Tables 15,15 and 16 (multivariable model adjusted for the effect of clustering).

**Table 11 Association between a MAT titre of ≥25 to any one of leptospiral serovars Copenhageni, Hardjo and Pomona and putative risk factors that were significant at p<0.2 in a univariable model, in farm dogs from the South Island of New Zealand.**

| **Variable** | **Level** | **Odds Ratio (95% C.I.)[5]** | **p-value** |
|---|---|---|---|
| Livestock Sero-prevalence | Low | *Ref* | *Ref* |
| | High | 3.90 (1.75-900) | 0.001 |
| Sex | Female | *Ref* | *Ref* |
| | Male | 0.41 (0.17-0.95) | 0.041 |
| Horses | Not present | *Ref* | *Ref* |
| | Present | 3.87 (1.67-8.77) | 0.020 |

[5] Confidence Interval

**Table 12 Association between a MAT titre of ≥25 to leptospiral serovar Hardjo and putative risk factors that were significant at p<0.2 in a univariable model, in farm dogs from the South Island of New Zealand.**

| **Variable** | **Level** | **Odds Ratio (95% C.I.)** | **p-value** |
|---|---|---|---|
| Livestock Sero-prevalence | Low | *Ref* | *Ref* |
| | High | 5.28 (2.12-14.16) | 0.005 |
| Sex | Female | *Ref* | *Ref* |
| | Male | 0.48 (0.19-1.18) | 0.119 |
| Horses | Not present | *Ref* | *Ref* |
| | Present | 3.21 (1.31-7.94) | 0.011 |
| Pigs | Not present | *Ref* | *Ref* |
| | Present | 0.45 (0.14-1.17) | 0.124 |
| Waterways | Not present | *Ref* | *Ref* |
| | Present | 0.12 (0.005-1.29) | 0.128 |

**Table 13 Association between a MAT titre of ≥25 to leptospiral serovar Copenhageni and putative risk factors that were significant at p<0.2 in a univariable model, in farm dogs from the South Island of New Zealand.**

| **Variable** | **Level** | **Odds Ratio (95% C.I.)** | **p-value** |
|---|---|---|---|
| Livestock Sero-prevalence | Low | *Ref* | *Ref* |
| | High | 3.38 (1.25-9.81) | 0.019 |
| Sex | Female | *Ref* | *Ref* |
| | Male | 0.28-(0.08-0.89) | 0.039 |
| Horses | Not present | *Ref* | *Ref* |
| | Present | 14.69 (4.79-55.53) | 0.00001 |

For serovar Pomona, age was the only significant variable on univariable analysis, with an Odds Ratio of 1.32 (95% C.I. 0.95-1.98) and p-value 0.11

**Table 14 Association between a MAT titre of ≥25 to any one of leptospiral serovars Copenhageni, Hardjo and Pomona and putative risk factors that were significant at p<0.05 in a multivariable model, in farm dogs from the South Island of New Zealand, adjusted for the effect of clustering of dogs on farms.**

| **Variable** | **Level** | **Odds Ratio (95% C.I.)** |
|---|---|---|
| Livestock Sero-prevalence | Low | *Ref* |
| | High | 2.69 (0.69-10.70)[6] *(adjusted for horses)* |
| Horses | Not present | *Ref* |
| | Present | 5.27 (0.78-21.12)[6] *(adjusted for livestock sero-prevalence)* |

The odds of a dog having a MAT titre of ≥25 to any serovar when living on a farm with horses was 5.27 (95% C.I: 1.32-21.09) times the odds of a dog having a MAT titre of ≥25 to any serovar when living on a farm without horses, adjusted for the effect of livestock prevalence and clustering of dogs on farms.

[6] OR includes 1 however this result was retained due to the presence of confounding between variables included in this table.

**Table 15 Association between a MAT titre of ≥25 to leptospiral serovar Hardjo and putative risk factors that were significant at p<0.05 in a multivariable model, in farm dogs from the South Island of New Zealand, adjusted for the effect of clustering of dogs on farms.**

| **Variable** | **Level** | **Odds Ratio (95% C.I.)** |
|---|---|---|
| Livestock Sero-prevalence | Low | *Ref* |
| | High | 3.30 (0.63-17.44)[6] *(adjusted for pigs and horses)* |
| Horses | Not present | *Ref* |
| | Present | 4.86 (1.72-14.38) *(adjusted for pigs and livestock sero-prevalence)* |
| Pigs | Not present | *Ref* |
| | Present | 0.47 (0.07-3.05)[6] *(adjusted for horses and livestock sero-prevalence)* |

The odds of a dog living on a farm with horses having a titre of ≥25 to Hardjo was 4.86 (95% C.I: 1.72-14.38) times the odds of a dog living on a farm without horses having a titre of ≥25 to Hardjo.

**Table 16 Association between a MAT titre of >25 to leptospiral serovar Copenhageni and putative risk factors that were significant at p<0.05 in a multivariable model, in farm dogs from the South Island of New Zealand, adjusted for the effect of clustering of dogs on farms.**

| Variable | Level | Odds Ratio (95% C.I.) |
|---|---|---|
| Livestock sero-prevalence | Low | *Ref* |
| | High | 3.89 (0.69-21.88)[7] *(adjusted for horses)* |
| Horses | Not present | *Ref* |
| | Present | 20.91 (3.46-126.24) *(adjusted for livestock sero-prevalence)* |
| High livestock sero-prevalence and Horses | Both not present | *Ref* |
| | Both present | 28.21 (1.06-754.72) |

[7] OR includes 1 however this result was retained as the interaction term between livestock sero-prevalence and horses was significant.

When serovar Copenhageni was considered individually, the presence of horses and high livestock prevalence not statistically significant when considered individually. There was no evidence of confounding, however the interaction term between livestock sero-prevalence and horses was significant (p=0.035). The odds of a dog having a MAT titre of $\geq$25 to serovar Copenhageni that was living on a farm with both horses and high livestock sero-prevalence was 28.2 (95% C.I: 1.06-754.70) times the odds of a dog having a titre of $\geq$25 to Copenhageni that was living on a farm without a high livestock sero-prevalence or horses, adjusted for the effect of clustering. When logistic regression was applied to serovar Pomona alone, only the age of the dog was significant at $p \leq 0.2$ but it did not remain significant at $p \leq 0.05$.

#### *4.4.4 Association between PCR results and putative risk factors*

Risk factors associated with positive urine PCR that were significant at $p \leq 0.2$ in a univariable model are given in Table 17.

**Table 17 Association between positive urine PCR and putative risk factors that were significant at $p \leq 0.2$ in a univariable model, in farm dogs from the South Island of New Zealand.**

| Variable | Level | Odds Ratio (95% C.I.) | p-value |
|---|---|---|---|
| Pigs | Not present | *Ref* | *Ref* |
| | Present | 2.12 (0.71-6.41) | 0.176 |
| Livestock sero-prevalence | Low | *Ref* | *Ref* |
| | High | 2.12 (0.70-6.93) | 0.190 |
| Sex | Female | *Ref* | *Ref* |
| | Male | 2.83 (0.89-10.92) | 0.096 |

Only the sex of the dog remained significant when a multivariable model was applied, however there was evidence of confounding between the sex of the dog and livestock sero-prevalence. The odds of a male dog having a positive urine PCR was 5.54 (95% C.I. 1.77-17.34) times the odds of a female dog having a positive urine PCR, adjusted for the effect of clustering of dogs on farms and for the effect of high livestock sero-prevalence (p=0.020). When only strong positive urine PCR results were considered, only the presence of pigs on the farm was significant in a univariable model; with an Odds Ratio of 2.49 (95% C.I: 0.69-9.34) and p-value 0.160. The association between the prevalence ratio of urinary shedding and the presence of deer, sheep, horses, cattle, vermin or waterways did not approach statistical significance. In this data set there was no statistically significant association found between a strong urine PCR result and any putative explanatory farm or dog level variable

### 4.5 Discussion

The study was designed to test the following principle hypotheses: that the prevalence of positive MAT titres >100 to serovars Hardjo, Copenhageni and Pomona in South Island dogs is similar to the prevalence of positive MAT titres >100 in New Zealand dogs reported in 2012; for Copenhageni 10.3 (95% C.I: 8.1-12.9)%, Hardjo 3.5 (95% CI: 2.3-5.3)% and Pomona 1.1 (95% C.I: 0.4-2.2)%, that the most prevalent serovar farm working dogs are exposed to is Hardjo, that the prevalence of positive urine PCR tests will be > 5%, and that positive urine PCR can occur with negative serology.

Urinary shedding of leptospires by apparently healthy dogs is a novel finding in New Zealand, which creates a significant concern for the management of leptospirosis on farms. The shedding identified in more than 12 (95% C.I: 5-24) % of dogs sampled was higher than previously reported in dogs from other countries (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003; Rojas, 2010). Eleven of the thirty farms in this study had at least one dog with a positive urine PCR. A significant association between serological titres and urinary shedding was demonstrated in this study; dogs with a positive MAT titre to any serovar, or with a positive MAT titre to Copenhageni were more than three times likely to have a strong positive urine PCR result than dogs with negative titres. It is interesting to note that of those dogs with a positive MAT and urine PCR (n=7), the PCR were mostly strong positives (6/7), whereas for those dogs with negative serology and positive urine PCR (n=9), only half of the PCR positives were strong positives (5/9), however these numbers are small. In the current study, urine shedding occurred with negative MAT titres adding weight to findings by Harkin *et al*. that the MAT is considered a poor predictor of urinary shedding in individual dogs (Harkin, Roshto, Sullivan, Purvis, & Chengappa, 2003) and therefore cannot be used to rule out urinary shedding. Serological surveys are an inaccurate predictor of zoonotic risk.

