Leptospirosis in dairy herds

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Abstract

The introduction and adoption of *Leptospira* vaccination in most New Zealand dairy herds in the 1980s was associated with a substantial reduction of the incidence of notified human leptospirosis cases in the population and notably among dairy farm workers. However, 80% of cases notified from 1999 to 2016 with a "farmer-type" occupation were dairy farmers, hence this occupational group continues to be at risk for leptospirosis.

Failure to vaccinate dairy herds can have serious public health consequences. An example is described in an opportunistic case study, chapter 3 of this thesis. Within the space of three months in early 2015, three workers from a dairy farm with an unvaccinated dairy herd were hospitalised with leptospirosis caused by serovars Hardjo and Pomona. In young and adult dairy cattle from this farm, Hardjo, Pomona, Copenhageni, Ballum and Tarassovi serovars were all detected serologically. While two of the diseased workers recovered, one remains affected four years after the event being unable to return to work.

These circumstances – the continuation of dairy workers among notified human cases and the potentially serious consequences from failure to vaccinate or to achieve effective immunity – have raised concerns about the effectiveness of the long–term vaccination programme in dairy herds. The concerns were further substantiated by an opportunistic pilot study (2011) that found evidence of *Leptospira* shedding in vaccinated dairy cattle.

Therefore, a large cross-sectional study of New Zealand dairy farms was conducted involving 200 dairy farms and 4,000 cows. Farms were randomly selected from the national database and blood and urine was collected from 20 cows per herd. Non-response (30%) was investigated by personal interview which indicated that selection bias (e.g. by selecting only farmers with vaccinated herds) was minimal, if not absent. Shedding was indicated by a positive qPCR at cow-level and by one or more shedders

per herd at herd-level. A serological response was considered positive when titres of the microscopic agglutination test (MAT) were at or above 48.

Overall shedding rates were 2.4% at cow- and 26.5% at herd-level. Seropositivity to Hardjo, Pomona and, when trivalent vaccines were used, Copenhageni, was most likely a response to vaccination. None of the vaccinal serovars were associated with urine shedding. However, there was a strong linear association at the cow-level between increasing MAT titres to Tarassovi and the likelihood of shedding. Serological evidence for exposure to Tarassovi was observed in 17% of cows and 74% of the herds. Few cows (1%) and 16% herds were sero-positive to Copenhageni when not vaccinated against this serovar which, however, was not associated with cow-level shedding. Similarly, the rodent-related serovar Ballum was not associated with shedding; with positive titres observed in 3% of cows and 38% of herds.

Studies in the 1970s and 1980s found little serological evidence of Tarassovi, so we conclude that this serovar has emerged, became endemic and is now probably causing most of the shedding in the dairy cattle population. Considering published evidence that a large proportion of notified cases in dairy farmers were Tarassovi, there is strong corroborative evidence that this serovar poses a public health risk for workers on dairy farms.

Our survey administered a questionnaire about vaccination practices and putative risk factors. All but one of the farmers had regular vaccination programmes for calves, heifers and cows using mostly bivalent (80%, 69%, 68% of farms, respectively) and some trivalent vaccines (20%, 31%, 32% of farms, respectively). Regardless of the almost universal practice of *Leptospira* vaccination in dairy cattle, fewer than 40% famers conformed with *Best Practice Guidelines* (2012) developed and propagated by the New Zealand Veterinary Association.

A further objective was a risk factor analysis (Chapter 6). One cow-level (age) and three herd-level (presence of sheep or dogs, herd size) factors were significantly associated with the risk of shedding. As 93% of the potential factors evaluated were at herd level,

and with only 200 herds included in the study, and the shedding rate being relatively low, the statistical power might have been too low to identify other herd-level determinants related to the management and environment of the farms. Nevertheless, a linear negative effect of age suggested that young cows were more likely to shed *Leptospira* than adult cows, and therefore increase the risk of infection for dairy workers.

Evidence from this thesis suggests that current *Leptospira* vaccination practices are effective for preventing the exposure of farm workers against the serovars most commonly incorporated in vaccines (Hardjo and Pomona), and the less common serovar Copenhageni. Thus, continuation with vaccination is supported. The public health risk arising from Tarassovi that has emerged, and evidence here that this serovar is widely present in the dairy cattle population, justifies raised awareness, the adoption of protection measures additional to vaccination, further research into the epidemiology of Tarassovi and an evaluation of the justification for its inclusion into vaccines. Dairy workers are advised to take extra care and precautions when milking and handling cows, especially first calving heifers irrespective of their vaccination status.

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"Whenever you are alone, remind yourself that the Almighty has sent everybody away so that it's only you and Him"-Mufti Menk. Hasbunallah wa ni'mal wakil.....(Allah is sufficient for us).

Declaration

This thesis was formatted as five independent research chapters suitable for journal publication. Therefore, concepts and/or methodology described in a chapter may be repeated in another chapter. My input as main author of this research was to plan and coordinate sampling of cows blood and urine, develop questionnaires to record relevant information, process samples in the laboratory and test them for antibodies and DNA against *Leptospira*, maintain the database, conduct systematic literature search, analyse data, and draft manuscripts reporting findings. People that contributed substantially to the research were made co-authors as listed at the beginning of each chapter.

List of abbreviations

B: *Leptospira borgpetersenii* sv. Ballum

C: *Leptospira interrogans* sv. Copenhageni

H: *Leptospira borgpetersenii* sv. Hardjo

H1 and H2: Herds 1 and 2

R1 and R2: Rising one-year-old and rising two-year-old, respectively

MAT: Microscopic agglutination test

P: *Leptospira interrogans* sv. Pomona

qPCR: Quantitative Polymerase Chain Reaction

T: *Leptospira borgpetersenii* sv. Tarassovi

List of publications

Benschop, J., Collins-Emerson, J., Maskill, A., O'Connor, P., Tunbridge, M., Yupiana, Y., & Weston, J. (2017). Leptospirosis in three workers on a dairy farm with unvaccinated cattle. *The New Zealand medical journal, 130*(1462), 102.

Heuer, C., Yupiana, Y., Collins-Emerson, J., Benschop, J., Weston, J., Wilkinson, D.,Valee, E., Wilson, P. (2017). *Leptospira* shedding in dairy herds: new findings. *Vetscript, 30,* 44-46.

Weston, J., Yupiana, Y., Benschop, J., Heuer, C., & Collins-Emerson, J. (2017). Preliminary Findings from the Nationwide Survey of Dairy Cattle in NZ Investigating Leptospirosis Vaccination Practices and Efficacy. *NZVA Dairy Cattle Vets Newsletter, 34,* 15-16.

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Chapter 1
Introduction

This general introduction describes brief reviews of production effects (milk production, abortion and other reproduction performance) of *Leptospira* infection and leptospirosis control in dairy cattle. The aims and structure of this thesis are also presented here.

1.1. Production effects of *Leptospira* infection in dairy cattle

Production effects of *Leptospira* infection in dairy cattle have been studied for many years in many countries. The effects could manifest as consequences of two different forms: clinical and subclinical infections. While the effects of clinical infections in cattle production such as abortions, and mastitis, associated with cessation of lactation and/or flaccid udders, were frequently reported, other effects such as perinatal death, premature birth and neonatal disease were rarely detected (Emanuel *et al*., 1964; Giles *et al.*, 1983; Ellis *et al.*, 1985). Few research reports were available on the impact of subclinical *Leptospira* infection on productivity. They focussed on reduced conception rates (Dhaliwal *et al.*, 1996b) and reduction in herd milk yield (Dhaliwal *et al.*, 1996a).

Abortion can occur from early pregnancy (0-3 months) (Elder *et al.*, 1985), 4 months of gestation (Te Brugge & Dreyer, 1985) or 6 months onwards (Ellis *et al.*, 1985; Hamali *et al.*, 2012). It has been suggested that infection with Pomona was associated with abortion during the last trimester (Howard, 1993). In contrast, a previous study showed abortion could occur at three months gestation (Elder *et al.*, 1985). While one study described *Leptospira* abortions in 2-3 year old heifers (Carter *et al.*, 1982), another one postulated that it was more prevalent in older cows (Hamali *et al.*, 2012). Different findings from these two reports might be related to the different level of exposure during pregnancy. Carter et al. (1982) showed that young animals had access

to effluent in a creek from a piggery up-stream from the farm and were hence exposed to *Leptospira*. Hamali et al. (2012) argued that biologically, older cows have greater chance of being affected by leptospirosis than heifers during their productive life. Two major serovars from the same species (*Leptospira interrogans)* were most frequently found as the infecting serovars (Pomona and Hardjo) in aborting dairy cattle. More rarely, other serovars were also regarded as potential causes for abortion. They included *L. interrogans* serovar Wolffi in Brazil (Langoni *et al.*, 1999), *L. interrogans* serovar Bratislava in Spain (Atxaerandio *et al.*, 2005), Grippotyphosa in Turkey (Carter *et al.*, 1982; Genc *et al.*, 2005) Butembo in Brazil (Saldanha *et al.*, 2007) and Canicola in Iran (Bahari *et al.*, 2011).

To confirm the infecting serovars, serologic tests such as MAT and bacteriologic tests such culture and dark field microscopy are usually used (Carter *et al.*, 1982). *Leptospira* shedding in urine is commonly associated with abortion. A study in New Zealand found that 50% of aborting dams had leptospiruria and only 0.7% in cows without *Leptospira* clinical sign (Carter *et al.*, 1982). However, if bacterial results could not be obtained, the confirmation was based on MAT titres of detected serovars. Abortion cases were often related to a high MAT titre ranging from 1:200 to 1: 50.000 (Carter *et al.*, 1982; Elder *et al.*, 1985). Beside *Leptospira spp*, some studies found co-infection with other pathogens in both aborting dams and aborted fetus. These pathogens included bovine viral diarrhoea (BVD) virus, infectious bovine rhinotracheitis (IBR) virus, *Brucella abortus, Toxoplasma gondii*, and *Neospora caninum* (Norton *et al.*, 1989; Murray, 1990; Escamilla *et al.*, 2007; Alejandro *et al.*, 2008; Genc *et al.*, 2005; Weston *et al.*, 2012).

In addition to abortion, clinical mastitis characterised by agalactia and flaccid udder were found to be the second most frequent effect in infected cattle (Sullivan & Callan, 1970; Davidson, 1971; Durfee & Allen, 1980; Higgins *et al.*, 1980; Giles *et al.*, 1983). *Leptospira interrogans* serovar Hardjo was predominant in such cases. While high MAT titres may be indicative of clinical *Leptospira* mastitis, a study of sub-clinical effects in the USA showed that there was no significant difference between cows with low (≤

1:30) and high (≥1:100) MAT titre 305-day milk yields. This might be because some of the high seropositive cows were due to past exposure that might have occurred before the current lactation (Dhaliwal *et al.*, 1996a). Other subclinical forms of *Leptospira* infection related to milk production have been recognized and indicated to cause reduction of 10% to 30% of annual expected milk in animals Pritchard (1986) in (Dhaliwal *et al.*, 1996a). Two other studies showed both mastitis and abortion in dairy herds with serologic evidence of *Leptospira interrogans* serovar Hardjo (Higgins *et al.*, 1980, Giles *et al.*, 1983).

In addition, perinatal deaths, premature calves and neonatal diseases have been reported in a dairy herd with an abortion storms where high titres to Pomona (MAT titre up to 1:30.000) and leptospires in urine were detected (Emanuel *et al.*, 1964). Furthermore, *Leptospira interrogans* serovar Hardjo was isolated from a premature calf (Giles *et al.*, 1983), and from calves with perinatal death in other dairy farms (Ellis *et al.*, 1985).

Leptospira infection in dairy cattle was also associated with decreased conception rates. A study showed a significantly lower proportion of pregnancy rate in cows with *Leptospira interrogans* serovar Hardjo with MAT titre ≥1:100 compared to seronegative cows (Dhaliwal *et al.*, 1996b). Similarly, serovar Hardjo was found in cows with longer time from calving to conception compared to seronegative cows (Guitian *et al.*, 1999). However, a previous study suggested that the negative effect of Hardjo on fertility was short-lived: a cohort study conducted by Dhaliwal *et al.* (1996c) showed a significant effect on reproductive performance during the year of diagnosis. Beside Hardjo, other serovars were also found in dairy farms in Brazil that experienced suboptimal reproduction. This includes *L. interrogans* serovar Bratislava and Grippotyphosa (Guitian *et al.*, 2001).

1.2. Leptospirosis control in dairy cattle

Some control measures have been used in dairy farms either as a response to an outbreak or as a regular prevention practice. These measures included vaccination,

prophylactic treatment, biosecurity and sanitation. Vaccination has been recognized as the main measure in outbreak as well as in endemic situations.

The efficacy of *Leptospira* vaccination in preventing urinary shedding in cattle is evident from a recent meta-analysis (Sanhueza *et al.*, 2018). This study showed that Hardjo vaccines were effective in reducing shedding due to natural and experimental infection by 82.1%. Vaccination programs in New Zealand using either bivalent (Hardjobovis and Pomona) (Mackintosh *et al.*, 1980c) or trivalent (Hardjo, Pomona and Copenhageni) (Flint & Liardet, 1980) have proven to be effective in preventing leptospiruria in naturally or experimentally infected dairy cows. While a recent case study of New Zealand dairy cows suggested that vaccination of infected animals may reduce but, likely not eliminate shedding (Yupiana *et al.*, 2019b), optimum prevention is achieved when vaccination precedes exposure (Hancock *et al.*, 1984b; Zimmerman *et al.*, 2013). Zimmerman (2013) showed that vaccination in young animals (one month old) significantly decreases urine shedding for 12 months after vaccination. Hence, vaccination is recommended to be administered at an early age. Even though the vaccination of infected animals may be somewhat effective, the use of antibiotics along with vaccination has been demonstrated to be more effective to reduce the infection rate in outbreak situations (Mughini-Gras *et al.*, 2014; Yupiana *et al.*, 2019b). Using antibiotic metaphylaxis in combination with vaccination will reduce renal infection up to the time that sufficient immunity from vaccination is achieved. One study showed, using streptomycin sulfate, 92% of the animals which were seropositives to *L. Butembo* (MAT titre$\geq$1:100) and had sign of infertility (return to heat after insemination) returned to reproductive life and became pregnant (Saldanha *et al.*, 2007). Furthermore, other antibiotics such as a single injection of oxytetracycline (20 mg/kg IM), tilmicosin (10 mg/kg, SC), or multiple injections of ceftiofur sodium (2.2 or 5 mg/kg, IM, once daily for 5 days, or 20 mg/kg, IM, once daily for 3 days) are effective in eliminating urinary shedding of leptospires (Alt *et al.*, 2001).

Other than vaccinating dairy cattle, vaccination of other species kept on dairy farms as well as vaccinating all newly introduced animals was suggested to effectively curtail

the spread of *Leptospira* in the herd. Some studies have shown that a higher risk of *Leptospira* infection was associated with risk factors such as being an open farm (e.g. purchasing or introducing animals from other farms), having pigs (Oliveira *et al.*, 2010) or dogs (Favero *et al.*, 2017) on the farm (Oliveira *et al.*, 2010; O'Doherty et al., 2014).

In some areas, rodents were regarded as source of transmission (Pimenta *et al.*, 2014), because rodents can spread *Leptospira* through surface water and concentrate feed. This led to the recommendation to reduce the exposure of cattle to rodents. Such practices may be enhanced by installing a tap water drinking system for cows, and feeding cows exclusively on pasture (Gädicke & Monti, 2013).

1.3. Thesis aim and structure

The overall goals of this thesis were to investigate the effectiveness of the current vaccination practices in preventing shedding, to provide evidence-based information for farmers on how to optimize vaccination in dairy cattle and how to control the exposure to other animals and humans. In order to achieve these goals, the following steps were undertaken: investigating the risk of *Leptospira* infection in an unvaccinated dairy farm, investigating shedding and seroprevalence of *Leptospira* in the dairy cattle population of New Zealand, exploring vaccination practices and assessing risk factors contributing to *Leptospira* shedding in New Zealand.

Data were collected from two sources: a study of leptospirosis in dairy animals of an unvaccinated herd and a nationwide survey of 200 dairy farms randomly selected from a national database. For the former, samples were collected from milking cows before and after vaccination combined with antibiotic treatment from March 2015 to January 2016. In the national survey, sampling and questionnaire interviews were completed between December 2015 and April 2016. Therefore, the thesis presents two contrasting situations of leptospirosis and *Leptospira* exposure in an unvaccinated herd (first study) and vaccinated herds (second study). In the second study, there was only one herd that had no *Leptospira* vaccination program implemented.

Chapter 2 is a systematic review that summarises information on leptospirosis and *Leptospira* exposure in dairy farmers (and people with dairy cattle exposure) and dairy cattle in New Zealand.

Chapter 3 describes the (first) epidemiological case study of *Leptospira* spp. in a dairy farming enterprise after three hospitalised human leptospirosis cases had occurred within three months in early 2015.

Chapter 4 reports *Leptospira* shedding and its association with five serovars of two *Leptospira* species (*L. borgpetersenii, L. interrogans*) in the New Zealand dairy cattle population.

Chapter 5 describes current *Leptospira* vaccination practices in New Zealand and how these practices comply with recommended best practice guidelines.

Chapter 6 explores the contribution of individual, geographical, management and herd demographics to urine shedding rates in dairy farms.

Implications of the findings of the thesis, a reflective critique of our research methodology, and suggestions for future studies are summarised in the general discussion (chapter 7).

References

Alejandro, C. I., Gustavo, R. L. C., Ivan, T. B. J., Manuel, X. C. V., Alejandro, C. J. C., Silvia, C. J. M., & Katherine, A. S. (2008). Presence of antibodies against brucellosis-leptospirosis and abortions in dairy cows in Mexico. *Journal of Animal and Veterinary Advances, 7*(12), 1640-1643.

Alt, D. P., Zuerner, R. L., & Bolin, C. A. (2001). Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar Hardjo. *Journal Of The American Veterinary Medical Association, 219*(5), 636-639.

Atxaerandio, R., Aduriz, G., Ziluaga, I., Esteban, J. I., Maranda, L. & Mainar-Jaime, R. C. (2005). Serological evidence of *Leptospira interrogans* serovar Bratislava infection and its association with abortions in cattle in northern Spain. *Veterinary Record, 156*(12), 376-380.

Bahari, A., Abdollahpour, G., Sadeghi-Nasab, A., Sattari Tabrizi, S., Yavari, M. & Dadmehr, B. (2011). A serological survey on leptospirosis in aborted dairy cattle in industrial farms of Hamedan suburb, Iran. *Iranian Journal of Veterinary Research, 12*(4), 337-339.

Carter, M. E., Cordes, D. O., Holland, J. T., Lewis, S. F., & Lake, D. E. (1982). Leptospirosis: II. Investigation of clinical disease in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*(9), 136-140.

Davidson, K. R. (1971). *Leptospira* Hardjo Infection in man associated with an outbreak in a dairy herd. *Australian Veterinary Journal, 47*(8), 408-408.

Dhaliwal, G. S., Murray, R. D., Dobson, H., Montgomery, J. & Ellis, W. A. (1996a). Effect of *Leptospira interrogans* serovar Hardjo infection on milk yield in endemically infected dairy herds. *Veterinary Record, 139*(13), 319-320.

Dhaliwal, G. S., Murray, R. D., Dobson, H., Montgomery, J. & Ellis, W. A. (1996b). Reduced conception rates in dairy cattle associated with serological evidence of *Leptospira interrogans* serovar Hardjo infection. *Veterinary Record, 139*(5), 110-114.

Dhaliwal, G. S., Murray, R. D. & Ellis, W. A. (1996). Reproductive performance of dairy herds infected with *Leptospira interrogans* serovar Hardjo relative to the year of diagnosis. *Veterinary Record, 138*(12), 272-276.

Durfee, P. T. & Allen, J. D. (1980). Serological titres of dairy cows over a 63-week period following natural infection with infection with *Leptospira interrogans* serovar Hardjo. *Australian Veterinary Journal, 56*(12), 574-579.

Elder, J. K., Pepper, P. M., Hill, M. W. M. & Ward, W. H. (1985). The significance of Leptospiral titres associated with bovine abortion. *Australian Veterinary Journal, 62*(8), 258-262.

Ellis, W. A., O'Brien, J. J., Bryson, D. G., & Mackie, D. P. (1985). Bovine leptospirosis: some clinical features of serovar Hardjo infection. *Veterinary Record, 117*(5), 101-104.

Emanuel, M. L., Mackerras, I. M. & Smith, D. J. W. (1964). The epidemiology of leptospirosis in North Queensland: I. General survey of animal hosts. *Epidemiology & Infection, 62*(4), 451-484.

Escamilla, H. P., Martínez, M. J. J., Medina, C. M. & Morales, S. E. (2007). Frequency and causes of infectious abortion in a dairy herd in Queretaro, Mexico. *Canadian Journal of Veterinary Research, 71*(4), 314-317.

Fávero, J.F., de Araújo, H.L., Lilenbaum, W., Machado, G., Tonin, A.A., Baldissera, M.D., Stefani, L.M. and Da Silva, A.S. (2017). Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. *Microbial pathogenesis*, *107*, pp.149-154.

Flint, S. H. & Liardet, D. M. (1980). A trivalent leptospiral vaccine with emphasis on a *Leptospira interrogans* serovar Hardjo component to prevent leptospiruria. *New Zealand Veterinary Journal, 28*(12), 263-266.

Gädicke, P. & Monti, G. (2013). Factors related to the level of occurrence of bovine abortion in Chilean dairy herds. *Preventive Veterinary Medicine, 110*(2), 183-189.

Genc, O., Otlu, S., Sahin, M., Aydin, F. & Gokce, H. I. (2005). Seroprevalence of brucellosis and leptospirosis in aborted dairy cows. *Turkish Journal of Veterinary & Animal Sciences, 29*(2), 359-366.

Giles, N., Hathaway, S. C. & Stevens, A. E. (1983). Isolation of *Leptospira interrogans* serovar Hardjo from a viable premature calf. *Veterinary Record, 113*(8), 174-176.

Guitian, F. J., Garcia-Pena, F. J., Sanjuan, M. L. & Yus, E. (2001). Serological study of the frequency of Leptospiral infections among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain. *Veterinary Microbiology, 80*(3), 275-284.

Guitian, J., Thurmond, M. C. & Hietala, S. K. (1999). Infertility and abortion among first-lactation dairy cows seropositive or seronegative for *Leptospira interrogans* serovar Hardjo. *Journal Of The American Veterinary Medical Association, 215*(4), 515-518.

Hamali, H., Joozani, R. J. & Abdollahpour, G. R. (2012). Serodiagnosis and molecular survey on Leptospiral abortions in the dairy cattle of Tabriz. *Iranian Journal of Veterinary Research, 13*(2), 120-125.

Hancock, G. A., Wilks, C. R., Kotiw, M. & Allen, J. D. (1984). The long term efficacy of a Hardjo-Pomona vaccine in preventing leptospiruria in cattle exposed to natural challenge with *Leptospira interrogans* serovar Hardjo. *Australian Veterinary Journal, 61*(2), 54-56.

Higgins, R. J., Harbourne, J. F., Little, T. W. & Stevens, A. E. (1980). Mastitis and abortion in dairy cattle associated with *Leptospira* of the serotype hardjo. *Veterinary Record, 107*(13), 307-310.

Langoni, H., De Souza, L. C., Da Silva, A. V., Luvizotto, M. C. R., Paes, A. C. & Lucheis, S. B. (1999). Incidence of leptospiral abortion in Brazilian dairy cattle. *Preventive Veterinary Medicine, 40*(3-4), 271-275.

Mackintosh, C. G., Marshall, R. B. & Broughton, E. S. (1980). The use of a Hardjo-Pomona vaccine to prevent leptospiruria in cattle exposed to natural challenge with *Leptospira interrogans* serovar Hardjo. *New Zealand Veterinary Journal, 28*(9), 174-177.

Mughini-Gras, L., Bonfanti, L., Natale, A., Comin, A., Ferronato, A., La Greca, E., Patregnani, T., Lucchese, L. and Marangon, S. (2014). Application of an integrated outbreak management plan for the control of leptospirosis in dairy cattle herds. *Epidemiology & Infection*, *142*(6), pp.1172-1181.

Murray, R. D. (1990). A field investigation of causes of abortion in dairy cattle. *Veterinary Record, 127*(22), 543-547.

Norton, J. H., Tranter, W. P. & Campbell, R. S. (1989). A farming systems study of abortion in dairy cattle on the Atherton Tableland. 2. The pattern of infectious diseases. *Australian Veterinary Journal, 66*(6), 163-167.

O'Doherty, E., Berry, D. P., O'Grady, L. & Sayers, R. (2014). Management practices as risk factors for the presence of bulk milk antibodies to salmonella, *Neospora caninum* and *Leptospira interrogans* serovar Hardjo in Irish dairy herds. *Animal, 8*(6), 1010-1019.

Oliveira, F., Azevedo, S.S., Pinheiro, S.R., Batista, C.S., Moraes, Z.M., Souza, G.O., Gonçales, A.P. and Vasconcellos, S.A. (2010). Risk factors associated with leptospirosis in cows in the state of Bahia, northeastern Brazil. *Pesquisa Veterinária Brasileira*, *30*(5), pp.398-402.

Pimenta, C., Castro, V., Clementino, I. J., Alves, C. J., Fernandes, L. G., Brasil, A. W. L., . . . Azevedo, S. S. (2014). Bovine leptospirosis in Para ba State: prevalence and

risk factors associated with the occurrence of positive herds. *Pesquisa Veterinaria Brasileira, 34*(4), 332-336.

Saldanha, G. B., Cavazini, N. C., Da Silva, A. S., Fernandes, M. B., Badke, M. R. T., & Pivetta, C. G. (2007). Positive serology for *Leptospira butembo* in bovine with reproductive problems. *Ciencia Rural, 37*(4), 1182-1184.

Sanhueza, J. M., Wilson, P. R., Benschop, J., Collins-Emerson, J. M., & Heuer, C. (2018). Meta-analysis of the efficacy of *Leptospira* serovar Hardjo vaccines to prevent urinary shedding in cattle. *Prev Vet Med, 153*, 71-76.

Sullivan, N. D., & Callan, D. P. (1970). Isolation of *Leptospira* Hardjo from cows with mastitis. *Australian Veterinary Journal, 46*(11), 537-539.

Te Brugge, L. A., & Dreyer, T. (1985). *Leptospira interrogans* serovar Hardjo associated with bovine abortion in South Africa. *Onderstepoort Journal of Veterinary Research, 52*(1), 51-52.

Weston, J. F., Heuer, C., Parkinson, T. J., & Williamson, N. B. (2012). Causes of abortion on New Zealand dairy farms with a history of abortion associated with Neospora caninum. *New Zealand Veterinary Journal, 60*(1), 27-34.

Yupiana, Y., Wilson, P. R., Weston, J. F., Vallee, E., Collins-Emerson, J. M., Benschop, J., . . . Heuer, C. (2019). Epidemiological investigation of *Leptospira* spp. in a dairy farming enterprise after the occurrence of three human leptospirosis cases. *Zoonoses And Public Health*, 2019.

Zimmerman, A.D., Springer, E.W., Barling, K.S., Buterbaugh, R.E., Pooley, R.D., Scholz, D.A., Rhoades, J.R. and Chase, C.C. (2013). Immunity in heifers 12 months after vaccination with a multivalent vaccine containing a United States Leptospira borgpetersenii serovar Hardjo isolate. *Journal of the American Veterinary Medical Association*, *242*(11), pp.1573-1577.

Chapter 2
A systematic review of leptospirosis in the New Zealand dairy industry

This chapter has been prepared as a manuscript for publication in the New Zealand Veterinary Journal

2.1. Summary

AIMS: To summarise information on leptospirosis and *Leptospira* exposure in dairy farmers (and people with dairy cattle exposure) and dairy cattle in New Zealand.

METHODS: A systematic review of leptospirosis and *Leptospira* exposure in dairy farmers (and people with dairy cattle exposure) and dairy cattle in New Zealand was conducted. A literature search using three international databases was performed for articles published between January 1900 and August 2018 and SciQuest for non-peer reviewed articles with no filters applied.

RESULTS: In total 51 articles were identified from 1950 to 2018 that investigated dairy cattle (n=34), dairy farmers/people with dairy cattle exposure (n=23) and both categories (n=6). The average annual incidence of leptospirosis in dairy farmers has decreased from 1100 per 100,000 (1970-1979) to 115.4 per 100,000 (1990-1998) and to 39.1 per 100,000 (1999-2016) with dairy farmers remaining the highest proportion of famer-type amongst cases through-out 1970 to 2016. From 1950 to 2018 there was a change in the relative proportion of cases attributed to the various serovar in dairy farmers. Whilst Hardjo remained predominant, the second most likely serovar associated with cases changed from Pomona to Tarassovi. However, the same observation could not be made in dairy cattle with *Leptospira* exposure or disease due to a difference in reporting system of human and cattle leptospirosis. The number of published articles in relation to disease in cattle peaked in the 1980s. Since 1990, articles in relation to cattle have predominantly been non-peer reviewed.

CONCLUSIONS: Dairy cattle remain an important host for *Leptospira* and for transmission to humans. A change in the relative proportion of cases attributed to the various serovar occurred in dairy farmers but could not be confirmed in dairy cattle. Thus, a nationwide survey in dairy cattle investigating all endemic serovars and further research in dairy farmers exploring potential risk factors need to be considered.

2.2. Introduction

Leptospirosis, caused by more than 250 serovars of pathogenic *Leptospira* spp., is one of the most widespread zoonotic diseases (Adler *et al.*, 2015; Evangelista & Coburn, 2010). The public health impact and economic losses in livestock of *Leptospira* infection are well recognized (Costa *et al.*, 2015; Sanhueza *et al.*, 2013; Vijayachari *et al.*, 2008). Leptospirosis in humans can range from a mild to a fatal disease that may include fever, headache, abdominal pain, meningitis and pulmonary haemorrhage (Levett, 2001). Clinical signs of leptospirosis in cattle include abortion, stillbirth, death, decreased milk production and infertility (Carter *et al.*, 1982; Dhaliwal *et al.*, 1996c). These clinical signs in cattle, however, may be rare especially when considering infection with a strongly host-adapted serovar such as Hardjo (Smith *et al.*, 1994).

The association between leptospirosis in humans and cattle contact, particularly dairy cattle is well established (Mackintosh *et al.*, 1982; Mwachui *et al.*, 2015). There is naturally more contact between humans and dairy cattle compared to contact between humans and beef cattle (Waitkins, 1986). For example, dairy cattle are milked at least daily while beef cattle have infrequent contact with humans. Thus, opportunities for both direct (e.g. urine spray whilst milking) and indirect transmission (e.g. while effluent spreading) of *Leptospira* are more likely in a dairy rather than a beef cattle setting. Leptospirosis in humans associated with dairy cattle has been reported in many countries for at least the last 60 years. For instance, the first identified human case in New Zealand was in dairy farmers in 1951(Bruere, 1952), in 1961 in the United States (Miller, 1961), in 1969 in the United Kingdom (Henderson, 1969), in 1971 in Australia (Davidson, 1971), and in 1977 in Israel (Shenberg *et al.*, 1977).

In New Zealand, leptospirosis was known as “dairy-farm fever” and was a common occurrence before the introduction of Hardjo and Pomona (bivalent) vaccines in the early 1980s (Marshall, 1987). While Hardjo and Pomona were the dominant serovars reported, six other serovars have been isolated in New Zealand that include *Leptospira*

borgpetersenii serovars Balcanica, Ballum and Tarassovi and *Leptospira interrogans* serovars Australis, Canicola and Copenhageni (Marshall & Manktelow, 2002). An average of 488 human cases was reported annually between 1970 to 1979 (Mackintosh, 1981). The average annual number of notified cases was halved, five years after implementing vaccination in dairy herds (Marshall, 1987). Vaccination of pigs and biosecurity and biosafety measures were also contributing factors to the decrease in the number of human cases (Ryan *et al.*, 1982b).

However, despite long-term vaccination using bivalent (Marshall & Chereshsky, 1996b) and some trivalent vaccines (Hardjo/Pomona/Copenhageni) in many dairy herds, the incidence of human leptospirosis in the New Zealand population is still higher than in other temperate countries (Hartskeerl *et al.*, 2011; Thornley *et al.*, 2002), with an incidence of 2.96 cases per 100,000 for in 2016 (ESR 2017). Furthermore, leptospirosis in dairy farmers associated with vaccinated dairy herds is still observed (McLean, 2014). In addition, an approximate two-fold increase in dairy production since the 1990s has been associated with an increase in herd size and a rise in employment within the dairy sector (LIC and DairyNZ Limited, 2014; NZIER, 2017; StatsNZ, 2013). As a result, infection pressures within herds might have changed and the number of people being exposed to *Leptospira* in a dairy setting has increased.

Long-term vaccination and farming changes are expected to be having an effect on leptospirosis and exposure in the dairy industry therefore we need the data to understand how the disease and exposure is changing. We conducted a systematic review of peer-reviewed and non-peer reviewed literature to summarise data on leptospirosis and *Leptospira* exposure in dairy farmers and dairy cattle in New Zealand.

2.3. Methods

2.3.1. Search strategy

Three international databases (Web of Science, Scopus and PubMed) were searched for articles published between January 1900 to August 2018 using key words

‘*leptospir**‘ AND *‘dairy herd*’* OR ‘*dairy farm*’* OR ‘*dairy cattle’* OR *‘dairy cow*’* OR ‘*milking cow*’* OR ‘*dairy worker*’* OR ‘*occupational’* OR ‘*human*’* AND ‘*Zealand’*. Other articles were searched on SciQuest with key words ‘leptospir*’ AND ‘dairy’. SciQuest is a database covering veterinary and animal sciences from New Zealand and Australian peer-reviewed and non peer-reviewed publications. Key words and the search strategy were determined after consultation with a Massey University librarian. Further articles were retrieved following co-author suggestions.

2.3.2. Eligibility criteria

Inclusion criteria. Any published articles (peer and non-peer reviewed), also unpublished articles which contain information on the following:

1. leptospirosis in dairy farmers or those exposed to dairy cattle; or
2. leptospirosis in dairy cattle; or
3. exposure to *Leptospira* in apparently healthy humans and/or dairy cattle

Exclusion criteria for Title and Abstract. When the title or abstract indicated that the article was a review, editorial, letter to editor, opinion or commentary without original data, these were excluded. Otherwise, articles went to full-text review.

Exclusion criteria during full-text review. Articles were excluded if there was no information about dairy cattle or dairy farmers. If the article did not explicitly mention “dairy” then supporting information, such as breed or management practices from the article was used to assist with the decision. If more than one article reported the same information, the more comprehensive article was retained.

2.3.3. Data Collection process

The data collection process was conducted in three steps; selecting abstract/title by the lead author (YY), reviewing full text articles (YY) and, when required, review by co-author (JB). In the first step, titles and abstracts were extracted and compiled in EndNote (Thomson Reuters, Philadelphia, PA, USA). In the second step: the full text of

articles was reviewed if available. If the full text could not be retrieved, and there was sufficient information within the abstract, these articles were included.

Data were extracted and presented in four tables, as leptospirosis in dairy cattle (Table 2.4-1), *Leptospira* exposure in dairy cattle (Table 2.4-2), leptospirosis in dairy farmers (Table 2.4-3) and *Leptospira* exposure in dairy farmers (Table 2.4-4). For the purposes of this review, dairy farmers were defined as dairy farmers and people with exposure to dairy cattle. Whenever available, information about study years, location, and serovars were reported in each table. For the purpose of this study, the eight serovars which have been isolated in New Zealand were reported. If provided, details about the age groups of dairy cattle was presented as calves (cattle <12 months) or cows (female cattle >12 months).

2.3.4. Case definition

Evidence of leptospirosis/*Leptospira* exposure was assessed in each study, and categorized as "probable" or "confirmed" (leptospirosis) and "positive" (*Leptospira* exposure), according to the following criteria.

1. For leptospirosis in dairy farmers the case definitions from the New Zealand Ministry of Health (Ministry of Health, 2018) was used and adapted as below:
    a. An eligible case was defined as a clinically compatible illness in humans.
    b. A confirmed case required a clinically compatible illness and at least one of the following: (i) isolation of leptospires from a clinical specimen; (ii) detection of leptospiral nucleic acid from a clinical specimen (iii) a fourfold or greater rise in leptospiral microscopic agglutination titre (MAT) between acute and convalescent serum ;(iv) a single antibody titre of $\geq$ 400 in the MAT for any serovar.
2. For leptospirosis in dairy cattle,
    a. A probable case was defined as a clinically compatible disease in dairy cattle

b. A confirmed case requires a clinically compatible disease and at least one of the following: (i) isolation of leptospires from a clinical specimen; (ii) detection of leptospiral nucleic acid from a clinical specimen; (iii) a single antibody titre of $\geq$ 1000 in the MAT for any serovar (Adler *et al.*, 2015).

3. For *Leptospira* exposure in human and dairy cattle, a positive MAT (as defined by the individual studies, from a titre of 24 upwards) or PCR in apparently healthy animals were considered as indicative of infection or previous infection. An assessment of the cut-points of MAT titres was performed to determine the risk of misclassification bias.

2.4. Results

In total, 442 articles were retrieved on first screening, including 52 from PubMed, 84 from Scopus, 181 from Web of Science, and 125 from SciQuest. After identifying 133 duplicates, 41 final articles that met the inclusion and exclusion criteria were progressed to a full analysis. A further ten articles were retrieved following co-author suggestion (Figure 2.4-1). In total, 51 eligible articles (peer-reviewed and grey literature) were selected: 34 articles on dairy cattle, 23 on dairy farmers and 6 on both dairy cattle and dairy farmers. Figures 2.4-2 and 2.4-3 show the distribution of publication dates of eligible articles. The highest percentage of the articles reporting leptospirosis and *Leptospira* exposure in dairy farmers and in dairy cattle was found between 1981-1985 (Figures 2.4-1 and 2.4-2). There were more peer reviewed articles than non-peer reviewed about dairy farmers (Figure 2.4-2). In dairy cattle, non-peer reviewed articles were predominant (Figure 2.4-1.)

2.4.1. Leptospirosis in dairy cattle

Table 2.4-1 provides details about 23 articles reporting clinical leptospirosis in dairy cattle from nine regions, but two articles did not specify the location. Clinical leptospirosis in dairy cattle was first reported in 1951 in 8 calves from a herd in the

South Island in which Pomona was identified as the infecting serovar (Bruere, 1952). In 1952, leptospirosis cases in in dairy cattle were first reported in the North Island where Pomona was also associated with the cases in 350 calves and 12 adult cows from 76 herds in Northland (Ensor & McClure, 1953). In addition, Auckland and Waikato were identified as the most frequent regions reporting leptospirosis from 1953 to 2018 while more recent articles reported cases in Hawkes Bay.

Production effects on cows were reported in 14 articles, 10 of those were abortion cases. Eight of the 10 articles reporting abortion identified Pomona, one reported Hardjo and Tarassovi (Ministry for Primary Industries, 2018) and one did not specify the serovar associated with the cases (Horner, 1988). An MPI report (2018) on a disease outbreak in 100 mixed-age cows reported nine abortions and five deaths. Six adult cows had titres of 50 to 800 to serovar Tarassovi and three of these six also had titres to Hardjo (800 to >1600). A number of cows had ticks on them, and *Theileria orientalis* was detected by PCR in serum from all five cows tested. This parasite was finally concluded as the cause of abortion but the additional role of *Leptospira* infection was recognized (Ministry for Primary Industries, 2018). The remaining four of 14 articles reporting production effects identified as agalactia with or without mastitis in cows with Hardjo (Ministry for Primary Industries, 1977a, 1977b, 1980; Orr, 1994). Eleven articles reported leptospirosis in calves, where eight cases were due to Pomona (Bruere, 1952; Cordes *et al.*, 1982; Ensor & McClure, 1953; Hill *et al.*, 2007; Ministry for Primary Industries, 1974, 2011, 2013; Orr, 1994), two to Ballum (Ministry for Primary Industries, 1977b; Varney & Gibson, 2008) and one to Copenhageni (Dodd & Brakenridge, 1960).

Two articles reported investigations of *Leptospira* infection during 1975-1977 on Waikato herds where clinical leptospirosis was suspected (Carter *et al.*, 1982; Cordes *et al.*, 1982). In one article, clinical leptospirosis (haemoglobinuria) was reported only in one of 250 herds and in another article (Cordes *et al.*, 1982), abortion was reported in 11 of 21 herds (Carter *et al.*, 1982). Both articles reported Pomona as the dominant serovar.

Vaccination status of the herds was recorded in six of the 22 articles (four vaccinated and two unvaccinated). Of the four articles that reported leptospirosis in vaccinated herds (Hill *et al.*, 2007; Ministry for Primary Industries, 1974; Varney & Gibson, 2008; Weston *et al.*, 2012), one reported the use of Pomona (Ministry for Primary Industries, 1974) and one reported a combination of Pomona, Hardjo and Copenhageni vaccine (Weston *et al.*, 2012). Two others had no information on the vaccine type used (Hill *et al.*, 2007; Varney & Gibson, 2008).

2.4.2. *Leptospira* exposure in dairy cattle

Table 2.4-2 details 14 articles that reported *Leptospira* exposure in apparently healthy dairy cattle. The majority of the articles were published before the 1990s (69%). These articles mostly came from studies performed in the Waikato, Manawatu, Taranaki and Otago regions. Five serovars were reported in the studies (Hardjo, Pomona, Ballum Copenhageni and Tarassovi), with Hardjo and Pomona dominating. Of the 13 articles, six determined the prevalence of *Leptospira* exposure, five by sero-prevalence and one by shedding prevalence (Bahaman *et al.*, 1984; Bolger, 1984; Neilson, 1984; Parramore *et al.*, 2011; Price, 1984).

Vaccination status was stated in eight studies (Bahaman *et al.*, 1984; Bolger, 1984; Carter *et al.*, 1982; Gibson & Varney, 2008; Marshall *et al.*, 1996; Neilson, 1984; Parramore *et al.*, 2011; Price, 1984). Hardjo with high MAT titres ($\geq$ 1600) was found in two healthy vaccinated cows following diagnosis of leptospirosis in two farm workers from a farm in Auckland (Gibson & Varney, 2008). Leptospiruria in 3.8% of cows and 29.5% of herds was detected in a study of 34 farms in Manawatu and 10 farms in Southland and Waikato Island that undertook a vaccination program (Parramore *et al.*, 2011). Hardjo was detected by MAT in 79% of unvaccinated herds in Otago (Thomas *et al.*, 1994).

Three types of study design were used in the articles reporting *Leptospira* exposure in apparently healthy cattle including cross-sectional, case series and a case control study.

2.4.3. Leptospirosis in dairy farmers

Sixteen eligible articles that described leptospirosis in dairy farmers are reported in Table 2.4-3. There were more articles reporting cases in the North Island compared to the South Island with Waikato predominating. Seven serovars were reported in human leptospirosis (Hardjo, Pomona, Copenhageni, Ballum, Tarassovi, Canicola and Australis) with Hardjo and Pomona identified as the predominant serovars. The study design that was mostly used in the articles reporting human leptospirosis was that of a case series, with one case report.

Four of the 15 articles summarised notified cases spanning 1951-1979 (Mackintosh, 1981) and 1990-2018 (Nisa *et al.*, 2018b; Thornley *et al.*, 2002; Wael *et al.*, 2018). The average annual incidence in humans has decreased from 15.7/100,000 in 1970-1979 (Mackintosh, 1981) to 4.4/100,000 in 1990-1998 (Thornley *et al.*, 2002) to 1.96/100,000 in 1999-2016 (Nisa *et al.*, 2018b) and to 1.6/100,000 in 2010-2015 (Wael *et al.*, 2018). Occupation data were recorded in at least 90% (Mackintosh, 1981), 83.2% (Thornley et al., 2002), 93% (Nisa *et al.*, 2018b) and 94% (Wael et al., 2018) of the notified cases. In relation to farming occupations, three articles differentiated dairy from other farmers (Mackintosh, 1981; Nisa *et al.*, 2018b; Thornley *et al.*, 2002). However in the study conducted by Wael *et al.* (2018), there was no differentiation of farmer type. Amongst farmer cases with type of farming recorded, the proportion of dairy farmers was 90% in 1970-1979 (Mackintosh, 1981), 80% in 1990-1998 (Thornley et al., 2002) and 80% in 1999-2016 (Nisa *et al.*, 2018b). The average annual incidences of leptospirosis in dairy farmers were 272.2 per 100,000 in 1951-1975, 1100.0 per 100,000 in 1970-1979 (Mackintosh, 1981), 115.4 per 100,000 in 1990-1998 (Thornley et al., 2002) and 39.1 per 100,000 in 1999-2016 (Nisa *et al.*, 2018b).