In this study, male dogs were greater than 5 times more likely to have a positive urinary PCR compared to female dogs, when the multivariate model was adjusted for the effect of clustering of dogs on farms, and the sero-prevalence in livestock. This observation supports the similar findings of increased risk of exposure in male dogs reported in the literature (van den Broek, Thrusfield, Dobbiet, & Ellisi, 1991; Rentko, Clark, Ross, & Schelling, 1992; Ward, Glickman, & Guptill, 2002; Ward, Guptill, Prahl, & Wu, 2004; R. I. Miller, Ross, Sullivan, & Perkins, 2007). Potential reasons for the increased exposure of male dogs include their increased propensity for roaming and sniffing/licking behaviours. The same findings were not repeated when only strong positive PCR results were considered; therefore the significance of sex on urinary shedding should be interpreted with caution.

PCR detects both leptospiral organisms and cell free DNA and the presence of either is considered significant. The TaqVet qPCR kit used in this study was used according to commercial detection protocols, a factor which places some limitations on interpretation of the data. Results with a Ct value between 37 and 45 were considered weak positives. The kit manufacturer, Laboratoire Service International, has conducted non-peer reviewed validation of the kit for up to 45 cycles under controlled conditions, however the repeatability of weak positive samples in a commercial laboratory has not been rigorously tested. The kit has been used to detect leptospiral DNA in previous studies (Ayral *et al.*, 2014; Zilber *et al.*, 2014), and has been validated for use on canine urine by the manufacturer. Potential false positive PCR due to contamination at sampling or at the laboratory, or due to non-specific causes are considered unlikely as new sampling equipment was used for each dog. The PCR was not batch processed at the laboratory, and a negative control was used to confirm result validity. A positive control was not used. False negative results due to intermittent shedding of the organism, deterioration of the sample during transport and storage, or because of low numbers of organisms are considered more likely than false positives. The PCR results were not quantified by normalising against a housekeeping gene or standard curve.

No MAT titres >100 to Copenhageni were identified in this study, in contrast to the prevalence of 10.3% suggested by a previous serosurvey (Harland *et al.*, 2012), however the populations of dogs sampled in each study were different. This study was conducted on farm dogs rather than urban dogs (a significant number of Christchurch dogs presumed to be urban were included in the 2012 survey). The lower prevalence may also be due to seasonal or annual variations in prevalence, although samples were obtained for this study during the autumn and winter, so exposure during the spring-autumn period were likely to have been detected serologically. No dogs from the South Island West Coast were included in the current study, and the prevalence of positive titres may be different in this region which has a higher rainfall and a known prevalence of leptospirosis in livestock (B.R.Jones pers.comm.). None of the dogs in this study had received a leptospiral vaccine, therefore confounding with vaccinal titres is not a concern, this is in contrast to the 2012 survey where no data were available on the vaccination status of the dogs surveyed.

Interestingly there are many more positive (>25) titres to Copenhageni (n=15) and Hardjo (n=20) in comparison to Pomona (n=4). While titres of <100 are not considered indicative of current infection or recent exposure, if the titres in this study were all attributable to non-specific reactivity similar numbers of positive titres to each serovar might be expected, suggesting that the titres to Copenhageni and Hardjo in this study are significant, although the number and contribution to background noise of saprophytic organisms is not quantified. These positive results may be indicative of true historical exposure as opposed to non-specific reactivity, and may be related to the prevalence of leptospiral infection with Copenhageni and Pomona within the livestock the dogs are in contact with.

This study did not show a clear association between the prevalence of leptospiral titres in livestock and serological and PCR results in dogs, aside from the significant interaction term of horses and livestock together on the same farm. Sero-prevalence in livestock was frequently significant on univariable analysis ($p \leq 0.2$) but was no longer significant in the multivariable model. Farms were classified as “High” livestock sero-prevalence if > 1/20 livestock animals had a positive titre to Pomona, or > 6/20 had a positive titre to Hardjo. This classification was based on previous work that found beef herds had a median prevalence of positive titres to Hardjo of 51%, and a median prevalence of positive titres to Pomona of 15% (Dreyfus, 2013). Misclassification bias may have resulted in an incorrect association or underestimation of the association between livestock sero-prevalence and serology or PCR results in these dogs. With a larger study size (more farms) a significant association between livestock prevalence and canine serological results or PCR may be demonstrated. This association may vary between geographical regions, therefore further investigation into these associations on North Island farms is warranted. Demonstrating a significant effect of putative risk factors on serological results and urinary shedding in this dataset was limited where the

proposed risk factors were present on the majority of farms (such as the presence of cattle, vermin and waterways). A much larger study population would be required to demonstrate a statistically significant effect of such risk factors, and for demonstrating any significant interaction or confounding effect between these risk factors.

There was a statistically significant association between positive titres (≥25) to serovar Copenhageni in dogs and the interaction term that included the presence of horses together with high livestock sero-prevalence, OR 28.28 (95% C.I; 1.06-754.42). Although the association between positive serology in dogs and the presence of horses on farms should be interpreted cautiously, it is noteworthy. While sporadic infection of horses with a number of leptospiral serovars has been reported in New Zealand, it is considered unlikely that horses are a maintenance host for serovars in this country (Hilbink & Penrose, 1990). More likely, horses are serving as a proxy for other farm factors that have a significant role in exposure of dogs to leptospires. Such factors may include the geographic region and the farm topography where horses are more commonly used, dogs roaming free where horses are being used in preference to motorbikes, or the storage of hard feed for horses being associated with increased rodent numbers. No other studies demonstrating an association between positive serology in dogs and the presence of horses on the same property were identified in the literature, and the implications of this finding for humans is uncertain; future studies into the role of horses in maintaining or shedding leptospires would clarify this further.

This study has provided more complete data for farm dogs (rather than urban dogs), and for dogs resident in the South Island of New Zealand, when compared to the 2012 study (Harland *et al.*, 2012). Dogs from the west coast of the South Island were underrepresented in this study. Nevertheless, the small sample size in this pilot study has provided estimates of prevalence of positive serologic titres and urinary shedding, but larger numbers of dogs would need to be sampled to support these findings, and draw stronger conclusions about the associations between urinary shedding and serological status of dogs with livestock sero-prevalence and on farm risk factors.

The high prevalence of shedding leptospiral DNA by dogs in this study is of significant concern, and similar studies should be undertaken in North Island dogs to confirm the prevalence of urinary shedding by dogs nationwide, and also the duration of shedding. More work is needed to identify the infecting serovar(s) that are shed in canine urine in this country, via culture or typing of amplified DNA products. This information will enable development of vaccines, and vaccination and control recommendations.

### 4.6 Conclusions

Urinary shedding of leptospires was identified in more than 12 (95% C.I: 5-24) % of South Island farm dogs. This finding is of concern because shedding will contribute to the persistence of leptospirosis on farms. While direct transmission of leptospirosis from dogs to humans is not commonly reported, the potential for zoonotic infection is real either through direct infection, or indirectly through a dogs' contribution to the maintenance of leptospires in the environment and circulating within a herd with subsequent infection of susceptible animals and exposure of humans on that farm.

In this study the prevalence of MAT titres $\geq$100 in South Island farm working dogs was lower than that reported previously in 2012. Of the three serovars tested (Hardjo, Pomona and Copenhageni), the serovar these dogs most frequently had positive titres to was Hardjo (18 (95% C.I: 10-30) %) followed by Copenhageni (14 (95% C.I: 8-24) %). Positive titres to Copenhageni were associated with high livestock prevalence together with the presence of horses on the same farm. While positive MAT titres were associated with urinary shedding, this study also demonstrated the presence of urinary shedding in apparently healthy dogs with negative MAT titres. The prevalence of positive MAT titres of $\geq$100 in South Island dogs to serovar Hardjo was 1 (95% C.I: 0-2)%, lower than that previously reported for North Island dogs. No titres $\geq$100 were identified to serovars Copenhageni or Pomona. The hypothesis that the prevalence of titres $\geq$100 to serovars Hardjo, Copenhageni and Pomona in South Island dogs is similar to that reported in New Zealand dogs in 2012 is rejected. The most prevalent serovar identified on serology (titres $\geq$25) was serovar Hardjo with a prevalence of 18 (95% C.I: 10-30)%, however the prevalence of Copenhageni titres was only slightly lower at 14 (95% C.I: 8-24)%. The hypothesis that the most prevalent serovar farm working dogs are most commonly exposed to is Hardjo is accepted. The prevalence of strong positive urine PCR tests was 12 (95% C.I: 5-24) %. The hypothesis that the prevalence of positive urine PCR tests in dogs would be $\geq$ 5% is accepted. Dogs with positive urine PCR and negative serology were identified. The hypothesis that positive urine PCR can occur with negative serology is accepted.

Establishment of the prevalence and duration of urinary shedding in dogs nationwide is required. In addition, identification of the serovar (s) that are shed in canine urine in New Zealand is necessary to provide complete epidemiological data. Further work is also needed to confirm any significant association between a high prevalence in livestock and the serological status of dogs on the same farm.

### 4.7 Acknowledgements

The author acknowledges the technical advice and diagnostic testing provided by NZVP, mEpiLab and the Epicentre. Thanks to Juan Sanhueza for collaborating with sampling and to all veterinarian and technical staff involved with sample collection. Funding for the study was generously provided by the Wairarapa Vet Association, the Companion Animal Society Companion Animal Council, the Massey University Research Fund, the LA Alexander Trust, the Centre for Service and Working Dog Health and the Phyllis Irene Grey Trust.

# Chapter 5
# Conclusion

## 5 Conclusion

Leptospirosis is a disease of significance worldwide and in New Zealand, affecting dogs, livestock, and humans. Leptospires are a cause of both severe clinical disease, and subclinical infection either of which contribute to shedding of leptospires in urine and the persistence of leptospires in the environment. In New Zealand the epidemiology of leptospirosis is significantly different to the epidemiology in other countries.

The nationwide seroprevalence survey in this work has confirmed a significant prevalence of positive titres to serovar Copenhageni in dogs throughout New Zealand, in both the North and South Islands, although it remains unclear whether the positive titres are related to natural exposure or, in some cases, from vaccination. Exposure to serovar Copenhageni is common for dogs in New Zealand, and the risk of dogs being exposed to serovar Copenhageni, and the requirement for vaccination against serovar Copenhageni, cannot be determined by geographical location of a dog or the breed group. In this study, small breeds did not have a lower risk of seropositivity to Copenhageni than farm working breeds, but farm working dogs appeared to be at increased risk for exposure to serovar Hardjo.

Serovar Pomona causes severe clinical disease in both pet and working farm dogs in New Zealand. Dogs of breeds typically used as farm working dogs are at an increased risk for exposure to serovar Hardjo, although whether or not this serovar causes significant clinical disease in farm dogs, or if infection results in prolonged urinary shedding after exposure, is yet to be determined. There is a need for a protective vaccine against infection with Pomona to be available for dogs deemed to be at risk of infection with this serovar, and consideration should be given to including serovar Hardjo in this vaccine if infection by this serovar can be demonstrated to cause clinical disease or urinary shedding of leptospires. WSAVA vaccination guidelines state that leptospirosis vaccines are non-core vaccines, and as such should only be used where the increased risk of exposure in a given region is known, or where a dog's lifestyle places it at increased risk (M. J. Day, Horzinek, & Schultz, 2007). Veterinarians therefore need to balance the risk the of adverse vaccine reactions (which appear to be less frequent with modern leptospiral vaccines (Lunn, 2015)) with the regional prevalence of serovars and risk assessment for the individual dog on a case by case basis.

Acute, subclinical and chronic leptospirosis in New Zealand dogs are likely to be significantly underdiagnosed. Veterinarians investigating clinical cases need to maintain a high index of suspicion for leptospirosis both for classical presentations and for less commonly seen syndromes, and should consider MAT testing for Pomona and Hardjo in addition to Copenhageni for cases where the clinicopathological picture is consistent with leptospirosis. Concurrent IgM ELISA serology and PCR testing on blood and/or urine may expedite a diagnosis and reduce the number of undiagnosed cases; therefore the use of multiple diagnostic tools in suspected cases is recommended. Undertaking convalescent serology and confirmation of the infecting serovar by urine culture and serotyping is strongly encouraged.

There is an association between the presence of horses on farms that have high livestock sero-prevalence, and positive MAT titres in farm dog on the same properties. Horses are considered unlikely to be a direct source of infection, and are more likely a proxy for other on farm activities that contribute to increased exposure to the dogs, although it is unclear what that associated activity might be; further studies on the role of horses in maintaining or shedding of leptospires would clarify their impact on leptospiral epidemiology further. A clear association between other farm risk factors or high livestock sero-prevalence and positive serology or urine PCR in farm dogs could not be clearly demonstrated.

Significant urinary shedding has been demonstrated in New Zealand dogs, with more than 12 (95% C.I: 5-24) % of South Island dogs tested having a positive urine PCR for leptospiral DNA. Dogs shedding leptospires in their urine are likely to be contributing to the epidemiology and the maintenance of leptospirosis on farms. It is speculated that these dogs may be serving as maintenance hosts for serovars other than Canicola, and contributing to the maintenance of this disease in a farm environment and therefore they are a zoonotic risk. Urinary shedding of leptospires by dogs does occur with low or negative MAT titres. Although there is an association between positive serology and urinary shedding, serology cannot be used as a predictor of urinary shedding in any individual dog.

Dogs from the west coast of the South Island were under represented in the farm working dog study, and only a small number of urban South Island dogs were included in the sero-prevalence survey. Further work is needed to determine the prevalence and persistence of urine shedding by New Zealand dogs nationwide, especially dogs in the North Island. Culture and typing or sequencing identification of the infecting serovars is required to make firm vaccination or control recommendations. Further study should be undertaken to confirm the prevalence of positive titres to leptospirosis in farm dogs in other areas of New Zealand, and to confirm any significant association between the serological status of farm dogs with livestock sero-prevalence and on farm risk factors with larger numbers of dogs included in the study.

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### 6.1 Appendix 1 Abbreviations

| | |
|---|---|
| CI | Confidence Interval |
| Ct | Cycle Threshold |
| DNA | Deoxyribonucleic Acid |
| ELISA | Enzyme Linked Immunosorbent Assay |
| FAO | Food and Agriculture Organisation |
| GEE | Generalised Estimating Equations |
| LPS | Lipopolysaccharide |
| MAT | Microscopic Agglutination Test |
| NZVP | New Zealand Veterinary Pathology |
| OIE | World Organisation for Animal Health |
| OR | Odds Ratio |
| PCR | Polymerase Chain Reaction |
| Ref | Reference Value |
| RNA | Ribonucleic Acid |
| Spp. | Species |
| WHO | World Health Organisation |
| WSAVA | World Small Animal Veterinary Association |

## 6.2 Appendix 2 Age, sex, breed, region and MAT titres for leptospiral serovars Ballum, Copenhageni, Pomona and Hardjo in 2005 for canine sera from the North and South Islands of New Zealand in 2005.