2.4.4. *Leptospira* exposure in apparently healthy dairy farmers

There were seven eligible articles on *Leptospira* exposure in dairy farmers published from 1980-2015 (Table 2.4-4) (Bettelheim & Fogg, 1986b; Blackmore & Schollum, 1982b; Fang *et al.*, 2014a; Mackintosh *et al.*, 1980d; Metcalfe *et al.*, 1981; Sanhueza *et*

al., 2015). Of the seven articles, one study was conducted in veterinary students (Fang *et al.*, 2014a) and one in veterinarians (Sanhueza *et al.*, 2015). The region most frequently studied was Manawatu (3/7 articles). Overall, Hardjo and Pomona were found to be the dominant serovars. All studies were cross-sectional in design.

An article was published in 1986 about a cross-sectional study conducted in three regions of the South Island (Canterbury, Otago and Southland). Blood samples were collected from 329 dairy farmers and in-contact cattle from unvaccinated herds in Canterbury (74 herds), Southland (67 herds) and Otago (57 herds). Amongst dairy farmers, serological reactions to Hardjo, Tarassovi, Copenhageni, Pomona and Australis were identified with Hardjo as the dominant serovar. Time spent milking cows in hours per week and herd size were related to dairy farmer seropositivity (MAT cut-point of $\geq$25) to serovar Hardjo. There were no details provided about how the analysis was performed. Cattle were only serologically tested for Hardjo and the majority of the herds were positive.

Two articles used chi-square analysis to evaluate the individual and on-farm risk factors associated with *Leptospira* exposure in dairy farmers (Blackmore & Schollum, 1982a; Mackintosh *et al.*, 1980d). The exposure in humans was measured as seropositivity to serovars Hardjo and Pomona with MAT cut-point of $\geq$24. Factors significantly associated with *Leptospira* exposure in humans included duration of milking, vaccination status of dairy herds, having a herringbone shed rather than a walk through shed, wearing shorts, keeping pigs on farms, herd size, being male and a history of leptospirosis in workers.

Two articles investigated exposure in the veterinary profession. Fang *et al.* (2014a) collected blood samples from 302 Massey University veterinary students. Sera were tested for antibodies to serovar Hardjo, Pomona and Ballum. Despite being exposed to dairy cattle within the curriculum (80% of students) and outside the curriculum (20.3% of students) all students were seronegative with MAT cut-point of 48. Sanhueza *et al.* (2015) recruited 277 veterinarians who were blood tested for serovars Hardjo,

Pomona, Ballum, Copenhageni, and Tarassovi antibodies: 211 veterinarians had contact histories with dairy cattle. Seropositivity to Pomona (2.5%) and Hardjo (2.2%) were predominant, followed by Ballum and Copenhageni (0.4%) and no seropositivity to Tarassovi with a MAT cut-point of 48. There was no association between time spent in contact with dairy cattle and sero-status to *Leptospira*.

2.4.5. Laboratory Tests

For clinical leptospirosis in cattle, dark field microscopy (DF) or culture isolation (CI) were used in seven of ten articles published between 1953-1994, while PCR was used in five of six articles published between 2007-2018. Almost all of the studies used serological tests (MAT).

In articles that reported leptospirosis and *Leptospira* exposure, the number of serovars examined varied between articles. Amongst the 34 articles, there was variation in relation to serovars examined. While all reported H and P, only two reported on five serovar (H,P,C,B,T) (Mackintosh *et al.*, 1982; Ministry for Primary Industries, 1977b), with 11 reporting either Copenhageni, Ballum and Tarassovi. In contrast, the majority of articles reporting leptospirosis or *Leptospira* exposure (16/23) in humans investigated all five serovars between 1971 to 2016.

2.4.6. MAT titre cut-point in exposure studies

Twelve of the 13 articles that reported *Leptospira* exposure in dairy cattle, used MAT. MAT is known for its complexity in terms of how the test is performed and how results are interpreted and is subject to a degree of subjectivity. Therefore, the cut-point chosen can potentially lead to misclassification of results. In six articles the cut-point was not defined, while it was defined in six others; three used $\geq$24, one used $\geq$50, and two used $\geq$200. Furthermore, seven articles that reported *Leptospira* exposure in dairy farmers used MAT with cut-points of $\geq$24 (3), $\geq$25 (1), $\geq$48 (2), and $\geq$100 (1). Two articles that used a cut-point of $\geq$200 to determine *Leptospira* exposure in animals could potentially classify exposed animals as unexposed (Carter *et al.*, 1982; Cordes *et*

al., 1982). A similar misclassification could also occur in one article about dairy farmers that used a cut-point of 100 (Metcalfe *et al.*, 1981).

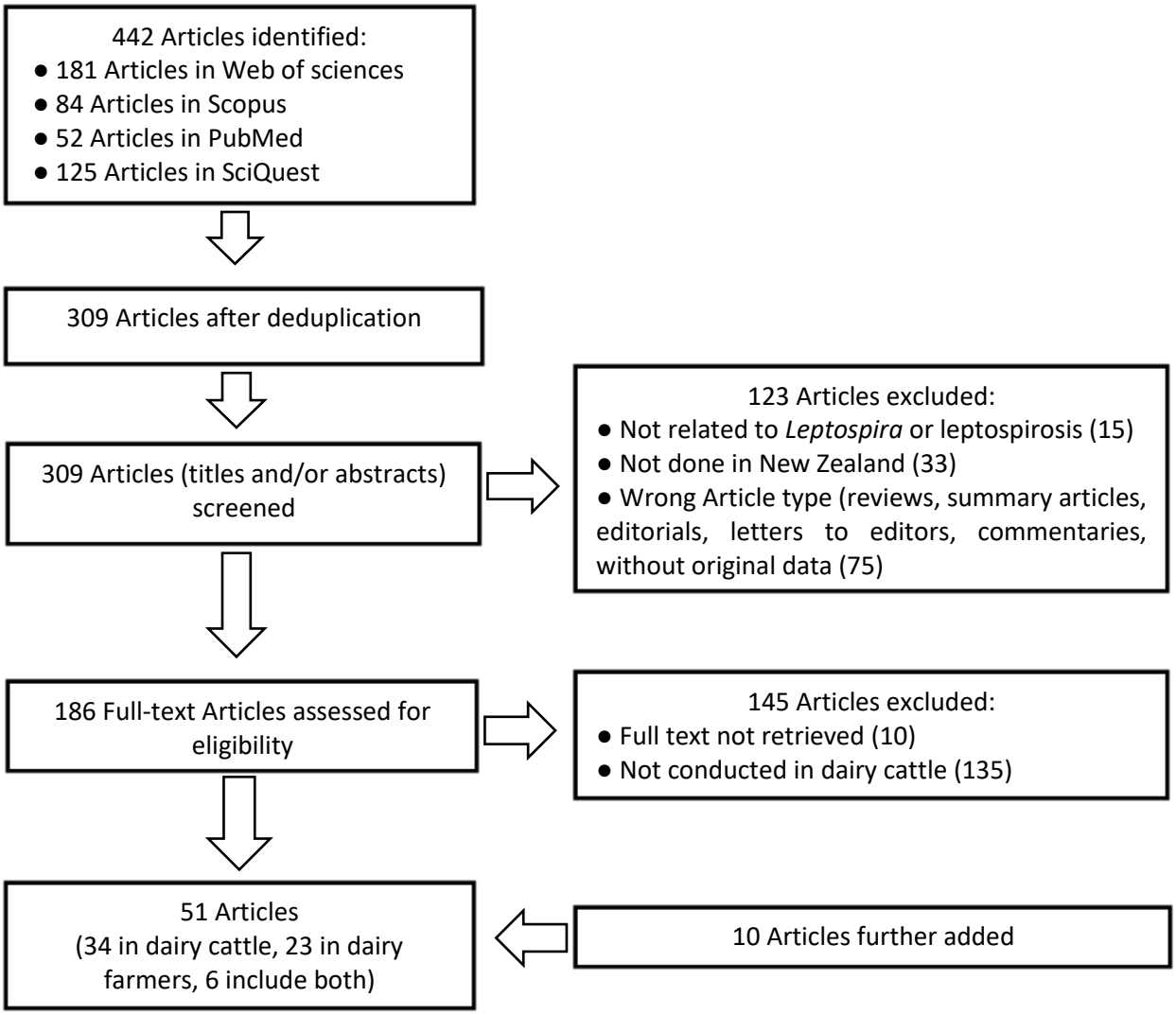

Figure 2.4-1. Flow diagram of the systematic review and identification of articles

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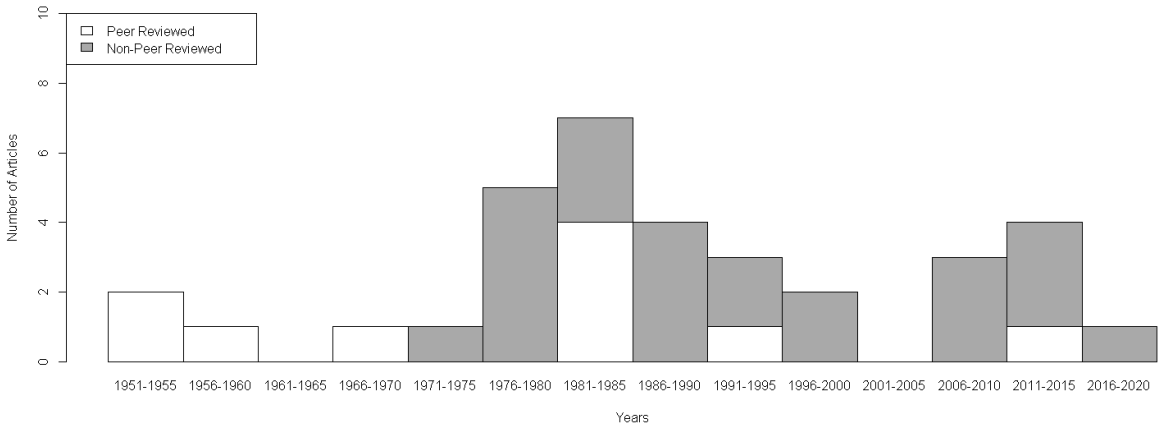

Figure 2.4-2. Number of articles (peer reviewed and non-peer reviewed) on leptospirosis and *Leptospira* exposure in dairy cattle in New Zealand that were retrieved from 1951 to 2018.

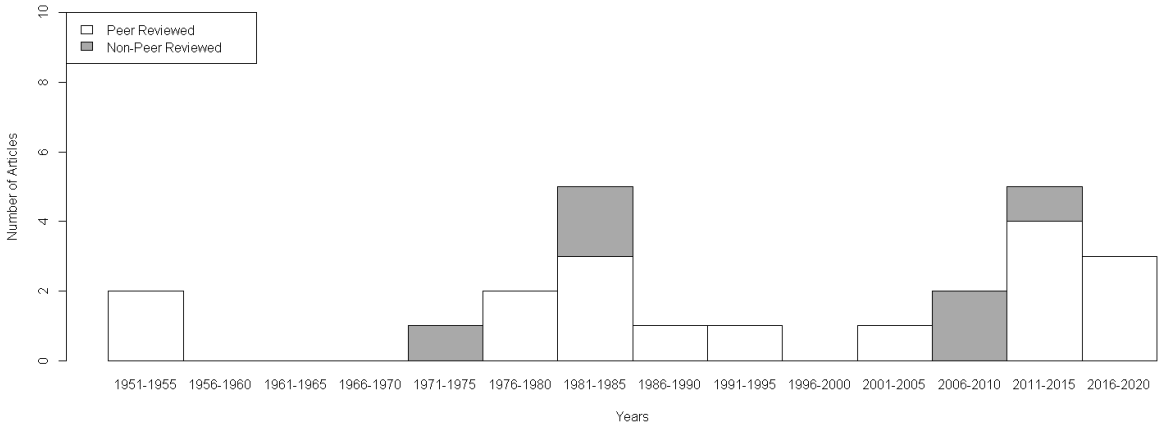

Figure 2.4-3. Number of articles (peer reviewed and non-peer reviewed) on leptospirosis and *Leptospira* exposure in dairy farmers in New Zealand that were published from 1951 to 2018.

Table 2.4-1 Articles reporting leptospirosis in dairy cattle in New Zealand

Citation	Study year (s)	Locations	Clinical signs	Diagnostic test	No. of probable case (cattle or/and herds)	No. of Confirmed cases (cattle or/and herds)	Serovar Positive (no. of Cattle)
Bruere, 1952	1951	NR	Death, Jaundice, haemoglobinuria	Gross pathology, MAT	8 calves	NR	P(5)
Ensor,1953	1952	Northland	Jaundice, anaemia, haemoglobinuria, death	Gross pathology, MAT	350 calves, 12 cows in 76 herds	11 herds	P (NR)
Dodd, 1960	1956-1958	Auckland Waikato	Jaundice, haemoglobinuria, death	Gross pathology, histopathology, dark field, culture, MAT	50 calves in 13 herds	NR	C (NR)
Moller, 1967	1964-1966	Waikato	Abortion	(≥1000 for P)	31 cows	All cows	P (all cows)
MPI, 1974	1974	Auckland	Haemoglobinuria, death	Gross pathology, dark field, MAT	*29 calves in 2 herds	1 calf	P,H (1 calf)
Carter, 1982	1975-1977	Waikato	Abortion	MAT (≥200 for P), dark field, culture	60 cows in 16 herds	NR	P (51 cows) H (19 cows)
MPI, 1977a	1977	Canterbury	Agalactia, mastitis	Dark field and MAT	2 herds	NR	H (NR)
MPI, 1977b	1977	Waikato	Agalactia	Dark field and MAT (≥2000)	10 cows	5	H (5)
				Dark field and MAT	1 calf	1	B (1)
				Culture, MAT, gross pathology,	2 herds	2	C (NR)
Cordes, 1982	1977	Waikato	Haemoglobinuria	Dark field and MAT >200 for H and P	*3 calves 1 herd	NR	P (3)
MPI, 1980	1980	Northland	Agalactia, mastitis	MAT	6 cows in one herds	NR	H (6 cows)
Horner, 1988	1984-1987	Auckland	Abortion	Histopathology, MAT	22 cows	NR	P (NR)
Gill, 1990	1989	Auckland, Canterbury, Otago	Abortion	MAT	46 cows	NR	P (NR)
Orr, 1990	1989	North Island	Death, dullness	Histopathology, MAT	17 calves	NR	P (NR)
McKenzie, 1989	1989	North Island	Abortion	MAT	**2 herds	NR	P (NR)
Thornton, 1992	1991	NR	Abortion	MAT	11 cows	NR	P (NR)
Thornton, 1996	1995	NR	Abortion	MAT	6 cows	NR	P (NR)
Orr, 1994	1994	NR	Agalactia, pyrexia	MAT for H, P and C	2 herds	1 cow	H (9 cows)

Hill,2007	2007	Hawke's Bay	Jaundice, Haemoglobinuria	Gross pathology, culture, dark field, PCR	*1calf	1	NR
			Haemoglobinuria, death	PCR, MAT for P and C	**7 (calves)	1	P (7)
			Haemoglobinuria, diarrhoea	PCR	1 calf	1	NR
#Varney, 2008	2008	Manawatu	Jaundice,anaemia, haemoglobinuria, death	Gross pathology,culture, dark field, PCR, MAT	*1(calf)	1	B (1)
MPI, 2011	2011	Hawke's Bay	Jaundice,anaemia, haemoglobinuria, death	MAT	2 calves	1	P (1)
Weston, 2012	NR	Taranaki CanterburyS outhland	Abortion	MAT for H and P	* 8 cows	1	P,H (8)
#MPI, 2013	2013	Wairarapa	Abortion	PCR, gross pathology,	3 cows	1	NR
		Auckland	Pyrexia, jaundice	MAT	1 cow	1	H and P (1)
		Southland	Haemoglobinuria, death	PCR, MAT for P , B and C	2 calves	1	P (1)
MPI, 2018	2018	Hawkes Bay	Abortion, death, jaundice	MAT	14 cows	3	H (3) T (6)

H:Hardjo ; P: Pomona; C:Copenhageni; B:Ballum; T: Tarassovi; *:originated from vaccinated herd; H:Hardjo ; **:originated from unvaccinated/poorly vaccinated herd; NR: Not reported: # both human and cattle cases were reported,

Table 2.4-2.Articles reporting *Leptospira* exposure in dairy cattle in New Zealand

Citation	Study year(s)	Locations	Diagnostic test	Number of cattle or/and herds tested	No.(%) positive	Serovar Positive
Hellstrom,1978	1975-1976	Manawatu	MAT (≥24 for H,P,C,B,T)	960 cows	717 (74.7)	H
					68(7.1)	P
					27(2.8)	C
					36(4.1)	B
					63(6.6)	T
MPI, 1978	1978	Waikato	CFT and MAT (for H, P, C, B, T)	5910 cows	1273 (21.5)	H
				6411 cows	592(9.2)	P
				1813 cows	32(1.8)	C
				257 cows	0(0.0)	B
				397 cows	1(0.3)	T
Carter, 1982	1975-1977	Waikato	MAT (≥200 for H,P,C,B)	**1562 cows	374 (23.9)	H
				(herds without abortions)	321 (20.5)	P
				**2642 cows	662 (25.0)	H
				(herds with abortions)	974 (36.9)	P
Mackintosh, 1982	NR	Manawatu	MAT (≥24 for H,P,C,B,T)	520 cows	345 (66.3)	H
					27 (5.2)	P
					23 (4.4)	C
					14 (2.9)	B
					25 (4.8)	T
Cordes, 1982	1977	Waikato	MAT ≥200 for H,P; darkfield; culture	7500 cows	936 (12.5)	H
					260 (3.5)	P
				250 herds	131 (52.4)	H
					15 (6.0)	P
Bolger, 1984	NR	Otago	MAT	**57 herds	54 (95.0)	H
					4 (7.0)	P
Neilson, 1984	NR	Gisborne	MAT for H, P	**25 herds	24 (96.0)	H
					8 (32.0)	P
Price, 1984	NR	Hawke's Bay	MAT	**20 herds	20 (100.0)	H

					15 (75.0)	P
Bahaman, 1984	1979-1980	Taranaki	MAT (≥24 for H,P)	**891 cows	551 (61.8)	H
					23(2.6)	P
				10 herds	10 (100.0)	H
					8 (80.0)	P
Thomas, 1994	NR	Otago	MAT	NR (herds)	NR(79.0)	H
Marshall, 1996	1996	NR	MAT (≥48) for H,P	**10 herds	9(90)	H
				*10 herds	8(80)	H
#Gibson, 2008	2008	Auckland	MAT for H, P	*4 cows	4 (100.0)	H
Varney, 2008	2008	Taranaki	MAT (≥50 for P,C,B)	20 calves	7 (35.0)	B
				8 calves	4 (50.0)	
Parramore, 2011	2011	Manawatu and the South Island	PCR and dark field	*445 cows	17(3.8)	NA
				*44 herds	13 (29.5)	NA

H:Hardjo ; P: Pomona; C:Copenhageni; B:Ballum; T: Tarassovi; *:originated from vaccinated herd; H:Hardjo ; **:originated from unvaccinated/poorly vaccinated herd; NR: Not reported: #: both human and cattle cases were reported; NA: Not applicable

Table 2.4-3.Summary of articles reporting leptospirosis in dairy farmers in New Zealand

Citation	Study year/s	Location	Diagnostic test	No. of eligible case	No. of Confirmed cases	Serovar Positive
Bruere,1952	1951	NR	MAT	3	NR	P
Mackintosh,1981	1951-1975	Nationwide	MAT (≥ 200 and ≥300), culture	1042		H
				1286		P
				19		C
				7	NR	B
				10		T
				1		Ca
				1		A
				356		NR
	1970-1979	Nationwide	NR	4390	NR	NR
Kirschner, 1954	1952-1953	Nationwide	NR	87	NR	P,C,Ca
Christmas,1974	1971-1972	Waikato	Culture (blood) and MAT	47	13	P
					22	H
MPI, 1977	1977	Canterbury	MAT	2	NR	H
Ryan, 1982	NR	Waikato	MAT	**19	NR	H,P
				*2		
Thornley, 2002	1990-1998	Nationwide	MAT	212	212	NR
#Thomas, 1994	1992-1993	Otago	MAT (≥ 400)	4	3	H
					56	H
Nisa, 2018	1999-2016	Nationwide	MAT, PCR	≈137	5	P
					25	B
					51	T
					5	H
					0	P
Cowie, 2012	2004-2010	Waikato	MAT, PCR	≈25	1	C
					5	B
					11	T

					3	NR
#Gibson, 2008	2008	Auckland	Serology test	2	NR	NR
#Varney, 2008	2008	Taranaki	MAT	1	1	B
Mclean, 2014	2010	Wairarapa	MAT,PCR, ELISA	**3	2	H
Wael, 2018	2010-2015	Nationwide	MAT,PCR, ELISA	A	NR	NA
MPI, 2013	2013	Wairarapa	MAT	1	NR	P
Benschop,2017	2015	Manawatu	MAT,PCR, ELISA	**3	2	P
					1	H

*:contact with vaccinated herd; H:Hardjo ; **:contact with unvaccinated/poorly vaccinated herd; H:Hardjo ;P: Pomona; C:Copenhageni; B:Ballum; T: Tarassovi; A: Australis; Ca: Canicola;≈:89-93% confirmed cases;‡: no differentiation on the occupation of the cases; #: both human and cattle cases were reported; NR: Not reported

Table 2.4-4.Summary of articles reporting *Leptospira* exposure in dairy farmers in New Zealand

Citation	Study year(s)	Locations	MAT cut-points	Number tested	No.(%) positive	Serovar Positive
Metcalfe,1981	1976-1978	Auckland	CFT (polyvalent complement sera) and MAT (≥100 for H,P,C,B,T)	58	1 (1.7)	C
		Taranaki		62	22 (35.5)	H
					7 (53.8)	H
		Waikato		13	1(7.7)	T
					1(7.7)	B
Mackintosh, 1980b	NR	Manawatu	MAT (≥24 for H,P,C,B,T)	213	48 (22.5)	H
					29 (13.6)	P
					12 (5.6)	H & P
					4 (1.9)	C
					3 (1.4)	B
Blackmore, 1982a	NR	Waikato, Wairarapa, Northland, Hawkes Bay	MAT (≥24 for H,P,C,B,T,A)	308	89 (28.9)	H
					73 (23.7)	P
					10 (3.2)	C
					2 (0.6)	B
					8 (2.6)	T
					4 (1.3)	A
					49 (15.9)	multiple
Blackmore, 1982b	NR	Manawatu	MAT (≥24 for H,P,C,B,T)	193	110 (57.0)	H
					67 (34.7)	P and T
					15 (7.8)	B and C
Bettelheim, 1986	NR	Canterbury	CFT, MAT (≥25 for H,P,C,B,T,Ca, A)	136	12 (8.8)	H
					2 (1.5)	P
					1 (0.7)	C
					2 (1.5)	T
		Otago		76	11(14.5)	H
					1 (1.3)	C

					3 (3.9)	T
					22 (18.8)	H
					3 (2.6)	C
		Southland		117	3 (2.6)	T
					3 (2.6)	A
Fang, 2014	2010-2011	Manawatu	MAT (≥48 for H,P)	302	0 (0.0)	
					6 (2.5)	H
					7 (2.2)	P
Sanhueza, 2015	2012	Nationwide	MAT (≥48 for H,P,C,B,T)	277	1 (0.4)	C
					1 (0.4)	B
					0 (0.0)	T

H: Hardjo ; P: Pomona; C:Copenhageni; B:Ballum; T: Tarassovi; A: Australis; Ca: Canicola; NR: Not reported

2.5. Discussion

This systematic review is the first to summarise data on leptospirosis and pathogenic *Leptospira* spp. exposure in dairy farmers and dairy cattle in New Zealand. Although the average annual incidence in humans has decreased from 15.7/100,000 in 1970-1979 (Mackintosh, 1981) to 4.4/100,000 in 1990-1998 (Thornley *et al.*, 2002) and to 1.96/100,000 in 1999-2016 (Nisa *et al.*, 2018b), the annual incidence of leptospirosis in New Zealand remains high compared to other temperate countries. However, this figure might be still underestimated due to a misdiagnosis of non-specific symptoms in mild cases of leptospirosis such as ILI (influenza like illness). Being off work for three or more days with flu signs has been associated with *Leptospira* exposure (Dreyfus *et al.*, 2015a; Sanhueza *et al.*, 2015).

The decreasing trend of leptospirosis in all humans is seen also in dairy farmers. The average annual incidence of this disease in dairy farmers has decreased from 1100/100,000 in 1970-1979 (Mackintosh, 1981) to 115.4/100,000 per 100,000 (1990-1998) (Thornley *et al.*, 2002) and to 39.1 per 100.000 (1999-2016) (Nisa *et al.*, 2018b). The two most recent estimates of annual incidence in dairy farmers are likely

underestimations as the information on animal type being farmed amongst those in farming occupations was not recorded in 273/539 (51%) cases in 1990-1998 (Thornley *et al.*, 2002) and 412/646 (64%) cases in 1999-2016 (Nisa *et al.*, 2018b).

Despite the decreasing trend of leptospirosis incidence over time in dairy farmers, dairy farmers remained the largest farmer-type within farming occupations amongst cases. Approximately 90% of human leptospirosis cases in 1970-1979 were dairy farmers (Mackintosh, 1981). The proportion declined to 21% in 1990-1998 (Thornley *et al.*, 2002) and then dropped to 14% in 1999-2016 (Nisa *et al.*, 2018b). Assuming that the proportion of cases in dairy farmers, amongst those where animal type was identified, was similar to those where animal type was not identified, the true annual incidence of notification in dairy farmers is estimated to be approximately 233.8/100,000 in 1990-1998 and 107.9/100,000 in 1999-2016.

Serovars reported in the general human population changed between 1952 and 2016. From 1952-1975, Pomona was the predominant serovar followed by Hardjo. In 1990-2016, Hardjo became the major serovar followed by Pomona in conjunction with an increase in serovar Ballum. Furthermore, data from 2010-2015 showed that Ballum has over- taken Pomona as the second major serovar. Similarly, changes over time in the serovars causing disease have also been identified in the dairy farming population. From Pomona followed by Hardjo as the predominant serovars in 1952-1979, a change to Hardjo followed by Tarassovi as the major serovars was observed in 1999-2016. A study of notified cases in the Waikato DHB between 2004-2010 (Cowie & Bell, 2012) reported Tarassovi was the predominant serovar in dairy farmers. The change over time in the pattern of the predominant serovars in dairy farmers is likely associated with the long-term vaccination of more than 90% of dairy herds with Hardjo and Pomona and some with Copenhageni (Heuer *et al.*, 2012; Marshall, 1987). While all vaccine serovars decreased in dairy farmers, Hardjo remained the dominant Serovar (Nisa *et al.*, 2018b). As antibodies against Hardjo cannot be differentiated from Balcanica by MAT, we suggest that a proportion of seropositivities to Hardjo in dairy farmers were actually due to Balcanica. However this is unlikely, as previous studies on

experimental and natural infection in cattle with Balcanica show this serovar is sporadic in cattle and is unlikely to be endemic (Mackintosh *et al.*, 1980b). In addition, as Hardjo and Balcanica are from the same serogroup, Hardjo infected or vaccinated cattle may be resistant to infection with Balcanica (Mackintosh, 1981). In New Zealand, Balcanica is an adapted serovar in possums (*Trichosurus vulpecula*) (Hathaway *et al.*, 1978; Mackintosh *et al.*, 1980b).

In addition to the emergence of Tarassovi in dairy farmers, the rise of Ballum has also been identified. Ballum has been the third most common serovar nationwide in dairy farmers from 1999-2016 (Nisa *et al.*, 2018b). In the dense dairy farming area of Waikato, the number of dairy farmers with Ballum infection was second only to Tarassovi (Cowie & Bell, 2012). The emergence of serovar Ballum in dairy farmers raises questions about the current role of wildlife species such as mice, rats and hedgehogs in transmitting *Leptospira* to dairy cattle. Transmission from the wildlife species to dairy farmers is also possible through indirect contact between humans and wildlife urine via contaminated feed such as calf meal, palm kernel and maize silage.

The changing pattern of the predominant serovar in dairy farmers has been observed but a similar observation could not be made in dairy cattle in this review due to potentially a large number of *Leptospira* infections in cattle not being investigated and *Leptospira* positive cattle were not reported in the MPI journal Surveillance. While underestimation of human leptospirosis likely occurred, the underestimation of cases in dairy cattle will be much higher. Human leptospirosis has been notifiable since 1952 and laboratory testing is government subsidised. Conversely, animal leptospirosis is not notifiable and the animal owner needs to pay for testing. In many studies in cattle, not all endemic serovars were studied, only Hardjo and Pomona were frequently investigated. While most studies in dairy farmers included all five major serovars; there are eight serovars routinely investigated for human cases (Hardjo, Pomona, Ballum, Copenhageni, Tarassovi, Canicola, Grippotyphosa, and Australis (Fang, 2014). MAT is suitable for *Leptospira* diagnosis in humans in New Zealand, as this test is highly specific in convalescent samples and acute samples with an MAT cut-point of $\geq$400

(Limmathurotsakul *et al.*, 2012). In this review, most of the cases in animals were retrieved from the Surveillance magazine that is published by the Ministry of Primary Industry (MPI). For Surveillance magazine, there are a number of reasons leptospirosis may be reported. This could include details of investigations of suspect exotic strains e.g. Canicola, or descriptions of leptospirosis as an endemic disease or of its diagnosis and control (J.Watts, personal communication, December 12, 2018). Some articles have reported other potential diseases with leptospirosis-like clinical signs in cattle that included Brucellosis (Moller *et al.*, 1967), *Neospora caninum* (Weston *et al.*, 2012), *Theileria orientalis* (Ministry for Primary Industries, 2018) in aborted cows and copper poisoning in the calves that had haemoglobinuria (Ministry for Primary Industries, 1974). The co-infection of these diseases with *Leptospira* infection, that share similar clinical presentations in cattle, might be the reason these cases were reported.

Due to under-reporting in cattle, mechanisms that increase the sensitivity of finding cases of leptospirosis are important. In the current study a 'one health' approach has been used to this end. Four articles explored the links between clinical leptospirosis in dairy farmers and infection in dairy cattle and reported findings of *Leptospira* infection in dairy cattle as a result of the follow up to the report of human cases (Benschop *et al.*, 2017; Gibson & Varney, 2008; Thomas *et al.*, 1994; Varney & Gibson, 2008). It was confirmed that there was an association between human cases and transmission from dairy cattle by comparing the serovar found in humans and cattle. In two articles, the same serovars were diagnosed from both human cases and cattle infection (Benschop *et al.*, 2017; Thomas *et al.*, 1994), while in two others, there was no specification on the serovar detected in humans (Gibson & Varney, 2008; Varney & Gibson, 2008). Other than increasing the sensitivity in detecting *Leptospira* infection in cattle, a 'one health' approach has been used to further establish the cause of disease in humans.

Few articles in this review conducted seroprevalence or *Leptospira* exposure studies in dairy farmers and dairy cattle, particularly after the 1990s. During this period, such studies were probably not highly prioritized, given the high uptake of dairy cattle vaccination and a reduction in human cases and clinical disease in cattle. Before

Leptospira vaccination became widespread in New Zealand, some seroprevalence studies in dairy cattle were conducted to investigate leptospirosis status in particular regions to promote the vaccination campaign. These studies were conducted in Otago (Bolger, 1984), Gisborne (Neilson, 1984), Hawkes Bay (Price, 1984) and Taranaki (Bahaman *et al.*, 1984) involving mostly unvaccinated dairy herds. Results from the studies showed high seropositivity to serovar Hardjo or Pomona ranging from 95-100% in herd level

Reviewing previously gathered information has some limitations. For example the lack of details in some articles including information about serovars, vaccination status of cattle, species of animal being farmed and MAT titre and cut-point, makes it difficult to draw inferences across studies. Collection and reporting of more comprehensive data can improve the use of information in the articles in the future. Determination of the MAT titre cut-point depends on the purpose of the study. Higher cut off points were used in clinical reports: 400 for humans and of 1000 for animals. However, there is no definition in determining the cut-point for *Leptospira* exposure in humans or animals. The cut- point used were varied among articles in this review, ranging from 24 to 200; larger variation was found for studies in animals. As there were no original data available, it was not possible for the seropositivity across studies to be reanalysed.

2.6. Conclusion

In conclusion, despite long-term vaccination of dairy herds in New Zealand, dairy farmers remain the single largest farming occupation with notified leptospirosis. Hardjo is still dominant but the increasing importance of serovars Tarassovi and Ballum in human cases, where no serovar specific intervention is available, is concerning. The role of dairy cattle in the maintenance of these “non-vaccine” serovars needs to be clarified. Furthermore, the contribution of potential risk factors in dairy herds and dairy farmers/people with dairy cattle exposure need to be re-assessed.

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References

Adler B, Paul NL., Caroline EC, Mathieu P, David AH, William AE, . . . Richard LZ. *Leptospira* and leptospirosis (B. Adler Ed. Vol. 387). *Verlag Berlin Heidelberg: Springer*. 2015

Anonymous. Ruakura Animal Health Laboratory Report. *Surveillance, 1,* 10-15, 1974

Anonymous. Lincoln Animal Health Laboratory Report. *Surveillance, 4,* 19-22, 19771

Anonymous. Ruakura Animal Health Laboratory Report. *Surveillance, 4,* 13-17, 1977b

Anonymous. Ruakura Animal Health Laboratory Report. *Surveillance, 5,* 12-17, 1978

Anonymous. Whangarei Animal Health Laboratory Report. *Surveillance, 7,* 10-11, 1980

Anonymous. Quarterly review of diagnostic cases:July to September 2011. *Surveillance, 38,* 16-25, 2011

Anonymous. Quarterly report of diagnostic cases July to Sept 2013. *Surveillance, 40,* 11-22, 2013

Anonymous. Leptospirosis. Retrieved 19 October 2018. 2018a from https://www.health.govt.nz/our-work/diseases-and-conditions/communicable-disease-control-manual/leptospirosis#casedef

Anonymous. Quarterly report of diagnostic cases: January to March 2018. *Surveillance, 45,* 15-24, 2018b

Bahaman AR, Marshall RB, & Hellstrom JSA. Serological study of bovine leptospirosis in the district of taranaki. *New Zealand Veterinary Journal, 32*(8), 134-136.,1984.

Benschop J, Collins-Emerson J, Maskill A, O'Connor P, Tunbridge M, Yupiana Y, & Weston J Leptospirosis in three workers on a dairy farm with unvaccinated cattle. *New Zealand Medical Journal, 130*(1462), 102-108, 2017

Bettelheim KA, & Fogg TR. Serological studies on leptospiral antibodies in dairy milkers in three regions of the South Island of New Zealand. *Comp Immunol Microbiol Infect Dis, 9*(4), 355-364, 1986

Blackmore DK, & Schollum LM.Risks of contracting leptospirosis on the dairy farm. *New Zealand Medical Journal, 95*(716), 649-652, 1982

Blackmore DK, & Schollum LM.The serological investigation of occupational exposure to leptospirosis. VA: International Symposia on Veterinary Epidemiology and Economics, 1982b

Bolger JF. An investigation in Otago to support a programme to vaccinate dairy cattle against leptospirosis as a means of safeguarding human health. *Surveillance, 11,* 7-7, 1984

Bolin CA. Leptospirosis in cattle: disease review and update. *Proc NAVC, 2005*, 6944-6946, 2005

Bruere AN. An association between leptospirosis in calves and man. *Australian Veterinary Journal, 28*(7), 174-174, 1952

Carter ME, Cordes DO, Holland JT, Lewis SF, & Lake DE. Leptospirosis: II. Investigation of clinical disease in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*(9), 1982

Christmas BW, Tennent RB, Philip NA, Lindsay PG. Dairy farm fover in New Zealand.[i] A local outbreak of human leptospirosis. New Zealand Medical Journal.;79(514):901-4, 1974

Cordes DO, Carter ME, Townsend KG, Lewis SF, & Holland JTS. Leptospirosis: I. Clinical investigation of the infection in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*(8), 1982

Costa F, Hagan JE, Calcagno J, Kane M, Torgerson P, Martinez-Silveira MS, . . . Ko, AI Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLoS Neglected Tropical Diseases, 9*(9), 2015

Cowie G, & Bell A A retrospective review of notified human leptospirosis cases in the Waikato region of New Zealand, 2004 to 2010. *New Zealand Medical Journal, 125*, 20-28, 2012Davidson KR. *Leptospira*-hardjo infection in man associated with an outbreak in a dairy herd. *Australian Veterinary Journal, 47*(8), 408, 1971

Dhaliwal GS, Murray RD, & Ellis WA. Reproductive performance of dairy herds infected with *Leptospira interrogans* serovar Hardjo relative to the year of diagnosis. *Veterinary Record, 138*(12), 272-276, 1996

Dodd DC, & Brakenridge DT, *Leptospira icterohaemorrhagiae* AB infection in calves. *New Zealand Veterinary Journal, 8*(4), 71-76, 1960

Dreyfus A, Heuer C, Wilson P, Collins-Emerson J, Baker M G, & Benschop J .Risk of infection and associated influenza-like disease among abattoir workers due to two *Leptospira* species. *Epidemiology And Infection, 143*(10), 2015

Ensor CR, & McClure TJ. Bovine leptospirosis in Northland. *New Zealand Veterinary Journal, 1*(3), 47-50, 1953

ESR 2016: The Institute of Environmental Science and Research Ltd. (2017). *Notifiable Diseases in New Zealand: Annual Report 2016*.

Evangelista KV & Coburn J. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiology, 5*(9), 2010

Fang, F. Leptospirosis diagnostics and exposure at the human and animal interface in New Zealand *(Doctoral dissertation, Massey University, Palmerston North, New Zealand)*, 2014

Fang, F., Benschop, J., Wilson, P. R., Collins-Emerson, J. M., Heuer, C., & Prattley, D. (2014). Seroprevalence and exposure to risk factors for leptospirosis among veterinary students at Massey University. *New Zealand Veterinary Journal, 62*(3), 130-135.

Gibson I, & Varney K. Quarterly review of diagnostic cases - July to September 2008. *Surveillance, 35,* 11-16, 2008

Gill J. Bovine abortion-laboratory network findings 1989. *Surveillance, 17,* 18, 1990

Hartskeerl RA, Collares-PereiraM, & Ellis WA Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clinical Microbiology and Infection, 17*(4), 494-501, 2011

Hathaway SC, Blackmore DK & Marshall RB. Serologic and cultural prevalence of *Leptospira interrogans* serovar Balcanica in possums (*Trichosurus vulpecula*) in New Zealand. *Journal of Wildlife Diseases, 14*(3), 1978

Hellstrom JS. Studies on some aspects of the epidemiology of bovine leptospirosis. *PhD thesis* , Massey University, Palmerston North, NZ, 1978.

Henderson RJ. Q fever and leptospirosis in dairy farming community and allied workers of Worcestershire. *Journal of Clinical Pathology, 22*(5), 1969

Heuer C, Benschop J, Stringer L, Collins-Emerson J, Sanhueza J, & Wilson PR. *Leptospirosis vaccination in NZ - Best Practice recommendations vaccine use to prevent human exposure*, 2012

Hill F, McSporran KD, Reichel MP, & TisdallDJ. Leptospiral infection can be confirmed by polymerase chain reaction. *Vetscript, 20,* 26-27, 2007

Horner GW. Bovine abortions - laboratory findings. *Surveillance, 15,* 17-17, 1988

Kirschner L. Recent Studies on Leptospirosis in New Zealand. Infection with a New Type (*Leptospira mitis*) Johnson (Syn. *Lept. hyos*.) in Man and Animals. New Zealand Medical Journal;53(294):119-28, 1954

Levett PN. Leptospirosis. *clinical microbiology reviews, 14*(2), 296–326, 2001

LIC and DairyNZ Limited. *New Zealand Dairy Statistics 2013-14*,2014 available at: www.dairynz.co.nz/dairystatistics

LimmathurotsakulD, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol, Y, ChierakulW., . . . Peacock SJ. Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clinical infectious diseases, 55*(3), 322-331, 2012

Mackintosh CG. *A study of the epidemiology and control of leptospirosis on dairy farms.* dissertation Massey University, 1981

Mackintosh CG., Marshall RB., & BlackmoreDK. *Leptospira interrogans* serovar balcanica in cattle. *New Zealand Veterinary Journal, 28*(12), 268-268, 1980a

Mackintosh CG, Schollum LM, Blackmore DK & Marshall RB. Epidemiology of leptospirosis in dairy farm workers in the Manawatu. Part II. A case study of high and low risk farms. *New Zealand Veterinary Journal, 30*, 73-36, 1982

Mackintosh, CG, Schollum LM, Blackmore DK, Willis AF, Cook NR, Stoke JCJ & Harris RE. Epidemiology of leptospirosis in dairy farm workers in the Manawatu Part I: A cross-sectional serological survey and associated occupational factors. *New Zealand Veterinary Journal, 28*(12), 245-250, 1980b

Marshall RB. A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. *Israel journal of veterinary medicine, 43*, 271-276, 1987

Marshall RB & Chereshsky A. Vaccination of dairy cattle against leptospirosis as a means of preventing human infections. *Surveillance, 23,* 27-28, 1996a

Marshall RB & Manktelow BW. Fifty years of leptospirosis research in New Zealand: a perspective. *New Zealand Veterinary Journal, 50*(3), 61-63, 2002

Marshall RB, Vermunt JJ, & Collins-Emerson J. Is leptospirosis vaccination working? *Vetscript, 9,* 4-5, 1996b

McKenzie. Animal Health Laboratory Network review of diagnostic cases - April to June 1989. *Surveillance, 16,* 23-24, 1989

McLean M, Ruscoe Q, Kline T, King C, Nesdale A. A cluster of three cases of leptospirosis in dairy farm workers in New Zealand. The New Zealand Medical Journal (Online). 127(1388), 2014

Metcalfe RV, Bettelheim KA, Densham EB, Pearce J, Sillars H & Thorn C. Studies on antibody levels to Brucella abortus, Toxoplasma gondii and *Leptospira* serogroups in sera collected in five locations of the North Island of New Zealand. *Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene - Abt. 1 Orig. A, 249*(4), 543-556, 1981

Miller NG. A serologic investigation of leptospiral infections in dairy farmers and cattle ranchers. *American Journal of Hygiene, 74*(2), 203, 1961

Moller K, Newling PE, Robson HJ, Hansen GJ, & Meursinge JA. A survey of abortions and long return intervals in dairy herds in the Huntly district. *New Zealand Veterinary Journal, 15*(8), 137-142, 1967

Mwachui MA, Crump L, Hartskeerl R, Zinsstag J & Hattendorf J. Environmental and Behavioural Determinants of Leptospirosis Transmission: A Systematic Review. *PLoS Neglected Tropical Diseases, 9*(9), 2005

Neilson FJA. Project Report AH 390 - Prevalence of leptospirosis in the dairy herds of the Gisborne veterinary district. *Surveillance, 11,* 8-8, 1984Descriptive Epidemiology of Human Leptospirosis in New Zealand: 1999 to 2016. (Nisa S, personal communication, September 10, 2018)

NZIER. *Dairy trade's economic contribution to New Zealand* available at: https://nzier.org.nz/static/media/filer_public/29/33/29336237-3350-40ce-9933-a5a59d25bd31/dairy_economic_contribution_update_final_21_february_2017.pdf, 2017

Orr MB. Animal Health Laboratory Network review of diagnostic cases - October to December 1989. *Surveillance, 17,* 3-4, 1990

Orr MB. Animal Health Laboratory Network review of diagnostic cases - January to March 1994. *Surveillance, 21,* 3-6, 1994

Price MC. Project Report AH 396 - Survey of the prevalence of leptospirosis in dairy herds in the Hastings veterinary district. *Surveillance, 11,* 7-7, 1984

Ryan TJ, SceatsJ, Penniket JH, & Maguire MA. Leptospirosis study seeks link between herd vaccination and human health status. *Surveillance, New Zealand, 8*(4), 7-10, 1982

Sanhueza JM, Heuer C, & West D. Contribution of *Leptospira*, Neospora caninum and bovine viral diarrhea virus to fetal loss of beef cattle in New Zealand. *Preventive Veterinary Medicine, 112*(1-2), 90-98, 2013

Sanhueza JM, Heuer C, Wilson PR, Benschop J, & Collins-Emerson JM. Prevalence and risk factors for *Leptospira* exposure in New Zealand veterinarians. *Epidemiology Infection, 143*(10), 2015

Shenberg E, Bitnun S, & Birnbaum SH. Leptospirosis among dairy workers in the valley of Jezreel: first recorded isolation of *Leptospira* Hardjo in Israel. *Israel Journal of Medical Sciences, 13*(4), 377-384, 1977

StatsNZ. Dairy export prices and volumes move upwards over 20 years. avaialable at: http://archive.stats.govt.nz/tools_and_services/newsletters/price-index-news/oct-13-dairy-exports.aspxs,2013

Thomas, M. C., Chereshsky, A., & Manning, K. (1994). An outbreak of leptospirosis on a single farm in east Otago. *New Zealand Medical Journal, 107*(982), 290-291.