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15567 | 12 | SBTX | 4 | Canterbury | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14453 | 10 | SBTX | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14519 | 6 | HUNT | 3 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14962 | NA | LAB | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 15117 | 9 | FOT | 2 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16239 | 10 | BOX | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16430 | 2 | JRT | 2 | Central Hawke's Bay District | 5 | 1 | 24 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 16734 | 6 | RHR | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16857 | 11 | RHR | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 17342 | 13 | MDOG | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 17407 | 11 | RHR | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 11603 | 7 | Udog | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 11664 | NA | gsh | 4 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12575 | 6 | Head | 3 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13437 | 9 | HEAD | 3 | Central Hawke's Bay District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 1 | 1 | F |
| 15312 | 14 | XBR | 4 | Central Otago District | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 15733 | 3 | WOR | 3 | Central Otago District | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | M |
| 15877 | 5 | BEA | 4 | Central Otago District | 3 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 24 | 0 | 0 | M |
| 12260 | 15 | blu | 4 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12656 | 12 | Bic | 1 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12793 | 5 | HUNT | 3 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 1 | 1 | F |
| 12890 | 11 | BIC | 1 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13277 | 12 | LAB | 4 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 14344 | 11 | FOTX | 2 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14634 | 13 | POO | 1 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 15065 | 9 | CHIH | 1 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15241 | NA | COLX | 4 | Christchurch City | 2 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 16700 | 9 | MALT | 1 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17135 | 14 | SPAN | 1 | Christchurch City | 2 | 0 | 0 | 0 | NA | 0 | 0 | 0 | NA | 0 | 0 | F |
| 17584 | 6 | FOT | 2 | Christchurch City | 2 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | M |
| 12012 | 6 | bic | 1 | Dunedin City | 3 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12021 | 10 | new | 4 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12423 | 14 | dal | 4 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12482 | 15 | FOTX | 2 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12721 | 4 | RotX | 4 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | MC |
| 13097 | 14 | GOL | 4 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13457 | 8 | COLX | 4 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 15634 | 3 | HEEL | 4 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17009 | 11 | KING | 1 | Dunedin City | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12973 | 7 | poo | 1 | Gore District | 4 | 0 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 14789 | 7 | POO | 1 | Gore District | 4 | 0 | 0 | 0 | 48 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 16735 | 7 | POO | 1 | Gore District | 4 | 0 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 15022 | 8 | HUNT | 3 | Hastings District | 5 | 1 | 0 | 0 | 0 | 0 | 48 | 0 | 48 | 0 | 0 | M |
| 15600 | 11 | AUC | 4 | Hastings District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15678 | 9 | POOT | 1 | Hastings District | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16118 | 7 | SBT | 4 | Hastings District | 5 | 1 | 48 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | M |
| 16337 | 3 | KING | 1 | Horowhenua District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13875 | 11 | JRTX | 2 | Horowhenua District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 11524 | 10 | mdog | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 48 | 0 | 24 | 0 | 0 | 0 | 0 | F |
| 11668 | 5 | gda | 4 | Kapiti Coast | 9 | 1 | 48 | 0 | 192 | 0 | 0 | 0 | 768 | 1 | 1 | MC |
| 12145 | 7 | mdog | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12287 | 10 | bult | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12297 | 8 | poom | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 12341 | 9 | box | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12453 | 15 | JRT | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12523 | 7 | Hunt | 3 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 12596 | 5 | LAB | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 24 | 0 | 1 | M |
| 12760 | 12 | LAB | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12774 | 10 | COS | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | NA | 0 | 0 | M |
| 12886 | 5 | SHT | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12938 | 11 | BEA | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12990 | 10 | Kee | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12991 | 11 | SCT | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13574 | 6 | DOB | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13590 | 6 | JRT | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13599 | 1 | AUT | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13691 | 6 | MINS | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13750 | 5 | LABX | 4 | Kapiti Coast | 9 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13804 | 6 | MINS | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | F |
| 13885 | 9 | BOX | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 13969 | 6 | Rot | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 14298 | 3 | BIC | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14299 | 0.75 | SAM | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14369 | 11 | BOX | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14407 | 10 | BEA | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14524 | 12 | COS | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14577 | 10 | GSH | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | F |
| 14820 | 10 | AME | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | FS |
| 14896 | 10 | AME | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14972 | 13 | AUC | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14974 | 10 | SHS | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15066 | 17 | FOTX | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15358 | 10 | BOC | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 768 | 1 | 1 | M |
| 15448 | 13 | XBR | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15528 | 10 | BCO | 4 | Kapiti Coast | 9 | 1 | 24 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15610 | 12 | LABX | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15636 | 11 | BLU | 4 | Kapiti Coast | 9 | 1 | 96 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 1 | F |
| 15768 | 11 | XBR | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15827 | NA | FOT | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15865 | 10 | SPR | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16094 | NA | SBT | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 16139 | 7 | GSH | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16335 | 14 | WHT | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16351 | 16 | FOTX | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16353 | 11 | BOCX | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16599 | 14 | UDOG | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 16665 | 13 | GOL | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 16686 | 10 | COSX | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16848 | NA | UDOG | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 16859 | 1.5 | MDOG | 4 | Kapiti Coast | 9 | 1 | 768 | 1 | 48 | 0 | 0 | 0 | 24 | 0 | 1 | F |
| 16860 | 1.5 | MDOG | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17104 | 6 | MPOO | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 17119 | 15 | XBR | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17122 | 13 | LAB | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17251 | 10 | MDOG | 4 | Kapiti Coast | 9 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17263 | 12 | GSH | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17359 | 13 | FOT | 2 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17381 | 4 | GSH | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17603 | 11 | CORGX | 1 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17630 | 10 | GOL | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17632 | 12 | LAB | 4 | Kapiti Coast | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11677 | 10 | lab | 4 | Lower Hutt City | 9 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12049 | NA | Udog | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12058 | 4 | sbtx | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12091 | 6 | dac | 4 | Lower Hutt City | 9 | 1 | 48 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12251 | 11 | king | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12557 | 12 | TER | 2 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12720 | 8 | BOX | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12736 | 13 | SPR | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12786 | 8 | GSH | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12848 | 9 | boc | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 768 | 1 | 1 | FS |
| 12850 | 13 | BIC | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12864 | 12 | LAB | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13092 | 9 | BOX | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13107 | 13 | LAB | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13147 | 12 | DAC | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13165 | 8 | CORG | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 13168 | 4 | LAB | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13522 | 11 | GOL | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 13525 | 12 | HUNT | 3 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13539 | 12 | PEK | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13609 | 13 | SBT | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | F |
| 13622 | 11 | CORG | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13746 | 0.33 | KING | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13752 | 10 | GOL | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14069 | 10 | JRT | 2 | Lower Hutt City | 9 | 1 | 24 | 0 | 0 | 0 | 24 | 0 | 24 | 0 | 0 | M |
| 14099 | 8 | SCR | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | NA | 0 | NA | 0 | 0 | 0 | 0 | U |
| 14150 | 15 | BOCX | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14233 | 6 | BOX | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14240 | 11 | LABX | 4 | Lower Hutt City | 9 | 1 | 48 | 0 | 96 | 1 | 24 | 0 | 0 | 0 | 1 | F |
| 14307 | 10 | BUL | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14310 | 8 | GOL | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14320 | 13 | GOL | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14325 | 7 | HUNT | 3 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14413 | 6 | FOT | 2 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14417 | 11 | MDOG | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14479 | 13 | SBT | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14788 | 15 | LABX | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14903 | 13 | LAB | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14917 | 11 | PUG | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14919 | 9 | SHT | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 16368 | 0.12 | LAB | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16525 | 1.5 | DOB | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16528 | 6 | BOX | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16692 | 11 | GOL | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | F |
| 16886 | 14 | GOL | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 16996 | 12 | CAI | 2 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17144 | 9 | BULM | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17305 | 9 | LHA | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17377 | 14 | WEI | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17379 | 10 | COS | 1 | Lower Hutt City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17650 | 7 | RETR | 4 | Lower Hutt City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 11569 | 10 | spr | 4 | Manawatu District | 6 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 11633 | 14 | mdog | 4 | Manawatu District | 6 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 11730 | 8 | blu | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 11731 | 14 | fot | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12026 | NA | ter | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12146 | 6 | bocx | 4 | Manawatu District | 6 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 12398 | 12 | gsh | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12466 | 3 | RETR | 4 | Manawatu District | 6 | 1 | 24 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 12525 | 6 | Hunt | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 384 | 1 | 1 | M |
| 12532 | 14 | Bic | 1 | Manawatu District | 6 | 1 | NA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12651 | NA | Hunt | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 12662 | 10 | Lab | 4 | Manawatu District | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 12716 | 3 | LABX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12731 | 1 | Fot | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12759 | 13 | FOXT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13058 | NA | Labx | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 13078 | 9 | BCO | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13112 | 6 | ALM | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13129 | 11 | UDOG | 4 | Manawatu District | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 13200 | 16 | FOT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13201 | 0.14 | FOT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13316 | 5 | CHIH | 1 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13322 | 3 | SHED | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 1 | 1 | F |
| 13385 | 11 | SBTX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13398 | 10 | BIC | 1 | Manawatu District | 6 | 1 | 96 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 1 | MC |
| 13407 | 6 | FOT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13446 | NA | DAL | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13493 | 1 | bulm | 4 | Manawatu District | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 48 | 0 | 1 | M |
| 13606 | 9 | LAB | 4 | Manawatu District | 6 | 1 | 0 | 0 | 48 | 0 | 24 | 0 | 0 | 0 | 0 | MC |
| 13631 | 9 | HUNT | 3 | Manawatu District | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 13728 | 3 | BOX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13790 | NA | UDOG | 4 | Manawatu District | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 13812 | 9 | UDOG | 4 | Manawatu District | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 13944 | 8 | RotX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13984 | 11 | HUNT | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 14114 | 12 | HUNT | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | FS |
| 14179 | 13 | RHRX | 4 | Manawatu District | 6 | 1 | 0 | 0 | NA | 0 | NA | 0 | 0 | 0 | 0 | FS |
| 14357 | 17 | FOT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14440 | 14 | GSH | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14518 | 9 | COS | 1 | Manawatu District | 6 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14569 | 11 | FOT | 2 | Manawatu District | 6 | 1 | 384 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | F |
| 14637 | 10 | HEAD | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 14659 | NA | BOC | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14721 | 7 | AFF | 1 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14881 | 4 | BIC | 1 | Manawatu District | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 14959 | 17 | FOT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | MC |
| 15191 | 1.3 | DOB | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15192 | 6 | BCO | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | F |
| 15494 | 6 | AUT | 2 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15512 | 2 | ROT | 4 | Manawatu District | 6 | 1 | 24 | 0 | 192 | 1 | 0 | 0 | 24 | 0 | 1 | F |
| 15524 | NA | UDOG | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 16034 | 6 | SPR | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16163 | 8 | POM | 1 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16432 | 3 | KING | 1 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16440 | 11 | LABX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16462 | 12 | OES | 1 | Manawatu District | 6 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 16515 | 13 | BULM | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16542 | 0.58 | BOX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16631 | 3 | SHED | 3 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 384 | 1 | 1 | F |
| 16714 | 7 | GOL | 4 | Manawatu District | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 16913 | 11 | UDOG | 4 | Manawatu District | 6 | 1 | 0 | 0 | NA | 0 | NA | 0 | NA | 0 | 0 | F |
| 17038 | 11 | BOX | 4 | Manawatu District | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17096 | 16 | CORG | 1 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | F |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17246 | 9 | LAB | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17268 | 6 | GSH | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17313 | 11 | LAB | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17323 | 12 | GSH | 4 | Manawatu District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17673 | 3 | MDOG | 1 | Manawatu District | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 11616 | 12 | lab | 4 | Marlborough District | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13318 | 10 | BOT | 4 | Marlborough District | 1 | 0 | 0 | 0 | 0 | 0 | 96 | 1 | 0 | 0 | 1 | M |
| 14329 | 1.42 | POI | 4 | Marlborough District | 1 | 0 | 0 | 0 | 96 | 1 | 0 | 0 | 24 | 0 | 1 | F |
| 12044 | 15 | fot | 2 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13257 | 13 | GSH | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13301 | 5 | PEK | 1 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13696 | 0.14 | LAB | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13727 | 11 | LAB | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13871 | 8 | WHT | 1 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14425 | 13 | LAB | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14446 | 5 | GDA | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14501 | 7 | FOT | 2 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15171 | NA | PUG | 1 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15173 | 12 | SHED | 3 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15747 | 1 | SPR | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15961 | 4 | BULTX | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16082 | 2 | GSH | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16481 | 3 | UDOG | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16658 | 4 | GOL | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17090 | 1 | LAB | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17260 | NA | LAB | 4 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17521 | 0.5 | POM | 1 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 17592 | 18 | POO | 1 | Masterton | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 17644 | 10 | BLU | 4 | Masterton | 8 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 17645 | 11 | POO | 1 | Masterton | 8 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16553 | 6 | NEW | 4 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16709 | 10 | XBR | 4 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 11551 | 9 | air | 1 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12124 | 10 | gre | 4 | Nelson City | 1 | 0 | 0 | 0 | 48 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 13914 | 8 | WOR | 3 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14928 | 9 | GSH | 4 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15404 | 13 | SCR | 1 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15482 | 7 | LABR | 4 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15939 | 4 | XBR | 4 | Nelson City | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | NA | 0 | 0 | M |
| 14239 | 6 | FOT | 2 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14362 | 8 | ROTX | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14418 | 10 | SBT | 4 | New Plymouth District | 7 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14486 | 15 | FOTX | 2 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15141 | 11 | RETR | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15304 | 10 | GOL | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16541 | 13 | LABX | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16909 | 14 | SIB | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 16910 | NA | LAB | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1536 | 1 | 1 | F |
| 17363 | 15 | SPR | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17504 | 9 | CHIH | 1 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 11689 | 14 | blu | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12150 | 12 | pug | 1 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12374 | 9 | labx | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12535 | 8 | Lab | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | MC |
| 12871 | 15 | CORG | 1 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13033 | NA | POI | 4 | New Plymouth District | 7 | 1 | 0 | 0 | NA | 0 | NA | 0 | 0 | 0 | 0 | U |
| 13039 | 7 | Chih | 1 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | FS |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13090 | 4 | LAB | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13372 | 11 | FOT | 2 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13382 | 9 | HEAD | 3 | New Plymouth District | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13387 | NA | HEAD | 3 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | U |
| 13527 | 11 | SHED | 3 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13621 | 9 | LAB | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | F |
| 13694 | 11 | DOB | 4 | New Plymouth District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 11568 | 12 | gol | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 192 | 1 | 1 | MC |
| 11570 | NA | shs | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 11604 | 14 | bocx | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 11717 | 14 | udog | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12047 | 13 | lab | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12141 | 4 | lab | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 12155 | 11 | shl | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12184 | NA | udog | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12208 | 11 | fot | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12225 | 10 | dac | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12268 | 11 | udog | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12270 | 14 | boc | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12334 | 2 | lab | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12337 | 11 | udog | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12433 | 13 | udog | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12470 | 10 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12517 | 9 | POM | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12519 | 9 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | MC |
| 12529 | 14 | Lab | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12594 | 14 | Bic | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12597 | 1.5 | PAP | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12619 | 16 | GSHX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12705 | 7 | LABX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | MC |
| 12818 | NA | HUNT | 3 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | U |
| 12818 | NA | HUNT | 3 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | U |
| 12822 | 9 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12898 | 13 | BOX | 4 | Palmerston North City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 12910 | 10 | SIB | 4 | Palmerston North City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12929 | 11 | FOT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12930 | 14 | GOL | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13000 | 11 | FOT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 13002 | 4 | GOL | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13252 | 6 | FOT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | F |
| 13475 | 10 | spr | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13480 | 10 | gol | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | FS |
| 13485 | 12 | gsh | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 384 | 1 | 0 | 0 | 1 | MC |
| 13506 | 15 | FOT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13569 | 4 | GSH | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 96 | 1 | 0 | 0 | 1 | FS |
| 13649 | 11 | BEA | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13718 | 12 | BOX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13777 | 9 | TER | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 24 | 0 | 1 | U |
| 13778 | 11 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13782 | 11 | BOC | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 13853 | 10 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13858 | 14 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13862 | 12 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14007 | 16 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14026 | 12 | LABX | 4 | Palmerston North City | 6 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14038 | 10 | jrt | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14120 | 13 | BIC | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | MC |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14126 | 13 | PEK | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14259 | 10 | AUT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14374 | 13 | SBT | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14375 | 4 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14429 | 5 | BOC | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14431 | 8 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14563 | 6 | CHO | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 14572 | 4 | POO | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14578 | 9 | BLU | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14595 | NA | WEI | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 14650 | 3 | GSH | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14716 | 14 | DAC | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14797 | 8 | CAI | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 14798 | 9 | BULTX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14800 | 6 | VIZ | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14949 | NA | ROT | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 24 | 0 | 1 | F |
| 15086 | 0.19 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15090 | NA | BOT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 15168 | 9 | MINS | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15179 | 6 | LHA | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15245 | 9 | CORG | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15248 | 5 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 15269 | 12 | BIC | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15280 | 15 | fot | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15334 | 10 | JRT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 15345 | 12 | COLX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 15502 | 8 | GOL | 4 | Palmerston North City | 6 | 1 | 24 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 15577 | NA | BOX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 15581 | 14 | FOT | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15819 | 14 | GOL | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15822 | 8 | KING | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15842 | 11 | SBT | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15948 | 10 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 15970 | 7 | BOX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 16035 | 8 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | FS |
| 16036 | 10 | MPOO | 1 | Palmerston North City | 6 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16042 | 9 | SPR | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16059 | 9 | BEA | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16164 | 10 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 48 | 0 | 0 | F |
| 16230 | 2 | CHIH | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 768 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 16308 | 7 | ROT | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | MC |
| 16413 | 9 | POO | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 16416 | 11 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 3072 | 1 | 0 | 0 | 1 | M |
| 16426 | 14 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16736 | 9 | BOX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16742 | 8 | FOTX | 2 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16829 | 15 | BLU | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16846 | 14 | BIC | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16940 | 7 | DOB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16949 | 2 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17027 | 12 | POO | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 384 | 1 | 1 | M |
| 17088 | 15 | COLX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17170 | 10 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17329 | 11 | UDOG | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | M |
| 17435 | 7 | ROTX | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17446 | 0.33 | LAB | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17449 | 13 | YOR | 1 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | MC |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17528 | 7 | GSP | 4 | Palmerston North City | 6 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16380 | 15 | XBR | 4 | Picton | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 15028 | 8 | CORG | 1 | Rangitikei District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16120 | 8 | HUNT | 3 | Rangitikei District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 16357 | 14 | DAC | 1 | South Taranaki District | 7 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16513 | 12 | ROT | 4 | South Taranaki District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | M |
| 16514 | 13 | FOTX | 2 | South Taranaki District | 7 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17124 | 9 | COL | 4 | South Taranaki District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15101 | 5 | BUL | 1 | South Taranaki District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15198 | 6 | KING | 1 | South Taranaki District | 7 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 14127 | 3 | UDOG | 4 | South Taranaki District | 7 | 1 | 0 | 0 | 48 | 0 | 384 | 1 | 48 | 0 | 1 | F |
| 14130 | 7 | SHED | 3 | South Taranaki District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | FS |
| 16312 | 6 | LABX | 4 | South Wairarapa | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14621 | 12 | JRT | 2 | Southland | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | F |
| 15424 | 7 | LAB | 4 | Southland | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 15735 | 1.5 | FOT | 2 | Southland | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16081 | 5 | MALT | 1 | Southland | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17307 | 17 | MALT | 1 | Southland | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14173 | 7 | BOX | 4 | Stratford District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 15305 | 1 | MALT | 1 | Stratford District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15461 | 13 | COS | 1 | Stratford District | 7 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | F |
| 15462 | 6 | BOC | 4 | Stratford District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15732 | 6 | SBT | 4 | Stratford District | 7 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 16001 | 11 | FOT | 2 | Stratford District | 7 | 1 | 0 | 0 | 0 | 0 | 96 | 0 | 192 | 1 | 1 | U |
| 16102 | NA | KING | 1 | Stratford District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 14075 | 9 | CORG | 1 | Stratford District | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15357 | 14 | BOC | 4 | Tararua District | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 384 | 1 | 1 | M |
| 15807 | 9 | LAB | 4 | Tararua District | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 11564 | 4 | pek | 1 | Tararua District | 8 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13131 | 13 | MDOG | 4 | Tararua District | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 384 | 1 | 1 | FS |
| 13567 | 5 | HEAD | 3 | Tararua District | 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 11557 | 10 | gol | 4 | Timaru | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15568 | 4 | SCR | 4 | Timaru | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16485 | 7 | UDOG | 4 | Timaru | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13949 | 11 | LABX | 4 | Wanganui District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14107 | 9 | LAB | 4 | Wanganui District | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | M |
| 15667 | 2 | GSP | 4 | Wanganui District | 6 | 1 | 384 | 1 | 0 | 0 | 0 | 0 | 384 | 1 | 1 | F |
| 15668 | 12 | ROT | 4 | Wanganui District | 6 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 16206 | 14 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | NA | 0 | 0 | F |
| 16271 | 0.33 | TERX | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16342 | 15 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16359 | 13 | SPAN | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16453 | 10 | CORG | 1 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 24 | 0 | 0 | 0 | 1 | F |
| 16455 | 6 | DOB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16456 | 7 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16457 | 5 | AUC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16458 | 13 | FOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16460 | 1 | JRTX | 2 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 192 | 1 | 1 | FS |
| 16488 | 17 | AUT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16547 | 9 | BOX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16561 | 10 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16629 | 16 | WHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 16633 | 13 | FOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16759 | 13 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16873 | 12 | GSHX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16876 | 17 | LABX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16921 | 3 | BCO | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 24 | 0 | 0 | M |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16930 | 6 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16952 | 11 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16964 | 0.58 | BUL | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16969 | 8 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17053 | 6 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17054 | 11 | BEA | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17098 | 10 | PEK | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 17101 | 2 | GSH | 4 | Wellington City | 9 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17128 | 10 | SPR | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17133 | 3 | TER | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17153 | 9 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17187 | 10 | ENS | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 24 | 0 | 0 | 0 | 1 | F |
| 17284 | 10 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17286 | 11 | XBR | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17371 | 14 | GEP | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17372 | 15 | BAS | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17408 | 14 | BOX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 17434 | 4 | MLM | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17455 | 4 | FOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17456 | 8 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17466 | 6 | ROT | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17470 | NA | BOX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17477 | 13 | SIB | 4 | Wellington City | 9 | 1 | 24 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 17480 | 6 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17481 | 7 | FOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17507 | 11 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 17525 | 13 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17526 | 9 | JRTX | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 17567 | 9 | CORG | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17568 | 16 | DOBX | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17571 | 6 | POOX | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 17661 | 2 | UDOG | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14129 | 12 | BOS | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14135 | 9 | BEA | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14138 | 13 | AUT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14154 | 9 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14155 | 11 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14200 | 13 | XBR | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | F |
| 14205 | 11 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14206 | 14 | FOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14212 | 9 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14217 | 7 | BOC | 4 | Wellington City | 9 | 1 | 48 | 0 | 192 | 1 | NA | 0 | 0 | 0 | 1 | M |
| 14223 | 7 | TERX | 2 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14314 | 5 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14315 | 9 | SCR | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14317 | 9 | SCR | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14365 | 13 | DOB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14377 | 8 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14378 | 6 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 14398 | 10 | POOT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14399 | 7 | POOT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14465 | 12 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14496 | 13 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 14587 | 8 | MPOO | 1 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 24 | 0 | 0 | F |
| 14591 | 11 | AKI | 1 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 14593 | 6 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14597 | 4 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14700 | 9 | POO | 1 | Wellington City | 9 | 1 | 24 | 0 | 1536 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 14701 | 11 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 1536 | 1 | 0 | 0 | 24 | 0 | 1 | M |
| 14734 | 7 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14740 | 13 | BULTX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14762 | 2 | GDA | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14764 | 7 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 14767 | 8 | SCR | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14774 | 2 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14807 | 11 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 14823 | 2 | POOX | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | NA | 0 | 0 | F |
| 14850 | 0.23 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14906 | 8 | NEW | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14908 | 10 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 14911 | 16 | TERX | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 14925 | 13 | COL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 14938 | 10 | JRT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14990 | 2 | LABX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15047 | 6 | KEL | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 15102 | 9 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15106 | 10 | BUL | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 15109 | 13 | SCT | 1 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15148 | 9 | WHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15186 | 11 | CCRET | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15209 | 11 | CHIH | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 15277 | 5 | LHA | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15278 | 8 | SHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15279 | 9 | bic | 1 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15281 | 8 | HUNT | 3 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15285 | 8 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15296 | 3 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15302 | 6 | BIC | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15366 | 2 | SHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15398 | 14 | COLX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15446 | 14 | SBTX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 15458 | 11 | HUNT | 3 | Wellington City | 9 | 1 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 15491 | 13 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 15496 | 6 | DOB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15515 | 8 | ROT | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15529 | 10 | BLU | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15536 | 11 | PIT | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 15584 | 11 | XBR | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 15654 | 12 | TPOO | 1 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 15687 | 12 | SIB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15692 | 9 | AUC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15706 | 9 | LABX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15757 | 12 | WHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | M |
| 15777 | 9 | LABX | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15785 | 3 | BOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15828 | 2 | COS | 1 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 15831 | 6 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15861 | 1 | BUL | 1 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 15862 | 13 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 15905 | 11 | XBR | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16018 | 10 | ROT | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16044 | 15 | SHL | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | M |
| 16050 | 2 | COS | 1 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 16069 | 3 | BLU | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16088 | 5 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16090 | 5 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16091 | 12 | POO | 1 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 16095 | 12 | LUR | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16098 | 11 | GSH | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16103 | 10 | SCT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 16113 | 10 | GOR | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16176 | 11 | JRT | 2 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 16186 | 11 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 16195 | 10 | CORG | 1 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 16203 | 11 | GRE | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 17389 | 9 | DOB | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 11503 | 11 | BOX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 11508 | 1.5 | BULM | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 11510 | 2 | lab | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 11556 | 0.75 | gol | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 11587 | 5 | lab | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 11592 | 12 | Udog | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 11595 | 7 | lab | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 11667 | 11 | boc | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 11671 | 10 | dac | 1 | Wellington City | 9 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 11698 | 7 | wei | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 11733 | NA | fotx | 2 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 24 | 0 | 0 | 0 | 0 | U |
| 11734 | 13 | dal | 4 | Wellington City | 9 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 11735 | 14 | bco | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12083 | 16 | dac | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12084 | 14 | sbtx | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12087 | 8 | lab | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | MC |
| 12216 | 17 | cos | 1 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12241 | 11 | boc | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12284 | NA | gshx | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12303 | 12 | box | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12306 | 7 | sbtx | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12307 | 5 | lab | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | MC |
| 12352 | 13 | jrt | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12356 | 9 | sht | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12357 | 7 | chih | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12360 | 0.92 | ter | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12362 | 13 | mdog | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | U |
| 12392 | 3 | ter | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12569 | 14 | FOT | 2 | Wellington City | 9 | 1 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 12618 | 15 | BOX | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 12682 | 10 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 12701 | 12 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 12805 | 15 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13027 | 15 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13043 | 7 | MDog | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 13075 | 6 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13211 | 12 | GOL | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13219 | 12 | BCO | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13242 | 13 | TER | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13250 | 8 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13333 | 10 | SCK | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13339 | 8 | MALT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13358 | 7 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13369 | 6 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | M |
| 13389 | 13 | WHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |

| Accession no. | Age_years | Breed code | Breed group | TA | Region | North Island | Ballum Titre | Ballum_pos | Copenhageni Titre | Cop_pos | Pomona Titre | Pom_pos | Hardjo Titre | Hard_pos | lepto_pos | Sex |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13390 | 11 | BOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 1 | MC |
| 13391 | 8 | MDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 24 | 0 | 0 | 0 | 1 | FS |
| 13404 | 13 | PEK | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13410 | 6 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13435 | 7 | boc | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13504 | 11 | SPR | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13505 | 12 | SPR | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | FS |
| 13509 | 7 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 13537 | 8 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13603 | 16 | CAI | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | MC |
| 13627 | 8 | FOT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 96 | 1 | 24 | 0 | 1 | MC |
| 13672 | 12 | SBT | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | FS |
| 13678 | 2 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 13706 | 2 | BOC | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13708 | 11 | NEW | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13734 | 1 | COS | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | MC |
| 13799 | NA | BOC | 4 | Wellington City | 9 | 1 | 24 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | FS |
| 13822 | 4 | UDOG | 4 | Wellington City | 9 | 1 | 0 | 0 | 192 | 1 | 48 | 0 | 48 | 0 | 1 | F |
| 13840 | 17 | DAC | 1 | Wellington City | 9 | 1 | 0 | 0 | 96 | 1 | 0 | 0 | 0 | 0 | 1 | F |
| 13884 | 10 | WHT | 1 | Wellington City | 9 | 1 | 0 | 0 | 384 | 1 | 0 | 0 | 24 | 0 | 1 | M |
| 13899 | 15 | SIB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13939 | 10 | JRT | 2 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13957 | 10 | SBTX | 4 | Wellington City | 9 | 1 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13959 | 3 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 13996 | 6 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |
| 13999 | 13 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14001 | 14 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | NA | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14006 | 12 | LAB | 4 | Wellington City | 9 | 1 | 0 | 0 | NA | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14046 | 14 | MPOO | 1 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | F |
| 14076 | 12 | SPR | 4 | Wellington City | 9 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | M |