Thornley CN, Baker MG, Weinstein P, & Maas EW. Changing epidemiology of human leptospirosis in New Zealand. *Epidemiology And Infection, 128*(01), 29-36, 2002

Thornton R. Animal Health Laboratory Network News. *Vetscript April,* 21-22, 1992

Thornton R. Bovine abortion diagnoses 1995. *Surveillance, 23,* 21-22, 1996

VarneyK, & Gibson I. Quarterly review of diagnostic cases - October to December 2007. *Surveillance, 35,* 11-15, 2008

Vijayachari P, Sugunan AP, & Shriram AN. Leptospirosis: an emerging global public health problem. *Journal of biosciences, 33*(4), 557-569, 2008

Wael F, Bruce M, Holt HR, Eltholth MM, & Merien F. Update on the status of leptospirosis in New Zealand. *Acta Tropica, 188*, 161-167, 2018

Waitkins S. Leptospirosis as an occupational disease. *British Journal of Industrial Medicine, 43*(11), 721-725, 1986

Weston JF, Heuer, C, Parkinson TJ, & Williamson NB. Causes of abortion on New Zealand dairy farms with a history of abortion associated with Neospora caninum. *New Zealand Veterinary Journal, 60*(1), 27-34, 2012

Wilson P, Heuer C, Benschop J, Collins-Emerson J, Parramore JJ, & Meenks MBRDoes long-term vaccination eliminate leptospiral shedding? A pilot study of New Zealand dairy cattle herds. *Vetscript, 26,* 16-16, 2013

Yupiana Y, Wilson PR, Weston JF, Vallée E, Collins-Emerson JM, Benschop J, . . . Heuer C. Epidemiological investigation of *Leptospira spp.* in a dairy farming enterprise after the occurrence of three human leptospirosis cases. *Zoonoses And Public Health*, 2019

Chapter 3
Epidemiological investigation of *Leptospira* spp. in a dairy farming enterprise after the occurrence of three human leptospirosis cases

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3.1. Summary

An epidemiological investigation was conducted in an unvaccinated dairy farming enterprise in which three workers on one of the milking herds (Herd 1) were diagnosed with leptospirosis due to serovars Hardjo (H) (n=2) and Pomona (P) (n=1) between January and March 2015. Blood and urine samples were collected from milking cows in Herd 1 (N=230) and Herd 2 (N=400), rising-one- (R1, N=125) and rising-two-year-old (R2, N=130) replacement heifers, and four pigs associated with Herd 1, in March 2015. Sera were tested using the MAT for serovars H, P, Copenhageni (C), Ballum (B) and Tarassovi (T), and urine samples were tested by qPCR. Seventy five percent of 109 cows in Herd 1 and 36% of 121 in Herd 2 were seropositive (≥48), predominantly to H and P, and 23% of 74 cows in Herd 1 and 1% of 90 cows in Herd 2 were qPCR positive. Fifty five percent of 42 R2 heifers were seropositive to T. No R1 and 17% of 42 R2 heifers were qPCR positive.

Subsequently, all cattle were vaccinated for H and P, and Herds 1 and 2 were given amoxicillin. After the booster vaccination, 7% of 91 in Herd 1, 2% of 82 in Herd 2 and 11% of 38 R1 heifers (sampled as R2) were PCR positive. After the amoxicillin treatment, no cows in Herd 1 and 5% of 62 cows in Herd 2 were urine PCR positive. Calves and pigs were seropositive to H, P, C and B.

Vaccination and antibiotic treatment appeared effective in reducing the risk of exposure of workers to vaccine serovars. However, evidence of non-vaccine serovars indicated that workers likely remain at risk of exposure to *Leptospira.*

3.2. Introduction

Leptospirosis is a zoonotic disease caused by pathogenic bacteria of the genus *Leptospira*. In New Zealand, six serovars belonging to two pathogenic species are known to be endemic in animals, namely *Leptospira borgpetersenii* serovars Hardjo (H), Ballum (B), Balcanica and Tarassovi (T) and *Leptospira interrogans* serovars Pomona (P) and Copenhageni (C) (Marshall & Manktelow, 2002). Cattle are considered to be maintenance hosts for serovar H and pigs for serovars P and T (Hathaway, 1981). Serovars Balcanica, Ballum and Tarassovi are maintained by wildlife(Hathaway, 1981). Accidental infection of humans from livestock commonly occurs in New Zealand (ESR, 2013-2017) but human-to-human infections are rarely reported globally (Adler & de la Peña Moctezuma, 2010) .

All serovars endemic in animals have been reported in human leptospirosis cases in New Zealand, with serovars H, P, B (Mackintosh et al., 1980b; Thornley et al.,2002) and T (ESR 2013-2017)reported most frequently. In the early 1980s, leptospirosis vaccination was initiated in dairy cattle and pigs in New Zealand due to high *Leptospira* transmission from these livestock to humans. Vaccination was associated with a significant decrease in the number of human cases (Marshall and Manktelow 2002).

Currently, approximately 95% of dairy herds in New Zealand use either a bivalent vaccine with serovars H and P or a trivalent vaccine with serovars H, P and C (Yupiana *et al.*, 2017b). In New Zealand, farmers have a legal requirement to protect workers from health and safety risks including zoonotic diseases. For leptospirosis, animal vaccination has been recommended as a long term strategy (WorkSafe New Zealand, 2015). However, leptospirosis cases are still reported in dairy farm workers (McLean, 2014). From 2012-2016, there were 376 reported cases of human leptospirosis in New Zealand among which 297 cases were in people working in high-risk occupations. Of those, 63% were farmers (ESR 2013-2017). Most reported being in contact with unvaccinated or poorly vaccinated herds (Christmas et al., 1974; McLean, 2014). There have been no recent published reports of epidemiological investigations of *Leptospira*

infection on farms where workers have been affected, or of the effectiveness of livestock vaccination programmes *per se* in minimising shedding and risk to workers.

This case study describes an epidemiological investigation of *Leptospira* infection and control in two unvaccinated dairy herds in a farming enterprise that had three cases of leptospirosis in workers within three months (Benschop *et al.*, 2017).

3.3. Materials and Methods

This was an opportunistic case study arising from clinical leptospirosis in three workers on a seasonal-supply dairy farming enterprise located in the lower North Island of New Zealand, diagnosed between January 25 and March 14, 2015 (Benschop *et al.*, 2017). Two cases were confirmed as H and one as P.

3.3.1. Farming enterprise and animals

The farming enterprise consisted of Herd 1 (H1) comprising adult (3-years and older) cows only and Herd 2 (H2) comprising adult cows and first lactation heifers, grazed separately without direct contact on adjacent areas (Farms 1 and 2, respectively, Figure 3.3-1). There were 230 milking cows in H1 grazing 130 hectares and milked in a rotary shed, and 400 milking cows in H2 grazing 190 hectares and milked in a herringbone shed. Rising one-year-old (R1) and pregnant R2 replacement heifers were managed on a third area (Farm 3, Figure 3.3-1) a short distance from the milking herd farms. Breeding bulls and pigs were present on Farm 1. There was no clinical evidence of leptospirosis in either cattle or pigs. Before the outbreak of leptospirosis in farm workers, *Leptospira* vaccination had not been undertaken for at least twenty years, and there was no rodent control programme in place. The three affected workers had been working solely with the cattle in H1.

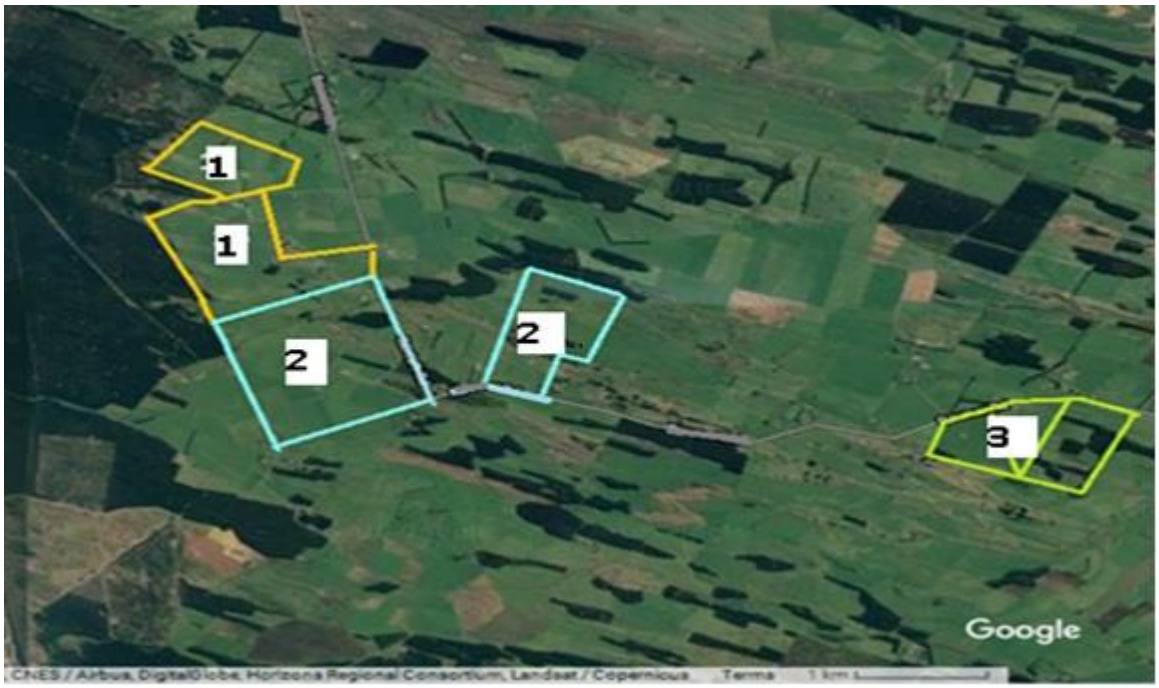

Figure 3.3-1.. Map showing location of Farm 1, grazing herd 1, Farm 2, grazing herd 2, and Farm 3, co-grazing rising one-year-old and rising two-year-old heifers from both herds 1 and 2.

3.3.2. Study design

On March 6, within a week of the second human confirmed case, initial screening was undertaken with blood and urine samples collected from a sample of adult milking cows in H1 and H2 to establish serological status. Positive MAT results prompted further sampling including rising 1-year-old (R1) and pregnant rising 2-year-old (R2) heifer replacements on Farm 3, and calves and pigs on Farm 1, as presented in Table 1.

For the initial screening, sample sizes were calculated to detect *Leptospira* urinary shedding, given an expected prevalence of 10%, at p=0.05, with 80% power, using PCR with sensitivity (Se) of 0.53 and specificity (Sp) of 0.96. Forty cows needed to be sampled from H1 and 45 cows from H2. Further power analyses were undertaken for each herd for testing the effectiveness of vaccination and antibiotic intervention on the reduction of shedding. To detect a reduction in *Leptospira* shedding from 30% before to 6% after intervention, with 80% power and 95% confidence, 60 animals sampled three times were required from H1. To detect a reduction in *Leptospira*

shedding from 20% before to 4% after vaccination and antibiotic treatment, with 80% power and 95% confidence, 80 animals sampled three times were required from H2. Additional sampling was therefore undertaken on March 18 and 19 to achieve the required power prior to intervention. Based on the assumption that the prevalence would be similar in R1 and R2 heifers, to detect a decrease in prevalence of shedding from 40% before to 4% after vaccination with 80% power and 95% confidence, 40 animals were required in each age category. Sampling four of the six pigs was sufficient to determine exposure rate, and sampling of 60 calves born July-August during the 2015 calving period was sufficient to investigate maternal antibody and/or early post-natal infection.

Thus, the first stage of the investigation involved collection of blood and urine samples from adult cows in H1 and H2 on March 6 and 12-13 days later. As there was no significant difference (Pearson's Chi-squared, >0.05) in seroprevalence for H and P between those sampling days (Table 3.3-1) the data were combined and designated as the initial investigation. Subsequent sampling episodes for H1 and H2 are referred to as "post-vaccination" (May 20/27 2015) and "post-vaccination/antibiotic" (Jan 19/20, 2016). Sampling of R1 and R2 heifers in March 2015 is referred to as "pre-vaccination". Sampling in November 2015 of those R1 heifers, which became R2 heifers in July/August is referred to as "post-vaccination".

Animal ethics approval was granted by the Massey University Animal Ethics Committee, protocol 15/27.

Table 3.3-1. Timeline for blood (B) and urine (U) sampling (number of samples in brackets), vaccination and antibiotic treatment for adult cows in Herds 1 (H1) and 2 (H2), and rising-one-year-old (R1) and rising-two-year-old (R2) heifers, calves (C; born August & September 2015), and pigs (P), in 2015 and 2016. * These animals have transitioned from R1 in July/August 2015.

Animal group	2015														2016	
	Mar,6	Mar,17	Mar,18	Mar,19	Mar,20	Mar,27	Mar,27	Apr,16	Apr,24	May,20	May,27	May,27	Oct,7	Nov,3	Jan,19	Jan,20
H1	B(41)	Sensitiser Vaccination		B(68)				Booster Vaccination				Antibiotic at drying off				B(85)
	U(41)			U(33)							U(91)					U(60)
H2	B(39)		B(82)												B(81)	
	U(22)		U(68)							U(89)					U(62)	
R1						B(41)	Sensitiser Vaccination		Booster Vaccination							
						U(41)										
R2						B(42)								B(38)*		
						U(42)								U(38)*		
C													B(61)			
P					B(4)											

3.3.3. Blood and urine collection

The sampling schedule is described in Table 3.3-1. For H1, H2, paired blood and urine samples were collected where possible. Post-vaccination, only urine samples were collected, targeting previously sampled cows where possible.

Blood samples were collected by venipuncture from the coccygeal vein in adult cattle, the jugular vein in calves, and by anterior vena cava puncture in pigs, into a 10 ml plain (red top) evacuated plastic tube without anticoagulant. Urine samples were collected into a 50 ml clean plastic container either from spontaneous urination or urination induced by stimulating the ventral vulva. Blood and urine samples were packed separately in plastic bags and taken in an insulated container on ice to the ^{m}Epilab, Massey University where they were processed within 24 hours of collection.

3.3.4. Vaccination and antibiotic treatment

Intervention involved both vaccination and treatment with antibiotic in H1 and H2, and vaccination alone in R1 and R2 heifers (Table 3.3-1).

Vaccination was by subcutaneous injection using a bivalent *Leptospira* vaccine (Leptoshield, Pfizer Animal Health, West Ryde, NSW, Australia) that contained antigens from serovars H and P. A single dose of long-acting amoxicillin (15 mg/kg, IM, Betamox LA, Noorbook, VIC Australia) was administered subcutaneously, delayed until the end of lactation to avoid milk withholding time and disposal.

3.3.5. MAT and qPCR

The MAT was performed at the ^{m}Epilab, Massey University. Blood samples were centrifuged at 1,300 *g* for 10 minutes and sera collected as supernatant. Thirty µL of each serum was mixed with 150 µL sterile standard saline into 96 well plates as a masterplate to make 1/6 dilution for testing. Master plates were then stored at -20°C. The remaining sera were stored at -80°C.

Serum samples were tested against serovars H, P, C, B and T. The MAT was performed as described by Fang et al. (2014), based on the method described by Faine (1982). Eight serial, two-fold dilutions were prepared in standard saline and ranged from 1:24 to 1:3072 (final dilution inclusive of antigen). A positive control using standard antisera against each serovar and a negative control using standard saline were prepared in a similar way. The dilutions were incubated with live cultures for 2 hours at 20-30 °C. A reciprocal titre of $\geq$1:48 test was considered positive. The end-point titre was the lowest dilution where approximately 50% or more of the leptospires were agglutinated or lysed.

Ten mL of each urine sample was centrifuged at 1,300 *g* for 10 minutes after which approximately 8 mL of supernatant was discarded using a transfer pipette (Raylab, Auckland, New Zealand). A 1.2 mL aliquot of the remaining urine and pellet was transferred into a 1.5 mL microfuge tube and centrifuged at 10,625 *g* for 20 minutes and then re-suspended in 200 µL PBS after discarding the supernatant. DNA extraction was performed using the QIAamp DNA mini kit (Qiagen) as per manufacturer's instructions. DNA was eluted in a final volume of 200 µL of elution buffer and stored at -20°C for Polymerase Chain Reaction (PCR) testing.

The qPCR assay was based on the method developed in the ^{m}EpiLab by Subharat et al. (2011) and refined by Fang et.al. (2014). Green-fluorescent nucleic acid stain SYTO9 was used as the intercalating dye. Primers 2For (5'-TGAGCCAAGAAGAAACAAGCTACA-3') and 504Rev (5'-MATGGTTCCRCTTTCCGAAGA-3') were used to amplify the *gyrB* gene. The 25-µL reaction included 2.5 µM SYTO9, 1× PCR buffer, 1.5 mM magnesium chloride ($MgCl_2$), 200 µM deoxyribonucleotide tri-phosphates, 5 pmol of 2For and 504Rev, 1 unit of Taq DNA polymerase, 2 µL of DNA extract, and double-distilled water (ddH_2O). Thermal cycling comprised initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 63°C for 20 sec, and extension at 72°C for 20 sec. Fluorescence readings were taken at the end of each extension cycle in the F1 (SYBR Green) channel. Melting curve analysis was performed by heating the PCR product from 78°C to 90°C and monitoring the fluorescence change every 0.2°C. The positive

control was serovar Pomona (laboratory strain), and distilled water was used as the negative control. Samples were considered positive, if a similar melting temperature (± 0.5°C) and a similar melting curve to the positive controls were produced.

3.3.6. Statistical analysis

All statistical analyses were performed using R version 3.3.2 (2016-10-31). Geometric mean titre (GMT) was calculated for positive samples. Student's t-test was used to compare the GMT between herds and between sampling times within herds, and Pearson's Chi-square with Yates' continuity correction was used to compare the proportion of positive to PCR and MAT between herds and sampling times. The 95% confidence intervals for proportions were calculated using Wilson's method (Newcombe, 1998).

3.4. Results

3.4.1. Herds 1 and 2

Initial investigation

Seroprevalence and GMT data from the initial investigation are presented in Table 3.4-1, with MAT titre distributions presented in Figures 3.4-1 and 3.4-2 Eighty-two cows (75%, 95% CI: 66-82%) in H1 and 43 (36%, 95% CI: 28-44%) cows in H2 were seropositive to at least one serovar. Cattle in H1 were positive against serovars H, P, C, B and T and cattle in H2 were positive against H, P, and B. The highest seroprevalence was for H and P in both herds. Urine qPCR prior to intervention (Table 3.4-2) was positive in 17 (23%, 95% CI: 15-34) cows in H1 and one (1%, 95% CI: 0-6) cows in H2

Post-intervention

Seroprevalence and GMT data from the post-intervention samplings are presented in Table 3.4-1 and MAT titre distributions are included in Figures 3.4-1 and 3.4-2. The proportion of cows seropositive to at least one serovar was lower after vaccination and antibiotic treatment in H1 (P=0.05). There was a reduction in seroprevalence for H in

H1 (p<0.001). There was no difference in within-herd seroprevalence between samplings for other serovars.

Table 3.4-1. Number of cows tested in herds 1 (H1) and 2 (H2) and % MAT positive (titre ≥48) (95% CI) to Hardjo (H), Pomona (P), Copenhageni (C), Ballum (B), Tarassovi (T) and overall, and geometric mean titre (GMT) (95% CI) of positive samples, at the initial investigation in March 2015 (Initial) and January, 2016, 8-10 months after vaccination and antibiotic treatment (post V/Ab)). January 2016 titres for Hardjo and Pomona are post-vaccination.

Herd	Sampling occasion	No. of sample	Seropositivity	Serovar					
				H	P	C	B	T	Overall
H1	Initial	109	Prev (%)	41	46	19	8	2	75
			(95%CI)	(33-51)	(37-55)	(13-28)	(4-15)	(0-6)	(66-82)
			GMT	186	435	117	56	272	
			(95%CI)	(136-255)	(293-646)	(76-179)	(44-71)	*(48-768)	
	Post-V/Ab	85	Prev (%)	17	51	11	2	4	61
			(95%CI)	(10-26)	(40-61)	(6-19)	(1-8)	(1-10)	(51-71)
			GMT	68	192	76	68	76	
			(95%CI)	(55-84)	(134-272)	(48-121)	*(48-96)	(28-206)	
H2	Initial	121	Prev (%)	31	16	0	1	0	36
			(95%CI)	(24-40)	(10-23)	(0-3)	(0-5)	(0-3)	(28-44)
			GMT	226	107	0	0	0	
			(95%CI)	(163-314)	(71-160)	NA	NA	NA	
	Post-V/Ab	81	Prev (%)	22	15	1	4	0	32
			(95%CI)	(15-32)	(9-24)	(0-7)	(1-10)	(0-5)	(23-43)
			GMT	100	102	96	60	0	
			(95%CI)	(72-138)	(68-152)	*(96)	(22-163)	NA	

The post-vaccination/antibiotic GMTs for serovar Hardjo in both H1 and H2 were significantly lower (*P*<0.001 and *P*=0.018, respectively) than at the initial investigation. The GMT for serovar Ballum in H2 was significantly higher (*P*<0.001) at the post-vaccination/antibiotic sampling.

Urine qPCR data post-intervention for H1 and H2 are presented in Table 3.4-2. The proportion of cows positive at the initial sampling was higher (p=0.006) than at the

post-vaccination sampling. No urine samples were qPCR positive at the post-vaccination/antibiotic sampling in H1, but five were positive in H2, likely due to infection with B according to serology (Table 3.4-1).

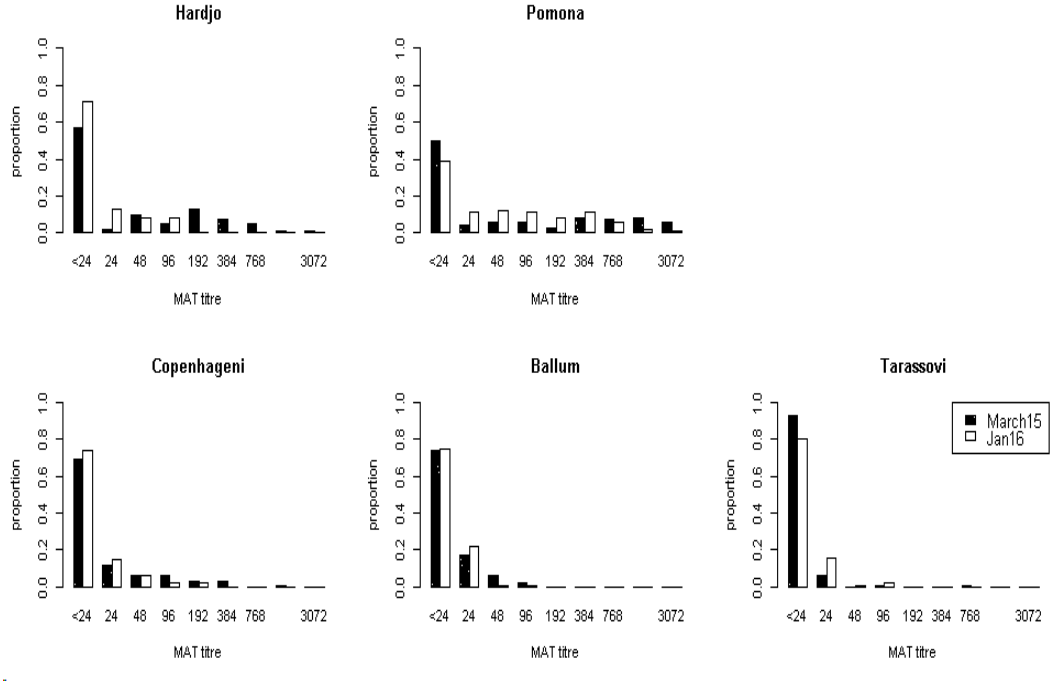

Figure 3.4-1. Proportion of cows in Herd 1 at each MAT titre for each serovar, at the initial sampling in March 2015 (n=109) and at the post-vaccination/antibiotic sampling in January 2016 (n=85). Note: January 2016 titres for Hardjo and Pomona are post-vaccination.

Table 3.4-2. Number of urine samples qPCR tested and proportion positive in March 2015 (initial), May 2015 (post-vaccination) and January 2016 (post-vaccination and antibiotic) in herds H1 and H2.

Herd	Sampling occasion	No. samples	% positive (95% CI)
	Initial	74	23 (15-34)
H1	Post vaccination	91	7 (3-14)
	Post vaccination/antibiotic	60	0 (0-6)
	Initial	90	1 (0-6)
H2	Post vaccination	89	2 (0-8)
	Post vaccination/antibiotic	62	5 (2-13).

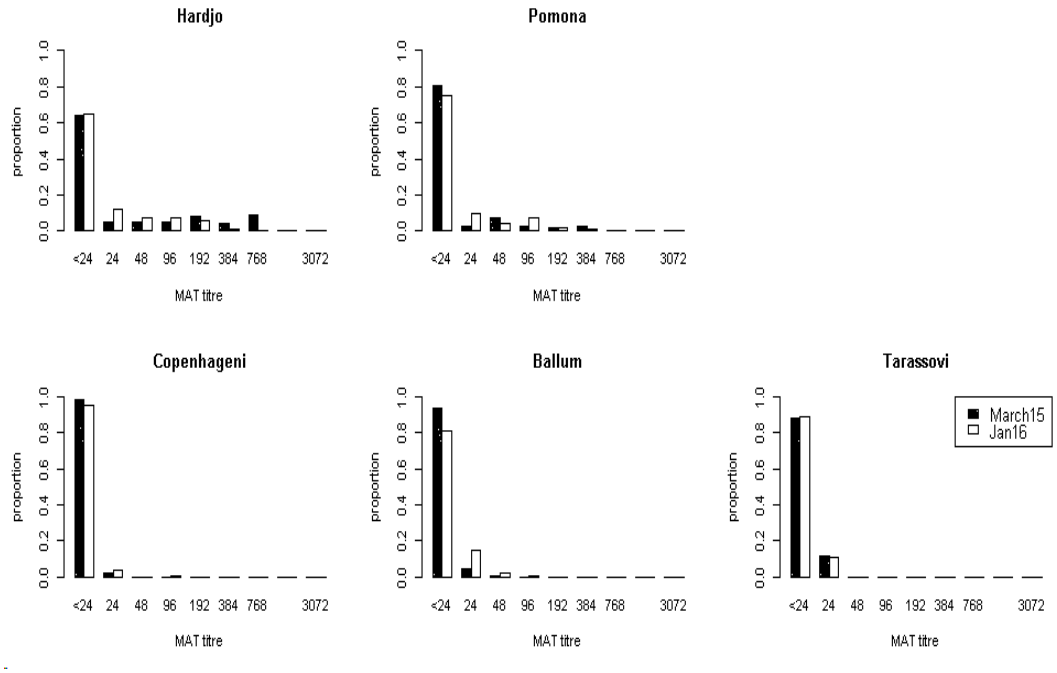

Figure 3.4-2. Proportion of cows in Herd 2 at each MAT titre for each serovar at the initial sampling in March 2015 (n=121) and at the post-vaccination/antibiotic sampling in January 2016 (n=81). Note: January 2016 titres for Hardjo and Pomona are post-vaccination.

3.4.2. Rising one- and two-year-old heifers

Pre-vaccination

Seroprevalence and GMT data are presented in Table 3.4-3 and the proportion at each titre is presented in Figures 3.4-3 and 3.4-4. While few R1 heifers were seropositive at the pre-vaccination screening, all were positive to at least one serovar post-intervention in November. None were positive for C or T at that sampling. The post-vaccination GMT in November was higher for H, P and B (P<0.001) than in March. Pre-vaccination screening showed that the majority of R2 heifers were seropositive to at least one serovar with 55% seropositive to T. The highest GMT was for T.

No urine sample collected from 41 R1 heifers in March 2015 was positive pre-vaccination but post-vaccination, as R2 heifers, four of 38 were positive, with serological evidence suggesting infection with B. Of 42 urine samples collected from R2

heifers in March 2015 pre-vaccination, 17% (95% CI: 5-28) were PCR positive with serological evidence suggesting infection with T.

Table 3.4-3. Number of Rising 1- (R1) and Rising two-year old (R2) heifers tested and % MAT positive (titre ≥48) (95% CI) to Hardjo(H), Pomona (P), Copenhageni (C), Ballum (B), Tarassovi (T), and overall, and geometric mean titre (GMT)(95% CI) of positives, pre-vaccination, in March (pre-vaccination) and November 2015 (post-vaccination).

Age Group	Sampling occasion	No. of samples	seropositivity	Serovar					
				H	P	C	B	T	Overall
R1	Pre-vaccination	41	Prev (%) (95%CI)	0 (0-9)	0 (0-9)	2 (0-13)	2 (0-13)	0 (0-9)	5 (1-16)
			GMT	0	0	48 0	48 0	0	
	Post-vaccination*	38	Prev (%) (95%CI)	97 (87-100)	76 (61-87)	0 (0-9)	73 (58-85)	0 (0-9)	100 (91-100)
			GMT	127 (102-158)	92 (77-109)	0	73 (59-90)	0	
R2	Pre-vaccination	42	Prev (%) (95%CI)	2 (0-12)	0 (0-8)	5 (1-16)	0 (0-8)	55 (40-69)	57 (42-71)
			GMT	48 (NA)	0	68 (1-5537)	0	230 (142-376)	

Note: November titres for Hardjo and Pomona are post-vaccination *These are categorized as R2 animals from July/August 2015.

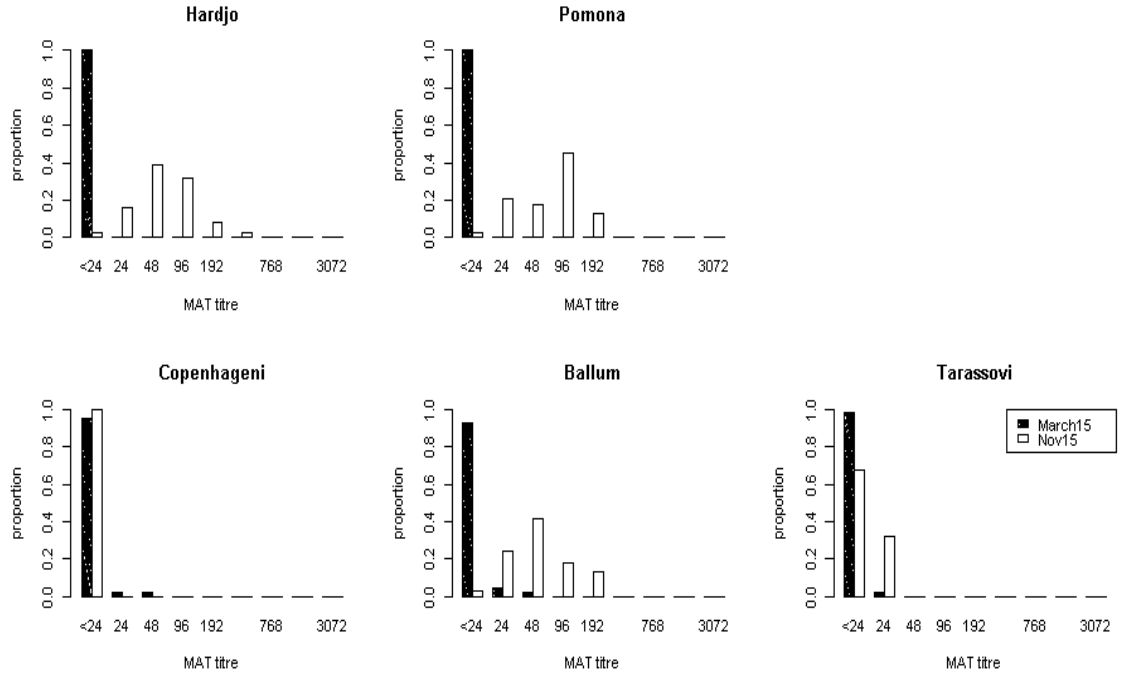

Figure 3.4-3. Proportion of rising one-year-old heifers at each MAT titre for each serovar in March 2015, pre-vaccination (Hardjo/Pomona) (n=41) and the same animals as R2 in November 2015, post-vaccination (n=38).

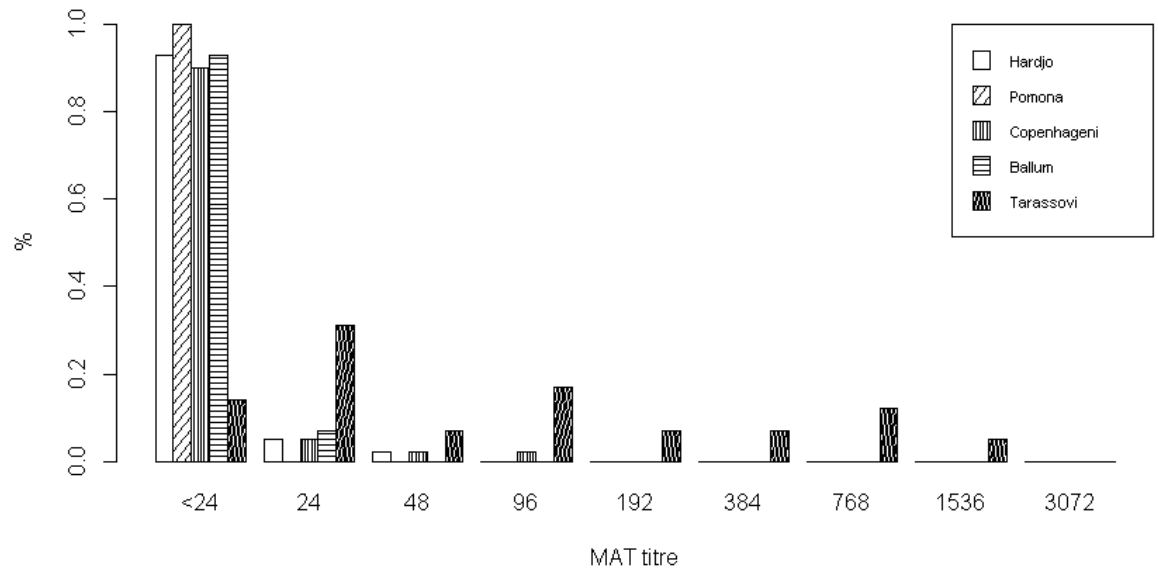

Figure 3.4-4. Proportion of R2 heifers at each MAT titre each serovar pre-vaccination in March 2015 (n=41)

3.4.3. Calves

Data are presented in Table 3.4.-4. Data suggest maternal antibody titres to H and P and recent exposure to B.

Table 3.4-4. Seroprevalence (95% CI) and MAT titre for each serovar in calves (n=61) born from Hardjo/Pomona vaccinated dams in July/August and sampled in October 2015. Note: these samples were prior to vaccination as calves.

Serovar tested	Seroprevalence (%) (95% CI)	GMT (95% CI)	MAT titre				
			48	96	192	384	768
Hardjo	36 (25-49)	75 (60-93)	11	8	3	0	0
Pomona	24 (16-37)	101 (69-146)	5	5	4	1	0
Copenhageni	2 (0.2-9)	48 (0)	1	0	0	0	0
Ballum	7 (3-16)	323 (113-926)	0	0	2	1	1
Tarassovi	0	0	0	0	0	0	0

3.4.4. Pigs

Data are presented in Table 3.4-5. Titres suggest recent exposure to P, C and B.

Table 3.4-5. MAT titres for five serovars from four pigs sampled in March 2015

Pig	MAT titre of serovar tested				
	Hardjo	Pomona	Copenhageni	Ballum	Tarassovi
1	0	1536	384	48	0
2	0	3072	192	24	0
3	48	1536	768	24	0
4	48	1536	1536	96	0

3.5. Discussion

This was an opportunistic epidemiological investigation of *Leptospira* spp. after three human leptospirosis cases amongst workers, and was designed to identify the sources of exposure to the workers, and to evaluate the effectiveness of vaccine and antibiotic interventions.

Since almost all dairy herds in New Zealand are vaccinated for serovars H and P (Yupiana *et al.*, 2017b) this was a rare opportunity to reinforce the link between failure to vaccinate and leptospirosis cases in workers. The study demonstrated a high *Leptospira* seroprevalence in cattle and pigs, and significant urinary shedding in cattle. Concurrence of serovars H and P between worker cases (Benschop *et al.*, 2017) and lactating cattle, strongly supports that transmission was from that source either directly or indirectly. This study demonstrated that vaccination, alone or in combination with antibiotic, was effective in reducing and possibly eliminating urinary shedding of vaccine serovars. However, evidence of shedding of serovars C, B and T, which are not in the vaccine used, demonstrated that workers remained at risk of exposure of *Leptospira per se,* and therefore, that other protective measures should be routinely adopted.

In New Zealand, H and P have historically been the predominant serovars found in leptospirosis cases among farm workers (Thornley *et al.*, 2002). This study confirms that the risk remains in unvaccinated herds. However, in addition to infection with H and P, recent reports (ESR 2013-2017) show an increasing proportion of cases associated with Ballum and Tarassovi, both of which were identified in this study, particularly in replacement heifers.

That all the worker cases were from H1 using the rotary milking shed, could suggest that this system may have inherently greater risk for transmission than the herringbone system used for H2. However, at the initial investigation, implemented immediately after notification of the disease among workers, the proportion of cows shedding *Leptospira* was 23 times higher in H1 than H2 despite that H1 had 42% fewer

cows milked. Extrapolation suggests that approximately 53 cows in H1 were shedding at the initial investigation compared with four in H2. This suggests that workers were infected as a result of the high challenge associated with the urinary shedding rate *per se* rather than inherent risk of rotary milking systems. Higher seroprevalence, and higher urinary shedding rate in H1 at the time of three worker cases within a short period, suggests that there was active epidemic infection in H1 likely related to recent exposure, whereas the serology and PCR results for H2 suggest endemic infection. Alternatively, exposure may have been by indirect contact with effluent since at least one worker reported gross contamination during effluent management. Other environmental exposure cannot be discounted.

During the initial investigation, serovars C and T were detected serologically in H1 but not H2. Serovar B was detected in both herds, at low prevalence. Exposure to wildlife might explain the differences as about 50% of Farm 1 was bordered by forest, while less than 10% of Farm 2 was bordered by forest. Various wildlife species are reservoir hosts for serovars C, B and T in New Zealand (Hathaway & Blackmore, 1981). Antibodies to those serovars were also variably observed in R1 and R2 heifers, with seroprevalence up to 55% for T, while seroprevalence was 2% for H in R2 and zero for P in both R1 and R2. These age-groups also had exposure to wildlife.

At the post vaccine/antibiotic sampling in January, it was notable that none of the cows sampled in H2 were positive to T despite that heifers, which were initially sampled as R2 and which were combined with H2 prior to calving, had a seroprevalence to T of 55% at the initial sampling. This suggests that this serovar had not been transmitted from the introduced heifers to the older cows in the herd, since only the latter were sampled after amalgamation. Higher than previously reported seroprevalence of T in dairy cattle has been identified only recently (Yupiana *et al.*, 2017b), so little is understood about its epidemiology, hence further study is required.

Despite a relatively high proportion of cattle being seropositive and some having high antibody titres, no signs of clinical leptospirosis were detected in any cattle age group

or pigs. One possible reason for this is that most cows were infected with serovar H, a cattle-adapted serovar for which infection is usually subclinical (Lilenbaum & Martins, 2014). However, serovar P, a non-adapted serovar in cattle, was also found in both herds, as were serovars C and B, and additionally T in H1. This suggests that herd immunity was sufficient to prevent clinical disease, but not shedding, or that these serovars were not particularly virulent in this herd.

Leptospira infection in pigs possibly occurred through transmission from cattle as both pigs and cattle on Farm 1 had serovars P and C. However, transmission from pigs to cattle, or concurrent exposure from an external source, particularly rodents in the case of C, cannot be discounted. Pomona is an adapted serovar in pigs (Adler & de la Peña Moctezuma, 2010). Copenhageni has also been detected in pigs in New Zealand (Kirschner, 1954). However, there is a possibility of cross-reaction between strains of P and C that could have contributed to these results from the pigs (Ryan 1978, cited in (Hellstrom, 1978) though given the observed distribution of titres, this appears unlikely.

High seroprevalence of T was found in R2 heifers at the initial investigation but seroprevalence in other groups was low. Additionally, C and B were present in heifers and PCR data suggest that some or all of these serovars were being shed in urine. These serovars could therefore pose a risk to workers directly, or subsequently, via amplification in older cows once those heifers were merged with the adult milking cows prior to calving. A recent study of 200 dairy herds in New Zealand has shown evidence of *Leptospira* shedding in 26.5% of herds and 2.4% of cows in vaccinated herds, with serological evidence for Tarassovi, and DNA evidence of a Tarassovi-like strain (Yupiana *et al.*, 2017b). Serological and PCR evidence from this herd is therefore not unlike that of many herds throughout New Zealand in which evidence is emerging for infection with this non-vaccination serovar. This is supported by recent evidence of this serovar in human cases (ESR 2013-2017) . Workers were therefore advised to practise protective measures such as wearing protective clothing during milking, covering wounds, avoiding direct contact with effluent, and protecting their face from

urine splash (Mackintosh et al., 1980b) rather than rely on vaccination and antibiotic treatment alone.

The PCR used in this study identified pathogenic *Leptospira* and did not differentiate between serovars. However, in New Zealand, since there are few serovars, with limited serological cross-reactivity between them, it has been proposed that parallel consideration of serology and urine PCR results allows reasonable specificity of diagnosis of serovar (Subharat *et al.*, 2011). Hence, it appears reasonable to suggest that the serovars shed in urine at the initial investigation were likely to be H and P, and that as the study progressed, C, B and T were also variably shed in urine, particularly in heifers.

Serological data for H and P from calves may represent maternal antibody, but exposure cannot be excluded as titres of 192-384 are unlikely to represent maternal antibody 2-3 months after birth, and are potentially predictive of active infection in dairy cattle (Yupiana et al., 2017). Serological evidence suggests environmental exposure to B, and when combined with results from other age groups, suggests that this organism may be prevalent in mice, its reservoir host species. The presence of antibodies to C and P suggest these serovars may also be circulating in wildlife endemic to the farm. A recent survey of wildlife in the proximity of this farm confirmed a high prevalence of C in mice (Moinet *et al.*, 2017).

Leptospira vaccination *per se* is efficacious in preventing renal colonization and urinary shedding (Mackintosh et al., 1980a), particularly if vaccination occurs prior to exposure. Long-term vaccination programmes, which are implemented in more than 95% of dairy herds with bivalent (H and P) or trivalent (H, P and C) vaccines in New Zealand, are effective in preventing shedding in adult cows (Yupiana *et al.*, 2017b). In H1, reduction in shedding was observed after bivalent vaccination alone, and elimination of shedding was observed after vaccination and antibiotic. However, in H2 there was an increase in prevalence of shedding after each intervention. In this herd, serological evidence suggests that the shedding was likely due to non-vaccine serovars,

particularly B but possibly also C, since there was an increase in seroprevalence from the initial sampling for these serovars. Serological observation of C in cattle and pigs on Farm 1, would have justified the use of a trivalent vaccine containing that serovar rather than the bivalent vaccine chosen by the farmer.