### 6.3 Appendix 3 Frequency of positive and negative Microscopic Agglutination Test titres for canine sera from the North and South Islands of New Zealand for serovars Ballum, Copenhageni, Pomona and Hardjo in 2005.

| | Maximum dilution showing more than 50% agglutination: | <1:24 (negative) | 1:24 | 1:48 | 1:96 | 1:192 | 1:384 | 1:768 | 1:1536 | 1:3072 | Total (all dilutions) | Total usable sera | Unusable |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *ballum* | North Is | 570 | 16 | 13 | 2 | 0 | 2 | 1 | 0 | 0 | 34 | 603 | 1 |
| | South Is | 50 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 51 | 0 |
| totals | | 620 | 16 | 14 | 2 | 0 | 2 | 1 | 0 | 0 | 35 | 654 | 1 |
| | | | | | | | | | | | | | |
| *copenhageni* | North Is | 459 | 30 | 45 | 27 | 26 | 8 | 1 | 2 | 0 | 139 | 598 | 6 |
| | South Is | 44 | 1 | 2 | 3 | 0 | 0 | 0 | 0 | 0 | 6 | 50 | 1 |
| totals | | 503 | 31 | 47 | 30 | 26 | 8 | 1 | 2 | 0 | 145 | 648 | 7 |
| | | | | | | | | | | | | | |
| *pomona* | North Is | 569 | 21 | 3 | 3 | 0 | 2 | 0 | 0 | 1 | 30 | 599 | 5 |
| | South Is | 48 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 51 | 0 |
| totals | | 617 | 23 | 3 | 4 | 0 | 2 | 0 | 0 | 1 | 33 | 650 | 5 |
| | | | | | | | | | | | | | |
| *hardjo* | North Is | 534 | 30 | 15 | 2 | 9 | 6 | 3 | 1 | 0 | 66 | 600 | 4 |
| | South Is | 40 | 6 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 9 | 49 | 2 |
| totals | | 574 | 36 | 16 | 3 | 10 | 6 | 3 | 1 | 0 | 75 | 649 | 6 |

## 6.4 Appendix 4 **Survey form completed at serum and urine sample collection from South Island farm dogs in 2013.**

**Canine Leptospirosis Survey** **Property identifier #........**

**Property owner name:** **Date:**

**Address:**

**Sampling veterinarian/clinic:**

**Are the sample dogs mainly:** **Urban** ☐ ☐ **Rural** ☐

**If rural, what farm type:** **Dairy** ☐ **Sheep/Beef** ☐ **Other** ☐

*Please specify:*

**Do these dogs have potential contact with the following;** **Horses** ☐

**Rats** ☐ **Mice** ☐ **Hedgehogs** ☐ **Pigs** ☐

**Sheep** ☐ **Cattle** ☐ **Deer** ☐ **Natural waterways** ☐

**Inclusion criteria;** Dogs must be over 3 months of age, and normally resident on this property with owner consent to be included in this survey. A maximum of 8 dogs to be sampled on each property; if more than 8 eligible dogs are resident on the property, the dogs are to be listed in alphabetical order by name, and the first 8 dogs in the list sampled.

**Dog owner/agent signature(s).......................................................................................................**

DOG #1 Name..................... Owner............................

Age.............. Breed...................................... Sex...................

**Housing:** **Run/tethered** ☐ **Fenced** ☐ **Free-roaming** ☐ **Indoors only** ☐

**Has this dog been unwell in the last 12 months??** YES ☐ NO ☐

**Please record history of previous illness on back of this sheet.**

**Has this dog been vaccinated against Leptospirosis?** YES ☐ NO ☐

*(Please include any off label administration of livestock vaccines)*

**Vaccination date and type:……………………………**

DOG #2 Name…………………. Owner……………………….

Age………….. Breed………………………………. Sex……………….

**Housing:** **Run/tethered** ☐ **Fenced** ☐ **Free-roaming** ☐ **Indoors only**

**Has this dog been unwell in the last 12 months??** YES ☐ NO ☐

**Please record history of previous illness on back of this sheet.**

**Has this dog been vaccinated against Leptospirosis?** YES ☐ NO ☐

*(Please include any off label administration of livestock vaccines)*

**Vaccination date and type:……………………………**

DOG #3 Name…………………. Owner……………………….

Age………….. Breed………………………………. Sex……………….

**Housing:** **Run/tethered** ☐ **Fenced** ☐ **Free-roaming** ☐ **Indoors only**

**Has this dog been unwell in the last 12 months??** YES ☐ NO ☐

**Please record history of previous illness on back of this sheet.**

**Has this dog been vaccinated against Leptospirosis?** YES ☐ NO ☐

*(Please include any off label administration of livestock vaccines)*

**Vaccination date and type:……………………………**

Appendix 5 Age, sex, MAT titres to leptospiral serovars Pomona, Hardjo and Copenhageni and Urine PCR results for South Island farm dogs sampled in 2013.

| Farm | Dog name | Dog ID | POM titre | HAR titre | COP titre | PCR result[8] | Stock Prev[9] | sex.m1 | age.y | cattle | sheep | pig | deer | vermin | water | horse |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inv | Bill | Inv 1 | Neg | Neg | Neg | 0 | 1 | 1 | 1.5 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| Inv | Kate | Inv 2 | Neg | Neg | Neg | 0 | 1 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| Inv | Tweed | Inv 3 | Neg | Neg | Neg | 0 | 1 | 1 | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| Inv | Lee | Inv 4 | Neg | Neg | Neg | 0 | 1 | 0 | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| Inv | Tike | Inv 5 | Neg | Neg | Neg | 1 | 1 | 1 | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| Inv | Finn | Inv 6 | Neg | Neg | Neg | 0 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| Mos | Flash | Mos 1 | Neg | Neg | Neg | 0 | 1 | 0 | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ16 | Boff | aj16 1 | 25 | 25 | Neg | 2 | 1 | 1 | 6 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ16 | Black | aj16 2 | Neg | 25 | Neg | 0 | 1 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ16 | Clover | aj16 3 | Neg | 25 | Neg | 0 | 1 | 0 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ16 | Jeff | aj16 4 | Neg | 25 | Neg | 1 | 1 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ16 | Todd | aj16 4 | Neg | 25 | Neg |  | 1 | 1 | 12 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ15 | Sid | aj15 1 | Neg | Neg | 25 | 0 | 0 |  | 6 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ15 | Meg | aj15 2 | Neg | Neg | 25 | 0 | 0 |  | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ15 | Tui | aj15 3 | Neg | Neg | Neg | 0 | 0 |  | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ25 | Clare | aj25 1 | Neg | 25 | Neg | 0 | 0 | 0 | 8 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| AJ25 | Jane | aj25 2 | Neg | Neg | 25 |  | 0 |  | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| AJ25 | Rhum | aj25 3 | Neg | 25 | Neg | 0 | 0 |  | 9 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| AJ27 | Blaze | aj27 1 | Neg | Neg | Neg | 2 | 0 | 1 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ27 | Brew | aj27 2 | Neg | Neg | Neg | 0 | 0 | 1 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ27 | Daisy | aj27 3 | Neg | Neg | Neg | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ27 | Meg | aj27 4 | Neg | Neg | Neg | 0 | 0 | 0 | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ27 | Merk | aj27 4 | Neg | Neg | Neg | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ27 | Toy | aj27 4 | Neg | Neg | Neg | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ14 | Blackie | aj14 1 | Neg | Neg | Neg | 0 | 0 | 1 | 5 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Boss | aj14 2 | Neg | Neg | Neg | 0 | 0 | 1 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Bourbon | aj14 3 | Neg | Neg | Neg | 0 | 0 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Briar | aj14 4 | Neg | Neg | Neg |  | 0 | 0 | 6 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Dee | aj14 5 | Neg | Neg | Neg |  | 0 | 0 |  | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Jess | aj14 6 | Neg | Neg | Neg |  | 0 | 0 |  | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Spotty | aj14 7 | Neg | Neg | Neg |  | 0 |  |  | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ14 | Tan Sally | aj14 8 | Neg | 200 | Neg |  | 0 | 0 |  | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ22 | Blue | aj22 1 | Neg | Neg | Neg |  | 0 | 1 | 6 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ22 | Wag | aj22 2 | Neg | Neg | Neg | 0 | 0 | 1 | 12 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ20 | Barley | aj20 1 | Neg | Neg | 25 | 0 | 1 | 0 | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ20 | Jack | aj20 2 | Neg | Neg | Neg | 1 | 1 | 1 | 8.5 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ20 | Shrek | aj20 3 | Neg | Neg | Neg | 0 | 1 | 1 | 9 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ20 | Bella | aj20 4 | Neg | Neg | Neg | 0 | 1 | 0 | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ20 | Bricks | aj20 5 | Neg | Neg | Neg | 0 | 1 | 0 | 0.9 | 1 | 1 | 1 | 1 | 1 | 1 |  |
| AJ31 | Boy | aj31 1 | Neg | Neg | Neg | 0 | 1 | 1 | 1.5 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ31 | Buss | aj31 2 | Neg | Neg | Neg | 1 | 1 | 1 | 8 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ31 | Jock | aj31 3 | 25 | Neg | Neg | 0 | 1 | 1 | 8 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ31 | Tess | aj31 4 | Neg | Neg | Neg | 1 | 1 | 0 | 8 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ31 | Rag | aj31 5 | 25 | Neg | Neg | 0 | 1 | 0 | 14 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ19 | Lacy | aj19 1 | Neg | Neg | Neg |  | 0 | 0 | 3 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ19 | Jess | aj19 2 | Neg | Neg | Neg |  | 0 | 0 | 5 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ19 | Tip | aj19 3 | Neg | Neg | Neg |  | 0 | 0 | 13 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ3 | Lou | aj3 1 | Neg | 25 | Neg |  | 0 | 0 | 8 | 1 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ3 | Pip | aj3 2 | Neg | Neg | Neg |  | 0 | 0 | 1.5 | 1 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ9 | Boss | aj9 1 | Neg | Neg | Neg | 0 | 0 | 1 | 12 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ9 | Glen | aj9 2 | Neg | Neg | Neg | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ9 | Walt | aj9 3 | Neg | Neg | Neg | 0 | 0 | 1 | 10 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ9 | Mac | aj9 4 | Neg | Neg | Neg | 0 | 0 | 1 | 5 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ9 | Jimmy | aj9 5 | Neg | Neg | Neg |  | 0 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ43 | Kate | aj43 1 |  |  |  | 0 | 0 | 0 | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ43 | Dee | aj44 2 | Neg | Neg | Neg |  | 0 | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ43 | Nell | aj45 3 | Neg | Neg | Neg |  | 0 | 0 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Alby | aj29 1 | Neg | Neg | Neg | 0 | 0 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Foz | aj29 2 | 25 | Neg | Neg | 0 | 0 | 0 | 10 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Smoke | aj29 3 | Neg | Neg | Neg | 0 | 0 | 1 | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Mot | aj29 4 | Neg | Neg | Neg | 0 | 0 | 1 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Mac | aj29 5 | Neg | Neg | Neg | 0 | 0 | 1 | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Lola | aj29 6 | Neg | Neg | Neg | 0 | 0 | 0 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ29 | Rad | aj29 7 | Neg | Neg | Neg | 0 | 0 | 1 | 11 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ6 | Drum | aj6 1 | Neg | Neg | Neg | 0 | 0 | 1 | 6 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ6 | Pard | aj6 2 | Neg | Neg | Neg | 0 | 0 | 1 | 10 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ7 | Chad | aj7 1 | Neg | Neg | Neg | 0 | 0 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Ben | aj7 2 | Neg | Neg | Neg | 0 | 0 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Sweep | aj7 3 | Neg | Neg | Neg | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Flo | aj7 4 | Neg | Neg | Neg | 0 | 0 | 0 | 1.4 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Hitch | aj7 5 | Neg | Neg | Neg | 2 | 0 | 1 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Mac | aj7 6 | Neg | Neg | Neg | 0 | 0 | 1 | 5 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Quid | aj7 7 | Neg | Neg | Neg | 0 | 0 | 1 | 8 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ7 | Starch | aj7 8 | Neg | Neg | Neg | 0 | 0 | 0 | 3 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| AJ5 | Jess | aj5 1 | Neg | Neg | 25 |  | 0 | 0 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ5 | Panda | aj5 2 | Neg | Neg | 25 |  | 0 | 1 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ5 | Jake | aj5 3 | Neg | Neg | Neg |  | 0 | 1 | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ5 | Bella | aj5 4 | Neg | Neg | Neg |  | 0 | 0 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ5 | Spot | aj5 5 | Neg | Neg | Neg |  | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AJ30 | NO ID | aj30 1 | Neg | Neg | Neg | 0 | 0 |  | 4 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| AJ30 | Ted | aj30 2 | Neg | Neg | Neg | 0 | 0 |  | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| AJ30 | Jess | aj30 3 | Neg | Neg | Neg | 0 | 0 |  | 8 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| AJ30 | Beck | aj30 4 | Neg | Neg | Neg | 0 | 0 |  | 5 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| AJ26 | Bruce | aj26 1 | Neg | Neg | Neg | 0 | 1 | 1 | 10 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ26 | Colombo | aj26 2 | Neg | Neg | Neg | 0 | 1 | 1 | 9 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ26 | Flick | aj26 3 | Neg | Neg | Neg |  | 1 | 0 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ13 | Ellie | aj13 1 | Neg | Neg | Neg |  |  | 0 | 8 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |

[8] PCR Result: 0=Negative 1=Weak Positive 2=Strong Positive
[9] Stock Seroprevalence: 0=Low 1=High

| AJ13 | Jaz | aj13 2 | Neg | Neg | Neg | 2 | | 0 | 11 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AJ13 | Mac | aj13 3 | Neg | Neg | Neg | 0 | | 1 | 6 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| **Farm** | **Dog name** | **Dog ID** | **POM titre** | **HAR titre** | **COP titre** | **PCR result[10]** | **Stock Prev[11]** | **sex.m1** | **age.y** | **cattle** | **sheep** | **pig** | **deer** | **vermin** | **water** | **horse** |
| AJ13 | Misty | aj13 4 | Neg | Neg | Neg | 0 | | 0 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ13 | Mitch | aj13 4 | Neg | Neg | Neg | 0 | | 0 | 10 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ1 | Jed | aj1 1 | Neg | Neg | Neg | | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ1 | Max | aj1 2 | Neg | Neg | Neg | | 1 | 1 | 8 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ17 | Dog1 | aj17 1 | Neg | Neg | Neg | | 0 | 1 | 8 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ17 | Dog2 | aj17 2 | Neg | Neg | Neg | | 0 | 0 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ17 | Dog3 Milk | aj17 3 | Neg | Neg | Neg | | 0 | 1 | 3 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ17 | Dog4 | aj17 4 | Neg | Neg | Neg | | 0 | 0 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ12 | Jae | aj12 1 | Neg | Neg | Neg | 0 | 0 | 1 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ12 | Katie | aj12 2 | Neg | Neg | Neg | 0 | 0 | 0 | 10 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ12 | Kim | aj12 3 | Neg | 50 | Neg | | 0 | 1 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ11 | Diesel | aj11 1 | Neg | Neg | Neg | 0 | 0 | 1 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ11 | Fog | aj11 2 | Neg | Neg | Neg | 0 | 0 | 1 | 5 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ11 | Jess | aj11 3 | Neg | Neg | Neg | | 0 | 0 | 4 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ11 | Meg | aj11 4 | Neg | Neg | Neg | | 0 | 0 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ11 | Nig | aj11 5 | Neg | Neg | Neg | 0 | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 0 |
| AJ28 | Soul | aj28 1 | Neg | 25 | 50 | 0 | 1 | 0 | 6 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| AJ28 | Barb | aj28 2 | Neg | Neg | 25 | 2 | 1 | 0 | 2 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| AJ28 | Meg | aj28 3 | Neg | 25 | 50 | 0 | 1 | 0 | 10 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| AJ45 | Rob | aj45 1 | Neg | 25 | 25 | 2 | 0 | 1 | 14 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ45 | Jack | aj45 2 | Neg | 25 | Neg | 2 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ45 | Spot | aj45 3 | Neg | Neg | Neg | 2 | 0 | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ45 | Chuck | aj45 4 | Neg | 25 | 25 | 2 | 0 | 1 | 4 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| AJ21 | Belle | aj21 1 | Neg | 50 | 25 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ21 | Romany | aj21 2 | Neg | 50 | 25 | 0 | 1 | 1 | 5 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ21 | Meg | aj21 3 | Neg | 50 | 25 | 0 | 1 | 0 | 12 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ21 | Spy | aj21 4 | Neg | 50 | 25 | 0 | 1 | 0 | 10 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ21 | Sway | aj21 5 | Neg | 50 | 25 | 0 | 1 | 0 | 7 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ32 | archie | aj32 1 | neg | neg | neg | 2 | 1 | 1 | 6 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ33 | dusty | aj32 2 | neg | neg | neg | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ34 | lady | aj32 3 | neg | neg | neg | 0 | 1 | 0 | 12 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ35 | molly | aj32 4 | neg | neg | neg | 0 | 1 | 0 | 2.5 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ36 | tui | aj32 5 | neg | neg | neg | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ37 | nettle | aj32 6 | neg | neg | neg | 0 | 1 | 0 | 10 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| AJ35 | lady | aj35 1 | 25 | 25 | 25 | | 1 | 0 | | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ35 | spot | aj35 2 | neg | 50 | 0 | 0 | 1 | 0 | | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ35 | lucky | aj35 3 | neg | 25 | 0 | 0 | 1 | 0 | | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ35 | billy | aj35 4 | neg | 25 | 50 | | 1 | 0 | | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ35 | chase | aj35 5 | neg | 25 | 25 | 2 | 1 | 0 | | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| AJ35 | sky | aj35 6 | neg | 25 | 0 | | 1 | 0 | | 1 | 1 | 0 | 1 | 1 | 1 | 1 |

[10] PCR Result: 0=Negative 1=Weak Positive 2=Strong Positive
[11] Stock Seroprevalence: 0=Low 1=High