Some studies have suggested that treatment with antibiotics in addition to vaccination is preferred to reduce *Leptospira* infection in cattle herds (Little et al., 1992; Mughini-Gras *et al.*, 2014). If used simultaneously, antibiotics should reduce or eliminate renal infection and therefore shedding, before animals have sufficient vaccine-induced immunity, as vaccines do not eliminate shedding in all animals in the short term (Mughini-Gras *et al.*, 2014). Immunity due to vaccination should prevent infection of subsequently exposed animals. Combinations of penicillin and streptomycin or streptomycin alone have been used widely, but ampicillin, amoxicillin and the third generation cephalosporins have also been used (Liegeon et al., 2018). In this study, a long-acting preparation of amoxicillin was chosen since Smith et al. (1997) demonstrated that this drug was effective in eliminating leptospires from the kidney following two and possibly one injection in cattle experimentally infected with serovar H. Treatment was given only to the milking cows because the greatest risk to workers was from this group. There was little evidence of vaccine serovars in replacement heifers, hence they were vaccinated prior to infection so immunity should have been protective. Antibiotic treatment was delayed until the end of lactation to avoid milk wastage and disposal problems.

No further human cases were seen on this property in the course of the investigation or to the time of manuscript submission.

3.6. Conclusion

In conclusion, the occurrence of leptospirosis in workers in this farming enterprise confirms that the risk of *Leptospira* infection with vaccine serovars in unvaccinated dairy cattle and exposure to dairy farm workers from cattle in New Zealand persists. This study also demonstrated that a combination of whole herd vaccination and

antibiotic treatment in adult cows was effective in decreasing and possibly eliminating urine shedding of vaccine serovars. It also confirmed, consistent with the study of Yupiana et al (2017), that serovars B and T which are not present in available vaccines may be shed in vaccinated herds, supporting that personal protective measures should continue to be adopted regardless of vaccination status of herds. This study also supports that investigation of the epidemiology and production impact of serovars not currently contained in vaccines is warranted.

3.7. Acknowledgements

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References

Adler, B., & de la Peña Moctezuma, A. (2010). *Leptospira* and leptospirosis. *Veterinary Microbiology, 140*(3), 287-296.

Benschop, J., Collins-Emerson, J., Maskill, A., O'Connor, P., Tunbridge, M., Yupiana, Y., & Weston, J. (2017). Leptospirosis in three workers on a dairy farm with unvaccinated cattle. *New Zealand Medical Journal, 130*(1462), 102-108.

Christmas, B. W., Tennent, R. B., Philip, N. A., & Lindsay, P. G. (1974). Dairy farm fever in New Zealand.[i] A local outbreak of human leptospirosis. *New Zealand Medical Journal, 79*(514), 901-904.

ESR 2013–2017: The Institute of Environmental Science and Research Ltd. (2017). *Notifiable Diseases in New Zealand: Annual Report 2012-2016*. Retrieved from https://surv.esr.cri.nz/.

Fang, F., Collins-Emerson, J. M., Heuer, C., Hill, F. I., Tisdall, D. J., Wilson, P. R., & Benschop, J. (2014). Interlaboratory and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle. *Journal of Veterinary Diagnostic Investigation, 26*(6), 734-747.

Hathaway, S. C. (1981). Leptospirosis in New Zealand: an ecological view. *New Zealand Veterinary Journal, 29*(7), 109-112. doi:10.1080/00480169.1981.34815

Hathaway, S. C., & Blackmore, D. K. (1981). Ecological aspects of the epidemiology of infection with leptospires of the Ballum serogroup in the black rat (Rattus rattus) and the brown rat (Rattus norvegicus) in New Zealand. *J Hyg (Lond), 87*(3), 427-436.

Hellstrom, J. S. (1978). *Studies on some aspects of the epidemiology of bovine leptospirosis.* (Doctoral dissertation, Massey University, Palmerston North, New Zealand). Retrieved from . https://mro.massey.ac.nz/handle/10179/296

Kirschner, L. (1954). Recent studies on leptospirosis in New Zealand; infection with a new type (*Leptospira* mitis) Johnson (Syn. Lept. hyos) in man and animals. *The New Zealand Medical Journal, 53*(294), 119-128.

Liegeon, G., Delory, T., & Picardeau, M. (2018). Antibiotic susceptibilities of livestock isolates of *Leptospira*. *International Journal of Antimicrobial Agents, 51*(5), 693-699. doi:10.1016/j.ijantimicag.2017.12.024

Lilenbaum, W., & Martins, G. (2014). Leptospirosis in Cattle: A Challenging Scenario for the Understanding of the Epidemiology. *Transboundary & Emerging Diseases, 61*, 63-68. doi:10.1111/tbed.12233

Little, T. W. A., Hathaway, S. C., Boughton, E. S., & Seawright, D. (1992). Development of a control strategy for *Leptospira*-Hardjo infection in a closed beef herd. *Veterinary Record, 131*(17), 383-386.

Mackintosh, C. G., Marshall, R. B., & Broughton, E. S. (1980). The use of a hardjo-pomona vaccine to prevent leptospiruria in cattle exposed to natural challenge with *Leptospira interrogans* serovar Hardjo. *New Zealand Veterinary Journal, 28*(9), 174-177.

Mackintosh, C. G., Schollum, L. M., Blackmore, D. K., Willis, A. F., Cook, N. R., Stoke, J. C. J., & Harris, R. E. (1980). Epidemiology of leptospirosis in dairy farm workers in the Manawatu Part I: A cross-sectional serological survey and associated occupational factors. *New Zealand Veterinary Journal, 28*(12), 245-250. doi:10.1080/00480169.1980.34769

Marshall, R. B., & Manktelow, B. W. (2002). Fifty years of leptospirosis research in New Zealand: a perspective. *New Zealand Veterinary Journal, 50*(3), 61-63.

McLean, M. (2014). A cluster of three cases of leptospirosis in dairy farm workers in New Zealand. *New Zealand Medical Journal, 127*(1388), 13-20.

Moinet, M., Nisa, S., Haack, N., Wilkinson, D. A., Oosterhof, H., Aberdein, D., . . . Benschop, J. (2017). *Is wildlife a source of Leptospira infection in livestock in*

New Zealand? Paper presented at the 2017 Conference Proceedings of the Large Animal Veterinarian Technicians Group of the NZVA, Wellington, NZ.

Mughini-Gras, L., Bonfanti, L., Natale, A., Comin, A., Ferronato, A., La Greca, E., . . . Marangon, S. (2014). Application of an integrated outbreak management plan for the control of leptospirosis in dairy cattle herds. *Epidemiology And Infection, 142*(6), 1172-1181. doi:10.1017/S0950268813001817

Newcombe, R. G. (1998). Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine, 17*(8), 857-872.

Smith, C. R., Corney, B. G., McGowan, M. R., McClintock, C. S., Ward, W., & Ketterer, P. J. (1997). Amoxycillin as an alternative to dihydrostreptomycin sulphate for treating cattle infected with *Leptospira borgpetersenii* serovar Hardjo. *Australian Veterinary Journal, 75*(11), 818-821. doi:10.1111/j.1751-0813.1997.tb15661.x

Subharat, S., Wilson, P. R., Heuer, C., & Collins-Emerson, J. M. (2011). Evaluation of a SYTO9 real-time polymerase chain reaction assay to detect and identify pathogenic *Leptospira* species in kidney tissue and urine of New Zealand farmed deer. *Journal of Veterinary Diagnostic Investigation, 23*(4), 743-752.

Thornley, C. N., Baker, M. G., Weinstein, P., & Maas, E. W. (2002). Changing epidemiology of human leptospirosis in New Zealand. *Epidemiology And Infection, 128*(01), 29-36. doi:10.1017/s0950268801006392

WorkSafe New Zealand. (2015). *Prevention and Control of Leptospirosis*. Retrieved from http://saferfarms.org.nz/assets/guides/WSNZ-1368-Prevention-and-Control-of-Leptospirosis-GPG-v5-0-FA1-LR.pdf

Yupiana, Y., Collins, J. C., Benschop, J., Weston, J., wilson, p., Vallee, E., & Heuer, C. (2017). *Nationwide survey of Leptospiral antibodies and shedding in New Zealand dairy herds.* Paper presented at the Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA Annual Conference, 2017 Conference, Wellington, New Zealand.

Chapter 4
Emerging *Leptospira* strain poses public health risk for dairy farmers in New Zealand

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4.1. Abstract

Leptospira infection in dairy cattle and leptospirosis in dairy farm workers were common in New Zealand prior to the introduction of dairy cattle vaccination in the 1980s. Despite widespread vaccination within the dairy industry, the long-term effectiveness of vaccination and current *Leptospira* exposure status remained unknown. A cross-sectional study was conducted from January-April 2016 to investigate the prevalence of pathogenic *Leptospira* spp. DNA in urine at cow and herd level, and its relationship to five *Leptospira* serovars known to be endemic. Two hundred dairy farms were randomly selected from the national database. Twenty paired blood and urine samples were collected on each farm from adult cows (n=4000). Sera were tested using the Microscopic Agglutination Test against serovars Hardjobovis (termed Hardjo), Pomona, Copenhageni, Ballum and Tarassovi with titres ≥48 being considered positive. Urine was tested using quantitative real-time PCR (qPCR) that amplifies the gryB gene. All but one herd had been vaccinated with a bivalent Hardjo/Pomona or trivalent vaccine incorporating Copenhageni. In total, 2.4% of cows were urine qPCR positive and 27% of farms had at least one urine qPCR positive cow. Overall 63% of cows were seropositive to one or more serovars: 44% for Hardjo, 28% for Pomona, 15% for Copenhageni (in vaccinated herds), and for unvaccinated cows: 1% for Copenhageni, and 3% for Ballum and 17% for Tarassovi. Of the 94 qPCR urine-positive cows, 51 were seropositive to Tarassovi, 3 to Ballum, 3 to Copenhageni, 24 to Hardjo, and 17 to Pomona, the latter two presumably reflecting vaccination titres. A strong association was found between shedding and serology for Tarassovi. While there was no evidence that current vaccination programmes were ineffective in protecting against their target serovars, serovar Tarassovi has apparently emerged in NZ dairy cattle. As Tarassovi is currently not included in vaccines and is prevalent in notified leptospirosis cases in dairy workers, we concluded that this serovar poses a public health risk.

4.2. Introduction

Leptospirosis is recognized as one of the most widespread zoonotic diseases worldwide and is caused by pathogenic species of *Leptospira* (Levett, 2001). Transmission of *Leptospira* from animals to humans usually occurs directly by contact with infected urine or indirectly from contaminated soil or water through broken skin or mucosa (Adler et al., 2015). Wildlife and domestic animals act as maintenance or spill-over hosts for different leptospiral serovars (Hathaway, 1981). Humans are accidental (spill-over) hosts and there are few recorded instances of human-to-human transmission (Bolin and Koellner, 1988; Doeleman, 1932). In New Zealand, six serovars belonging to two pathogenic species are known to be endemic in animals, namely, *Leptospira* borgpetersenii serovars Hardjo, Ballum, Balcanica and Tarassovi and *Leptospira* interrogans serovars Pomona and Copenhageni (Marshall and Manktelow, 2002). Hardjo is an adapted serovar for cattle, and Pomona and Tarassovi for pigs (Hathaway, 1981). Other serovars are maintained by wildlife, namely serovar Balcanica by possums (Trichosurus vulpecula) and wild deer (Cervus elaphus), serovar Ballum by house mice (Mus musculus), ship rats (Rattus rattus) and hedgehogs (Erinaceus europaeus) and serovar Copenhageni by Norwegian rats (Rattus norvegicus) (Hathaway, 1981).

The annual incidence of leptospirosis in humans in New Zealand is higher than in other temperate countries (Hartskeerl and Collares- Pereira, 2011; Thornley and Baker, 2002), with an average annual incidence of 1.96 cases per 100,000 for the period 1999–2016 (Nisa et al., 2018). The highest number of notified human leptospirosis cases in New Zealand history was recorded in 1971, with 860 cases (Mackintosh, 1981). During 1970–1979, approximately 90% of human cases reported were in dairy workers (Mackintosh, 1981). Historically, Hardjo and Pomona had been the most reported serovars in humans (Christmas and Tennent, 1974a, b).

In response to the high incidence of human cases, vaccination of dairy herds against Hardjo and Pomona, along with hygiene measures were implemented in the late 1970s

(Blackmore and Marshall, 1981; Ryan and Hellstrom, 1982). The average annual incidence fell from 15.7 per 100,000 in 1970–1979 to 4.4 per 100,000 in 1990–1998 (Marshall and Chereshsky, 1996; Thornley et al., 2002). Several authors stated that the propagation of vaccination of dairy cattle protected dairy workers against leptospirosis without, however, presenting evidence (Bettelheim and Fogg, 1986; Blackmore et al., 1981; Mackintosh and Schollum, 1982a, b; Ryan et al., 1982). In addition to the apparent effectiveness of the vaccination programme, experimental or intervention studies showed that vaccination using a bivalent (Hardjo and Pomona) or trivalent (with Copenhageni) vaccine could prevent leptospiruria or shorten the period of *Leptospira* shedding following natural and artificial challenge (Flint and Liardet, 1980; Mackintosh and Marshall, 1980; Marshall and Broughton, 1979).

More recently, farmers, meat workers, cattle exporters, hunters, and trappers have been identified as high-risk occupations, with 63% of recent notified cases being farmers (ESR Report, 2012-16) and particularly those in contact with unvaccinated or poorly vaccinated dairy herds (Benschop et al., 2017; McLean, 2014). Hardjo and Pomona are currently the most commonly reported serovars in human leptospirosis. However, the numbers of cases attributed to serovars Ballum (Thornley et al., 2002) and Tarassovi (Cowie and Bell, 2012) are increasing. Hardjo accounted for 42% of notified cases between 1999–2016 (460/1094), Pomona for 23% (249/1094), Ballum for 21% (231/1094) and Tarassovi for 9% (96/1094) (Nisa et al., 2018).

Although anecdotal evidence suggests that 90% of dairy herds in New Zealand are vaccinated against *Leptospira*, the incidence of human cases is still considered high by international standards (Heuer et al., 2012). In the absence of specifically designed studies investigating the extent of shedding in vaccinated dairy herds, a small-scale pilot study was conducted in 2011 (Wilson et al., 2013). That study involved 44 conveniently selected vaccinated farms with 10 adult milking cattle sampled per farm, and found evidence of *Leptospira* in 3.8% urine of cows on 29.5% of sampled farms. As serum samples were not collected it was not possible to determine which *Leptospira* serovars were associated with the infection.

This paper reports a national survey of randomly selected dairy herds conducted to assess the prevalence of qPCR positive urine (termed 'shedding') and its relationship with the serospecific prevalence of *Leptospira* in dairy cattle in New Zealand.

4.3. Materials & methods

4.3.1. Study design

A cross-sectional study was conducted from 5th January to 26th April 2016 to investigate Leptospira shedding and seroprevalence in dairy cattle in New Zealand. Sample size was calculated to estimate a shedding prevalence based on simple random sampling (Dohoo et al., 2009), and an a priori prevalence of 30% of herds and 4% of cows shedding with intra-class correlation (ICC) of 0.23 (Parramore and Meenks, 2011). Assuming perfect herd level sensitivity and specificity, 200 farms (20 cows per farm) were required to estimate the herd level prevalence with 95% confidence interval of±6.4%. At individual animal level, 4000 cows were required to estimate the sero-prevalence in cows with 95% confidence interval of ±1.4%. We timed the sampling (Jan-Mar) approximately 7–12 months after most dairy farmers booster-vaccinated adult cows (Apr-Jun) in order to allow time for titres to decrease.

4.3.2. Recruitment of farms

Dairy farms were randomly selected from the sampling frame, the national database of dairy farms in New Zealand (LIC & DairyNZ Limited, 2014). The selection was stratified by region and herd size (150–249, 250–349,350–649,>649 cows per herd). The percentage of herds in the sample was proportional to the regional population size. The herd size distribution in the sample of each region was equivalent to that of the population. On these conditions, DairyNZ Ltd. provided a list of 396 farms, representing the national distribution of farms by region and herd size. Where more than one herd was managed on a farm, only one of the herds was selected at the discretion of the farm veterinarian. The terms farm and herd are referred to synonymously.

To obtain a final sample of 200 herds, all of the 396 listed farmers were initially notified about the study via a letter including information about the study, the sampling details and the questions that would be asked during a phone interview. Fig. 1 provides details about reasons of 17 farmers who declined to participate. The remaining 379 farmers were contacted via phone by a trained team providing basic information and asking standard questions. This included reasons if participation was declined. Out of 379, 111 farms either had incorrect phone numbers or were not interested in participating. Reasons stated were apparently unrelated to any of the study parameters ('too busy', 'personal reasons', 'no specific reason'). When farmers consented to participate, they were given further details about the study and were asked for contact details of their farm veterinarian. The 69 farmers who did not want to participate were phoned again after the study and asked for the reason of non-compliance, as well as whether they vaccinated their cattle against leptospirosis. Finally, 200 farmers participated in the study (Figure 4.3-1).

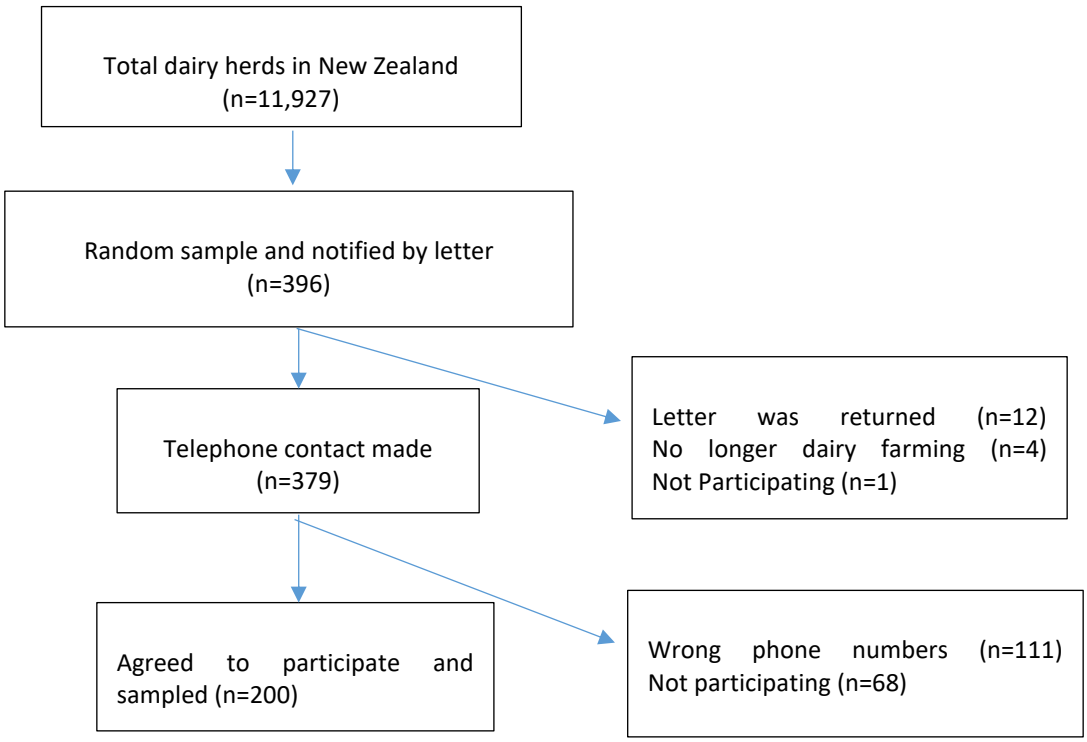

Figure 4.3-1. Farm selection process of a subsample from the national database supplied by DairyNZ of randomly selected herds stratified by size and region

4.3.3. Recruitment of veterinary practices

Veterinary practices nominated by participating farmers were contracted to collect blood and urine, and to supervise completion of a questionnaire. The veterinary practice names and other details given by the farmers were cross-checked with the Veterinary Council of New Zealand veterinary practice database. Farmers were contacted again to verify their veterinary practices contact details if anything was unclear. Emails outlining the project, sampling specifications and consent forms were sent to each nominated veterinary practice, with a follow-up by phone if no response was received after about 7 days. All 93 nominated veterinary practices agreed to participate and a contact veterinarian or veterinary technician was identified. Instructions for questionnaire completion, animal selection and sampling, and dispatch procedures were provided. A 3-month sampling schedule was distributed to ensure practice and laboratory logistical requirements were met. Nominated veterinarians contacted their clients, ensured that consent forms were signed, and undertook sampling and questionnaire completion. A week before the actual date of sampling, a package specific for each farm was sent to each vet practice. It contained the questionnaire, farm information sheet, sampling instructions, sampled animal data form and labels for blood and urine. The sampling forms and questionnaire can be provided on request from the corresponding author.

4.3.4. Sample and data collection

To ensure that 20 paired samples were obtained, 40 cows were selected using systematic random sampling as it was expected that urine samples would only be able to be collected from about half of the cows. Samples were collected at the end of milking. Urine samples were collected first, in a 50 ml clean/sterile plastic vial either from spontaneously urinating cows or induced urination by stimulating the ventral vulva. If the latter, faecal material was cleaned away by washing with warm water. A minimum of 10 mL of blood was collected by jugular or caudal vein venepuncture into a 10 mL plain (red top) evacuated plastic tube. Animal details including age, breed and

vaccination status were entered into the animal data form. Samples were identified with the labels provided and immediately put into an insulated box with ice or ice pads with blood and urine packed separately in watertight plastic bags. The cooled samples, completed questionnaires and individual animal data forms were sent in the same consignment immediately (on the day of collection) by courier to the laboratory at Massey University. Sampling was restricted to Monday – Thursday to ensure samples reached the laboratory within 24 hours of collection.

4.3.5. Vaccination status of herds

In brief, farmers of 145 (73%) herds stated that their cattle had been vaccinated with a bivalent (Hardjo H and Pomona P) vaccine and cattle of 54 (27%) herds with a trivalent vaccine that also included Copenhageni (C). 'Vaccination' meant that at least one age group (calves, heifers and cows) had received the vaccine at least once a year. No vaccine in New Zealand contains serovars Ballum (B) or Tarassovi (T).

4.3.6. Ethics

Manipulations performed on animals were approved by the Massey University Animal Ethics Committee, protocol 15/57.

4.3.7. Laboratory procedures

All samples were processed immediately upon receipt at the laboratory, mostly on the day following collection. Urine samples were centrifuged at 3,000 *g* for 10 minutes then at 12,000 *g* for 20 minutes. The supernatant was discarded by a transfer pipette and 200 µl of phosphate-buffered saline (PBS) was used to re-suspend the pellet. This mixture was used to extract DNA. Blood samples were centrifuged at 3,000 *g* for 10 minutes and sera collected as supernatant. Thirty microliter of each serum was mixed with 150 µl standard saline into 96 well plates as a master plate to make 1/6 dilution for testing. The master plates were then stored at -20°C. The remaining sera were stored at -80°C.

The DNA was extracted using an automated purification and extraction machine, KingFisher Flex Magnetic Particle Processors (Thermo Fisher Scientific, Life Technologies New Zealand Ltd., Auckland, New Zealand) and NucleoMagVET extraction kit as per manufacturer's instructions (Macherey-Nagel, Medi'Ray New Zealand, Auckland, New Zealand). DNA samples were stored at -20 °C.

The qPCR assay was based on the method used by Subharat *et al.* (2011) and refined by Fang *et al.* (2014b). KAPA SYBR FAST 2X qPCR Master Mix/Universal (Kapa biosystems, Sigma-Aldrich, Auckland, New Zealand, Cat. KK4600) was used to amplify the DNA. The assay was performed in a Rotor-Gene Q machine (Qiagen, Bio-Strategy Ltd, Auckland, New Zealand). Primers 2For (5'-TGAGCCAAGAAGAAACAAGCTACA-3') and 504Rev (5'-MATGGTTCCRCTTTCCGAAGA-3') were used to amplify the *gyrB* gene. The 20 µl reaction comprised of 10 µl KAPA SYBR FAST 2X qPCR Master Mix/Universal (Kapa biosystems, Sigma-Aldrich, Auckland, New Zealand, Cat. KK4600), 8.2 µl double distilled water (ddH_2O) and 5 pmol of 2For and 504Rev primers with the following thermal cycling conditions: initial denaturation at 95°C for 5 min, followed by 50 cycles of 95°C for 15 s, 55°C for 30 s and extension at 72°C for 30 s. Fluorescence readings were taken at the end of each extension cycle in the F1 (Sybr green) channel. Melting curve analysis was performed by heating the PCR product from 78°C to 90°C and monitoring the fluorescence change every 0.2°C. The positive control was a cloned fragment of gyrB from serovar Pomona (laboratory strain), and ddH_2O was used as the negative control. For the purpose of this study, the qPCR data have been used qualitatively only.

Serum samples were tested against serovars Hardjobovis (H), Pomona (P), Copenhageni (C), Ballum (B) and Tarassovi (T). The MAT was performed as described by Fang *et al.* (2014b), based on the method described by Faine (1982). Eight serial, two-fold dilutions were prepared in standard saline and ranged from 1:24 to 1:3072 (final dilution inclusive of antigen). A positive control using standard antisera against each serovar and a negative control using standard saline were prepared in a similar way. The dilutions were incubated with live cultures for 2 hours at 20-30 °C. A

reciprocal titre of ≥1:48 was considered positive (Blackmore *et al.*, 1982; Suwimonteerabutr *et al.*, 2005). The end-point titre was the lowest dilution where approximately 50% or more of the leptospires were agglutinated or lysed.

4.3.8. Data analysis

Prevalence data for serovars H and P, are for the 199 vaccinated herds. Serology data for cows and herds vaccinated or not vaccinated with serovar C were considered separately. A herd was considered to be *Leptospira* shedding positive if at least one cow was PCR positive and a herd was considered MAT seropositive if at least one cow was MAT positive. The 95% confidence intervals (95% CI) and the percentages at cow level for qPCR and MAT results were the predicted values of a generalised logistic mixed-effects model (GLM) with farm as the random effect. This was realised using generalised estimating equations (GEE). The overall prevalence of positive herds was derived from an intercept-only GLM model and regional herd prevalences from a GLM model with region as categorical covariate. Herd prevalences and 95% CI were calculated as predicted means.

The cow-level prevalence of shedding in urine was compared between seropositive and seronegative animals for each of the five serovars (GLM). The statistical procedure adjusted 95% CI for effect of clustering of cows within herd. Odds ratios of PCR positivity in MAT positive versus MAT negative cows for each serovar were calculated. For serovars that were significantly associated with shedding, GLM predicted shedding probabilities for titre step categories 1:48, 1:96, 1:192 1:384 and ≥1:768 were plotted to examine the shape of a possible dose-response relationship. Maps of farm locations and regional herd prevalences were generated using the R package.

Since only few cows were non-vaccinated to H and P, Pearson's Chisquare with and without Yates' continuity correction was used, rather than a mixed effects model, in a separate analysis without random effect to compare the serovar specific proportions of cows positive to MAT in the non-vaccinated herd to those in vaccinated herds.

We used the Kappa (K) statistic to evaluate pairwise dependencies between serovars (H, P, C, B, T). Any K values higher than 0.6 would indicate a dependence in the sense that being positive (or negative) to one serovar would predict the sero-status of another serovar. The hypothesis of a north to south gradient of shedding and serovar prevalence was evaluated using the Cochran-Armitage test for trend (Margolin, 1988). Data were allocated to nine regions ranked by latitude from north to south as shown in Table 4.3-1.

All statistical analyses were done using R version 3.3.2 (RStudio Team, 2016)

4.4. Results

4.4.1. Farms and vaccination practices

Of the 69 farmers who did not wish to participate, three farmers did not conduct vaccination, four farmers were no longer dairy farming and 11 farmers either did not know the answer or did not want to answer the question. The majority of the 200 participating farms (65%) were located in the North Island, with the highest number in Waikato (25%). The numbers of farms in categories of herd size and region are shown in Table 4.4-1 Herd size and regional distributions were similar to dairy population statistics (LIC and DairyNZ Ltd., 2018) with a shift towards larger herds (40% in sample vs. 17% in the population) and a slight over-representation of Taranaki (13% vs. 10%) and the Manawatu/Hawkes Bay/Wairarapa group (15% vs. 9%, resp.). However, these herd sizes were all non-significantly different from population averages ($p > 0.10$). The age of sampled cows ranged from two to 16 years with 85% of cows between 2 and 7 years.

Only 1 of the 200 farms did not have a current *Leptospira* vaccination programme. Briefly, all farms with a vaccination programme vaccinated calves, replacement heifers and adult cows. For calves, 80% of responders used a bivalent (H/P) and 20% a trivalent vaccine (H/P/C). For heifers, 69% of responders used a bivalent and 31% used a trivalent vaccine, and for cows, 68% used a bivalent, 32% used a trivalent vaccine.

4.4.2. Urinary shedding prevalence

Cow-level

Ninety-four cows were urine qPCR positive to *Leptospira spp.* (2.4%; 95% CI: 1.8–3.1). The prevalence of PCR positives in herds with at least one positive cow was 8.9% (95% CI: 7.5–10.5). Regions with the highest proportion of cows that were urine qPCR positive were Northland and Bay of Plenty in the North Island, and West Coast in the South Island (Table 4.4-2). There was a statistically significant trend of decreasing shedding prevalences from north to south (p=0.008). None of the 20 cows in the non-vaccinating herd was qPCR positive. After accounting for clustering (GEE), the frequencies of qPCR positives in bivalent (2.0%, 95% CI 1.3–3.1%) and trivalent vaccinated herds (3.5%, 95% CI 2.2–5.6%) were similar (p > 0.2).

Herd-level

Fifty-three farms had at least one cow with a urine sample positive for *Leptospira* spp. By qPCR (26.5%, 95% CI: 18.9–35.9). As for cows above, the regions with the highest proportion of farms that had at least one urine qPCR positive sample were Northland, Bay of Plenty and West Coast (Table 4.4-2; Figure 4.4-1). As at cow level, there was a decreasing trend of herd level shedding prevalences from north to south, which was significant despite relatively low statistical power (p=0.03).

4.4.3. Seroprevalence

Cow-level

Seroprevalence data for each serovar (C considered separately for vaccinated and non-vaccinated cows) are presented in Table 4.4-3. In the vaccinated cows, the predominant serovar was H followed by P and C. Seroprevalence for H was significantly higher than for P and C in vaccinated cows (p < 0.001). For non-vaccine serovars, seroprevalence to T was higher than B and C (p < 0.001). In the nonvaccinated herd, seroprevalence was 20% for H and 5% for P (p= 0.3).

The distributions of reciprocal MAT titres to serovar H, P, C (stratified by vaccination status), B and T in individual cows are presented in Figure 4.4-2. Seroprevalences decreased from north to south for Tarassovi, but not for other serovars ($p < 0.001$). The trend appeared to depend strongly on high prevalence of cows being seropositive to Tarassovi in Northland (33%) and Bay of Plenty (27%) compared to other regions to the South of these regions (9–18%). The Yates corrected frequencies of seropositive cows to any of the serovars in the non-vaccinated herd were not statistically different to those of the 199 vaccinated herds. Non-vaccine serovars B, C, T were independent in MAT as Kappa values for pairwise comparisons were<0.1. The highest K value was 0.33 for the H–P pair, thus low agreement even though the cows were exposed to vaccination for the two serovars at the same time. This was most likely caused by a difference in vaccine titre duration.

Herd-level

Herd-level data are shown in Table 4.4-4. In vaccinated herds, seropositivity to H was predominant followed by P and C. There was no significant difference between the seroprevalence to H and P at herd level ($p=0.2$). For non-vaccine serovars, seroprevalence for T was higher than for B and C ($p < 0.001$). There were no significant north to south trends in the herd level seroprevalence of any serovar.

4.4.4. Association between shedding in urine and serology

Using a reciprocal titre of ≥1:48 as positive, the odds ratio of urine PCR positivity in MAT positive versus MAT negative cows was 0.43 (95% CI: 0.26-0.71) for H, 0.61 (95% CI: 0.25–1.49) for P, 0.72 (95% CI: 0.20–2.57) for C (in vaccinated cows), 1.01 (95% CI: 0.30–3.45) for B, and 5.52 (95% CI: 3.41–8.92) for T. There were insufficient qPCR positive animals at this cut-off for C in unvaccinated cows to test for association with shedding.

Due to its strong positive association at titre cut-off 1:48, serovar T was evaluated at increasing titre steps. Figure 4.4-3. suggests an exponential increase of the predicted

shedding probability as titres increase with significant differences between titres steps 1:96 or 1:192 over ≤1:48, and 1:384 or ≥1:768 over 1:96.

Table 4.4-1. Number of participating dairy farms by herd size category and region.

Farm Region	**Number of herds in each size category**				**Total number of herds**
	150-249	**250-349**	**350-649**	**650+**	
Northland	0	5	8	5	18
North Waikato	4	5	7	9	25
South Waikato	3	4	8	10	25
Bay of Plenty	1	0	4	2	7
Taranaki	1	11	8	6	26
Manawatu-Wanganui/ Wairarapa/Hawkes Bay	1	5	16	7	29
The North Island	**10**	**30**	**51**	**39**	**130**
Nelson/Marlborough/ West Coast	0	2	3	4	9
Canterbury/North Otago	0	5	7	21	33
Southland	2	1	10	15	28
The South Island	**2**	**8**	**20**	**40**	**70**
Total number of herds	**12**	**38**	**71**	**79**	**200**

Table 4.4-2. Number of cows and herds sampled from each region, number of qPCR positive samples (N pos) at herd and individual cow level and percentage positive (%) with 95% confidence intervals (95%CI) adjusted for the effect of clustering of cows within herd.

Region	**Number of cows/herds sampled**	**Urine qPCR**			
		Cows		**Herds**	
		Npos	**%(95%CI)**	**Npos**	**%(95%CI)**
Northland	360/18	15	4(2-8)	9	50(29-71)
North Waikato	500/25	17	3(2-7)	9	36(20-55)
South Waikato	500/25	14	3(1-6)	6	24(11-43)
Bay of Plenty	140/7	6	4(2-10)	4	57(25-84)
Taranaki	520/26	4	1(0.2-3)	2	8(2-24)
Manawatu-Wanganui/ Wairarapa/Hawkes Bay	580/29	8	1(1-3)	6	21(10-38)
Nelson/Marlborough/ West Coast	180/9	8	4(2-11)	5	63(31-86)
Canterbury/North Otago	660/33	9	1(1-3)	5	15(7-31)
Southland	560/28	13	2(1-5)	7	25(13-43)
Total	4000/200	94	2(2-3)	53	27(19-36)

Table 4.4-3. Percentage (and 95% confidence intervals (95%CI)) of cows serologically positive for vaccine serovars Hardjo and Pomona (vaccinated cows only, n=199) and Copenhageni (when trivalent vaccine was used) and non-vaccine serovars Copenhageni (when bivalent vaccine was used), Ballum and Tarassovi, , adjusted for the effect of clustering within herds.

Region	N bivalent/N trivalent vaccinated	Vaccine serovars (% and 95% CI)			Non-vaccine serovars (% and 95% CI)		
		Hardjo	Pomona	Copenhageni	Copenhageni	Ballum	Tarassovi
Northland	360/80	52(43-61)	25(17-35)	3(1-7)	1(1-3)	3(1-8)	33(23-44)
North Waikato	500/240	38(28-48)	25(17-35)	21(11-35)	2(1-5)	6(3-9)	17(10-28)
South Waikato	500/280	55(46-64)	35(29-42)	25(16-37)	NA	3(2-6)	18(12-27)
Bay of Plenty	140/100	38(21-59)	21(9-42)	10(4-22)	3(1-10)	5(2-14)	27(15-45)
Taranaki	520/220	45(36-55)	20(15-27)	6(3-12)	1(0.2-2)	3(2-7)	12(8-17)
Manawatu-Wanganui/Wairarapa/Hawkes Bay	580/0	46(35-57)	34(26-43)	NA	0.3(0.1-1)	3(2-6)	9(6-14)
Nelson/Marlborough/Wes Coast	180/0	43(28-60)	32(19-48)	NA	1(0.1-3)	3(1-8)	14(8-25)
Canterbury/North Otago	640/100	44(35-52)	33(26-41)	13(5-29)	1(0.5-2)	2(1-3)	18(12-25)
Southland	560/40	38(28-48)	23(18-29)	5 (1-19)	1(0.2-2)	3(2-6)	17(11-25)
Total	3980/1060	45(43-46)	28(27-30)	15(11-21)	1(0.5-1)	3(3-4)	17(15-20)

Table 4.4-4. Percentage (and 95% confidence intervals (95% CI) of herds serologically positive for vaccine serovars Hardjo and Pomona (vaccinated herds only, n=199) and Copenhageni (when a trivalent vaccine was used), and non-vaccine serovars Copenhageni (when a bivalent vaccine was used), Ballum and Tarassovi

Region	N bivalent/N trivalent vaccinated	Vaccine serovars (% and 95% CI)			Non-vaccine serovars (% and 95% CI)		
		Hardjo	Pomona	Copenhageni	Copenhageni	Ballum	Tarassovi
Northland	18/4	100(80-100)	94(74-99)	50(15-85)	29(12-55)	33(16-56)	89(67-97)
North Waikato	25/12	96(80-99)	88(70-96)	67(39-86)	31(13-58)	48(30-67)	72(52-86)
South Waikato	25/14	100(87-100)	100(87-100)	86(60-96)	0(0-3)	44(27-63)	76(57-89)
Bay of Plenty	7/5	86(49-97)	71(36-92)	60(23-88)	50(9-91)	43(16-75)	100(65-100)
Taranaki	26/11	96(81-99)	100(87-100)	55(28-79)	13(4-38)	35(19-54)	62(43-78)
Manawatu-Wanganui/Wairarapa/Hawkes Bay	29/0	100(88-100)	97(83-99)	NA	7(2-2)	41(26-59)	62(44-77)
Nelson/Marlborough/Wes Coast	9/0	100(70-100)	89(57-98)	NA	11(2-43)	33(12-64)	78(45-94)
Canterbury/North Otago	32/5	100(89-100)	100(89-100)	60(23-88)	21(10-40)	30(17-470	76(59-87)
Southland	28/2	100(88-100)	96(82-99)	50(9-91)	12(4-29)	32(18-51)	79(60-90)
Total	199/53	98(96-99)	95(92-98)	66(54-77)	16(10-23)	38(33-42)	74(68-80)

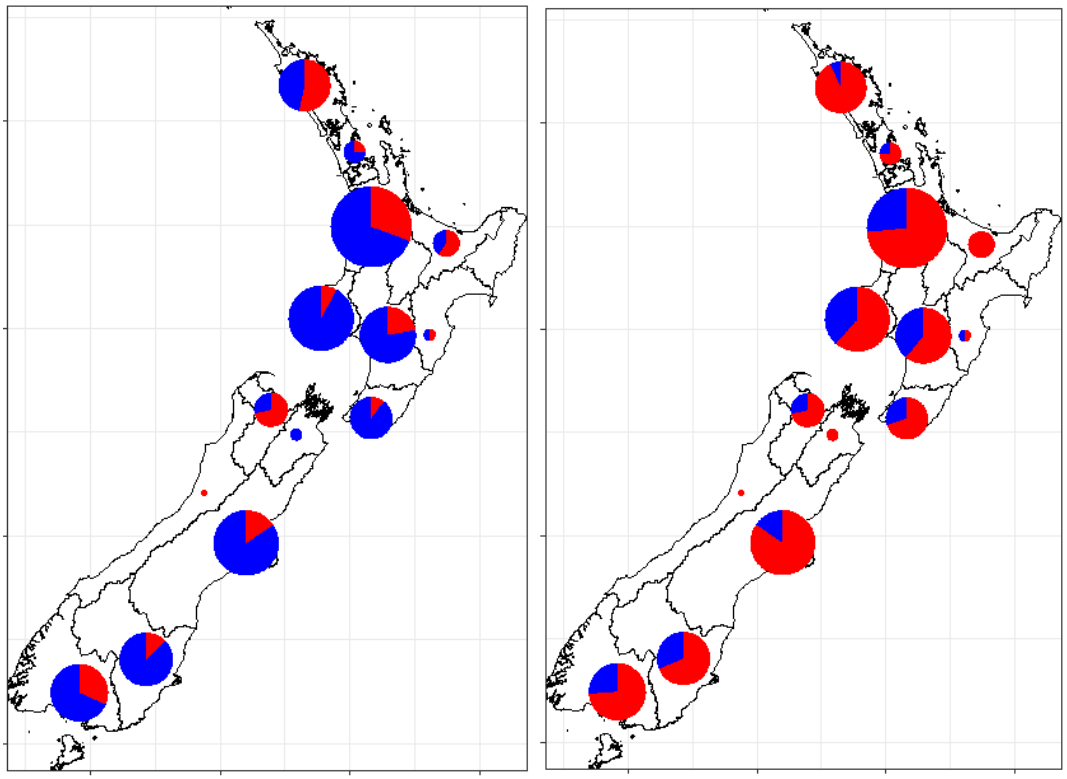

Figure 4.4-1 Left: proportion of herds with at least one PCR positive cow (red), or with all cows being PCR negative (blue), and right: proportion of herds with at least one cow seropositive (≥48) to Tarassovi (red), or with all cows being seronegative to Tarassovi (blue). Size of the circles represents the relative number of herds in a region.

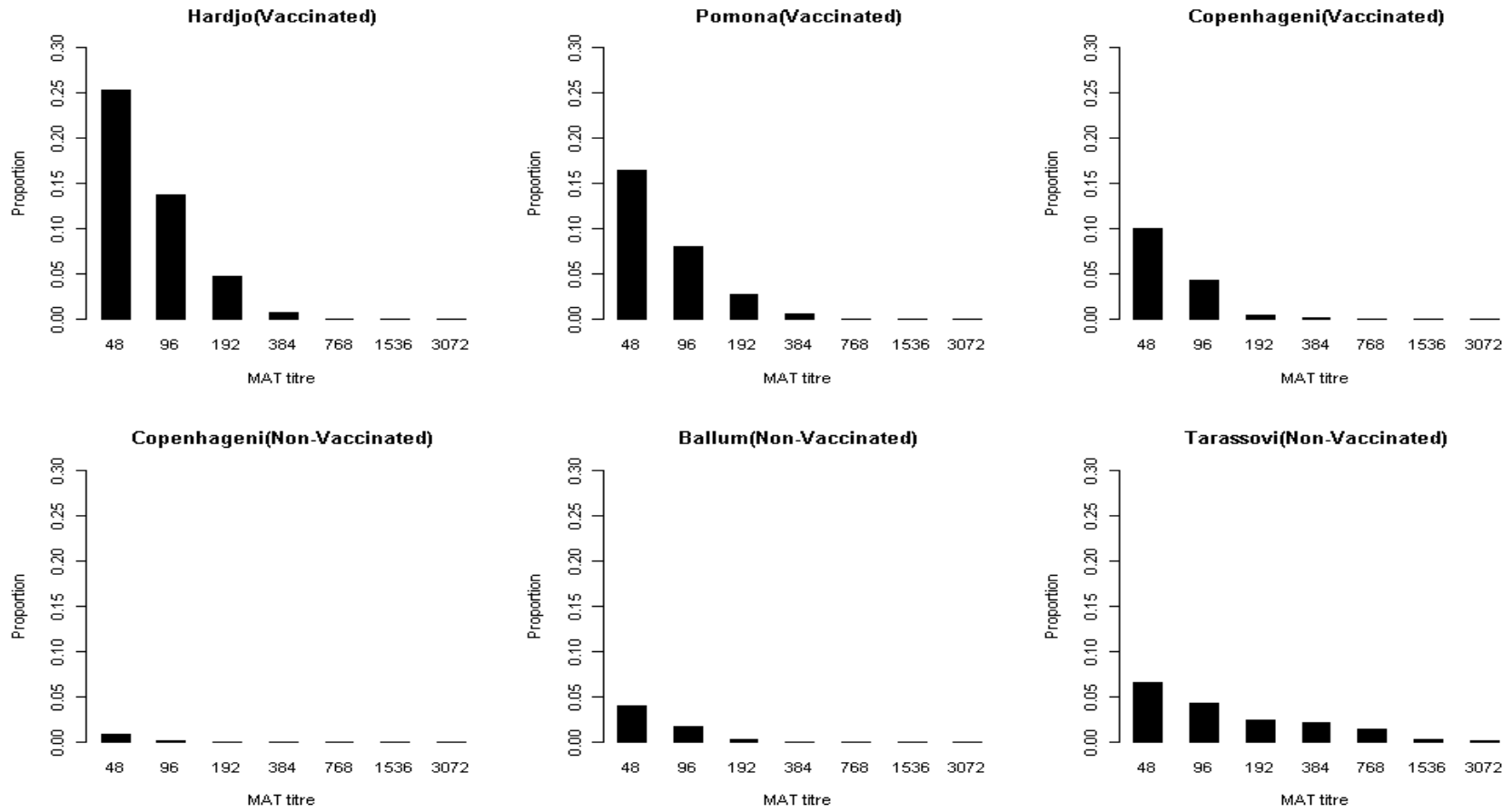

Figure 4.4-2. Proportion of cows at each MAT titre of 48 or more to serovars Hardjo and Pomona in vaccinated cows (n=3980), Ballum and Tarassovi. (n=4000), and Copenhageni, (vaccinated) (n=1060) and non-vaccinated) (n=3072)

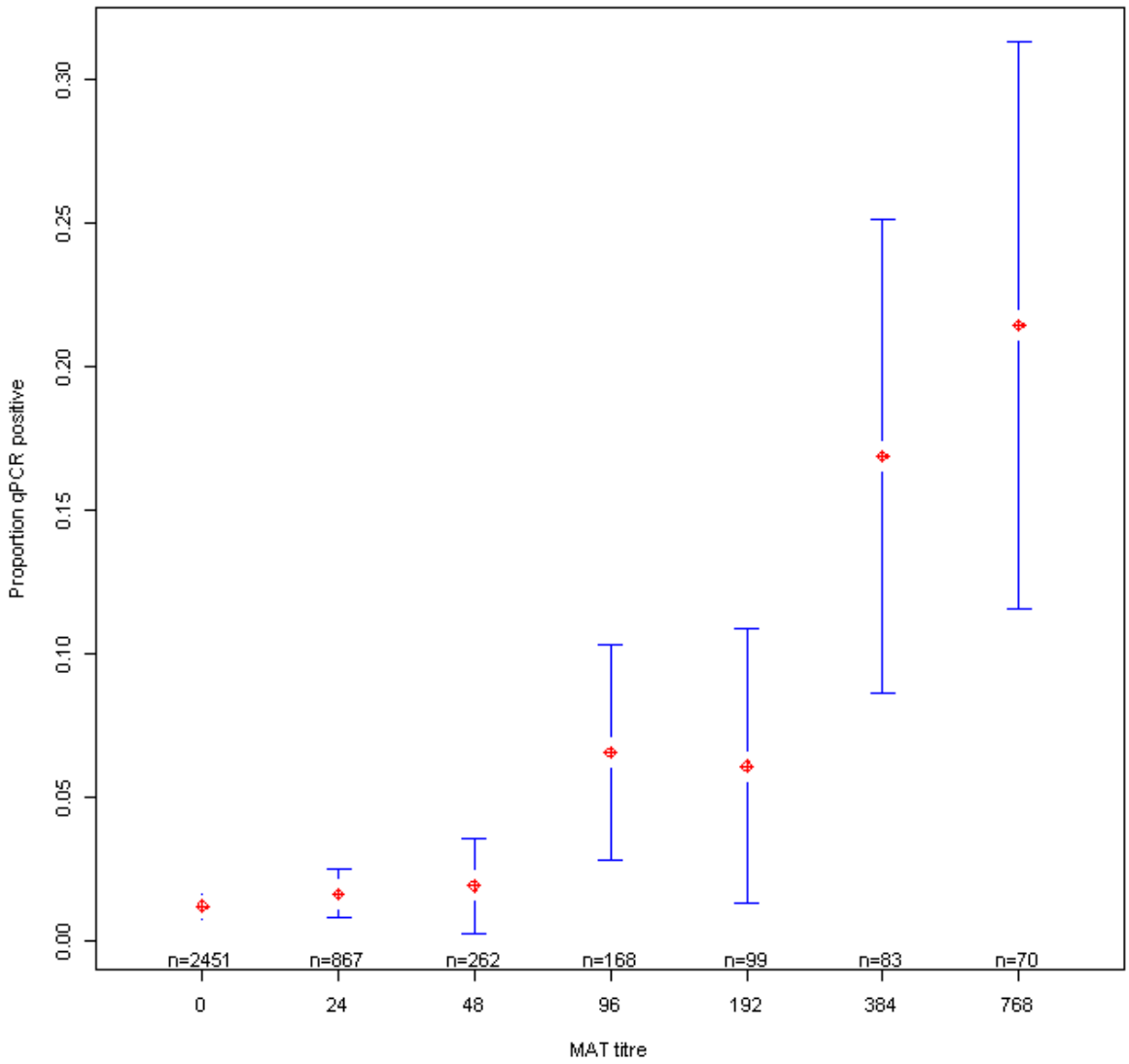

Figure 4.4-3. Number of cows and proportion (with % confidence interval bars) urine qPCR positive at each MAT titre (0 to 768 or higher) to serovar Tarassovi.

4.5. Discussion

This is the first nationwide, stratified random survey to investigate urinary shedding and seroprevalence of *Leptospira* on vaccinated dairy farms in New Zealand. There was no evidence that long-term vaccination programmes for H and P were ineffective in reducing or eliminating shedding. On the other hand, evidence was inconclusive that vaccination practices on New Zealand dairy farms were protective. Serological evidence indicated that cows were naturally exposed to T and B, and for C in herds not vaccinating against C. The sero-prevalence of T and its strong association with shedding suggests that this is an emerging serovar in dairy cattle, which is a novel and important finding from this study.

The prevalence of *Leptospira* shedding was similar to that of a pilot study (Parramore et al., 2011) that prompted this definitive study. Our estimates of 26.5% (95% CI: 18.9–35.9) of herds and 2.4% (95% CI: 1.8–3.1) of cows showing evidence of shedding were similar to those observed in that study. If it is considered that the qPCR for detecting leptospiral DNA in urine of deer was 97% sensitive and 99% specific (Subharat et al., 2011), and this is assumed to be similar in cattle, the true prevalence might have been somewhat overestimated: adjusting the cow level shedding prevalence for lack of test accuracy resulted in a median of 1.7%; a similar adjustment for herd level prevalence resulted in a median of 20.4% (data not shown). In this calculation, the herd sensitivity of detecting herds with 10% shedders when sampling 20 animals was 87% with 81% herd specificity for correctly classifying herds with no shedders. Thus with a perfect test, there would be at least one in five herds shedding *Leptospira* with an approximately 8.5% (1/0.204 * 1.7%) shedding prevalence of cows in infected herds.

The association between shedding and serology for T was strong and highly significant with a dose-response effect such that the proportion of cows shedding *Leptospira spp.* increased almost exponentially with the MAT titre. Since a high MAT titre indicates recent infection (Mackintosh et al., 1980) and the prevalence of shedding decreases with time after infection, this association strongly suggests that the serovar being shed

was often T. The absence of reported clinical disease such as abortion, together with the relatively large proportion of seropositive cows (17%) and herds (74%) support the assertion that T may be highly adapted to dairy cattle. Hence, we propose that dairy cattle are a likely reservoir host for this serovar in New Zealand.

Tarassovi was first detected in cattle in New Zealand in 1953 (Kirschner, 1954) with three of 100 cows being seropositive in an abattoir survey in the Otago region. From 1968–1982, two studies reported this serovar in cattle, with seroprevalence in cows ranging from 0 to 6% at a similar titre cut-off to that used in this study (Mackintosh and Schollum, 1982a; Ryan and Marshall, 1976). Clinical manifestation of this serovar in cattle has not been detected in New Zealand (Hellstrom, 1978; Ryan and Marshall, 1976), which is consistent with questionnaire data for the survey conducted with the present study (data not presented). A recent study reported a similar T seroprevalence in beef cows (95%CI: 15–22%) and herds (95%CI: 73–94%) (Mannewald et al., 2017) as observed in our study of dairy cattle. Thus, there is a difference over time between these two periods suggesting that T has emerged during the 36 years since it was last reported.

The proposition that T is an emerging serovar is supported by human notified leptospirosis case data. Cowie and Bell (2012) also demonstrated that T was the dominant serovar among leptospirosis cases in dairy workers in Waikato from 2004-2010. In that report, dairy workers were 7-fold as likely to test positive for T as were dry stock farmers or abattoir workers. A further report of 1556 human leptospirosis cases in New Zealand showed that T was the predominant serovar in dairy farm workers between 1999 and 2016 (Nisa et al., 2018). These findings in conjunction with the earlier mentioned lower prevalence estimates 30–40 years ago clearly demonstrate that T is an emerging public health risk, and that the risk is particularly high, relatively, for people in close contact with dairy cattle.

Since 99.5% of sampled cows were from herds with a current vaccination programme, antibody titres to vaccine serovars likely reflect vaccination (Tripathy and Smith, 1975),

since H and P are in all available *Leptospira* vaccines in New Zealand. Titres and prevalence observed are consistent with those reported earlier for vaccinated herds (Mackintosh and Marshall, 1980; Mackintosh et al., 1980). Nevertheless, it cannot be fully discounted that natural exposure may be occurring in a small number of instances, since titres of 384 and 792 recorded may reflect exposure, and qPCR does not distinguish serovar in shedding cows. Genetic sequencing, would be needed for verification, and to precisely quantify the effectiveness of vaccination for these serovars. While herd-level seroprevalence for H and P was similar, cow level seroprevalence for H was higher than for P. This is consistent with a longer half-life of titres to H than P (Dreyfus et al., 2014).

The lack of association, or for H a marginally protective association, between shedding and prevalence of serovars contained in vaccines support the notion that vaccination programmes may have been effective in reducing or preventing the shedding of *Leptospira* in dairy cows. Had vaccination been ineffective, MAT positive cows would be expected to have a higher PCR prevalence than MAT negative cows. However, the data for serovar H suggested to the contrary that MAT negative cows had 3% and Mat positive cows 1% PCR prevalence. Earlier studies in cattle suggested that urine PCR prevalence increased with MAT titres to H or P (Fang et al., 2014) as shown in this study for T. The presence or absence of T did not bias these relationships because T was unrelated to H, P and C (Kappa all<0.1). Under the assumption that natural exposure of cattle to H, P, and/or C had a booster effect on top of vaccination and that this increased MAT titres, the prevalence of high titres to H, P and C would have been higher than observed. Assuming further that vaccines would have failed to protect against shedding of these serovars, the urine-PCR prevalence would have been high too. Since, this was not observed, there was no evidence suggesting that the observed urine shedding of cows was predominantly due to one of the vaccine-serovars. We therefore propose that there was no evidence that current vaccination practices were ineffective for protecting workers against exposure to serovars H and P from dairy cattle. Nevertheless, the survey could not conclusively demonstrate that vaccination was effective.

Among the non-vaccine serovars, seroprevalences for T of 74% at herd and 17% individual cow levels were substantially higher than those for C in non-vaccinated herds or B. The lowest prevalence was for C with 1% of non-vaccinated cows being seropositive. Seroprevalences for non-vaccine serovars C and B were similar to those reported from surveys in 1973 and 1974 when 480 dairy and beef cows were randomly sampled from 158 herds and 11 regions in New Zealand (Hellstrom, 1978). At that time, T was predominant among the nonvaccine serovars that also included B and C, but at lower prevalence (< 5%) than in our study (17%). For C and B, we observed slightly higher-cow-level seroprevalence than from that report. However, a recent New Zealand study of mostly non-vaccinated beef cattle showed seroprevalance estimates of 12–16% for B and 11–15% for C (Mannewald et al., 2017). The higher seroprevalences of beef cattle to B and C in that study compared to our estimates in dairy cattle may be associated with the environment on beef farms such as hilly terrain and the presence of other livestock species notably sheep, and possibly rodents and other wildlife species. A greater understanding of the epidemiology of this serovar in different environments is required. MAT testing for the present and the Mannewald et al (2017) studies was undertaken in the same laboratory under identical conditions as for this study, hence testing bias is unlikely to have materially affected inferences.

While there was a range of shedding prevalence between regions, differences were not statistically significant. This is consistent with observation by Hellstrom (1978) of similarity between regions. However, there were significant trends of decreasing cow and herd level shedding prevalences from north to south when regions were sorted by latitude, and a similar trend for seroprevalence to T. This was also similar to the observation from the Hellstrom (1978) survey in which the prevalence of T at both herd and cow levels was also highest in these two regions, albeit at a three-fold lower prevalence than in our study. The warmer climate of the Northern areas of New Zealand may explain these trends.

Participating farms were randomly selected from the national database. Stratification was undertaken to achieve a representative distribution of farms by region and herd

size. As a result of the selection process, the distribution of number of farms for each herd size category was not exactly proportional. However, this slight deviation from a true and perfect subset of the herd size distribution did not affect our estimates of shedding and serology prevalence because herd size categories were not significantly associated with shedding prevalence. Furthermore, all 69 farmers who did not wish to participate in the study stated reasons unrelated to vaccination per se. Nevertheless, some degree of response bias cannot be ruled out. Hence, there is limited likelihood that sampling bias would materially alter the conclusions of this study.

The MAT has been used widely as a diagnostic tool to detect antibodies against *Leptospira*. This test is a serogroup-specific assay, so that cross-reactivity between serovars in the same serogroup of *Leptospira* commonly occurs (Levett, 2001). However, compared with most other places, where more serogroups and serovars of *Leptospira* circulate, there are only five serovars (H,P,C,B,T) with different serogroup detected in cattle New Zealand (Marshall and Manktelow, 2002). Of the five serovars tested, there was no evidence of cross-reaction in the data: Kappa values of agreement were all smaller than 0.1 indicating no agreement, thus being seropositive for one serovar had no bearing on an animal being positive for another serovar. Nevertheless, molecular confirmation or culture, particularly for Tarassovi, is needed to confirm serovar identification. This work is ongoing.

4.6. Conclusion

The findings from this study demonstrate that dairy cows in New Zealand not uncommonly shed leptospires in urine but that they are unlikely to be of vaccine serovars. The probable cause of shedding, supported by the data, is that T is the main *Leptospira* organism shed, consistent with reports of Tarassovi in notified human cases, especially in dairy workers. Data support the proposition that T is now at a higher prevalence than observed 30–40 years ago, which in combination with human notified case data suggests it is an emerging serovar in New Zealand dairy cattle. The isolation and molecular description of serovar T is now needed, as is an evaluation of

its inclusion in future vaccines for dairy cattle in New Zealand, along with investigation of its epidemiology.

4.7. Acknowledgements

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References

Adler, B., Levett, P.N., Cameron, C.E., Picardeau, M., Levett, D.A.Ha.P.N., A.Ellis, W., et al., 2015. In: In: Adler, B. (Ed.), *Leptospira* and Leptospirosis Vol. 387 Springer, Verlag Berlin Heidelberg.

Benschop, J., Collins-Emerson, J., Maskill, A., O'Connor, P., Tunbridge, M., Yupiana, Y., Weston, J., 2017. Leptospirosis in three workers on a dairy farm with unvaccinated cattle. N. Z. Med. J. 130 (1462), 102.

Bettelheim, K.A., Fogg, T.R., 1986. Serological studies on leptospiral antibodies in dairy milkers in 3 regions of the south island of New Zealand. Comp. Immunol. Microbiol. Infect. Dis. 9 (4), 355–364. https://doi.org/10.1016/0147-9571(86)90048-2.

Blackmore, D.K., Bahaman, A.R., Marshall, R.B., 1982. The epidemiological interpretation of serological responses to leptospiral serovars in sheep. N. Z. Vet. J. 30 (4), 38–42.

Blackmore, D.K., Marshall, R.B., MacKintosh, C.M., 1981. Alternative strategies for the control of leptospirosis in dairy herds. N. Z. Vet. J. 29 (1-2), 19–20. https://doi.org/10.1080/00480169.1981.34780.

Bolin, C.A., Koellner, P., 1988. Human-to-human transmission of *Leptospira interrogans* by milk. J. Infect. Dis. 158 (1), 246–247.

Christmas, B.W., Tennent, R.B., Philip, N.A., Lindsay, P.G., 1974a. Dairy farm fever in New Zealand.[i] A local outbreak of human leptospirosis. N. Z. Med. J. 79 (514), 901–904.

Christmas, B.W., Till, D.G., Bragger, J.M., 1974b. Dairy farm fever in New Zealand isolation of L Pomona and L Hardjo from a local outbreak. N. Z. Med. J. 79 (514), 904–906.

Cowie, G., Bell, A., 2012. A retrospective review of notified human leptospirosis cases in the Waikato region of New Zealand, 2004 to 2010. N. Z. Med. J. 125 (1358), 20–27.

Doeleman, F., 1932. Ziekte van Weil, rechstreeks overgebracht van mensch op mensch. Ned. Tijdschr. 76, 5057.

Dohoo, I.R., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd ed. AVC Incorporated Charlottetown, Canada.

Dreyfus, A., Benschop, J., Collins-Emerson, J., Wilson, P., Baker, M.G., Heuer, C., 2014. Sero-prevalence and risk factors for leptospirosis in abattoir workers in New Zealand. Int. J. Environ. Res. Public Health 11 (2), 1756–1775. https://doi.org/10.3390/ijerph110201756.

ESR Report 2012-16. The Institute of Environmental Science and Research Ltd. (2012-2016). Notifiable Diseases in New Zealand: Annual Report 2012-2016. (https://surv.esr.cri.nz/).

Faine, S., 1982. Guidelines for the Control of Leptospirosis. World Health Organization,

Geneva, Switzerland.

Fang, F., Collins-Emerson, J.M., Heuer, C., Hill, F.I., Tisdall, D.J., Wilson, P.R., Benschop, J., 2014. Interlaboratory and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle. J. Vet. Diagn. Investig. 26 (6), 734–747.

Flint, S., Liardet, D., 1980. A trivalent leptospiral vaccine with emphasis on a *Leptospira interrogans* serovar Hardjo component to prevent leptospiruria. N. Z. Vet. J. 28 (12), 263–266.

Hartskeerl, R.A., Collares-Pereira, M., Ellis, W.A., 2011. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clin. Microbiol.Infect. 17 (4), 494–501. https://doi.org/10.1111/j.1469-0691.2011.03474.x.

Hathaway, S.C., 1981. Leptospirosis in New Zealand: an ecological view. N. Z. Vet. J. 29(7), 109–112. https://doi.org/10.1080/00480169.1981.34815.

Hellstrom, J.S., 1978. Bovine Leptospirosis in New Zealand. (Doctor of Philosophy).Massey University, Massey University.

Heuer, C., Benschop, J., Stringer, L., Collins-Emerson, J., Sanhueza, J., Wilson, P., 2012. Leptospirosis Vaccination in NZ - Best Practice Recommendations Vaccine Use to Prevent Human Exposure. Palmerston North, New Zealand: Institute of Veterinary, Animal and Biomedical Sciences, Massey University.

Kirschner, L., 1954. Recent studies on leptospirosis in New Zealand; infection with a new type (*Leptospira mitis*) Johnson (Syn. Lept. hyos) in man and animals. N. Z. Med. J.53 (294), 119–128.

Levett, P.N., 2001. Leptospirosis. Clin. Microbiol. Rev. 14.

LIC and DairyNZ Limited, 2018. New Zealand Dairy Statistics 2013-18. Hamilton, New Zealand. Retrieved from. www.dairynz.co.nz/dairystatistics.

Mackintosh, C.G., 1981. A Study of the Epidemiology and Control of Leptospirosis on Dairy Farms: a Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Veterinary Pathology and Public Health at Massey University. Massey University.

Mackintosh, C.G., Marshall, R.B., 1980. Serological Titres Resulting From Leptospiral Vaccine.

Mackintosh, C.G., Marshall, R.B., Broughton, E.S., 1980. The use of a hardjo-pomona vaccine to prevent leptospiruria in cattle exposed to natural challenge with *Leptospira interrogans* serovar Hardjo. N. Z. Vet. J. 28 (9), 174–177.

Mackintosh, C.G., Schollum, L.M., Blackmore, D.K., Marshall, R.B., 1982a. Epidemiology of leptospirosis in dairy farm workers in the Manawatu. Part II. A case study of high and low risk farms. N. Z. Vet. J. 30, 73–76.

Mackintosh, C.G., Schollum, L.M., Blackmore, D.K., Marshall, R.B., 1982b. Epidemiology of leptospirosis in dairy farm workers in the Manawatu. Part II. A case study of high and low risk farms. N. Z. Vet. J. 30, 73–76.

Mannewald, A., Collins-Emerson, J., Dreyfus, A., Sanhueza, J.M., Wilson, P.R., Benschop, J., Heuer, C., 2017. Seroprevalence and distribution of *Leptospira interrogans* serovar Copenhageni and *L. borgpetterseni* serovars Tarassovi and Ballum in Beef Cattle, Sheep and Deer in New Zealand. Proc. 10th Meeting of the Internaitonal Leptospirosis Society.

Margolin, B.H., 1988. 'Test for trend in proportions'. In: In: Kotz, S., Johnson, N.L., Read, C.B. (Eds.), Encyclopedia of Statistical Sciences, volume 9. John Wiley & Sons, New York, pp. 334–336.

Marshall, R.B., Broughton, E., Hellstrom, J., 1979. Protection of cattle against natural challenge with *Leptospira interrogans* serovar Hardjo using a hardjo-pomona vaccine. N. Z. Vet. J. 27 (6), 114–116.

Marshall, R.B., Chereshsky, A., 1996. Vaccination of dairy cattle against leptospirosis as a means of preventing human infections. Surveillance (Wellington) 23, 27–28.

Marshall, R.B., Manktelow, B.W., 2002. Fifty years of leptospirosis research in New Zealand: a perspective. N. Z. Vet. J. 50 (3), 61–63.

McLean, M., 2014. A cluster of three cases of leptospirosis in dairy farm workers in New Zealand. New Zealand medical journal (Online) 127 (1388), 13–20.

Nisa, S., Wilkinson, D.A., Paine, S., Cullen, K., Wright, J., Baker, M.G., J., B, 2018. Descriptive Epidemiology of Human Leptospirosis in New Zealand: 1999 to 2016.

Parramore, J.J., Meenks, M.B.R., Wilson, P.R., Heuer, C., Weston, J.F., 2011. The Effectiveness of Longterm Vaccination of Dairy Cattle on New Zealand Farms. Palmerston North.

RStudio Team, 2016. RStudio: Integrated Development for R. RStudio, version 1.0.44. Inc., Boston, MA. URL http://www.rstudio.com/.

Ryan, T.J., Hellstrom, J.S., Penniket, J.H., 1982. Leptospirosis in dairy farmers: prevention by vaccination of cattle. N. Z. Vet. J. 30 (7), 107–108. https://doi.org/10.1080/00480169.1982.34900.

Ryan, T.J., Marshall, R.B., 1976. Isolation of a Leptospire Belonging to Serogroup Tarassovi.

Subharat, S., Wilson, P.R., Heuer, C., Collins-Emerson, J.M., 2011. Evaluation of a SYTO9 real-time polymerase chain reaction assay to detect and identify pathogenic *Leptospira* species in kidney tissue and urine of New Zealand farmed deer. J. Vet.Diagn. Investig. 23 (4), 743–752.

Suwimonteerabutr, J., Chaicumpa, W., Saengjaruk, P., Tapchaisri, P., Chongsa-nguan, M.,Kalambaheti, T., et al., 2005. Evaluation of a monoclonal antibody–based dot-blot ELISA for detection of *Leptospira spp* in bovine urine samples. Am. J. Vet. Res. 66 (5),762–766.

Thornley, C.N., Baker, M.G., Weinstein, P., Maas, E.W., 2002. Changing epidemiology of human leptospirosis in New Zealand. Epidemiol. Infect. 128 (01), 29–36. https://doi.org/10.1017/s0950268801006392.

Tripathy, D., Smith, A., Hanson, L., 1975. Immunoglobulins in cattle vaccinated with leptospiral bacterins. Am. J. Vet. Res. 36 (12), 1735–1736.

Wilson, P.R., Heuer, C., Benschop, J., Collins-Emerson, J., Parramore, J.J., Meenks, M.B.R., 2013. Does long-term vaccination eliminate leptospiral shedding? A pilot study of New Zealand dairy cattle herds. Vetscript 26, 16.

Chapter 5
Leptospira spp. vaccination practices on New Zealand dairy farms

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5.1. Abstract

A cross-sectional study of 200 randomly selected dairy farms stratified by herd size and region throughout New Zealand was conducted to investigate bivalent *Leptospira interrogans* sv. Pomona (P) and *L. borgpetersenii* sv. Hardjo (H) and trivalent (including *L. interrogans. sv.* Copenhageni (C)) vaccination practices and their effectiveness in dairy herds in New Zealand. This paper describes vaccination practices and evaluates conformity with best practice guidelines using data from a questionnaire administered by participating veterinary practices.

Leptospira vaccination programmes had been implemented on 99.5% (95% CI: 97.2-99.9) of farms, and 89.4% of those had programmes running for five years or more. Most farmers used bivalent vaccines while19.6%, 31.3% and 32.1% used trivalent vaccines on calves, heifers and cows, respectively. A higher proportion of farmers in the North Island used trivalent vaccines than in South Island. The 7-in-1 combined bivalent H, P and clostridial vaccine was used more commonly than *Leptospira*-only vaccines in calves than in other age groups. Approximately 60% of farmers purchased only vaccinated animals and about 30% were unsure of the vaccination status of purchased cattle. Sixty one percent of farmers had other livestock on their farms and of them, 42% vaccinated some or all for *Leptospira.* A similar proportion of vaccine administration was by veterinary service providers and farm staff. *Leptospira* vaccines were administered always or sometimes with other animal remedies on 15.8% and 47.9% of farms, respectively. Most farmers had not made changes to their vaccination programme in the previous five years. Timing of first vaccination of calves ranged from two weeks to 10 months of age, with 60% vaccinating by four months. Approximately half of the farms timed vaccinations for calves and heifers, and 93% for cows according to best practice guidelines. It is concluded that there is almost universal adoption of *Leptospira* vaccination in dairy cattle, but there are areas to address in terms of following best practice guidelines and refinement of vaccination programmes, particularly with respect to timing of vaccination in calves.

5.2. Introduction

Leptospira infection in cattle occurs worldwide and affects production via abortion, stillbirth, and reduced milk production [1]. Infected cattle can transmit *Leptospira* to humans through direct contact with infected urine or indirectly through the contaminated environment [2, 3]. Therefore, farm [4] and meat workers [5] are at high risk of exposure to *Leptospira* from cattle. To control this disease in cattle and reduce the risk of human infection, vaccination has been conducted in many countries including New Zealand [6], Canada [7], the United States of America [8], and Brazil [9].

A recent meta-analysis, mainly based on artificial challenge studies, showed that *Leptospira* vaccination was approximately 89% effective in preventing *Leptospira borgpetersenii* serovar Hardjo (H) shedding in cattle [10]. For optimum efficacy, vaccination should precede infection. *Leptospira* infection in calves under five months of age have been reported by some studies in New Zealand [11, 12]. A five-week-old calf from a Southland dairy farm showed infection with *Leptospira interrogans* serovar Pomona (P) and PCR positive urine [13]. Calves first vaccinated at one month of age can be protected against *Leptospira* colonization 12 months after vaccination [8]. Thus, vaccination at an early age is recommended. In New Zealand, widespread *Leptospira* vaccination in dairy herds has been implemented for almost 40 years. Initially, bivalent (H and P) were used to protect both cattle and humans [14] because of the high prevalence in cattle and high incidence of leptospirosis due to those serovars in dairy workers [15]. Recent anecdote suggests that more than 90% of New Zealand dairy herds are vaccinated with either bivalent or trivalent (including *L. interrogans sv.* Copenhageni (C)) vaccines [16]. There are six serovars serologically detected from cattle in New Zealand including H, P and C along with *Leptospira b.* serovars Ballum (B), Balcanica (Ba) and Tarassovi (T) of which all but T have been isolated from cattle [17, 18]. Serovars H, P, C, B are routinely tested and reported in humans [19-23]. The current routine diagnostic tests do not distinguish between H and Ba, as both serovars are very closely related serologically and genetically. A recent summary of notification data from 1999 to 2016 demonstrate an increase in the incidence of leptospirosis in

dairy workers due to T and B [24] suggesting research is needed to better understand transmission pathways, and if associated with livestock, to inform consideration of inclusion of these two serovars in vaccines.

To promote optimum protection from *Leptospira* infection, the New Zealand Veterinary Association (NZVA) developed "Leptosure" [25], a risk evaluation programme to assist veterinarians in developing control strategies with their dairy farming clients. More specific guidelines were published by the NZVA's Dairy Cattle Special Interest Branch, describing "best practice" protocols for ruminant vaccination with the main goal of protecting humans [16], and by default, protecting animal health and production. These guidelines were specifically designed for New Zealand's seasonal pastoral dairy production system.

Despite the long-term adoption of *Leptospira* vaccination, there has been no evaluation of vaccination programme efficacy in reducing or eliminating shedding of vaccine serovars or conformity with best practice guidelines. This paper presents a descriptive analysis of *Leptospira* vaccination practices and evaluation of conformity with best practice guidelines, as part of a broader study of dairy cattle *Leptospira* vaccination programmes in New Zealand [26].

5.3. Materials & methods

5.3.1. Study design

A cross-sectional study of *Leptospira* shedding in urine and seroprevalence in pastoral farmed seasonal supply dairy cattle in New Zealand was conducted from January to April 2016 to evaluate the implementation and effectiveness of vaccination programmes in reducing or eliminating *Leptospira* shedding, establish conformity with vaccination best practice guidelines, and to re-evaluate the epidemiology of *Leptospira per se* in dairy cattle. Full details of the study including sample size calculation, farm recruitment, sample and data collection and laboratory testing are presented in Yupiana *et al.,* (2019). Briefly, 200 commercial dairy farms were randomly selected

from a national database after stratifying by region and herd size. The farm's veterinary service provider was recruited to collect samples and administer a questionnaire. Between January and April 2016, paired urine and blood samples were collected from 20 randomly selected adult cows per herd, and analysed by PCR and Microscopic Agglutination, respectively, at the Hopkirk Institute, Massey University.

5.3.2. Farm vaccination data collection

A survey was conducted using a pre-tested questionnaire that included contact details for the farmer and veterinary service provider, general and farm demographic information, vaccination practices and farm management practices. The questionnaire was pre-circulated to participating farmers, and final completion was by face-to-face interview by the veterinary service provider (veterinary practice veterinarian or veterinary technician) at the time of sample collection [26].

A record of vaccine brand and type (bivalent or trivalent) used in each herd during the past five years was obtained from participating veterinary service provider databases to either verify farmer response or provide missing or unknown information about the vaccine(s) used.

The *Leptospira* vaccine brands used by farmers in this study included "Leptavoid 2" (MSD Animal Health), "Lepto 2-way" and "Lepto 3-way" (Virbac New Zealand), "Leptoshield 2", "Leptoshield 3", and "Ultravac 7 in 1" (Zoetis Animal Health). The latter vaccine contains antigen to five clostridial species along with *Leptospira* H and P. For the purposes of this paper, vaccines used were categorised as either bivalent or trivalent, except where specific reference is made to use of the 7-in-1 vaccine.

5.3.3. Definitions

Age groups were calves (males and females <12 months), heifers (females 12 -24 months), adult cows (>24 months), yearling bulls (12 to 24 months) and adult bulls (>24 months). The number of years a farmer had vaccinated was categorised as <5, 5-10, 10-20 and >20 years.

Best practice (BP) guidelines against which farmer's vaccination practices were evaluated are based on those of the New Zealand Veterinary Association's recommendation [16] as summarised in Table 5.3-1. The BP guidelines differentiate vaccination practices between "high-risk" farms, being those with previously unvaccinated cattle, access to potentially contaminated water (e.g. flooded pasture), or where replacement stock are returning from locations where they grazed with other cattle of unknown vaccination status and "low-risk" farms.

Table 5.3-1. Best practice guidelines for dairy cattle *Leptospira* vaccination on high- and low-risk farms against which farmers' vaccination practices were evaluated (based on Heuer et al 2012).

	High risk farms	**Low risk farms**
1st vaccination (sensitizer)	10-14 weeks after start of calving	10-18 weeks after start of calving
2nd vaccination	14-18 week old, 4 weeks after 1st vaccination	14-22 weeks old, 4 weeks after 1st vaccination
3rd vaccination	Optional, 6 months after sensitizer	none
1st booster	5-7 months after 2nd calf vaccination when 10 months old (May), or as soon as convenient thereafter, to align with adult stock.	5-7 months after 2nd calf vaccination when 10 months old (May), or as soon as convenient thereafter, to align with adult stock
Annual booster (adult cows)	Lactating herd at dry-off (May) 1st calving cows: 32 months of age (May)	Lactating herd at dry-off

5.3.4. Data analysis

Data were analysed at herd level using R version 3.3.2 (2016-10-31). The percentage and the 95% Confidence Interval (95%CI) for dairy farmers' responses to the questions

about vaccine types, administration of vaccine (veterinary service providers and farm staff), conformity with BP guidelines and vaccination status of purchased cattle were calculated using Wilson's method [27]. Pearson's Chi-square without Yates' continuity correction was used to compare the percentage of farmers who used bivalent vs. trivalent vaccine in calves, heifers and adult cows and the percentage of farmers who used trivalent in calves vs heifers and adult cows. Pearson's Chi-square with Yates' continuity correction was used to compare the percentage of farmers who used bivalent vs. trivalent vaccine in the North and the South Islands in calves, heifers and adult cows. The potential confounding effect of veterinary service provider was not tested because few attended more than two farms.

5.4. Results

The response rate related to vaccination practices in calves, heifers and adult cows varied from 78%-100%, with 63% of farmers answering all questions. Of the 200 dairy farms surveyed, 199 (99.5%: 95% CI: 97.2-99.9) farms had a current vaccination programme against *Leptospira*. *Leptospira* vaccination had been administered for more than 20 years on 39 % of the farms, and less than 5 years on 10% of those farms with a vaccination programme (Figure 5.4-1). The non-vaccinated herd had been vaccinated until 2014 and no reason was given for ceasing vaccination.

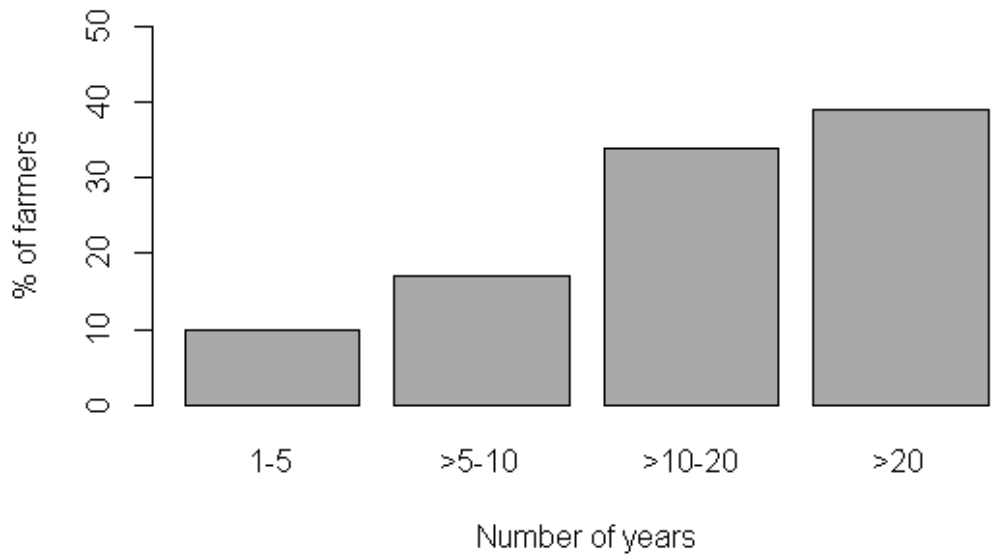

Figure 5.4-1. The number of years that farmers had implemented a *Leptospira* vaccination programme for their dairy cattle (n=199)

The number of respondents and the percentage of those using a 7-in-1, other bivalent, or trivalent vaccine in calves, heifers and adult cows is presented in Table 5.4-1. Twenty, 36 and 34, farmers did not stipulate the vaccine used in calves, heifers, and adult cows, respectively. Most respondents used bivalent vaccines in all age classes, though a higher percentage of farmers gave a trivalent vaccine to heifers and adult cows than to calves ($p<0.05$). Of the bivalent vaccine users for calves, the majority used the 7-in-1 vaccine. Of 161 famers who responded, 41.6% (95%CI; 34.3%-49.3%) vaccinated bulls at the same time as cows. A higher percentage of farmers in the North Island used a trivalent vaccine for calves, heifers and adult cows than those in the South Island ($p<0.001$) (Table 5.4-2).

Table 5.4-1. Number of dairy farmers (N) responding to the question of which vaccine types were used, and the percentage (and 95% CI) of those who used 7-in-1 (Clostridial + *Leptospira* borgpeterseni serovar Hardjo (H) and L. interrogans serovar Pomoa (P)), other bivalent (H and P) and trivalent (H, P and L interrogans serovar Copenhageni (C)), in calves, heifers and adult cows in New Zealand in the five years prior to 2016.

Age group	**N**	**Bivalent**			**Trivalent**
		7-in-1	**Other**	**Total**	
		% (95% CI)	**% (95% CI)**	**% (95% CI)**	**% (95% CI)**
Calves	179	55.3 (48.0-62.4)	25.1 (19.4-32.0)	80.4 (74.0-85.6)	19.6 (14.4-26.0)
Heifers	163	16.6(11.6-23.0)	52.1(44.5-60.0)	68.7 (61.2-75.3)	31.3 (24.7-38.8)
Adult cows	165	14.5 (10.0-20.7)	111(74.0-88.1)	67.9 (60.4-74.5)	32.1 (25.5-39.6)

Table 5.4-2. Number of dairy farmers (N) in the North and South Islands responding to the question of which vaccine types were used and the percentage (and 95% CI) of those which were trivalent (*Leptospira* borgpeterseni serovar Hardjo (H) and L. interrogans serovar Pomoa and L interrogans serovar Copenhageni (C)), in calves, heifers and adult cows in the five years prior to 2016.

	North island			South island		
Age group	N	Bivalent	Trivalent	N	Bivalent	Trivalent
		% (95% CI)	% (95% CI)		% (95% CI)	% (95% CI)
Calves	115	72.2 (63.4-79.5)	27.8 (20.5-36.6)	64	95.3 (87.1-98.4)	4.7 (1.6-12.9)
Heifers	104	56.7 (47.4-65.8)	43.3 (34.2-52.9)	59	89.8 (79.5-95.3)	10.2 (4.7-20.5)
Adult cows	107	57.0 (47.5-66.0)	43.0 (34.0-52.5)	58	87.9 (77.1-94.0)	12.1 (6.0-22.9)

The vaccination status of purchased heifers, adult cows and bulls is presented in Table 5.4.3. Of farmers responding, the majority purchased vaccinated animals. Between a quarter and a third of farmers were unsure about the vaccination status of various classes of stock purchased.

Table 5.4-3. Number of dairy farmers (N) who purchased only vaccinated, some vaccinated or unvaccinated heifers, adult cows and bulls or who were unsure about vaccination status, in the five years prior to 2016.

Vaccination status of purchased cattle	Heifers		Adult cows		Bulls	
	N	% (95%CI)	N	% (95%CI)	N	% (95%CI)
All vaccinated	68	60.7(51.5-69.3)	70	63.6 (54.3-72.0)	87	65.4 (57.0-73.0)
Some vaccinated	2	1.8 (0.5-6.3)	3	2.7 (0.9-7.7)	2	1.5 (0.4-5.3)
None vaccinated	6	5.4 (2.5-11.2)	6	5.5 (2.5-11.4)	10	7.5 (4-13.3)
Unsure	36	32.1 (24.2-41.3)	31	28.2 (20.6-37.2)	34	25.6 (18.9-33.6)
Total	112		110		133	

Changes in vaccination practices are presented in Table 5.4-4. The majority of farmers continued the same vaccination practice over the previous five years. Change in vaccination practices were mostly related to the timing rather than vaccine type and brands, or vaccination personnel. The most common changes were to adopt a third vaccination of calves within their first 12 months, changing of months of 1st and 2nd vaccination, and the interval from 1st to 2nd vaccination.

On 15.8% and 47.9% of farms, *Leptospira* vaccines were always or sometimes, respectively, administered at the same time as other animal remedy treatments, while 36.3% of farmers never combined *Leptospira* vaccination with other treatments.

In total, 61.1% (95% CI: 53.9-67.8) of farmers managed other livestock (beef cattle, sheep, pigs and deer) on their farm and 42.2% (95% CI: 35.3-49.4) vaccinated some or all of those species.

Table 5.4-4. Description of changed vaccination practices, and number of farmers (n=31/180) who changed each practice for calves, heifers and cows during the 5 years prior to 2016.

Practice change	Age group		
	Calves	**Heifers**	**Adult Cows**
Timing/schedule			
First to second vaccination (getting both shorter and longer)	14		
Booster in heifers added		6	
Annual booster in adult cows			7
Vaccine type			
Bivalent to trivalent	1		
Trivalent to bivalent	1		
Vaccine brand	3	1	1
Vaccine administration			
Veterinary service provider to farm staff	3		
Farm staff only to farm staff and veterinary service provider	1		
Other			
Adopting 3rd calf vaccination	8		
Adopting heifer booster		1	

The percentage of responding farmers administering first vaccination to calves at various ages is shown in Figure 5.4-2, and the month of first vaccination of calves is shown in Figure 5.4-3. Sixty percent of farmers first vaccinated calves before the age of four months. The latest that calves received first vaccination was 10 months of age. First vaccination of calves was carried out during every month of the year, but November and December were the most common months for the first vaccination to be administered.

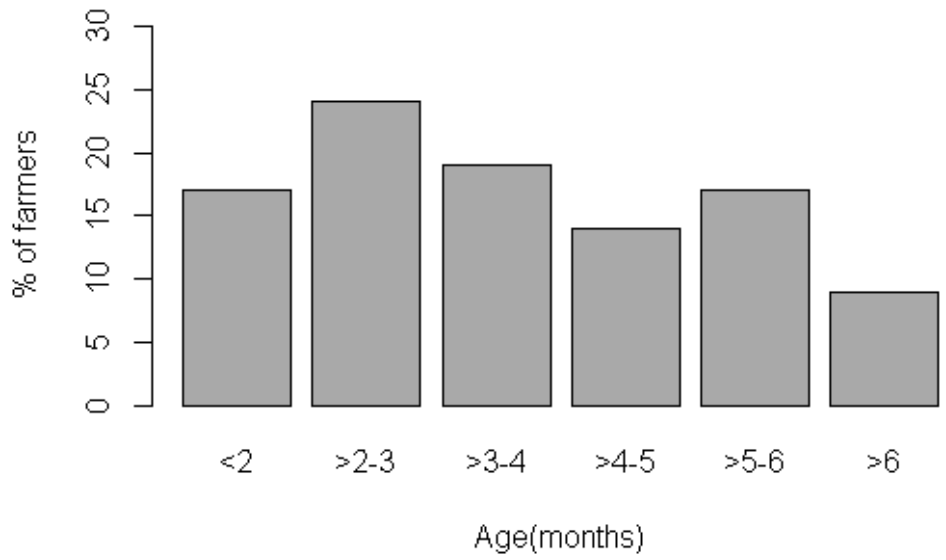

Figure 5.4-2. Percentage of dairy farmers responding (n=178) who vaccinated calves for the first time at different ages.

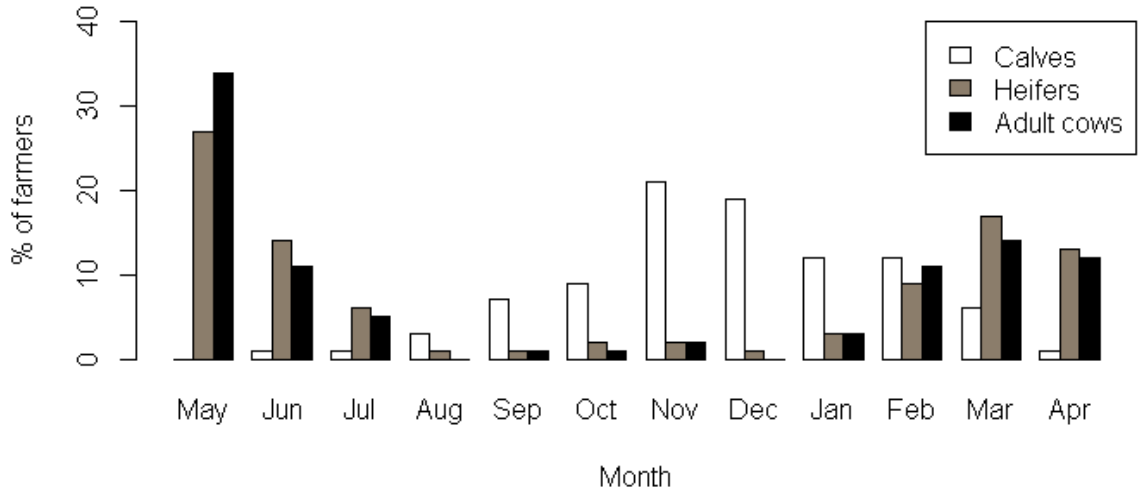

Figure 5.4-3. Percentage of dairy farmers responding and the month in which vaccinations were administered; the first vaccination to calves (n=170), booster to heifers (n=189) and annual booster to adult cows (n=189).

The number and percentage of farms where *Leptospira* vaccination had been conducted in accordance with BP guidelines are shown in Table 6. Approximately half of farms had calves first vaccinated according to BP guidelines. Most calves received booster vaccinations in accordance with BP. A third vaccination of calves was conducted on a third of high risk farms. Booster vaccination of heifers was conducted according to BP guidelines on almost half of farms and annual booster of adult cows was conducted according to BP guidelines on 92.7% of farms. Overall, 18.7%, (95% CI: 13.4-25.6) of high risk farms and 20.0% (95% CI: 8.9-39.1) of low risk farms had conformed to BP guidelines for all age groups combined (excluding the recommended third vaccination in calves). May was the most common month for booster vaccination of heifers and adult cows (Figure 5.4-3) in accordance with BP guidelines.

Conformity with the BP guidelines was higher for the first vaccination of calves and annual booster for heifers when the programme was administered by farm staff than by veterinary service providers." (Table 5.4-5).

Table 5.4-5. Number (N) and percentage (and 95%CI) of dairy farmers responding on who's vaccination programmes did and did not conform with *Leptospira* vaccination best practice (BP) guidelines (see Table 1), stratified for high-risk (HR) and low-risk (LR) farms, for calves, heifers and cows, and vaccine administration.

Vaccination episode	Conformity with BP guideline	Vaccine administration					
		Veterinary service provider		Farm staff		Overall[+]	
		N	% (95%CI)	N	% (95%CI)	N	% (95%CI)
1st calf vaccination HR	Yes	25	32.1 (22.7-43.0)	40	*51.3 (40.4-62.1)	67	41.4 (34.1-49.1)
	No	53	67.9 (56.9-77.3)	38	49.4 (38.9-59.9)	95	58.6 (50.9-65.9)
1st calf vaccination LR	Yes	8	66.7 (39.1-86.2)	5	38.4 (17.7-64.5)	15	55.6 (37.3-72.4)
	No	4	33.3 (13.8-60.9)	8	61.5 (35.5-82.2)	12	44.4 (27.6-62.7)
2nd calf vaccination	Yes	60	86.9 (77.0-92.9)	72	78.3 (68.8-85.5)	144	80.9 (74.5-86.0)
	No	9	13.0 (7.0-22.9)	20	21.7 (14.5-31.2)	34	19.1 (14.0-25.5)
3rd calf vaccination HR	Yes	0	0(0-8)	1	2.3 (0.4-11.8)	1	57.1 (44.1-69.2)
	No	12	27.3 (16.3-41.8)	31	70.5 (55.8-81.8)	62	42.9 (30.8-55.9)
1st annual booster (Heifer)	Yes	28	33.3 (24.2-43.9)	41	56.2 (44.8-66.9)	72	43.6 (36.3-51.3)
	No	56	66.7 (56.1-75.8)	32	43.8 (33.0-55.2)	93	56.4 (48.7-63.7)
Annual booster adult cows	Yes	89	92.7 (85.7-96.4)	71	93.4 (85.5-97.2)	171	92.9 (88.2-95.8)
	No	7	7.3 (3.6-14.3)	5	6.6 (2.8-14.5)	13	7.1(4.2-11.7)

[+] Includes farmers who did not stipulate service provider

5.5. Discussion

The purpose of this paper was to describe *Leptospira* vaccination practices in randomly selected dairy herds in New Zealand, and how they conform to best practice (BP) guidelines. *Leptospira* vaccination programmes are almost universally adopted, and have been implemented on many dairy farms for decades. This paper reports the *Leptospira* vaccines used and the manner in which vaccination programmes are applied. This report appears to be novel since no literature could be found which describes vaccination programmes for diseases in pastoral livestock similar to the content of this paper.

Data presented here were from a randomly selected dairy herd population and there was a high response rate to all questions, hence results are likely to be a robust representation of *Leptospira* vaccination practices in the New Zealand dairy industry. Despite *Leptospira* vaccination being voluntary, data suggest that at least 97.2% of dairy farmers vaccinate. These data are likely representative results as of the 69 non-responders, only three farmers did not administer vaccination for their herd. This confirms a high level of awareness of the occupational health implication and legal requirement to take reasonable steps to protect workers from zoonotic disease and/or of the animal health and production effects due to this infection. It also suggests confidence in the effectiveness of vaccination. This level of awareness and confidence was likely prompted by research undertaken in the late 1970s and early 1980s, reviewed by Marshall and Manktelow (2002). The reduction in incidence of human cases, predominantly dairy workers, from 21.8 per 100,000 in 1979 to 5.9 per 100,00 in 1992 was attributed largely to vaccination, complemented by other protective measures for dairy workers [29, 30]. The number of notified cases remains low, although an average of 39.1 cases per 100,000 people associated with dairy farming has been notified annually between 1999-2016 [24], suggesting that vaccination is not fully protective, and/or that other serovar(s) are present among dairy cattle. Observations by Yupiana *et al.,* (2019) support the latter explanation.

More farmers use bivalent than trivalent vaccines. This might be due to the availability of the vaccine since there are more bivalent vaccine brands than trivalent. Economic factors may be influential but are unlikely to be a major reason since the cost of trivalent vaccines is only marginally higher than for bivalent vaccines. Furthermore, the decision for the vaccine type used was largely not the farmer's choice but the veterinarian's. Previous New Zealand studies have detected *Leptospira* shedding and exposure to serovar Copenhageni in dairy herds [11, 31], but at a lower prevalence than H and P. The decision by veterinarians and farmers to use trivalent vaccines may be associated with evaluation of the risk of transmission from other species such as Norway rats (*Rattus norvegicus*) [32].

This study found a significantly higher percentage of trivalent vaccine used in the North Island. Data collected in the questionnaire on observation of rodents [26] showed rats were observed on a higher percentage of farms in the North Island. The Leptosure^{TM}–programme recommends evaluation of environmental risks such as rodents when making decisions about vaccination usage and programmes.

Age at first vaccination was a potentially important component of this study since a pilot study identified that shedding of leptospires in urine was positively and significantly associated with later timing of first calf vaccination [33]. Studies in New Zealand have shown that calves under five months of age can be infected by *Leptospira* [11, 12]. A surveillance report of leptospirosis in a five-week-old calf from a Southland dairy farm showed infection with Pomona and PCR positive urine [13]. Another study demonstrated that calves vaccinated at one month old and booster vaccinated 4 weeks later had reduced leptospiruria following challenge 12 months after vaccination [8]. However, an experimental study on 45 calves demonstrated a poorer serological response in calves vaccinated at three months of age than at six months of age [34], although MAT antibody cannot be used as a direct measure to assess the protection against *Leptospira.* More definitive research into the timing of first vaccination, the impact of maternally derived antibody on vaccine efficacy and the

effectiveness of current vaccination practices is required to better inform decisions around timing of first vaccination.

Most farmers implemented their vaccination programme consistently from year to year. Of those who changed their practices, most related to the timing and schedule for calf vaccination. Early vaccination was strongly advocated by veterinarians in New Zealand in recent years. Insufficiently detailed information was available to ascertain whether the change was an advancement or delay in the age of first vaccination. Inferences cannot be made since age at first vaccination, based on the month, is correlated with timing of calving.

More than 60% of farmers administered the *Leptospira* vaccine sometimes or always at the same time as other animal remedy treatments. This was likely to reduce time and cost of multiple animal handling. The questionnaire did not enquire as to which additional treatments were used. However, there is a limited number of other vaccines used in dairy cattle in New Zealand, and there is little evidence that a small number of multiple vaccines given simultaneously will materially impair the immune response to the *Leptospira* vaccine [35]. It is unlikely that simultaneous treatments for internal or external parasites would negatively influence immunological response to *Leptospira* vaccines.

Data showed that approximately 40% of farmers purchased or introduced heifers, cows and bulls, of which some or none were vaccinated, or their vaccination status was unknown. This is a potential risk for exposure of herds to infection via shedding by introduced animals. The question of purchasing stock was primarily for assessment of this as a risk for shedding. However, it was a shortcoming of the questionnaire that farmers were not asked whether they vaccinated or quarantined purchased stock. An earlier study in the Hauraki Plains region involving 450 dairy herds showed that 25% of farmers planned to purchase milking stock replacements, but few enquired about vaccination status [6]. Previous studies identified that being an open farm, that is, one that brought in stock, was a risk factor for *Leptospira* infection in dairy workers and

dairy cattle [36, 37] suggesting that purchased stock may have been carriers and shedders.

Questionnaires are often subject of a range of biases [38]. Recall bias was recognized as likely being the most important of potential biases in this study. Vaccine brand and type used were validated using veterinary practice data. The period over which a herd had been under a vaccination programme may also have been subject to speculation, or admission bias in the event of short duration. This was minimised by categorising data to four-time intervals. Conversely there might be an underestimation of the number or years that a vaccination programme had been implemented as the questionnaire could only address the current farmer/manager's recollection. There will be some herds that had vaccinated for almost 40 years, given that the vaccine was first available in the early 80s [28]. Questions that were not answered by some participants in this study could produce biased estimates, e.g. name of vaccines used. However, this may not influence the internal validity, as missing data are assumed to be not related to vaccination status of the herds.

Leptospira vaccines in New Zealand are registered as veterinary medicines requiring veterinary prescription, although non-veterinarians are permitted to administer the vaccine if they have been trained in the correct technique and the prescribing veterinarian is satisfied to authorize/prescribe and dispense the product for farmer administration [39]. This study found that the proportion of farmers who had veterinary service providers or farm staff administer the vaccine was similar for calves and heifers but a higher proportion of adult cow herds were vaccinated by veterinary service providers. Data showed a significantly greater conformity with BP guidelines for first calf and heifer vaccination when vaccines were administered by farm staff. The reasons for this are likely to be complex involving a multitude of logistics and management factors and may or may not necessarily reflect familiarity with the guidelines. For example, the relatively low conformity for heifer vaccination could be explained because heifers are frequently away from the "home" farms, and not under direct management of the farmer when guidelines suggest the annual vaccination

should take place. An earlier report noted concern about heifer vaccination and in some cases this age group was not vaccinated at all [6]. It appears that this concern persists.

While scientific articles have been used to inform BP guidelines where available, some non-validated or non-researched recommendations from pharmaceutical companies and unpublished data have been used. There is a lack of robust data available for some important aspects such as the impact of maternally derived antibody in calves on vaccine efficacy as highlighted by Heuer *et al.,* (2012), hence, a 'first principles' approach was taken with respect to some guidelines. Furthermore, there are no researched data elsewhere on the effectiveness of long-term *Leptospira* vaccination programmes as such in contrast with conventional studies of vaccine efficacy *per se* [10]. Evaluation of the outcome of programmes that do and do not conform to BP guidelines, in terms of *Leptospira* shedding in urine, was outside the scope of the present paper, but is addressed elsewhere [26]. These factors predicate that no inference should be drawn about the effectiveness of vaccination programmes that do or do not conform with BP guidelines until knowledge gaps on practices and outcomes are filled and guidelines are re-evaluated accordingly. Nevertheless, data here do suggest a need for further understanding of the reasons for non-conformity with BP guidelines to assist veterinarians to communicate science-based recommendations to achieve the best possible vaccine use and design of vaccination programmes under their supervision.

There was almost a universal adoption of vaccination of dairy cattle in New Zealand indicating a high level of awareness among dairy farmers about the importance of conducting vaccination in dairy herds to protect humans and/or livestock. Our data demonstrate which vaccines were used and when and how they are used. It informs the understanding of other aspects of vaccination programme implementation and control of *Leptospira* infection in dairy cattle. This study also demonstrated the current level of conformity with present BP guidelines, with some non-conformity possibly indicating knowledge among the veterinary profession and farmers about the scientific

uncertainties surrounding some elements of the guidelines. It also suggests opportunity to improve communication of guidelines by veterinary service providers to their dairy farmer clients.

5.6. Acknowledgements

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References

[1] Adler, Ben, ed. *Leptospira and leptospirosis*. Vol. 387. Springer, 2015.

[2] Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. Clinical microbiology and infection. 2011; 17(4):494-501.

[3] Levett PN. Leptospirosis: a forgotten zoonosis? Clinical and Applied Immunology Reviews. 2004;4(6):435-48.

[4] Dobinson J. Leptospirosis and the dairyfarmer. Leptospirosis and the dairyfarmer. 1967.

[5] Dreyfus A, Wilson P, Collins-Emerson J, Benschop J, Moore S, Heuer C. Risk factors for new infection with *Leptospira* in meat workers in New Zealand. Occup Environ Med. 2015;72(3):219-25.

[6] Ryan TJ, Hellstrom JS, Penniket JH. Leptospirosis in dairy farmers: prevention by vaccination of cattle. New Zealand veterinary journal. 1982; 30(7):107-8.

[7] Van De Weyer LM, Hendrick S, Rosengren L, Waldner CL. Leptospirosis in beef herds from western Canada: Serum antibody titers and vaccination practices. The Canadian Veterinary Journal. 2011;52(6):619.

[8] Zimmerman AD, Springer EW, Barling KS, Buterbaugh RE, Pooley RD, Scholz DA, Rhoades JR, Chase CC. Immunity in heifers 12 months after vaccination with a multivalent vaccine containing a United States Leptospira borgpetersenii serovar Hardjo isolate. Journal of the American Veterinary Medical Association. 2013;242(11):1573-7.

[9] Pereira MH, Cooke RF, Alfieri AA, Vasconcelos JL. Effects of vaccination against reproductive diseases on reproductive performance of lactating dairy cows submitted to AI. Animal reproduction science. 2013;137(3-4):156-62.

[10] Sanhueza JM, Wilson PR, Benschop J, Collins-Emerson JM, Heuer C. Meta-analysis of the efficacy of *Leptospira* serovar Hardjo vaccines to prevent urinary shedding in cattle. Preventive veterinary medicine. 2018;153:71-6.

[11] Dodd DC, Brakenridge DT. *Leptospira icterohaemorrhagiae* AB infection in calves. New Zealand Veterinary Journal. 1960;8(4):71-6.

[12] Ensor CR, McClure TJ. Bovine leptospirosis in Northland. New Zealand Veterinary Journal. 1953; 1(3):47-50.

[13] Anonymous. *Quarterly report of diagnostic cases July to Sept 2013.* Ministry for Primary Industries 2013; 40.

[14] Marshall RB. A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. Israel journal of veterinary medicine. 1987; 43:271-6.

[15] Blackmore DK, RB Marshall, and CM Mackintosh. Alternative strategies for the control of leptospirosis in dairy herds New Zealand Veterinary Journal, 29(1-2), 19-20.

[16] Heuer C, Benschop J, Stringer L, Collins-Emerson J, Sanhueza J, Wilson P. Leptospirosis in New Zealand: best practice recommendations for the use of vaccines to prevent human exposure. Massey University, Institute of Veterinary, Animal and Biomedical Sciences. 2012

[17] Hellstrom JS. *Studies on some aspects of the epidemiology of bovine leptospirosis* (Doctoral dissertation, Massey University);1978

[18] Mackintosh CG, Marshall RB, Blackmore DK. *Leptospira interrogans* serovar Balcanica in cattle. New Zealand veterinary journal. 1980;28(12):268.

[19] The Institute of Environmental Science and Research Ltd. *Notifiable Diseases in New Zealand: Annual Report 2012*. Porirua, New Zealand.

[20] The Institute of Environmental Science and Research Ltd. *Notifiable Diseases in New Zealand: Annual Report 2013*. Porirua, New Zealand.

[21] The Institute of Environmental Science and Research Ltd. *Notifiable Diseases in New Zealand: Annual Report 2014*. Porirua, New Zealand.

[22] The Institute of Environmental Science and Research Ltd. *Notifiable Diseases in New Zealand: Annual Report 2015*. Porirua, New Zealand.

[23] The Institute of Environmental Science and Research Ltd. *Notifiable Diseases in New Zealand: Annual Report 2016* Porirua, New Zealand.

[24] Descriptive Epidemiology of Human Leptospirosis in New Zealand: 1999 to 2016. (Nisa S, personal communication, September 10, 2018).

[25] Cranefield S. Protecting all New Zealanders against leptospirosis .Proceedings of the society of Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association 32, 2002:53-63

[26] Yupiana Y, Vallee E, Wilson P, Collins-Emerson J, Weston J, Benschop J, Heuer C. Emerging *Leptospira* strain poses public health risk for dairy farmers in New Zealand. Preventive Veterinary Medicine.2019;170:104727.

[27] Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. Statistics in medicine. 1998; 17(8):857-72.

[28] Marshall RB, Manktelow BW. Fifty years of leptospirosis research in New Zealand: a perspective. New Zealand Veterinary Journal. 2002; 50(sup3):61-3.

[29] Marshall RB, Chereshsky A. Vaccination of dairy cattle against leptospirosis as a means of preventing human infections. Surveillance. 1996; 23(1):27-8.

[30] Thornley CN, Baker MG, Weinstein P, Maas EW. Changing epidemiology of human leptospirosis in New Zealand. Epidemiology & Infection. 2002; 128(1):29-36.

[31] Mackintosh CG, Schollum LM, Blackmore DK. Epidemiology of leptospirosis in dairy farm workers in the Manawatu. Part II. A case-control study of high and low risk farms. New Zealand Veterinary Journal. 1982; 30(6):73-6.

[32] Hathaway SC, Blackmore DK, Marshall RB. Leptospirosis in free-living species in New Zealand. Journal of Wildlife Diseases. 1981 Oct;17(4):489-96

[33] Wilson P, Heuer C, Benschop J, Collins-Emerson J, Parramore JJ, Meenks MBR. Does long-term vaccination eliminate leptospiral shedding? A pilot study of New Zealand dairy cattle herds. Vetscript, 2013; 26, 16-16.

[34] Schollum LM, Marshall RB. Age and the ability of calves to respond to a leptospiral vaccine. New Zealand veterinary journal. 1985; 33(9):146-7.

[35] Wilson P, Mackintosh CG, Griffin JFT. Immunological responses to vaccines in deer: effect of multiple vaccines. Proceedings of the Deer Branch of the New Zealand Veterinary Association 18, 2001; 9-14.

[36] O'Doherty E, Berry DP, O'Grady L, Sayers R. Management practices as risk factors for the presence of bulk milk antibodies to Salmonella, *Neospora caninum* and *Leptospira interrogans* serovar hardjo in Irish dairy herds. animal. 2014; 8(6):1010-9.

[37] Oliveira F, Azevedo SS, Pinheiro SR, Batista CS, Moraes ZM, Souza GO, Gonçales AP, Vasconcellos SA. Risk factors associated with leptospirosis in cows in the state of Bahia, northeastern Brazil. Pesquisa Veterinária Brasileira. 2010;30(5):398-402.

[38] Choi BC, Pak AW. Peer reviewed: a catalog of biases in questionnaires. Preventing chronic disease. 2005;2(1).

[39] Anonymous.Worksafe.2018. available at https://worksafe.govt.nz/topic-and-industry/working-with-animals/prevention-and-control-of-leptospirosis/gpg/

Chapter 6
On-farm risk factors associated with *Leptospira* shedding in New Zealand dairy cattle

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6.1. Abstract

This study aimed to evaluate risk factors associated with shedding of pathogenic *Leptospira* species in urine at animal and herd levels. Two hundred dairy farms were randomly selected from the DairyNZ database. Urine samples were taken from 20 lactating, clinically normal cows in each herd between January and April 2016 and tested by real-time PCR using *gyrB* as the target gene. Overall, 26.5% of 200 farms had at least one PCR positive cow, and 2.4% of 4,000 cows were shedding *Leptospira* in the urine. Using a questionnaire, information about risk factors at cow and farm level was collected via face-to-face interviews with farm owners and managers. Animals on all but one farm had been vaccinated against Hardjo and Pomona, and cows on 54 of 200 (27%) farms had also been vaccinated against Copenhageni in at least one age group (calves, heifers and cows). Associations found to be statistically significant in univariate analysis (at $p<0.2$) were assessed by multivariable logistic regression. Factors associated with shedding included cattle age (OR= 0.82, 95% CI= 0.71-0.95), keeping sheep (OR= 5.57, 95% CI= 1.46-21.25) or dogs (OR= 1.45, 95% CI= 1.07-1.97), and managing milking cows in a single as opposed to multiple groups (OR= 0.45, 95% CI= 0.20 – 0.99). We conclude that younger cattle were more likely to be shedding *Leptospira* than older cattle and that the presence of sheep and dogs was associated with an increased risk of shedding in cows. Larger herds were at higher risk of having *Leptospira* shedders. However, none of the environmental risk factors that were assessed (e.g. access to standing water, drinking water source), or wildlife abundance on-farm, or pasture were associated with shedding, possibly due to low statistical power, given the low overall shedding rate.

6.2. Introduction

Leptospirosis is one of the most widespread bacterial diseases caused by approximately 250 serovars of pathogenic *Leptospira* spp [1]. Both animals and humans can be infected by *Leptospira*. Infected animals can shed *Leptospira* into the environment intermittently via urine for up to 40 weeks after initial detection [1, 2]. Transmission between animals and from animals to humans can occur through direct contact with infected urine or indirectly through contamination of the environment, via open wounds or the mucous membranes of eyes, nose and mouth [1].

Before the introduction of extensive vaccination with bivalent (Hardjo and Pomona) vaccines in New Zealand dairy herds in the 1980s, human leptospirosis cases in dairy farm workers related to those serovars were commonplace with an average annual incidence of 1,100 notified cases per 100,000 of the resident population in 1970-1979 [3, 4]. During that period, clinical leptospirosis with leptospiruria in cattle was frequently diagnosed and largely associated with Pomona infection [5, 6]. However, *Leptospira* shedding was also identified in subclinically infected cattle [5, 7, 8]. Carter et al. (1982) and Cordes et al. (1982) found 0.7% and 0.4% of dairy cows, respectively, in Waikato farms were shedding *Leptospira* without showing any clinical signs. The true percentage of cattle shedding *Leptospira* might have been higher since the detection was based on microscopy and culture techniques. Current molecular techniques have higher sensitivities especially at the acute phase [9]. A small pilot study in 2011 involving 44 vaccinated dairy herds showed *Leptospira* shedding, detected by qPCR and/or dark-field microscopy in 4% of 445 vaccinated dairy cows and in 30% of herds [10]. However, there were no data collected on the infecting serovar/s.

That preliminary study prompted a nationwide survey of dairy herds conducted from 2015-2016 which found a similar animal- and herd-level shedding prevalence. This study identified that of five serovars tested, Tarassovi was the only one positively associated with shedding [11]. A recent review of the epidemiology of notified human leptospirosis cases in New Zealand from 1999 to 2016 found Tarassovi to be the

second most frequent serovar infecting dairy farm workers after Hardjo [12]. In the Waikato region, a high density dairy farming area, Tarassovi was the dominant serovar in notified cases of leptospirosis in dairy farm workers [13].

Investigation of risk factors for *Leptospira* exposure in dairy farm workers and people with dairy contact was conducted in New Zealand more than thirty years ago. A cross-sectional study in the Manawatu region [14] and a wider study involving the Waikato, Manawatu, Northland, Bay of Plenty, and Wairarapa regions [15] showed increased time spent in the dairy shed, wearing shorts during milking, keeping pigs for sale, male gender, a previous history of leptospirosis in farm workers, a known clinical history of leptospirosis in cattle, increased size of the milking herd, and no vaccination of the herd against leptospirosis, as being risk factors associated with seropositivity to *Leptospira* in workers. However, these associations were analysed without adjustment for confounding.

While there have been no studies investigating risk factors for *Leptospira* infection in dairy cattle in New Zealand, studies in other countries suggested several risk factors. These included large herd size [16 - 18], the presence of other animals such as sheep, goats, swine, dogs and rodents on farm [19-22], the purchase or introduction of cattle [20, 23], increasing age of cattle [22] and surface water for drinking [16].

Based on the recent research and human notified case data as above, the serovar distribution in New Zealand dairy cattle appears to have changed since studies in the 70s and 80s and the adoption of vaccination. This supported the need to re-evaluate risk factors associated with shedding of *Leptospira* in dairy herds to better inform current measures to control *Leptospira* in dairy herds, and consequently reduce exposure to workers. Therefore, this study aimed to identify herd- and cow-level risk factors associated with *Leptospira* shedding in dairy cattle farms in New Zealand.

6.3. Methods

6.3.1. Study design

A cross-sectional study of *Leptospira* shedding in urine and seroprevalence in dairy cattle in New Zealand was conducted from 5th January to 26th April 2016, primarily to evaluate the effectiveness of vaccination programmes for reducing *Leptospira* shedding, but also to re-evaluate the epidemiology of *Leptospira* on dairy farms. Full details of the study including sample size calculation, farm recruitment, sample and data collection and laboratory testing are presented in Yupiana *et al.,* (2019). Briefly, 20 adult cows from 200 randomly selected dairy herds, stratified by herd size and region throughout New Zealand, were blood and urine sampled by the farm's veterinary service provider. This study focused on urine shedding only. Urine samples were analysed by qPCR using *gyrB* as a target gene for *Leptospira* DNA as described by Subharat et al. (2011) and Fang et al. (2014). Manipulations performed on animals were approved by the Massey University Animal Ethics Committee, protocol 15/57.

6.3.2. Farm data collection

Information on possible risk factors was collected using a pre-tested questionnaire (Supplementary Material 1). Data collected included general and farm demographic information, vaccination practices including vaccine/s used and vaccination protocol and timing, herd size categorised as 0-270, 271-462, 463-592 and >592 lactating cows, the environment including drinking water sources, access to standing or flood water and wildlife abundance, the presence of pigs, sheep, deer, dogs, whether leptospirosis had occurred in farm workers, and whether clinical leptospirosis was recently detected in animals. The questionnaires were completed at the time of sample collection by the veterinary service provider by face-to-face interview.

The information from the questionnaires was manually entered into a Microsoft Access database.

6.3.3. Statistical analysis

All statistical analyses were done using R version 3.3.2 (2016-10-31). Statistical significance was accepted at $p < 0.05$.

The relationship between herd- and cow-level putative risk factors associated with the urine PCR result (positive or negative) was analysed at the individual animal level using logistic regression with a random effect for farm to adjust for unmeasured confounders at herd level and for correlation of the response within herd.

Continuous predictors of shedding were checked for collinearity. If the Pearson's correlation coefficient between two continuous variables was greater than 0.9, only one of the two variables was retained. The decision was based on biological plausibility and the strength of the crude association with the outcome [27]. The linearity assumption for continuous predictors was tested by exploring the nature of the relationship between a continuous predictor and the outcome. If linearity was not a reasonable assumption, the variable was split into categories and factorised. The likelihood ratio test (LRT) was used to decide whether a variable or factor was significant in the model. A preferred model was determined by the lowest AIC (Akaike information criterion) [27]. The relationship between herd- and individual-level putative risk factors and the outcome was analysed in three steps. Firstly, the odds ratio for each variable was screened individually. Secondly, variables with a P-value of 0.2 or below were included to develop the final multivariable model by backward elimination. In this step, variables with P-value > 0.05 were excluded from the final model. Finally, initially non-significant variables were again added one by one to the final model to check if any of them had initially been confounded to non-significance. The criterion for retention was based on the statistical significance of the predictor [27]. Confounding was evaluated by assessing the change in a coefficient or its standard error by more than 20% before and after removing a suspected confounder from the model [27]. Biologically plausible interaction terms among all the variables in

the final model were tested [27]. We calculated odds ratios by exponentiating the regression coefficients and the endpoints of their 95% confidence intervals.

6.4. Results

6.4.1. Descriptive statistics

In total, 200 dairy farms participated in the study; 65% (n = 130) were in the North Island and 35% (n = 70) in the South Island. The mean herd size was 462 milking cows, (range 130 to 2201). This is about 10% higher than the average herd size of 419 in New Zealand in 2015/2016 [28]. The median age of sampled cows was 4 years, with a range of 2-16 years. In total, 68% of participating farms introduced replacement cows into their herds within the previous 5 years. Other animals kept on surveyed farms included sheep (33%), beef cattle (32%), pigs (20.5%) and dogs (76%). Wildlife such as rats, mice, and possums were seen on 24.5% of the farms. The proportion of farmers who often saw rats (P=0.02) and mice (P=0.008) around milking sheds was significantly higher in the North Island than in the South Island. Troughs were the only permanent water sources for cows on 71.5% of farms, but on the remainder, cows could also access ponds, streams, valley dams and/or ditches.

In total, 94 cows (2.4%) from 53 herds (26.5%) were urine qPCR positive. There was no significant difference in *Leptospira* shedding prevalence between the North Island (27.7%) and the South Island (24.3%).

All but one farmer had conducted vaccination against serovars Hardjo and Pomona, and 54 of 199 (27%) farmers additionally vaccinated at least one age group against serovar Copenhageni. The only unvaccinated farm in this study was included in the statistical analysis.

6.4.2. Factors associated with *Leptospira* shedding

Univariate analysis

Table 6.4-1 shows one cow-level and eight herd-level risk factors that were unconditionally associated with shedding at *P*< 0.2. At the animal level, there was a significant negative linear relationship between age and shedding. At the herd level, significant variables associated with shedding were region, breed of cow, keeping sheep or dogs on the farm, herd size (higher in large herds), and vaccine type used in heifers and adult cows. Managing cows as a single mob as opposed to multiple mobs was significantly associated with a lower shedding risk. The effect of region was only marginally significant, but herds in Northland, Bay of Plenty and West Coast regions had higher shedding levels than Taranaki. Similarly, Friesian-Jersey crossbred cows appeared to be more prone to shedding while breed overall was only marginally significant. No other risk factors from the questionnaire were associated with shedding.

Table 6.4-1. Unconditional associations between potential risk factors and *Leptospira* shedding status (P-value < 0.2)

Risk factor		Level of observation	No. of herds or cows	Odds Ratio	95% Confidence Interval		*P*-value
					Lower	Upper	
Region	Taranaki	herd	26	Ref.			
	Northland		18	6.58	1.36	31.83	
	North Waikato		25	4.35	0.95	19.85	
	South Waikato		25	3.49	0.75	16.18	
	Bay of Plenty		7	7.30	1.03	51.78	
	Lower North Island		29	1.79	0.37	8.64	
	West Coast		9	6.99	1.12	43.70	
	Canterbury/North		33	1.54	0.32	7.36	

	Otago						
	Southland		28	2.80	0.45	17.49	0.085
Breed	Friesian	herd	1520	Ref.			
	Friesian-Jersey		1460	2.22	1.03	4.77	
	Jersey		580	0.97	0.32	2.92	
	Other		140	0.46	0.04	5.30	0.105
Are all milking cows on the property managed as one mob/group	No	herd	56	Ref.			
	Yes		136	0.36	0.18	0.74	0.005
Keep sheep on the farm	No	herd	137	Ref.			
	Yes		63	2.34	1.15	4.77	0.019
Sizes of the milking herd	0-270	herd	50	Ref.			
	>270-462		67	1.09	0.41	2.87	
	>462-592		33	3.47	1.24	9.73	
	>592		50	2.20	0.83	5.81	0.041
Age of milking cows	Cont.	cow	3360	0.82	0.73	0.93	0.001
Vaccine type for heifers	2way	herd	111	Ref.			
	3way		51	2.25	0.98	5.17	0.055
Vaccine type for cows	2way	herd	111	Ref.			
	3way		43	1.95	0.88	4.31	0.098
Number of dogs kept on the farm	Cont.	herd	200	1.12	0.96	1.30	0.147

Multivariate analysis

One animal-level and three herd-level risk factors remained in the final model (Table 6.4-2). Older cows were less likely to shed with the odds decreasing by 18% for every additional year of age. Keeping sheep with no dogs on farms increased the odds of cows shedding *Leptospira,* additional dogs with no sheep kept on the farm increased the odds of shedding, having both sheep and dogs on farm increased the risk of shedding and managing milking cows in a single rather than multiple groups reduced the odds of a cow shedding.

Table 6.4-2. Final logistic regression model with a random effect for herd showing associations between *Leptospira* shedding status and potential risk factors.

Risk factor		**Coef.**	**Odds Ratio**	**95% Confidence Interval**		***P*-value**
Age of milking cows (years)	Continuous	-0.19	0.82	0.71	0.95	0.007
Sheep on the farm	No (ref.)					
	Yes	1.72	5.57	1.46	21.25	0.012
No. of dogs on the farm	Continuous	0.37	1.45	1.07	1.97	0.016
Sheep on the farm * number of dogs on the farm	Continuous	-0.41				0.036
Are all milking cows on the property managed as one group	No (ref.)					
	Yes	-0.79	0.45	0.2	0.99	0.049

6.5. Discussion

This is the first report describing risk factors for *Leptospira* shedding in dairy cows in New Zealand. This analysis was prompted by the observation that cows in 26.5% of dairy herds shed *Leptospira* in urine, largely associated with serovar Tarassovi, which is not in *Leptospira* vaccines in New Zealand [11]. The study showed serological evidence for Tarassovi in 75% of herds and 17% of cows. Theoretically, the shedding might be due to other non-vaccine serovars (Copenhageni and Ballum). However, this is unlikely due to the lack of an association between serology and urine PCR. Shedding

due to vaccine serovars Hardjo and Pomona is highly unlikely because again, they were not associated with shedding and vaccination against these serovars was deemed to be efficacious [24].

This paper was intended to provide a better understanding about the risk profile of cows and herds with respect to shedding. Thus, we hypothesised that factors other than vaccination would explain the shedding rates. Of particular interest were putative infection sources such as drinking water sources, access to standing water on pasture, rivers, valley dams or floodwater, and exposure to wildlife or other domestic animals. The risk factors identified were younger age, larger herds and the presence of sheep or dogs on farms, though the risk appeared to be less when both sheep and dogs were present on-farm.

Few recorded potential risk factors were significant in the final regression model. One explanation is the low prevalence of shedding (2.4%) in cows, resulting in low statistical power for a logistic regression analysis. The absence of statistical significance is therefore poor evidence that non-significant potential factors pose no risk. For example, risk associated with exposure to water and wildlife, which are biologically plausible, might well be undetectable using the approach employed here. Hence, while the survey was appropriate to identify a larger number of risk factors had the shedding prevalence been higher, in the event, identification of risk factors was constrained by low power. Nevertheless, there were a few factors that the study was able to identify.

Studies elsewhere suggested a relationship between the seroprevalence in cattle and the presence of cervids [20] and sheep/goat [22]. Our data demonstrated that the presence of sheep on dairy farms was a risk factor for *Leptospira* shedding in cows. *Leptospira* shedding in sheep in New Zealand was a common finding [29, 30], and sheep farmers rarely vaccinate against L*eptospira*. Fang et al. (2015) has shown that urinary shedding and seropositive rates were 31% and 21%, respectively, in sheep and in cattle. In New Zealand, sheep are regarded to be a reservoir host for Hardjo [30].

However, antibodies to Pomona [31], Copenhageni, Ballum and Tarassovi [32] have also been detected. Mannewald et al., (2019) recently showed 14% seroprevalence to Tarassovi in sheep. This was higher than 2.6% using the same MAT cut-point reported 30 years prior [33], suggesting a change in epidemiology of this serovar, consistent with recent data for dairy cattle [11] and humans [12]. However, isolation of this serovar in sheep has not been reported. Thus, the role of sheep as a source of Tarassovi transmission on dairy farms cannot be confirmed without new data.

An increased number of dogs on-farm was associated with an increased risk of having one or more shedding cows in the herd. Favero et al. (2017) also found that cattle were more likely to be seropositive to *Leptospira* when dogs had access to pasture. In New Zealand, a study investigating *Leptospira* antibody against Hardjo, Pomona, Copenhageni and Ballum in dogs showed a significant association between seropositivity to Hardjo and farm working dogs as opposed to other breeds [34] suggesting a possible transmission to cows from dogs. Tarassovi was not tested in Harland's study. This serovar was isolated from pigs and dogs in New Zealand about 40 years ago [35, 36]. Hence, dogs could theoretically be a source of infection for dairy cattle. However, transmission to dairy cattle is considered unlikely due to the low concentration of leptospires in urine and the poor survival of *Leptospira* in dog urine [3]. Having few dogs and hundreds of cows on a typical dairy farm, it is much more plausible that leptospires are transmitted from cows to dogs, not the other way round as suggested by the association in our study.

Sheep and dogs on-farm being a risk factor for shedding in cows may be a spurious association, considering the relatively small numbers of sheep (median=6) and dogs (median=2) kept on the farms. Unmeasured factors related to having other animals on the farms might have contributed to the higher risk of shedding in cows. For example, in our study, we found there were correlations between the presence of sheep and presence of beef cattle and/or pigs on the farms and, an increased number of dogs was also associated with the presence of cattle and/or pigs. These associations are not readily explainable.

Serological evidence supports that Tarassovi is adapted to pigs [36]. However, we did not find an association between the presence of pigs and *Leptospira* shedding in dairy cattle, despite 22% of the dairy farms having pigs. Possibly, pigs were not infected by this serovar, or were generally kept in confined places with limited or no direct contact with cattle. Furthermore, the average number of pigs kept on the farm was two, with a maximum of eight, suggesting they were kept for consumption and slaughtered relatively young, limiting risk of exposure to sufficient challenge. A study has suggested that transmission of members of the *L. borgpetersenii* serogroup requires direct host-to-host contact due to poor survival in the environment [38]. Therefore, we infer that pigs were an unlikely source of infection for dairy cattle, which would also explain the non-significant effect in our study. In addition, a previous study has shown that keeping pigs for human consumption rather than for sale was not considered a risk factor for leptospirosis in humans [14].

The presence of rodents is usually associated with a contaminated environment with *Leptospira* [39] that potentially increased risk of *Leptospira* transmission to other mammals. In our data however, an association between the presence of rodents and infection of cows could not be established. This might be because the shedding we observed in dairy cows was not related to a serovar adapted to rodents. In New Zealand, Ballum is the usual serovar identified in rodents. Previous studies reported that 28-30% of rodents were seropositive to Ballum [40, 41], but only 3% of cows in our study had this serovar and this was not related to shedding [11]. Tarassovi was rarely found in rodents [41] which again are consistent with the lack of an association between rodents and shedding in cows. A study in an urban environment in Brazil showed a significant association between the presence of rodents and seropositivity to *Leptospira spp.* including Hardjo and Icterohaemorrhagiae in cows [21]. However, since serovar/host relationships of *Leptospira* are highly specific for country and urban vs rural environments, overseas studies may bear little relevance to New Zealand.

Access to surface water, has been commonly associated with *Leptospira* infection [42] but was not a significant factor in our study despite there being 29% of farms where

cows had access to water sources other than troughs. A possible explanation is that Tarassovi, the likely infecting serovar, survives for relatively short periods in water [38]. Therefore, surface water might not be an important source for transmission of circulating Tarassovi on dairy farms. Surface water may be a higher risk source of infection when rodents are common. A study in Brazil found a significant association between access to streams and seropositivity of animals to Hardjo/Wolffi or Icterohaemorrhagiae [16]. The authors inferred that rodents carrying Icterohaemorrhagie might have contaminated the water and that this exposed animals to the bacteria. Similarly, another study in Brazil showed a significant association between flooded pasture and seropositivity of animals to serovar Hardjo [21]. In contrast, a study of beef cattle in Ireland did not find the presence of a river as a risk factor [18]. Clearly, differences in the epidemiology of *Leptospira* infection are influenced by different environmental factors.

The risk of shedding linearly decreased with cow age from 2 to 6+ years. This may be a function of exposure time. If exposure is more or less constant, older cattle would be exposed repeatedly and be expected to develop a stronger cell mediated immune response (CMI) over time [43]. Consistent with our finding, a study in Waikato [7] has shown 69% of shedders (vs. 31% in the population) were two- and three-year-olds and the other 31% ranged from four to nine years old. Another study suggested that heifers were infected after their introduction into the milking herd [44]. The authors reported a lower proportion of clinical cases in cows having had four or more lactations than in younger cows, supporting an age effect in the epidemiology of infection and disease.

The finding that younger cows were more likely to shed than older cows potentially poses a higher risk of exposure for farmers and farm workers while milking first lactation heifers. In New Zealand, young cattle are often grazed away from the farm until they are old enough to enter the milking herd as heifers. As vaccination status of these animals may be uncertain they may be at greater risk of infection from the vaccinal serovars and present a risk. Heifers that were introduced to the adult herd

and milked for the first time may suffer a relatively high level of stress. They may be more likely to kick the cups off and urinate [45] increasing the likelihood of exposure of workers.

Large herds were more likely to harbour shedders than small herds. As for most pathogens, several studies [17, 18, 23] have shown that large herd size was associated with a higher risk of *Leptospira* transmission in cattle due to more frequent contact between infectious and susceptible animals. Large herds also have more contact with other herds through purchases and contract heifer grazing than small herds, hence are more likely to introduce shedders than small herds. Thus, larger herds are more likely to circulate and maintain the bacteria in the dairy population.

While some of the associations discussed above may be biologically plausible, a cross-sectional study such as this can only generate hypotheses about possible causal pathways. Exposure could have occurred at any time and did not necessarily precede the time of infection and shedding. For example, milking cows might have been infected before contact with sheep or dogs. Hence caution must be exercised when interpreting results from a cross-sectional study such as this. A longitudinal study design would be more appropriate to investigate the epidemiology and risk factors for shedding of the serovar/s identified in dairy cattle in New Zealand.

6.6. Conclusion

In summary, we conclude that younger dairy cows are more likely to shed *Leptospira* on New Zealand dairy farms. Farm workers may use this information to take extra care and precautions when milking first calving heifers. While the presence of sheep and dogs was positively associated with shedding in cows, the biological plausibility of these species as risk factors requires further study.

6.7. Acknowledgements

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References

1. Evangelista KV and Coburn J (2010) *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiology, 5*:9, 1413-1425.

2. Leonard F *et al.* (1993) Association between cessation of leptospiruria in cattle and urinary antibody levels. *Research in Veterinary Science, 55*:2, 195-202.

3. Mackintosh CG (1981) A study of the epidemiology and control of leptospirosis on dairy farms (dissertation): Palmerston North, New Zealand: Massey University.

4. Marshall RB (1987) A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. *Israel journal of veterinary medicine, 43*, 271-276.

5. Carter ME *et al.* (1982) Leptospirosis: II. Investigation of clinical disease in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*:9, 136-140.

6. Ensor CR and McClure TJ (1953) Bovine leptospirosis in Northland. *New Zealand Veterinary Journal, 1*:3, 47-50.

7. Cordes DO *et al.* (1982) Leptospirosis: I. Clinical investigation of the infection in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*:8, 122-124.

8. Dodd DC and Brakenridge D T (1960) *Leptospira icterohaemorrhagiae* AB infection in calves. *New Zealand Veterinary Journal, 8*:4, 71-76.

9. Musso D and La Scola B (2013) Laboratory diagnosis of leptospirosis: A challenge. *Journal of Microbiology Immunology and Infection, 46*:4, 245-252.

10. Wilson P *et al.* (2013) Does long-term vaccination eliminate leptospiral shedding? A pilot study of New Zealand dairy cattle herds. *Vetscript, 26:11,* 16-16.

11. Yupiana Y *et al.* (2019). Emerging *Leptospira* strain poses public health risk for dairy farmers in New Zealand. *Preventive Veterinary Medicine,* 104727.

12. Descriptive Epidemiology of Human Leptospirosis in New Zealand: 1999 to 2016. (Nisa S, personal communication, September 10, 2018).

13. Cowie G and Bell A (2012) A retrospective review of notified human leptospirosis cases in the Waikato region of New Zealand, 2004 to 2010. *New Zealand medical journal (Online)*.

14. Mackintosh CG *et al.* (1980) Epidemiology of leptospirosis in dairy farm workers in the Manawatu Part I: A cross-sectional serological survey and associated occupational factors. *New Zealand Veterinary Journal, 28*:12, 245-250.

15. Blackmore DK and Schollum LM (1982) Risks of contracting leptospirosis on the dairy farm. *New Zealand Medical Journal, 95*:716, 649-652.

16. Campos AP *et al.* (2017) Seroprevalence and risk factors for leptospirosis in cattle, sheep, and goats at consorted rearing from the State of Piaui, northeastern Brazil. *Tropical animal health and production, 49*:5, 899-907.

17. Miyama T *et al.* (2018) Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan. *Preventive Veterinary Medicine, 149*, 15-20.

18. Ryan EG *et al.* (2012) Herd-level risk factors associated with *Leptospira* Hardjo seroprevalence in Beef/Suckler herds in the Republic of Ireland. *Veterinary Ireland Journal, 2*(9), 1-10.

19. Favero JF *et al.* (2017) Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. *Microbial Pathogenesis, 107*, 149-154.

20. Oliveira F *et al.* (2010) Risk factors associated with leptospirosis in cows in the state of Bahia, northeastern Brazil. *Pesquisa Veterinaria Brasileira, 30*:5, 398-402.

21. Pimenta C *et al.* (2014) Bovine leptospirosis in Para ba State: prevalence and risk factors associated with the occurrence of positive herds. *Pesquisa Veterinaria Brasileira, 34*:4, 332-336.

22. Schoonman L and Swai ES (2010) Herd-and animal-level risk factors for bovine leptospirosis in Tanga region of Tanzania. *Tropical animal health and production, 42*:7, 1565-1572.

23. O'Doherty E *et al.* (2014) Management practices as risk factors for the presence of bulk milk antibodies to Salmonella, Neospora caninum and *Leptospira interrogans* serovar hardjo in Irish dairy herds. *Animal, 8*:6, 1010-1019.

24. Sanhueza JM *et al.* (2018). Meta-analysis of the efficacy of *Leptospira* serovar Hardjo vaccines to prevent urinary shedding in cattle. *Preventive Veterinary Medicine, 153*, 71-76.

25. Subharat S *et al.* (2011) Evaluation of a SYTO9 real-time polymerase chain reaction assay to detect and identify pathogenic *Leptospira* species in kidney tissue and urine of New Zealand farmed deer. *Journal of Veterinary Diagnostic Investigation, 23*:4, 743-752.

26. Fang F *et al.* (2014) Interlaboratory and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle. *Journal of Veterinary Diagnostic Investigation, 26*:6, 734-747.

27. Dohoo IR, Martin W and Stryhn H (2009) *Veterinary epidemiologic research* ,2nd edn. Canada: AVC Incorporated Charlottetown.

28. LIC and DairyNZ Limited (2016) New Zealand Dairy Statistics 2015-16. Available at https://www.dairynz.co.nz/publications/dairy-industry/new-zealand-dairy-statistics-2015-16/

29. Fang F *et al.* (2015) Shedding and seroprevalence of pathogenic *Leptospira spp*. in sheep and cattle at a New Zealand abattoir. *Zoonoses And Public Health, 62*:4, 258-268.

30. Vallee E *et al.* (2015). Serological patterns, antibody half-life and shedding in urine of *Leptospira spp*. in naturally exposed sheep. *New Zealand Veterinary Journal, 63*:6, 301-312.

31. Dreyfus A *et al.* (2018) Seroprevalence and herd-level risk factors for seroprevalence of *Leptospira spp*. in sheep, beef cattle and deer in New Zealand. *New Zealand Veterinary Journal, 66*:6, 302-311.

32. Mannewald A *et al.* (2019) Prevalence of atypical *Leptospira* serovars in New Zealand's pastoral livestock (Thesis):. Uppsala, Sweden: Uppsala University

33. Blackmore DK, Bahaman AR and Marshall RB (1982a) The epidemiological interpretation of serological responses to leptospiral serovars in sheep. *New Zealand Veterinary Journal, 30*:4, 38-42.

34. Harland AL *et al* (2013) A serological survey of leptospiral antibodies in dogs in New Zealand. *New Zealand Veterinary Journal, 61*:2, 98-106.

35. Mackintosh CG, Blackmore DK and Marshall R B (1980a) Isolation of *Leptospira Interrogans* serovars Tarassovi and Pomona from Dogs. *New Zealand Veterinary Journal, 28*:5, 100-100.

36. Ryan T and Marshall R (1976) Isolation of a leptospire belonging to serogroup tarassovi. *New Zealand Veterinary Journal,* 24:9 212-213.

37. Hathaway SC (1981) Leptospirosis in New Zealand: an ecological view. *New Zealand Veterinary Journal, 29*:7, 109-112.

38. Bulach DM *et al.* (2006) Genome reduction in *Leptospira* borgpetersenii reflects limited transmission potential. *Proc Natl Acad Sci U S A, 103*:39, 14560-14565

39. Adler *et.al* (2015) *Leptospira* and Leptospirosis Vol. 387. Berlin: Verlag Berlin Heidelberg: Springer.

40. Hathaway SC and Blackmore DK (1981) Ecological aspects of the epidemiology of infection with leptospires of the Ballum serogroup in the black rat (*Rattus rattus*) and the brown rat (*Rattus norvegicus*) in New Zealand. *J Hyg (Lond), 87*:3, 427-436.

41. Moinet M *et al.* (2017) Is wildlife a source of *Leptospira* infection in livestock in New Zealand? *The 2017 Conference Proceedings of the Large Animal Veterinarian Technicians Group of the NZVA Wellington, NZ*.

42. Mwachui MA *et al.* (2015) Environmental and Behavioural Determinants of Leptospirosis Transmission: A Systematic Review. *PLoS Negl Trop Dis, 9*:9.

43. Fraga TR, Barbosa AS and Isaac L (2011) Leptospirosis: Aspects of Innate Immunity, Immunopathogenesis and Immune Evasion From the Complement System. *Scandinavian Journal of Immunology, 73:*5, 408-419.

44. Durfee PT and Allen JD (1980) Serological titers of dairy-cows over a 63-week period following natural infection with *Leptospira interrogans* serovar Hardjo. *Australian Veterinary Journal, 56*:12, 574-579.

45. Jago J and Kerrisk K (2011) Training methods for introducing cows to a pasture-based automatic milking system. *Applied Animal Behaviour Science, 131*(3-4), 79-85.

Chapter 7
General Discussion

7.1. Introduction

Even after the large scale implementation of vaccination of dairy herds in the 1980s, leptospirosis remains an important zoonotic disease for occupationally exposed dairy workers in New Zealand. This thesis intended to answer queries about the infecting serovars, vaccination practices and likely factors involved with reducing or increasing the risk of human exposure arising from dairy farming.

The large cross-sectional study presented in this thesis (Chapter 4) was designed to follow the lead of an opportunistic pilot study that seemed to demonstrate that *Leptospira* continued to be shed in dairy herds despite vaccination (Wilson *et al.*, 2013). In addition, an opportunity arose unexpectedly to investigate the case of *Leptospira* infections in three dairy cattle workers on an unvaccinated dairy farm. The possible serious consequences of such a situation and factors associated with shedding are described in Chapters 3 and 6, respectively.

Overall, the findings from our study suggests that vaccination programs in dairy herds are effective, despite the relatively low degree of farmers conforming to BP (best practice recommendation for the use of vaccines) guidelines. Overall, 18.7% (95% CI: 13.4-25.6) of high risk farms and 20.0% (95% CI: 8.9-39.1) of low risk farms conformed with BP guidelines for vaccination of all age groups of animals combined (chapter 5). Another important finding from this study was the emergence of serovar Tarassovi. Tarassovi was the only serovar among those tested that had a significant and positive association with shedding. This serovar has been regarded rare and had therefore not been included in test panels of leptospirosis studies since about 30 years.

The lack of a positive association between shedding and any of the serovars included in vaccines (Hardjo, Pomona, Copenhageni) supports the notion that long-term

vaccination on dairy farms is generally effective in reducing or preventing the shedding of these serovars in dairy cattle. Chapter 3 demonstrated that Hardjo and Pomona were the dominant serovars on a dairy farm in the absence of vaccination. It is therefore postulated that vaccination shall be continued to prevent the re-emergence of Hardjo and Pomona and their serious negative impact on the occupational exposure and health of dairy farm workers as exemplified in Chapter 3.

The findings of the emergence of Tarassovi in dairy cattle (chapter 4) was in line with findings in dairy farmer case notifications of leptospirosis: Hardjo was still the dominant serovar, however, Tarassovi has overtaken Pomona as the second most dominant infecting serovar in dairy farmers but not in other occupational groups (dry stock farmers, abattoir workers). This is a strong call for alternative strategies other than vaccination to protect dairy farm workers against contracting leptospirosis in their work place. Chapter 6 informs about factors contributing to *Leptospira* shedding in dairy cattle. It firstly showed that shedding was highest in first calving heifers and reduced linearly with increasing age. Secondly, increasing herd size and the presence of sheep and an increasing number of dogs were associated with an increase in the risk of shedding.

This chapter discusses the overall research findings of the thesis. They are summarised under the headings 'Leptospirosis in dairy farmers', 'Tarassovi in vaccinated dairy herds' and 'Leptospirosis control'. This chapter then adds a critical review of experimental designs and methodologies, suggests areas for future research, and recommends actions to be taken to control leptospirosis on dairy farms.

7.2. Leptospirosis in dairy farmers

Leptospirosis was a frequent disease of dairy farmers in New Zealand before *Leptospira* vaccination of dairy herds became a widely adopted practice. On average, 488 human cases were notified every year from 1970 to 1979, of which 90% were dairy workers (Mackintosh, 1981). Following an increasing voluntary adoption of vaccination in the 1980s, there was a remarkable drop of the annual leptospirosis incidence among

dairy farmers: cases dropped tenfold from the 1970s to the 1990s (Mackintosh, 1981; Thornley *et al.*, 2002) and threefold from 1998 to 2016 (Nisa *et al.*, 2018b).

The overall decrease of leptospirosis cases in dairy farmers was largely due to an absolute reduction in the number of cases due to serovars Hardjo and Pomona. This was likely caused by vaccination practised by more than 95% of dairy farmers in New Zealand (chapter 5). Despite a tendency of decline, Hardjo is still the most frequently infecting serovar in this occupational group (Nisa *et al.*, 2018b). This may be related to continued exposure of workers in a small proportion of poorly or unvaccinated dairy herds (Benschop *et al.*, 2017; McLean, 2014). Chapter 3 is an example of such a situation where Hardjo and Pomona were the infecting serovars of three hospitalised human cases on an unvaccinated dairy farm (Benschop et al., 2017). Another outbreak of three human cases in a poorly vaccinated dairy farm was reported earlier (McLean, 2014). These are strong signals that unvaccinated herds might contribute significantly to human cases despite less than 5% of herds not effectively vaccinated.

The dominance of Hardjo in dairy farmers could also be due to a serologic cross-reaction to serovar Balcanica. Serovar Balcanica belongs to the same serogroup as serovar Hardjo and is antigenically similar making it indistinguishable in MAT. Therefore, vaccines against Hardjo may also provide cross-protection against Balcanica. While Hardjo and Balcanica can be distinguished by bacterial restriction-endonuclease DNA analysis (BRENDA) (Robinson *et al.*, 1982), this technique is currently not performed for the diagnosis of *Leptospira* infection in New Zealand for research or case confirmation. Genomic sequencing has been widely used for characterization of *Leptospira* and can be used as an alternative (Adler *et al.*, 2015), but is expensive and analysis is time consuming. Balcanica has been demonstrated to be adapted to possums (Hathaway, 1981; Marshall *et al.*, 1976). It has also been isolated from cattle (Mackintosh *et al.*, 1980b) but limited transmission between cattle has been shown (Mackintosh *et al.*, 1981). This and the fact that Hardjo vaccines may cross-protect against Balcanica suggest that this serovar is probably not established in dairy cattle. Since *Leptospira* shedding in vaccinated cattle (chapter 4)

was not associated with Hardjo titres, we believe that Balcanica infection may not even occur in vaccinated herds.

In the past 20 years, four major serovars were detected in notified leptospirosis cases that involved dairy farmers in New Zealand (Nisa *et al.*, 2018b). The dominant serovars in dairy farmers were Hardjo and Tarassovi at similar frequencies, followed by Ballum and Pomona. This was also supported by the findings in chapter 4 where shedding in vaccinated dairy cattle was associated with Tarassovi. Tarassovi is more frequent in dairy than dry stock farmers or abattoir workers due to the high exposure to urine during milking to of an average of 450 cows twice a day (2-4 hours of exposure/day+person). The two regions with the highest number of dairy farms, Waikato and Taranaki, also had the highest number of human cases with Tarassovi infection in dairy farmers. A summary of notification data from 2004 to 2010 in the Waikato again showed that Tarassovi was the highest serovar in dairy farmers (Cowie & Bell, 2012).

There was an increasing trend of serovar Ballum in the notified cases data: Ballum increased in dairy and beef farmers but not in meat workers (Nisa *et al.*, 2018b). While in these data, about 3% of dairy cattle had evidence of exposure to Ballum (Chapter 4), there was no association with *Leptospira* in their urine, either because Ballum was less prone than Tarassovi for being excreted or because the impact of Ballum on shedding was too small to become significant in the data (*i.e.* low statistical power). A recent study showed that more than 90% of over 100 mice and rats captured on a dairy and a beef farm were serologically positive for Ballum (Moinet *et al.*, 2017). Therefore, a strong exposure to Ballum from rodents appears to exist in dairy and beef farms, and this may expose both animals and humans directly, rather than humans being infected with Ballum indirectly by rodents through cattle. However, there is no obvious biologically plausible reason for the finding of an increasing trend of Ballum in workers on dairy and beef farms as opposed to abattoir workers or other human cases from non-livestock environments where rodents and hedgehogs might also be abundant.

Risk factors for *Leptospira* seropositivity were previously studied in dairy farmers before vaccination became widely implemented in dairy herds. These factors included duration of milking, vaccination status of dairy herds, having a herringbone rather than a walk through milking parlour, wearing shorts, keeping pigs on farms, herd size, being male and a history of leptospirosis in workers Mackintosh *et al.* (1980d). However, almost all of these factors have drastically changed since 1980. Almost all dairy farms have by now been vaccinated for many years, walk through sheds were replaced by herringbone or rotary sheds, raising pigs on dairy farms is no longer a common practice and herd size has increased about fourfold. These substantial changes might have caused a different environment to the extent that the risk factor scenario supported the emergence of other dominant infecting serovars such as the new Tarassovi strain.

7.3. Tarassovi in vaccinated dairy herds

The first observation of Tarassovi in humans and animals was reported by Kirschner in 1953. In this report, antibodies to Tarassovi were detected in three people with clinical symptoms and contact with pigs and/or cattle. Furthermore, examination of cattle and pig sera from an abattoir in Dunedin found 3% of cattle and 6% of pigs had Tarassovi with MAT titres higher than 150 (Kirschner, 1954). Tarassovi was isolated for the first time in 1976 from one of 80 pigs sampled at an abattoir that originated from farms in Waikato and Wellington (Ryan & Marshall, 1976). Transmission between pigs was also shown in this study. Furthermore, in 1978, Tarassovi isolates were recovered from four healthy dogs in kennels in South Auckland (Mackintosh *et al.*, 1980a). There was no dog-to-dog transmission suggested in this outbreak. This indicated that dogs may not serve as reservoir hosts to this serovar. Genomic investigation using restriction endonuclease showed that DNA fragment patterns of the isolates from the dogs were identical to those from the pigs (Mackintosh, 1981).

Serological evidence of Tarassovi has been found in deer (Flint *et al.*, 1988; Wilson *et al.*, 1998) and sheep (Blackmore *et al.*, 1982; Mannewald *et al.*, 2019) with MAT titre

greater than 48. Wildlife species such as Norway rats, ship rats, possums, hedgehogs (Hathaway *et al.*, 1981) and mice (Moinet *et al.*, 2017) also showed serological evidence of Tarassovi at a cut-off of ≥24 . Hathaway et al. (1981) argued that most of the serologic reaction to Tarassovi in wildlife species might be due to cross-reactivity to other dominant serovars (Hardjo and Ballum). In addition, Tarassovi has never been isolated from deer,sheep and wildlife species in New Zealand. Hence, deer, sheep and wildlife species may not have played an important role in Tarassovi circulation and transmission.

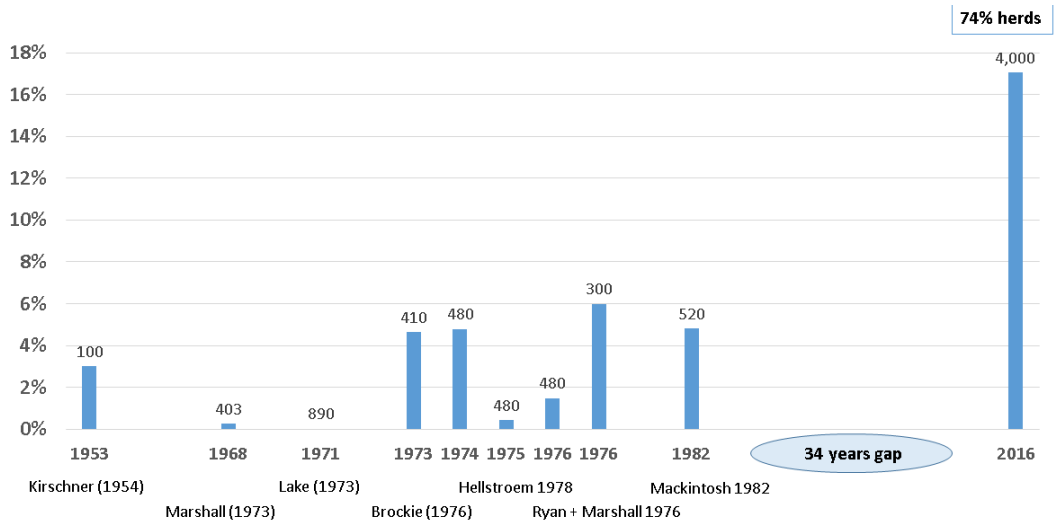

Figure 7.3-1 The percentage of seropositivity to serovar Tarassovi and the number of cattle sampled from 1953 to 2016 in New Zealand (adapted from (Yupiana et al., 2017a))

In cattle, there was a 34-year gap where Tarassovi was not investigated (Figure 7.3-1). In unvaccinated cattle (beef and dairy), this serovar was detected as the third most common serovar in cattle with prevalence of 4.3% of 480 cattle (Hellstrom, 1978). The author indicated that proportion of Tarassovi titres might represent cross-reactions occurring in sera with high titres against either Hardjo or Pomona. Similarly, recent serological testing of stored beef cattle sera from 2009 survey of dry stock farms showed that seroprevalence to Tarassovi (18%) was again the third after Hardjo and Pomona (Dreyfus *et al.*, 2018; Mannewald *et al.*, 2019). However, the prevalence was four-fold as high as the prevalence of the mixed beef/dairy cattle survey in 1978 (Hellstrom, 1978). Findings in vaccinated dairy cattle (Chapter 4) found a similar

prevalence in dairy cattle as Mannewald et al. (2019) in beef cattle (18% vs 17%). The most important finding from this study is that only Tarassovi had a strong positive association with urine shedding. A high MAT titre against Tarassovi was detected, ranging from 48 to 3072 (chapter 4).

The increasing number of Tarassovi in cattle has raised questions around this serovar adapting to cattle as a reservoir host. Previous studies have suggested some characteristics to define maintenance hosts and reservoir hosts (Hathaway, 1981; Haydon *et al.*, 2002; Marshall & Manktelow, 2002). The definition varies among studies but mainly agree on some criteria such as the ability of a pathogen to colonise in the tissue of a host species, a high susceptibility of the host to infection (low infective dose), the host shedding viable organism for long periods, and that the pathogen is tolerated by the host, hence does not kill it or cause serious disease. The Tarassovi findings in 74% of herds and 17% of animals and that this is strongly associated with shedding, with the animals displaying no clinical signs or abortions suggests that dairy cattle are likely to maintain Tarassovi in the population. However, Tarassovi has never been isolated from cattle in New Zealand. In our study, approximately 40% of urine-positive farms had more than one cow shedding *Leptospira*. Thus it appears likely that transmission between cattle occurred. However, experimental transmission studies would be required to conclusively demonstrate this.

In this study, there were 94 cows that were PCR positive. Amplicon sequencing on the 94 PCR positive samples revealed that nine resembled Ballum, eight Copenhageni, seven Hardjo, one Pomona, one a mix of Copenhageni and Ballum. The majority of 55 samples had an identical sequence that could not be conclusively identified. It was therefore labelled 'agent X', i.e. a possible new strain. The remaining 13 samples had poor sequencing results and information about the serovar or serogroup could not be derived. Phylogenetical analysis using the gyrase B region identified the new sequence of 'agent X' as a member of the *Leptospira borgpetersenii* species while serologically, most of these samples had titres against serovar Tarassovi. Further analysis was conducted on the association between the sequencing results and MAT results (cut-

point ≥96) for the 55 “new strain” amplicons. This cut-off was chosen for analysis as an agglutinating titre of ≥96 is considered significant for the diagnosis of *Leptospira* infection (Grooms & Bolin, 2005). In total, 76% of them were serologically associated with Tarassovi, 4% with Pomona and the remaining 20% were not associated with any tested serovar. A study in Australia had a similar finding regarding the identification of a new member of Tarassovi serogroup (Corney *et al.*, 2008). In Corney study, an isolate was collected from a heifer’s urine on a dairy farm that was serologically negative for all serovars. Serologically, the isolate produced titres against the *Leptospira borgpetersenii* serovar Tarassovi. However, gene analysis of a PCR using 16S rRNA and DNA gyrase subunit B identified the isolate as a member of the species *Leptospira weilii*.

7.4. Leptospirosis control

The aim of leptospirosis control in dairy cattle in New Zealand is to protect both dairy farmers and dairy cattle. It has been suggested that an effective way to control leptospirosis in cattle is to integrate antibiotic therapy, vaccination and management of the environment (Martins & Lilenbaum, 2017). While vaccination and management of the environment play important roles in the success of regular control program in cattle, antibiotic treatment should be limited to an outbreak situation. Antibiotic treatment was found to be crucial to forego legal consequences when an outbreak in humans occurred and dairy cattle were not vaccinated against *Leptospira* (chapter 3). Dairy farmers as the employers have to meet their obligation under the Health and Safety in Employment Act (1992) to take effective measures for the protection of their workers against *Leptospira* infection, for example by vaccinating cattle.

One main indicator of *Leptospira* vaccine efficacy is the elimination of *Leptospira* shedding in animals. Efficacy of *Leptospira* vaccine in reducing shedding in cattle has been widely studied. The efficacy is usually assessed by comparing the prevention of shedding in vaccinated vs unvaccinated animals after challenge either in artificial or natural challenge conditions. A meta-analysis involving eight clinical trials was

conducted to evaluate the efficacy of the Hardjo commercial vaccines in preventing shedding in cattle (Sanhueza *et al.*, 2018). This study showed that in experimental settings, the vaccines were effective in reducing shedding due to natural or artificial challenge by 82.1%. There was some variation in this study, such as the vaccine type used (monovalent vs multivalent), challenge setting (artificial vs natural), and age, which can influence vaccine efficacy. Despite the variations, the overall estimated vaccine efficacy was deemed sufficient to reduce disease incidence in animals. In chapter 4 of our study, we suggest that vaccination of Hardjo and Pomona and some Copenhageni on dairy farms in New Zealand might have been effective in reducing the shedding in the cattle. Even though vaccination might just have decreased the period of shedding to the extent that the study had not enough power to show a significant association, we postulate that shedding was not caused by vaccine serovars.

There are several factors influencing vaccine effectiveness, including vaccine, hosts, humans and environment (Rashid *et al.*, 2009). Vaccine factors include the antigen strain, the potency and preservation of vaccine. In New Zealand, three serovars (Hardjo, Pomona and Copenhageni) are included in *Leptospira* vaccines for livestock despite six serovars have been isolated from animals (Hardjo, Pomona, Copenhageni, Ballum, Tarassovi and Balcanica). As there is no cross-reaction among those serovars except between Hardjo and Balcanica, vaccination is currently no option for controlling Ballum or Tarassovi infection in livestock. Host determinants for vaccine effectiveness include maternal antibody, concurrent disease, immune system dysfunction and breed. It has been shown that cattle can be infected as young as five-weeks-old (Ministry for Primary Industries, 2013) thus vaccination at an early age is highly recommended. However, the timing of vaccination has to consider when the maternal antibody wanes as this mostly occurs by 6 months of age (Hellstrom, 1978). A technical report has suggested that maternal antibody could interfere with the development of immune protection from vaccination Ankenbauer-Perkins (2000) in (Heuer *et al*. (2012)). However, authors of more recent studies concluded that maternal antibodies may not interfere with immunity derived from vaccination (Woolums, 2007; Cortese,

2011). Best practice guidelines therefore recommend to vaccinate calves as early as possible (Heuer *et al.*, 2012).

While other host factors cannot easily be controlled, human factors such as vaccine handling and timing of vaccination can. In New Zealand, either veterinarian/veterinary technicians or farmers/farm workers administer *Leptospira* vaccines (chapter 5). However, there are currently no data about the effect of vaccine handling by either of these groups on vaccine effectiveness. Best practice guidelines aim at vaccinating calves as early as four months old. Even though approximately 60% of the dairy farmers in our study conformed with these recommendations (chapter 5), none of the vaccine serovars was associated with urine shedding. Thus, it appears that vaccines and vaccination is effective.

A meta-analysis has shown that under controlled environments, *Leptospira* vaccine is effective in preventing urinary shedding (Sanhueza *et al.*, 2018). However, in the natural environment, factors such as the presence of other domestic (Subharat *et al.*, 2012a) or wild animal species and access to surface water on the farms have been indicated to increase the exposure of *Leptospira* in animals and humans (Mwachui *et al.*, 2015). These factors are commonly found on dairy farms in New Zealand. This study implies that the influence of these factors does not decrease the effectiveness of vaccines.

In 2012, best-practice recommendations for the vaccination of livestock to protect humans was developed by Massey University on behalf of the New Zealand Veterinary Association (Heuer *et al.*, 2012). They provide details about vaccination schedules considering animal age, calendar months and the interval between boosters for both high- and low-risk scenarios. Chapter 5 describes the vaccination practices in dairy herds in New Zealand and compare them with the recommended best practice protocols. A high level of dairy farmer compliance for vaccination in general was found. At least 90% of dairy farmers stated that they vaccinated their stock regularly once in 12 months. A previous study concluded that the motivation of vaccinating dairy cattle

depended to a large extent on the advice provided by the farm veterinarian (Brown *et al.*, 1985). Vaccinating calves early was introduced by best practice guidelines as a cornerstone of recommended practices to achieve optimal vaccine effectiveness. About 60% farmers followed this recommendation. However, more awareness about the guidelines is desirable among veterinarians and farmers.

In the absence of a vaccine for the recently emerged serovar Tarassovi, options for protecting workers include the use of personal protective equipment (PPE) during milking (apron, boots, glasses), being aware of and reducing direct contact with splashing urine, and washing hands before smoking and after close contact with cows and especially urine. The risk associated with Ballum, a serovar that is increasingly found in notified human cases, firstly appears to be far lower than Tarassovi in dairy cattle based on a prevalence of 3% (vs 17% for Tarassovi). Secondly, humans appear to get exposed to Ballum by direct contact with rodents or hedgehogs, or indirectly by environmental contamination of surface water on pasture and of drinking water by rodents. Thus, control measures would have to aim at reducing contact with wildlife species, especially rodents. However, controlling wildlife is challenging because different species of wildlife require different control strategies (Martins & Lilenbaum, 2017). The understanding about the role of different species of wildlife is still limited. For example, little is known about the prevalence of shedders among rabbits, a widely abundant wildlife species in addition to rodents, hedgehogs and possums. Gaining a better understanding about wildlife habitats and their proneness to *Leptospira* infection is therefore essential for designing effective control measures. A current case study on a dairy farm found a substantial proportion of mice and hedgehogs with serologic evidence of serovar Ballum (Moinet *et al.*, 2017). Rather than controlling the infection in each of the wildlife species, the limitation of interactions between wildlife, livestock and humans may be a more realistic target, for example by building wildlife-safe fences.

The possible contribution of domestic animals other than dairy cattle to the risk of shedding in dairy cows was evaluated in chapter 6. There was an association between

the presence of sheep and the increasing number of dogs with the rate of shedding in dairy cows. However, these data did not show significant associations between the presence of pigs and beef cattle to the risk of shedding in dairy cows. This may be attributed to the generally small number of such domestic species on dairy farm and may indicate an overall small if not zero risk. Sheep, beef cattle and deer are known to be infected by Hardjo and Pomona at high prevalence in New Zealand dry stock farms (Dreyfus *et al.*, 2018). In addition, the non-vaccine serovar Tarassovi is present at similar prevalence in beef and dairy cattle. It was also found at quite high proportions in sheep and deer (Mannewald *et al.*, 2019). Therefore, vaccination of all domestic ruminant livestock is encouraged while co-grazing of dairy cattle with these species is not recommended.

7.5. Methodology critiques

7.5.1. Use of the Microscopic Agglutination test

MAT has been widely used to detect *Leptospira* antibodies and can be used to differentiate serogroups but not always serovars (Levett, 2001). Only if the circulating serovars all belong to different serogroups can MAT reliably differentiate *Leptospira* serovars. In New Zealand, six serovars have been isolated in animals namely *Leptospira interrogans* serovars Copenhageni and Pomona and *L. borgpetersenii* serovars Balcanica, Hardjo, Tarassovi and Ballum (Marshall & Manktelow, 2002). Among these, five serovars which belonged to five different serogroups were investigated (Levett, 2001). Therefore, serovar information that was found in this study using MAT can be regarded as reliable.

Observing 99% negative MAT results for Copenhageni and 97% for Ballum suggest that these are minimum estimates of specificity for those serovars. The other serovars occurred at higher prevalence, most probably due to true positives. It is therefore believed that MAT has a high specificity. Moreover, a cut-point of $\geq$48 was used to determine seropositivity (Chapter 3 and 4). Using a lower cut-point would increase the

sensitivity but decrease the specificity and give rise to false positives. A previous study has suggested that using a cut-point of ≥48 rather than ≥24 has minimized non-specificity (Blackmore *et al.*, 1982). In conclusion, it is believed that the utility of MAT was high.

PCRs have been used in many studies to detect *Leptospira* using urine samples from various types of animals including cattle (Fang *et al.*, 2015; Hernandez-Rodriguez *et al.*, 2011; Otaka *et al.*, 2012), sheep (Fang *et al.*, 2015) and deer (Subharat *et al.*, 2012b). Evidence showed that the PCR assay is more sensitive compared to traditional methods such as culture and dark-field microscopy (O'Keefe, 2002) as this assay can detect both viable and dead bacteria. In our study, we used a qPCR assay that detects the DNA gyrase subunit B (*gyrB*) gene. Compared with the conventional method, qPCR is faster and less sensitive to contamination (Picardeau, 2013). Using deer urine, Subharat et al.(2011) revealed a sensitivity and specificity of 96.7% and 100% respectively using the same target gene (DNA *gyrB)*. Observing 97.6% negative PCR results from our samples suggest that these are minimum estimates of specificity for this assay. Therefore, the value of qPCR for diagnosis of *Leptospira* in analysing urine samples was recognized.

7.5.2. Method of herd selection for the population survey (Chapter 4)

The 396 survey farms were randomly selected from a national database (DairyNZ core database). This number is nearly double the participants required in this study (n=200) in order to anticipate a situation if a low response rate occurs. In this study, 111 farmers could not be contacted due to incorrect phone numbers. From those randomly selected and contacted by phone, 68 were not willing to participate. Because this could potentially pose a selection bias, the reason of non-compliance was asked and could verify this to be unrelated to any of the study variables in 42 of the farms. Thus, we were confident that the 200 participating farmers were a representative subset of the population. Consequently, it is considered that the survey had high external validity.

Dairy farms were stratified by region and herd size to achieve equivalence to the national herd. However, the actual proportion of farmers participating within regions and herd sizes were not exactly the same as the proportions in our original plan. A slightly higher percentage of farms with larger herd size than we expected, participated in this study. Nevertheless, the analysis showed that the proportion of shedding did not significantly differ between herds of different size. Therefore, it is concluded that this difference did not affect the interpretation of the result.

7.5.3. Questionnaire design

A questionnaire with closed, mostly quantitative type of questions was developed for this study. In general, this type of questions are easier to answer and can produce consistent responses (Dohoo *et al.*, 2009). A relatively small proportion of questions in this study were designed as open questions. However, as these open questions were simple and not seeking sensitive information, the style and the skill of the interviewers (farm's veterinary service providers) may not influence the answers. In addition, before being administered, the questionnaire was pre-tested with three farmers in two dairy farms to assess the clarity of the questionnaire and the suitability to the participant.

7.6. Recommendations for dairy farmers

The strong survey outcome of a lack of an association between vaccine serovars (Hardjo, Pomona and Copenhageni) and shedding increased the confidence that vaccination against leptospirosis, was effective in the study herds, and therefore in the dairy farming population. Consequently, one extension message was to continue vaccinating dairy cattle. As less than half of the farmers and/or veterinarians followed best practice guidelines for vaccination, this was pointed out in these messages.

Another valid and credible conclusion was the causal effect that the widely distributed, new *Leptospira* strain had on shedding. The relatively small proportion of Ballum positive cows on some dairy farms indicated another potential human health hazard,

presumably due to the presence of infected rodents. The resulting recommendations are, firstly, for veterinarians to discuss this issue with their dairy farming clients and possible measures for preventing infection such as reducing exposure to urine, e.g. by using protective clothing and glasses (re. Tarassovi), and secondly, controlling wildlife species, especially rodents. Hence, the following recommendations were propagated to farmers:

- *Continue vaccination in dairy cattle and livestock animals on dairy farms conforming with best practice. For optimum result, vaccination should be conducted as early as possible to precede infection in young animals.*
- *Control rodents and wildlife by assessing the farm environment and identifying the species of rodents and wildlife present on the farm.*
- *Use boots, aprons, glasses and avoid contact with urine and other risky material such as aborted material, effluent, and surface water on dairy farms.*
- *Wash hands after milking and before smoking or eating/drinking during breaks.*
- *Consult your veterinarian regarding leptospirosis control on your farm.*

7.7. Future research

7.7.1. Tarassovi's molecular structure

After completion of this study, preliminary sequence analyses were then conducted. Since the sequencing work was not part of this thesis, it could not be considered in the relevant chapter (4).

The analyses were done on PCR amplicons specific for pathogenic *Leptospira* on cattle urine collected from the survey. A multiple sequence alignment compared DNA sequences from our samples with sequences of serovar Hardjo, Pomona, Copenhageni, Ballum, Tarassovi and Balcanica in New Zealand. The analysis of the results showed that the DNA sequences that were associated serologically with Tarassovi had no similarity with the DNA of any of the six serovars found in New Zealand. However, 76% of the amplicons labelled as 'agent X' were related to Tarassovi of species *L.*

borgpetersenii. This is strong indication that a new *Leptospira* strain with MAT-reactivity to Tarassovi has emerged. Nevertheless, only a partial fragment of the *gyrB* gene with approximately 500 bp was amplified and sequenced from these samples. To gain a comprehensive understanding of the molecular features of this "new strain", an isolate is needed for whole genome analysis.

7.7.2. Research for Tarassovi-vaccine development

Notification data of human leptospirosis in New Zealand shows an increasing number of dairy farmers were infected with serovar Tarassovi (Nisa et al., 2018). This was supported by finding of this study that Tarassovi was positively and significantly associated with *Leptospira* shedding in vaccinated dairy cattle in New Zealand. It also suggests that Tarassovi might have become adapted to dairy cattle (chapter 4). While the risk of infection with Tarassovi appears to be increasing in dairy farmers, a *Leptospira* vaccine with this serovar for dairy cattle is not available, something that needs urgent attention. Currently, Tarassovi vaccine in New Zealand is available only for pigs. Vaccine development is a complex and long process that commonly includes several exploratory and pre-clinical stages to progress through to market availability. In the exploratory stage, potential antigens are identified and described. This is followed by assessing the safety and the immunogenicity of the antigen candidates in-vitro (The College of Physicians of Philadelphia, 2019). To be readily available in the market, veterinary products have to follow a registration process (Ministry for Primary Industries, 2017). For the registration application, a vaccine candidate has to be supported by scientific data that includes the quality, effectiveness and safety of the product on target animals (Ministry for Primary Industries, 2017). Therefore, further research is required to facilitate the development of a vaccine using a recently circulated serovar that is intended for cattle use.

7.7.3. Potential production effects related to Tarassovi infection

Production effects of *Leptospira* infection in cattle may be caused by abortion, stillbirth and a decrease of milk production. Abortion and stillbirth were mostly associated with

serovar Pomona (chapter 2) while Hardjo may be associated with decreased milk production and mastitis (chapter 2). Symptoms related to infection with other serovars such as Copenhageni and Ballum have been detected in calves. However, none of the surveyed farmers who had Tarassovi positive cows in their herds reported such adverse production effects. Further research is therefore required to explore the impact of Tarassovi infection on production traits.

7.7.4. Locating *Leptospira* in the dairy farm environment

The survival of *Leptospira* in the environment primarily depends on soil moisture, humidity, drinking water source, sun exposure, and pH. On dairy farms, *Leptospira* is likely to be found on wet pasture, effluent, and standing water. Preliminary work discovered *Leptospira* in the dairy farming environment (Nisa *et al.*, 2018a). This research has successfully cultured *Leptospira* from surface water (streams, ponds and troughs). We recommend to conduct a large scale, representative survey to locate the likely sources of *Leptospira* in the dairy farm environment. This would contribute to a better understanding and management of biosafety and biosecurity to reduce human and animal exposure to *Leptospira*.

Reference

Adler, B., & de la Peña Moctezuma, A. (2010). *Leptospira* and leptospirosis. *Veterinary Microbiology, 140*(3), 287-296.

Adler, B. (Ed.). (2015). *Leptospira and leptospirosis* (Vol. 387). Springer.

Alejandro, C. I., Gustavo, R. L. C., Ivan, T. B. J., Manuel, X. C. V., Alejandro, C. J. C., Silvia, C. J. M., & Katherine, A. S. (2008). Presence of Antibodies Against Brucellosis-Leptospirosis and Abortions in Dairy Cows in Mexico. *Journal of Animal and Veterinary Advances, 7*(12), 1640-1643.

Alt, D. P., Zuerner, R. L., & Bolin, C. A. (2001). Evaluation of antibiotics for treatment of cattle infected with *Leptospira borgpetersenii* serovar Hardjo. *Journal Of The American Veterinary Medical Association, 219*(5), 636-639.

Anonymous. (2018). Worksafe. Retrieved from https://worksafe.govt.nz/topic-and-industry/working-with-animals/prevention-and-control-of-leptospirosis/gpg/

Atxaerandio, R., Aduriz, G., Ziluaga, I., Esteban, J. I., Maranda, L., & Mainar-Jaime, R. C. (2005). Serological evidence of *Leptospira interrogans* serovar Bratislava infection and its association with abortions in cattle in northern Spain. *Veterinary Record, 156*(12), 376-380.

Bahaman, A. R., Marshall, R. B., & Hellstrom, J. S. (1984). A serological study of bovine leptospirosis in the district of taranaki. *New Zealand Veterinary Journal, 32*(8), 134-136. doi:10.1080/00480169.1984.35096

Bahari, A., Abdollahpour, G., Sadeghi-Nasab, A., Sattari Tabrizi, S., Yavari, M., & Dadmehr, B. (2011). A serological survey on leptospirosis in aborted dairy cattle in industrial farms of Hamedan suburb, Iran. *Iranian Journal of Veterinary Research, 12*(4), 337-339.

Benschop, J., Collins-Emerson, J., Maskill, A., O'Connor, P., Tunbridge, M., Yupiana, Y., & Weston, J. (2017). Leptospirosis in three workers on a dairy farm with unvaccinated cattle. *New Zealand Medical Journal, 130*(1462), 102-108.

Bettelheim, K. A., & Fogg, T. R. (1986a). Serological studies on leptospiral antibodies in dairy milkers in 3 regions of the south island of New Zealand. *Comparative Immunology Microbiology and Infectious Diseases, 9*(4), 355-364. doi:10.1016/0147-9571(86)90048-2

Bettelheim, K. A., & Fogg, T. R. (1986b). Serological studies on leptospiral antibodies in dairy milkers in three regions of the South Island of New Zealand. *Comparative Immunology, Microbiology and Infectious Diseases, 9*(4), 355-364. doi:10.1016/0147-9571(86)90048-2

Blackmore, D. K., Bahaman, A. R., & Marshall, R. B. (1982). The epidemiological interpretation of serological responses to leptospiral serovars in sheep. *New Zealand Veterinary Journal, 30*(4), 38-42.

Blackmore, D. K., Marshall, R. B., & MacKintosh, C. M. (1981). Alternative strategies for the control of leptospirosis in dairy herds. *New Zealand Veterinary Journal, 29*(1-2), 19-20. doi:10.1080/00480169.1981.34780

Blackmore, D. K., & Schollum, L. M. (1982a). Risks of contracting leptospirosis on the dairy farm. *New Zealand Medical Journal, 95*(716), 649-652.

Blackmore, D. K., & Schollum, L. M. (1982b). *The serological investigation of occupational exposure to leptospirosis*. VA: International Symposia on Veterinary Epidemiology and Economics.

Bolger, J. F. (1984). *An investigation in Otago to support a programme to vaccinate dairy cattle against leptospirosis as a means of safeguarding human health*.

Bolin, C. A., & Koellner, P. (1988). Human-to-human transmission of Leptospira interrogans by milk. *Journal of Infectious Diseases, 158*(1), 246-247.

Brown, W. B., Kissling, R., & Rysewyk, A. G. v. (1985). *Evaluation of a dairy cattle leptospirosis vaccination awareness campaign for dairy farmers* (Vol. ISVEE 4: Proceedings of the 4th International Symposium on Veterinary Epidemiology and Economics, Singapore): International Symposia on Veterinary Epidemiology and Economics.

Bruere, A. N. (1952). An association between leptospirosis in calves and man. *Australian Veterinary Journal, 28*(7), 174-174.

Bulach, D.M., Zuerner, R.L., Wilson, P., Seemann, T., McGrath, A., Cullen, P.A., Davis, J., Johnson, M., Kuczek, E., Alt, D.P. and Peterson-Burch, B. (2006). Genome reduction in Leptospira borgpetersenii reflects limited transmission potential. *Proceedings of the National Academy of Sciences*, *103*(39), pp.14560-14565.

Campos, Â.P., Miranda, D.F.H., Rodrigues, H.W.S., Lustosa, M.D.S.C., Martins, G.H.C., Mineiro, A.L.B.B., Castro, V., Azevedo, S.S. and de Sousa Silva, S.M.M. (2017). Seroprevalence and risk factors for leptospirosis in cattle, sheep, and goats at consorted rearing from the State of Piauí, northeastern Brazil. *Tropical animal health and production*, *49*(5), pp.899-907.

Carter, M. E., Cordes, D. O., Holland, J. T., Lewis, S. F., & Lake, D. E. (1982). Leptospirosis: II. Investigation of clinical disease in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*(9), 136-140. doi:10.1080/00480169.1982.34915

Choi, B. C. K., & Pak, A. W. P. (2005). Peer reviewed: a catalog of biases in questionnaires. *Preventing chronic disease, 2*(1).

Christmas, B. W., Tennent, R. B., Philip, N. A., & Lindsay, P. G. (1974a). Dairy farm fever in New Zealand.[i] A local outbreak of human leptospirosis. *New Zealand Medical Journal, 79*(514), 901-904.

Christmas, B. W., Till, D. G., & Bragger, J. M. (1974b). Dairy farm fever in New Zealand isolation of *L .Pomona* and *L.Hardjo* from a local outbreak. *New Zealand Medical Journal, 79*(514), 904-906.

Cordes, D. O., Carter, M. E., Townsend, K. G., Lewis, S. F., & Holland, J. T. S. (1982). Leptospirosis: I. Clinical investigation of the infection in dairy cattle in the Waikato district of New Zealand. *New Zealand Veterinary Journal, 30*(8), 122-124. doi:10.1080/00480169.1982.34907

Corney, B. G., Slack, A. T., Symonds, M. L., Dohnt, M. F., McClintock, C. S., McGowan, M. R., & Smythe, L. D. (2008). *Leptospira weilii* serovar Topaz, a new member of the Tarassovi serogroup isolated from a bovine source in Queensland, Australia.

International Journal of Systematic and Evolutionary Microbiology, 58, 2249-2252. doi:10.1099/ijs.0.64884-0

Cortese, V. (2011). *Immunology and vaccination of dairy cattle*. Chichester, UK: John Wiley & Sons.

Costa, F., Hagan, J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S., Stein, C., Abela-Ridder, B. and Ko, A.I. (2015). Global morbidity and mortality of leptospirosis: a systematic review. *PLoS neglected tropical diseases*, *9*(9), p.e0003898.

Cowie, G., & Bell, A. (2012). A retrospective review of notified human leptospirosis cases in the Waikato region of New Zealand, 2004 to 2010. *New Zealand Medical Journal, 125*, 20-28

Cranefield, S. (2002). *Protecting all New Zealanders against leptospirosis.* Paper presented at the Proceedings of the Society of Sheep and Beef Cattle Veterinarians of the New Zealand Veterinary Association.

Davidson, K. R. (1971). Leptospira-hardjo infection in man associated with an outbreak in a dairy herd. *Australian Veterinary Journal, 47*(8), 408. doi:10.1111/j.1751-0813.1971.tb09234.x

Dhaliwal, G. S., Murray, R. D., Dobson, H., Montgomery, J., & Ellis, W. A. (1996a). Effect of *Leptospira interrogans* serovar Hardjo infection on milk yield in endemically infected dairy herds. *Veterinary Record, 139*(13), 319-320.

Dhaliwal, G. S., Murray, R. D., Dobson, H., Montgomery, J., & Ellis, W. A. (1996b). Reduced conception rates in dairy cattle associated with serological evidence of *Leptospira interrogans* serovar Hardjo infection. *Veterinary Record, 139*(5), 110-114.

Dhaliwal, G. S., Murray, R. D., & Ellis, W. A. (1996c). Reproductive performance of dairy herds infected with *Leptospira interrogans* serovar Hardjo relative to the year of diagnosis. *Veterinary Record, 138*(12), 272-276.

Dobinson, J. (1967). *Leptospirosis and the dairyfarmer*.

Dodd, D. C., & Brakenridge, D. T. (1960). *Leptospira icterohaemorrhagiae* AB infection in calves. *New Zealand Veterinary Journal, 8*(4), 71-76. doi:10.1080/00480169.1960.33383

Doeleman, F. (1932). Ziekte van Weil, rechstreeks overgebracht van mensch op mensch. *Ned Tijdschr Geneeskd, 76*, 5057.

Dohoo, I. R., Martin, W., & Stryhn, H. (2009). *Veterinary epidemiologic research* (2nd ed.): AVC Incorporated Charlottetown, Canada.

Dreyfus, A., Benschop, J., Collins-Emerson, J., Wilson, P., Baker, M. G., & Heuer, C. (2014). Sero-prevalence and risk factors for leptospirosis in abattoir workers in New Zealand. *International Journal of Environmental Research and Public Health, 11*(2), 1756-1775. doi:10.3390/ijerph110201756

Dreyfus, A., Heuer, C., Wilson, P., Collins-Emerson, J., Baker, M. G., & Benschop, J. (2015a). Risk of infection and associated influenza-like disease among abattoir workers due to two *Leptospira* species. *Epidemiology And Infection, 143*(10), 2095-2105. doi:10.1017/s0950268814002477

Dreyfus, A., Wilson, P., Benschop, J., Collins-Emerson, J., Verdugo, C., & Heuer, C. (2018). Seroprevalence and herd-level risk factors for seroprevalence of *Leptospira spp*. in sheep, beef cattle and deer in New Zealand. *New Zealand Veterinary Journal, 66*(6), 302-311. doi:10.1080/00480169.2018.1507770

Dreyfus, A., Wilson, P., Collins-Emerson, J., Benschop, J., Moore, S., & Heuer, C. (2015b). Risk factors for new infection with *Leptospira* in meat workers in New Zealand. *Occupational and Environmental Medicine, 72*(3), 219-225. doi:10.1136/oemed-2014-102457

Durfee, P. T., & Allen, J. D. (1980). Serological titers of dairy cows over a 63-week period following natural infection with Leptopsira interrogans serova Hardjo. *Australian Veterinary Journal, 56*(12), 574-579. doi:10.1111/j.1751-0813.1980.tb02601.x

Elder, J. K., Pepper, P. M., Hill, M. W. M., & Ward, W. H. (1985). The significance of leptospiral titres associated with bovine abortion. *Australian Veterinary Journal, 62*(8), 258-262. doi:10.1111/j.1751-0813.1985.tb14246.x

Ellis, W. A., O'Brien, J. J., Bryson, D. G., & Mackie, D. P. (1985). Bovine leptospirosis: some clinical features of serovar hardjo infection. *Veterinary Record, 117*(5), 101-104.

Emanuel, M. L., Mackerras, I. M., & Smith, D. J. W. (1964). The epidemiology of leptospirosis in North Queensland: I. General survey of animal hosts. *Epidemiology & Infection, 62*(4), 451-484.

Ensor, C. R., & McClure, T. J. (1953). Bovine leptospirosis in Northland. *New Zealand Veterinary Journal, 1*(3), 47-50.

Escamilla, H. P., Martínez, M. J. J., Medina, C. M., & Morales, S. E. (2007). Frequency and causes of infectious abortion in a dairy herd in Queretaro, Mexico. *Canadian Journal of Veterinary Research, 71*(4), 314-317.

ESR 2013–2017: The Institute of Environmental Science and Research Ltd. (2017). *Notifiable Diseases in New Zealand: Annual Report 2012-2016*. Retrieved from https://surv.esr.cri.nz/

ESR 2016: The Institute of Environmental Science and Research Ltd. (2017). *Notifiable Diseases in New Zealand: Annual Report 2016*. Retrieved from www.surv.esr.cri.nz

Evangelista, K. V., & Coburn, J. (2010). *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiology, 5*(9), 1413-1425. doi:10.2217/fmb.10.102

Faine, S. (1982). *Laboratory methods. In: Guidelines for the control of Leptospirosis Laboratory methods.World Health Organization Geneva, Switzerland*

Fang, F. (2014). *Leptospirosis diagnostics and exposure at the human and animal interface in New Zealand (Doctoral dissertation, Massey University, Palmerston North, New Zealand).* Massey University. Retrieved from https://mro.massey.ac.nz/handle/10179/296

Fang, F., Benschop, J., Wilson, P. R., Collins-Emerson, J. M., Heuer, C., & Prattley, D. (2014a). Seroprevalence and exposure to risk factors for leptospirosis among veterinary students at Massey University. *New Zealand Veterinary Journal, 62*(3), 130-135. doi:10.1080/00480169.2013.862161

Fang, F., Collins-Emerson, J. M., Heuer, C., Hill, F. I., Tisdall, D. J., Wilson, P. R., & Benschop, J. (2014b). Interlaboratory and between-specimen comparisons of diagnostic tests for leptospirosis in sheep and cattle. *Journal of Veterinary Diagnostic Investigation, 26*(6), 734-747. doi:10.1177/1040638714548476

Fang, F., Collins-Emerson, J. M., Cullum, A., Heuer, C., Wilson, P. R., & Benschop, J. (2015). Shedding and seroprevalence of pathogenic *Leptospira spp.* in sheep and cattle at a New Zealand abattoir. *Zoonoses And Public Health, 62*(4), 258-268.

Fávero, J.F., de Araújo, H.L., Lilenbaum, W., Machado, G., Tonin, A.A., Baldissera, M.D., Stefani, L.M. and Da Silva, A.S. (2017). Bovine leptospirosis: Prevalence, associated risk factors for infection and their cause-effect relation. *Microbial pathogenesis*, *107*, pp.149-154.

Flint, S. H., Corner, R. J., & Marshall, R. B. (1988). A serological study of leptospirosis in domestic deer in new-zealand. *New Zealand Veterinary Journal, 36*(3), 151-152. doi:10.1080/00480169.1988.35515

Flint, S. H., & Liardet, D. M. (1980). A trivalent leptospiral vaccine with emphasis on a *Leptospira interrogans* serovar Hardjo component to prevent leptospiruria. *New Zealand Veterinary Journal, 28*(12), 263-266.

Fraga, T. R., Barbosa, A. S., & Isaac, L. (2011). Leptospirosis: Aspects of Innate Immunity, Immunopathogenesis and Immune Evasion From the Complement

System. *Scandinavian Journal of Immunology, 73*(5), 408-419. doi:10.1111/j.1365-3083.2010.02505.x

Gädicke, P., & Monti, G. (2013). Factors related to the level of occurrence of bovine abortion in Chilean dairy herds. *Preventive Veterinary Medicine, 110*(2), 183-189.

Genc, O., Otlu, S., Sahin, M., Aydin, F., & Gokce, H. I. (2005). Seroprevalence of brucellosis and leptospirosis in aborted dairy cows. *Turkish Journal of Veterinary & Animal Sciences, 29*(2), 359-366.

Gibson, I., & Varney, K. (2008). Quarterly review of diagnostic cases - July to September 2008. *Surveillance, 35,* 11-16.

Giles, N., Hathaway, S. C., & Stevens, A. E. (1983). Isolation of *Leptospira interrogans* serovar hardjo from a viable premature calf. *Veterinary Record, 113*(8), 174-176.

Grooms, D. L., & Bolin, C. A. (2005). Diagnosis of fetal loss caused by bovine viral diarrhea virus and *Leptospira spp*. *Veterinary Clinics of North America: Food Animal Practice, 21*(2), 463-472.

Guitian, F. J., Garcia-Pena, F. J., Sanjuan, M. L., & Yus, E. (2001). Serological study of the frequency of leptospiral infections among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain. *Veterinary Microbiology, 80*(3), 275-284. doi:10.1016/s0378-1135(01)00306-6

Guitian, J., Thurmond, M. C., & Hietala, S. K. (1999). Infertility and abortion among first-lactation dairy cows seropositive or seronegative for *Leptospira interrogans* serovar Hardjo. *Journal Of The American Veterinary Medical Association, 215*(4), 515-518.

Hamali, H., Joozani, R. J., & Abdollahpour, G. R. (2012). Serodiagnosis and molecular survey on leptospiral abortions in the dairy cattle of Tabriz. *Iranian Journal of Veterinary Research, 13*(2), 120-125.

Hancock, G., Wilks, C., Kotiw, M., & Allen, J. (1984a). The long term efficacy of a Hardjo-pomona vaccine in preventing leptospiruria in cattle exposed to natural challenge with Leptospira interrogans serovar hardjo. *Australian Veterinary Journal, 61*(2), 54-56.

Hancock, G. A., Wilks, C. R., Kotiw, M., & Allen, J. D. (1984b). The long term efficacy of a Hardjo-Pomona vaccine in preventing leptospiruria in cattle exposed to natural challenge with *Leptospira interrogans* serovar Hardjo. *Australian Veterinary Journal, 61*(2), 54-56. doi:10.1111/j.1751-0813.1984.tb07192.x

Harland, A.L., Cave, N.J., Jones, B.R., Benschop, J., Donald, J.J., Midwinter, A.C., Squires, R.A. and Collins-Emerson, J.M. (2013). A serological survey of leptospiral antibodies in dogs in New Zealand. *New Zealand veterinary journal*, *61*(2), pp.98-106.

Hartskeerl, R. A., Collares-Pereira, M., & Ellis, W. A. (2011). Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clinical Microbiology and Infection, 17*(4), 494-501. doi:10.1111/j.1469-0691.2011.03474.x

Hathaway, S. C. (1981). Leptospirosis in New Zealand: an ecological view. *New Zealand Veterinary Journal, 29*(7), 109-112. doi:10.1080/00480169.1981.34815

Hathaway, S. C., & Blackmore, D. K. (1981). Ecological aspects of the epidemiology of infection with leptospires of the Ballum serogroup in the black rat (Rattus rattus) and the brown rat (Rattus norvegicus) in New Zealand. *Journal of Hygiene, 87*(3), 427-436.

Hathaway, S. C., Blackmore, D. K., & Marshall, R. B. (1978). Serologic and cultural prevalence of *Leptospira interrogans* serovar Balcanica in possums (Trichosurus vulpecula) in New Zealand. *Journal of Wildlife Diseases, 14*(3), 345-350. doi:10.7589/0090-3558-14.3.345

Hathaway, S. C., Blackmore, D. K., & Marshall, R. B. (1981). Leptospirosis in Free-Living Species in New Zealand. *Journal of Wildlife Diseases, 17*(4), 489-496. doi:10.7589/0090-3558-17.4.489

Haydon, D. T., Cleaveland, S., Taylor, L. H., & Laurenson, M. K. (2002). Identifying reservoirs of infection: a conceptual and practical challenge. *Emerging Infectious Diseases, 8*(12), 1468-1473.

Hellstrom, J. S. (1978). *Studies on some aspects of the epidemiology of bovine leptospirosis.* (Doctor of Philosophy), Massey University. Retrieved from https://mro.massey.ac.nz/handle/10179/296

Henderson, R. J. (1969). Q fever and leptospirosis in dairy farming community and allied workers of Worcestershire. *Journal of Clinical Pathology, 22*(5), 511-&. doi:10.1136/jcp.22.5.511

Hernandez-Rodriguez, P., Diaz, C. A., Dalmau, E. A., & Quintero, G. M. (2011). A comparison between polymerase chain reaction (PCR) and traditional techniques for the diagnosis of leptospirosis in bovines. *Journal of Microbiological Methods, 84*(1), 1-7. doi:10.1016/j.mimet.2010.10.021

Heuer, C., Benschop, J., Stringer, L., Collins-Emerson, J., Sanhueza, J., & Wilson, P. R. (2012). *Leptospirosis vaccination in NZ - Best Practice recommendations vaccine use to prevent human exposure*. Retrieved from

http://www.massey.ac.nz/massey/fms/Colleges/College%20of%20Sciences/epicentre/Docs/Leptospirosis%20in%20NZ%20-%20Best%20Practice%20recommendations%20vaccine%20use%20to%20prevent%20human%20exposure.pdf

Higgins, R. J., Harbourne, J. F., Little, T. W., & Stevens, A. E. (1980). Mastitis and abortion in dairy cattle associated with *Leptospira* of the serotype hardjo. *Veterinary Record, 107*(13), 307-310.

Hill, F., McSporran, K. D., Reichel, M. P., & Tisdall, D. J. (2007). Leptospiral infection can be confirmed by polymerase chain reaction. *Vetscript, 20,* 26-27.

Horner, G. W. (1988). Bovine abortions - laboratory findings. *Surveillance, 15,* 17-17.

Jago, J., & Kerrisk, K. (2011). Training methods for introducing cows to a pasture-based automatic milking system. *Applied Animal Behaviour Science, 131*(3-4), 79-85.

Khairani-Bejo, S., Bahaman, A. R., Zamri-Saad, M., & Mutalib, A. R. (2004). survival of *Leptospira interrogans* serovar Hardjo in Malaysian environment. *Journal of Animal and Veterinary Advances, 3*(3), 123-129.

Kirschner, L. (1954). Recent studies on leptospirosis in New Zealand; infection with a new type (*Leptospira mitis*) Johnson (Syn. Lept. hyos) in man and animals. *New Zealand Medical Journal, 53*(294), 119-128.

Langoni, H., De Souza, L. C., Da Silva, A. V., Luvizotto, M. C. R., Paes, A. C., & Lucheis, S. B. (1999). Incidence of leptospiral abortion in Brazilian dairy cattle. *Preventive Veterinary Medicine, 40*(3-4), 271-275. doi:10.1016/S0167-5877(99)00020-3

Leonard, F., Quinn, P., Ellis, W., & O'Farrell, K. (1993). Association between cessation of leptospiruria in cattle and urinary antibody levels. *Research in Veterinary Science, 55*(2), 195-202.

Levett, P. N. (2001). Leptospirosis. *clinical microbiology reviews, 14*(2), 296–326. doi:10.1128/CMR.14.2.296–326.2001

Levett, P. N. (2004). Leptospirosis: a forgotten zoonosis? *Clinical and Applied Immunology Reviews, 4*(6), 435-448.

LIC and DairyNZ Limited. (2014). *New Zealand Dairy Statistics 2013-14*. Retrieved from www.dairynz.co.nz/dairystatistics

LIC and DairyNZ Limited. (2016). *New Zealand Dairy Statistics 2015-16*. Hamilton, New Zealand. Retrieved from https://www.dairynz.co.nz/publications/dairy-industry/new-zealand-dairy-statistics-2015-16/

Liegeon, G., Delory, T., & Picardeau, M. (2018). Antibiotic susceptibilities of livestock isolates of *Leptospira*. *International Journal of Antimicrobial Agents, 51*(5), 693-699. doi:10.1016/j.ijantimicag.2017.12.024

Lilenbaum, W., & Martins, G. (2014). Leptospirosis in Cattle: A Challenging Scenario for the Understanding of the Epidemiology. *Transboundary & Emerging Diseases, 61*, 63-68. doi:10.1111/tbed.12233

Limmathurotsakul, D., Turner, E.L., Wuthiekanun, V., Thaipadungpanit, J., Suputtamongkol, Y., Chierakul, W., Smythe, L.D., Day, N.P., Cooper, B. and Peacock, S.J. (2012). Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clinical infectious diseases*, *55*(3), pp.322-331.

Little, T. W. A., Hathaway, S. C., Boughton, E. S., & Seawright, D. (1992). Development of a control strategy for *Leptospira*-Hardjo infection in a closed beef herd. *Veterinary Record, 131*(17), 383-386.

Mackintosh, C. G. (1981). *A study of the epidemiology and control of leptospirosis on dairy farms: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Pathology and Public Health at Massey University.* Massey University.

Mackintosh, C. G., Blackmore, D. K., & Marshall, R. B. (1980a). Isolation of *Leptospira Interrogans* serovars Tarassovi and Pomona from Dogs. *New Zealand Veterinary Journal, 28*(5), 100-100. doi:10.1080/00480169.1980.34709

Mackintosh, C. G., & Marshall, R. B. (1980). Serological titres resulting from leptospiral vaccine. *New Zealand Veterinary Journal, 28*(8), 172. doi:10.1080/00480169.1980.34739

Mackintosh, C. G., Marshall, R. B., & Blackmore, D. K. (1980b). *Leptospira interrogans* serovar Balcanica in cattle. *New Zealand Veterinary Journal, 28*(12), 268-268. doi:10.1080/00480169.1980.34774

Mackintosh, C. G., Marshall, R. B., & Broughton, E. S. (1980c). The use of a Hardjo-Pomona vaccine to prevent leptospiruria in cattle exposed to natural challenge with *Leptospira interrogans* serovar Hardjo. *New Zealand Veterinary Journal, 28*(9), 174-177.

Mackintosh, C. G., Marshall, R. B., & Thompson, J. C. (1981). Experimental infection of sheep and cattle with *Leptospira interrogans* serovar Balcanica. *New Zealand Veterinary Journal, 29*(1-2), 15-19.

Mackintosh, C. G., Schollum, L. M., Blackmore, D. K., & Marshall, R. B. (1982). Epidemiology of leptospirosis in dairy farm workers in the Manawatu. Part II. A

case study of high and low risk farms. *New Zealand Veterinary Journal, 30*, 73-36.

Mackintosh, C. G., Schollum, L. M., Blackmore, D. K., Willis, A. F., Cook, N. R., Stoke, J. C. J., & Harris, R. E. (1980d). Epidemiology of leptospirosis in dairy farm workers in the Manawatu Part I: A cross-sectional serological survey and associated occupational factors. *New Zealand Veterinary Journal, 28*(12), 245-250. doi:10.1080/00480169.1980.34769

Mannewald, A., Collins-Emerson, J., Dreyfus, A., Sanhueza, J. M., Wilson, P. R., Benschop, J., Wilson, P.R., Verdugo, C., Emanuelson, U. Fayaz, A., Boqvist, S., Haack, N., Heuer, C.(2019). Seroprevalence and distribution of *Leptospira interrogans* serovar Copenhageni and L. borgpetterseni serovars Tarassovi and Ballum in Beef Cattle, Sheep and Deer in New Zealand. Manuscript submitted for publication.

Marshall, R. B. (1987). A nationwide experiment to control human leptospirosis by vaccinating dairy cattle. *Israel journal of veterinary medicine, 43*, 271-276.

Marshall, R. B., Broughton, E., & Hellstrom, J. (1979). Protection of cattle against natural challenge with *Leptospira interrogans* serovar Hardjo using a hardjo-pomona vaccine. *New Zealand Veterinary Journal, 27*(6), 114-116.

Marshall, R. B., & Chereshsky, A. (1996a). Vaccination of dairy cattle against leptospirosis as a means of preventing human infections. *Surveillance (Wellington), 23*, 27-28.

Marshall, R. B., & Chereshsky, A. (1996b). Vaccination of dairy cattle against leptospirosis as a means of preventing human infections. *Surveillance, 23,* 27-28.

Marshall, R. B., & Manktelow, B. W. (2002). Fifty years of leptospirosis research in New Zealand: a perspective. *New Zealand Veterinary Journal, 50*(3), 61-63.

Marshall, R. B., Manktelow, B. W., Ryan, T. O., & Hathaway, S. C. (1976). *Leptospira interrogans* serovar Balcanica from a possum. *New Zealand Medical Journal, 84*(568), 74-75.

Marshall, R. B., Vermunt, J. J., & Collins-Emerson, J. (1996). Is leptospirosis vaccination working? *Vetscript, 9,* 4-5.

Martins, G., & Lilenbaum, W. (2017). Control of bovine leptospirosis: Aspects for consideration in a tropical environment. *Research in Veterinary Science, 112*, 156-160. doi:10.1016/j.rvsc.2017.03.021

McLean, M. (2014). A cluster of three cases of leptospirosis in dairy farm workers in New Zealand. *New Zealand Medical Journal, 127*(1388), 13-20.

Metcalfe, R. V., Bettelheim, K. A., Densham, E. B., Pearce, J., Sillars, H., & Thorn, C. (1981). Studies on antibody levels to Brucella abortus, Toxoplasma gondii and *Leptospira* serogroups in sera collected in five locations of the North Island of New Zealand. *Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene - Abt. 1 Orig. A, 249*(4), 543-556.

Miller, N. G. (1961). A serologic investigation of leptospiral infections in dairy farmers and cattle ranchers. *American Journal of Hygiene, 74*(2), 203.

Ministry for Primary Industries. (1974). Ruakura Animal Health Laboratory Report. *Surveillance, 1,* 10-15.

Ministry for Primary Industries. (1977a). Lincoln Animal Health Laboratory Report. *Surveillance, 4,* 19-22.

Ministry for Primary Industries. (1977b). Ruakura Animal Health Laboratory Report. *Surveillance, 4,* 13-17.

Ministry for Primary Industries. (1980). Whangarei Animal Health Laboratory Report. *Surveillance, 7,* 10-11.

Ministry for Primary Industries. (2011). Quarterly review of diagnostic cases:July to September 2011. *Surveillance, 38,* 16-25.

Ministry for Primary Industries. (2013). Quarterly report of diagnostic cases July to Sept 2013. *Surveillance, 40,* 11-22.

Ministry for Primary Industries. (2017). Veterinary Medicines. Retrieved from https://www.mpi.govt.nz/processing/agricultural-compounds-and-vet-medicines/veterinary-medicines/

Ministry for Primary Industries. (2018). Quarterly report of diagnostic cases: January to March 2018. *Surveillance, 45,* 15-24.

Ministry of Health. (2018). Leptospirosis. Retrieved 19 October 2018 from https://www.health.govt.nz/our-work/diseases-and-conditions/communicable-disease-control-manual/leptospirosis#casedef

Miyama, T., Watanabe, E., Ogata, Y., Urushiyama, Y., Kawahara, N., & Makita, K. (2018). Herd-level risk factors associated with *Leptospira* Hardjo infection in dairy herds in the southern Tohoku, Japan. *Preventive Veterinary Medicine, 149*, 15-20. doi:10.1016/j.prevetmed.2017.11.008

Moinet, M., Nisa, S., Haack, N., Wilkinson, D. A., Oosterhof, H., Aberdein, D.,Russel, J.C., Vallee, E., Wilson, P., Collins-Emerson, J., Heuer, C., Benschop, J. (2017). *Is wildlife a source of Leptospira infection in livestock in New Zealand?* Paper

presented at the 2017 Conference Proceedings of the Large Animal Veterinarian Technicians Group of the NZVA Wellington, NZ.

Moller, K., Newling, P. E., Robson, H. J., Hansen, G. J., & Meursinge, J. A. (1967). A survey of abortions and long return intervals in dairy herds in the Huntly district. *New Zealand Veterinary Journal, 15*(8), 137-142. doi:10.1080/00480169.1967.33713

Mughini-Gras, L., Bonfanti, L., Natale, A., Comin, A., Ferronato, A., La Greca, E., Patregnani, T., Lucchese, L. and Marangon, S. (2014). Application of an integrated outbreak management plan for the control of leptospirosis in dairy cattle herds. *Epidemiology & Infection*, *142*(6), pp.1172-1181.

Murray, R. D. (1990). A field investigation of causes of abortion in dairy cattle. *Veterinary Record, 127*(22), 543-547.

Mwachui, M. A., Crump, L., Hartskeerl, R., Zinsstag, J., & Hattendorf, J. (2015). Environmental and Behavioural Determinants of Leptospirosis Transmission: A Systematic Review. *PLoS Neglected Tropical Diseases, 9*(9), e0003843. doi:10.1371/journal.pntd.0003843

Neilson, F. J. A. (1984). Project Report AH 390 - Prevalence of leptospirosis in the dairy herds of the Gisborne veterinary district. *Surveillance, 11,* 8-8.

Newcombe, R. G. (1998). Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in medicine, 17*(8), 857-872.

Niewiesk, S. (2014). Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies. *Frontiers in immunology, 5*, 446.

Nisa, S., Wilkinson, D. A., Edwards, M., Haack, N., Collins-Emerson, J., French, N. P., & Benschop, J. (2018a). *Metabarcoding with MinION: Pathogenic Leptospira in the Environment*. Paper presented at the 2018 One Health Aotearoa Symposium, Wellington.

Nisa, S., Wilkinson, D. A., Paine, S., Cullen, K., Wright, J., Baker, M. G., & J., B. (2018b). *Descriptive Epidemiology of Human Leptospirosis in New Zealand: 1999 to 2016. Unpublished manuscript*

Norton, J. H., Tranter, W. P., & Campbell, R. S. (1989). A farming systems study of abortion in dairy cattle on the Atherton Tableland. 2. The pattern of infectious diseases. *Australian Veterinary Journal, 66*(6), 163-167.

NZIER. (2017). *Dairy trade's economic contribution to New Zealand* Retrieved from https://nzier.org.nz/static/media/filer_public/29/33/29336237-3350-40ce-9933-

a5a59d25bd31/dairy_economic_contribution_update_final_21_february_2017.pdf

O'Doherty, E., Berry, D. P., O'Grady, L., & Sayers, R. (2014). Management practices as risk factors for the presence of bulk milk antibodies to Salmonella, Neospora caninum and *Leptospira interrogans* serovar Hardjo in Irish dairy herds. *Animal, 8*(6), 1010-1019. doi:10.1017/s175173111400055x

O'Keefe, J. S. (2002). A brief review on the laboratory diagnosis of leptospirosis. *New Zealand Veterinary Journal, 50*(1), 9-13.

Oliveira, F., Azevedo, S.S., Pinheiro, S.R., Batista, C.S., Moraes, Z.M., Souza, G.O., Gonçales, A.P. and Vasconcellos, S.A. (2010). Risk factors associated with leptospirosis in cows in the state of Bahia, northeastern Brazil. *Pesquisa Veterinária Brasileira*, *30*(5), pp.398-402.

Orr, M. B. (1994). Animal Health Laboratory Network review of diagnostic cases - January to March 1994. *Surveillance, 21,* 3-6.

Otaka, D., Martins, G., Hamond, C., Penna, B., Medeiros, M., & Lilenbaum, W. (2012). Serology and PCR for bovine leptospirosis: herd and individual approaches. *Veterinary Record-English Edition, 170*(13), 338.

Parramore, J. J., Meenks, M. B. R., Wilson, P. R., Heuer, C., & Weston, J. F. (2011). *The effectiveness of longterm vaccination of dairy cattle on New Zealand farms*. Massey University. Palmerston North.

Pereira, M. H. C., Cooke, R. F., Alfieri, A. A., & Vasconcelos, J. L. M. (2013). Effects of vaccination against reproductive diseases on reproductive performance of lactating dairy cows submitted to AI. *Animal Reproduction Science, 137*(3-4), 156-162. doi:10.1016/j.anireprosci.2012.12.011

Picardeau, M. (2013). Diagnosis and epidemiology of leptospirosis. *Médecine et maladies infectieuses, 43*(1), 1-9.

Pimenta, C.L., Castro, V., Clementino, I.J., Alves, C.J., Fernandes, L.G., Brasil, A.W., Santos, C.S. and Azevedo, S.S. (2014). Bovine leptospirosis in Para ba State: prevalence and risk factors associated with the occurrence of positive herds. *Pesquisa Veterinária Brasileira*, *34*(4), pp.332-336.

Price, M. C. (1984). Project Report AH 396 - Survey of the prevalence of leptospirosis in dairy herds in the Hastings veterinary district. *Surveillance, 11,* 7-7.

Rashid, A., Rasheed, K., & Akhtar, M. (2009). Factors influencing vaccine efficacy - a general review. *Journal of Animal and Plant Sciences, 19*(1), 22-25.

Robinson, A. J., Ramadass, P., Lee, A., & Marshall, R. B. (1982). Differentiation of subtypes within *Leptospira-Interrogans* serovars Hardjo, Balcanica and Tarassovi, by Bacterial Restriction-Endonuclease DNA Analysis (Brenda). *Journal of Medical Microbiology, 15*(3), 331-338. doi:10.1099/00222615-15-3-331

RStudio Team. (2016). Integrated Development for RStudio. http://www.rstudio.com/.

Ryan, E. G., Leonard, N., O'Grady, L., Doherty, M. L., & More, S. J. (2012). Herd-level risk factors associated with *Leptospira* Hardjo seroprevalence in Beef/Suckler herds in the Republic of Ireland. *Veterinary Ireland Journal, 2*(9), 1-10. doi:10.1186/2046-0481-65-6

Ryan, T. J., Hellstrom, J. S., & Penniket, J. H. (1982a). Leptospirosis in dairy farmers: prevention by vaccination of cattle. *New Zealand Veterinary Journal, 30*(7), 107-108. doi:10.1080/00480169.1982.34900

Ryan, T. J., & Marshall, R. B. (1976). Isolation of a leptospire belonging to serogroup tarassovi. *New Zealand Veterinary Journal, 24*(9), 212-213. doi:10.1080/00480169.1976.34320

Ryan, T. J., Sceats, J., Penniket, J. H., & Maguire, M. A. (1982b). Leptospirosis study seeks link between herd vaccination and human health status. *Surveillance, New Zealand, 8*(4), 7-10.

Saldanha, G. B., Cavazini, N. C., Da Silva, A. S., Fernandes, M. B., Badke, M. R. T., & Pivetta, C. G. (2007). Positive serology for *Leptospira butembo* in bovine with reproductive problems. *Ciencia Rural, 37*(4), 1182-1184.

Sanhueza, J. M., Heuer, C., & West, D. (2013). Contribution of *Leptospira*, Neospora caninum and bovine viral diarrhea virus to fetal loss of beef cattle in New Zealand. *Preventive Veterinary Medicine, 112*(1-2), 90-98. doi:10.1016/j.prevetmed.2013.07.009

Sanhueza, J. M., Heuer, C., Wilson, P. R., Benschop, J., & Collins-Emerson, J. M. (2015). Prevalence and risk factors for *Leptospira* exposure in New Zealand veterinarians. *Epidemiology Infection, 143*(10), 2116-2125. doi:10.1017/S0950268815000515

Sanhueza, J. M., Wilson, P. R., Benschop, J., Collins-Emerson, J. M., & Heuer, C. (2018). Meta-analysis of the efficacy of *Leptospira* serovar Hardjo vaccines to prevent urinary shedding in cattle. *Preventive Veterinary Medicine, 153*, 71-76. doi:10.1016/j.prevetmed.2018.02.015

Schollum, L. M., & Marshall, R. B. (1985). Age and the ability of calves to respond to a leptospiral vaccine. *New Zealand Veterinary Journal, 33*(9), 146-147.

Schoonman, L., & Swai, E. S. (2010). Herd-and animal-level risk factors for bovine leptospirosis in Tanga region of Tanzania. *Tropical animal health and production, 42*(7), 1565-1572.

Shenberg, E., Bitnun, S., & Birnbaum, S. H. (1977). Leptospirosis among dairy workers in the valley of Jezreel: first recorded isolation of *Leptospira* Hardjo in Israel. *Israel Journal of Medical Sciences, 13*(4), 377-384.

Smith, C., Ketterer, P., McGowan, M., & Corney, B. (1994). A review of laboratory techniques and their use in the diagnosis of *Leptospira interrogans* serovar Hardjo infection in cattle. *Australian Veterinary Journal, 71*(9), 290-294.

Smith, C. R., Corney, B. G., McGowan, M. R., McClintock, C. S., Ward, W., & Ketterer, P. J. (1997). Amoxycillin as an alternative to dihydrostreptomycin sulphate for treating cattle infected with *Leptospira borgpetersenii* serovar Hardjo. *Australian Veterinary Journal, 75*(11), 818-821. doi:10.1111/j.1751-0813.1997.tb15661.x

StatsNZ. (2013). Dairy export prices and volumes move upwards over 20 years. Retrieved 15 December 2018 2018 from http://archive.stats.govt.nz/tools_and_services/newsletters/price-index-news/oct-13-dairy-exports.aspxs

Subharat, S., Wilson, P., Heuer, C., & Collins-Emerson, J. (2012a). Longitudinal serological survey and herd-level risk factors for *Leptospira spp*. serovars Hardjo-bovis and Pomona on deer farms with sheep and/or beef cattle. *New Zealand Veterinary Journal, 60*(4), 215-222. doi:10.1080/00480169.2012.663323

Subharat, S., Wilson, P. R., Heuer, C., & Collins-Emerson, J. M. (2011). Evaluation of a SYTO9 real-time polymerase chain reaction assay to detect and identify pathogenic *Leptospira* species in kidney tissue and urine of New Zealand farmed deer. *Journal of Veterinary Diagnostic Investigation, 23*(4), 743-752.

Subharat, S., Wilson, P. R., Heuer, C., & Collins-Emerson, J. M. (2012b). Growth response and shedding of Leptospira spp. in urine following vaccination for leptospirosis in young farmed deer. *New Zealand Veterinary Journal, 60*(1), 14-20. doi:10.1080/00480169.2011.624985

Sullivan, N. D., & Callan, D. P. (1970). Isolation of *Leptospira* Hardjo from cows with mastitis. *Australian Veterinary Journal, 46*(11), 537-539.

Suwimonteerabutr, J., Chaicumpa, W., Saengjaruk, P., Tapchaisri, P., Chongsa-nguan, M., Kalambaheti, T., Ramasoota, P., Sakolvaree, Y. and Virakul, P. (2005). Evaluation of a monoclonal antibody–based dot-blot ELISA for detection of

Leptospira spp in bovine urine samples. *American journal of veterinary research*, *66*(5), pp.762-766.

Te Brugge, L. A., & Dreyer, T. (1985). Leptospira interrogans serovar hardjo associated with bovine abortion in South Africa. *Onderstepoort Journal of Veterinary Research, 52*(1), 51-52.

The College of Physicians of Philadelphia. (2019). Vaccine. Retrieved 2019 from https://www.historyofvaccines.org/content/articles/vaccine-development-testing-and-regulation

Thomas, M. C., Chereshsky, A., & Manning, K. (1994). An outbreak of leptospirosis on a single farm in east Otago. *New Zealand Medical Journal, 107*(982), 290-291.

Thornley, C. N., Baker, M. G., Weinstein, P., & Maas, E. W. (2002). Changing epidemiology of human leptospirosis in New Zealand. *Epidemiology And Infection, 128*(01), 29-36. doi:10.1017/s0950268801006392

Tripathy, D., Smith, A., & Hanson, L. (1975). Immunoglobulins in cattle vaccinated with leptospiral bacterins. *American Journal Of Veterinary Research, 36*(12), 1735-1736.

Vallee, E., Heuer, C., Collins-Emerson, J. M., Benschop, J., & Wilson, P. R. (2015). Serological patterns, antibody half-life and shedding in urine of *Leptospira spp*. in naturally exposed sheep. *New Zealand Veterinary Journal, 63*(6), 301-312. doi:10.1080/00480169.2015.1049668

Van De Weyer, L. M., Hendrick, S., Rosengren, L., & Waldner, C. L. (2011). Leptospirosis in beef herds from western Canada: Serum antibody titers and vaccination practices. *The Canadian Veterinary Journal, 52*(6), 619.

Varney, K., & Gibson, I. (2008). Quarterly review of diagnostic cases - October to December 2007. *Surveillance, 35,* 11-15.

Vijayachari, P., Sugunan, A. P., & Shriram, A. N. (2008). Leptospirosis: an emerging global public health problem. *Journal of biosciences, 33*(4), 557-569.

Wael, F., Bruce, M., Holt, H. R., Eltholth, M. M., & Merien, F. (2018). Update on the status of leptospirosis in New Zealand. *Acta Tropica, 188*, 161-167.

Waitkins, S. (1986). Leptospirosis as an occupational disease. *British Journal of Industrial Medicine, 43*(11), 721-725.

Weston, J. F., Heuer, C., Parkinson, T. J., & Williamson, N. B. (2012). Causes of abortion on New Zealand dairy farms with a history of abortion associated with Neospora caninum. *New Zealand Veterinary Journal, 60*(1), 27-34. doi:10.1080/00480169.2011.631171

Wilson, P., Heuer, C., Benschop, J., Collins-Emerson, J., Parramore, J. J., & Meenks, M. B. R. (2013). Does long-term vaccination eliminate leptospiral shedding? A pilot study of New Zealand dairy cattle herds. *Vetscript, 26,* 16-16.

Wilson, P., Mackintosh, C. G., & Griffin, J. F. T. (2001). *Immunological responses to vaccines in deer: effect of multiple vaccines.* Paper presented at the Proceedings of the Deer Branch of the New Zealand Veterinary Association.

Wilson, P., McGhie, J., Marshall, R. B., Audige, L. J. M., Collins-Emerson, J., Quankai, W., & Alley, M. R. (1998). Observations of leptospirosis in farmed deer. *New Zealand Veterinary Journal, 46*(4), 131-139. doi:10.1080/00480169.1998.36077

Woolums, A. R. (2007). *Vaccinating calves: new information on the effects of maternal immunity.* Paper presented at the proceedings AABP conference.

WorkSafe New Zealand. (2015). *Prevention and Control of Leptospirosis*. Retrieved from http://saferfarms.org.nz/assets/guides/WSNZ-1368-Prevention-and-Control-of-Leptospirosis-GPG-v5-0-FA1-LR.pdf

Yupiana, Y., Collins-Emerson, J. M., Benschop, J., Weston, J. F., Wilson, P. R., Vallee, E., & Heuer, C. (2017a). *Nationwide survey of dairy herds for Leptospira*. Paper presented at the Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA Annual Conference, 2017 Conference, Wellington.

Yupiana, Y., Collins-Emerson, J. M., Benschop, J., Weston, J. F., Wilson, P. R., Vallee, E., & Heuer, C. (2017b). *Nationwide survey of leptospiral antibodies and shedding in New Zealand dairy herds*. Paper presented at the Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA Annual Conference, 2017 Conference, Wellington.

Yupiana, Y., Vallee, E., Wilson, P., Collins-Emerson, J. M., Weston, J. F., Benschop, J., & Heuer, C. (2019a). *Emerging Leptospira strain poses public health risk for dairy farmers in New Zealand*. Manuscript sumbitted for publication.

Yupiana, Y., Wilson, P. R., Weston, J. F., Vallee, E., Collins-Emerson, J. M., Benschop, J., . . . Heuer, C. (2019b). *Epidemiological investigation of Leptospira spp. in a dairy farming enterprise after the occurrence of three human leptospirosis cases*.

Zimmerman, A. D., Springer, E. W., Barling, K. S., Buterbaugh, R. E., Pooley, R. D., Scholz, D. A., . . . Chase, C. C. L. (2013). Immunity in heifers 12 months after vaccination with a multivalent vaccine containing a United States *Leptospira borgpetersenii* serovar Hardjo isolate. *Journal Of The American Veterinary Medical Association, 242*(11), 1573-1577. doi:10.2460/javma.242.11.1573

Appendices

Appendix I: Farmer questionnaire

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A Study of Leptospirosis Vaccination in Dairy Cattle

FARMER'S CONSENT

I have read the "Farmer Information Sheet" I received by email and understand the project and my role as a participant. Any questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate and will make my animals available for urine, blood and milk sampling free of charge. I am willing to provide information for the questionnaire.

☐YES ☐NO

I give consent to researchers to access herd testing data for milk production and reproduction data of sampled cows during the 2014-15 season.

☐YES ☐NO ☐Not enrolled

If YES, please state the herd testing organisation (e.g. LIC): _____

I agree that the samples and data collected today may be used for testing for other animal diseases according to the confidentiality clause in the emailed "Farmer Information Sheet".

☐ YES ☐ NO

Signature: _____ **Date:** _____

Full Name - printed _____

_____

FARM QUESTIONNAIRE

This questionnaire collects information about the farm, leptospirosis, vaccination practices, and other risk factors for leptospirosis in dairy herds. Data will be used in conjunction with the analysis of blood and urine from cows, and a bulk tank milk sample.

The completed questionnaire MUST be returned to Massey University along with the Farmer's consent (above), "Sampled Animal Data Form" and samples. Please ensure that the most appropriate person on the farm is interviewed, to ensure that the most accurate data are provided.

CONTACT DETAILS

1. **Property name**: _____

2. **Farm address**: _____

_____

_____District/region: _____

3. **Dairy supplier number** : _____

4. **Contact person (name):** _____

The contact person is:

☐Owner ☐Manager ☐Sharemilker ☐Other_____

Phone & mobile: _____

Email: _____

5. **Person interviewed (name):** _____☐ same as above

   If not the same: ☐Owner ☐Manager ☐Sharemilker

   ☐Other_____

   Phone & mobile: _____

   Email: _____

6. **Veterinarian/Veterinary technician** (*name*): _____

   Phone & mobile: _____

   Email: _____

   Veterinary Practice: _____

   Address:_____

   _____

7. **Date of visit for sample collection**: _____

GENERAL FARM INFORMATION

8. **What is the size of this farm: i.e. milking platform**? _____hectares (effective)

9. **Farm management**

☐ Owner managed ☐ Manager employed ☐ Sharemilker

☐ Other, please state: _____

10. **How many years has the current manager been in charge of the herd**?

_____ years ☐UNSURE

11. **Are all milking cows on the property managed as one mob**?

☐YES ☐NO

12. **Calving pattern (*tick as applicable*)**:

☐ Spring: planned start of calving (date): _____

☐ Autumn: planned start of calving (date): _____

☐ Non- seasonal calving

13. **Breed composition**:

_____% Friesian _____% Jersey _____% Friesian-Jersey Cross

_____% Other Breed ☐UNSURE

14. **Numbers of dairy cattle on this farm on the day of sampling**:

Age	Numbers		
	Total	On the milking platform	Away/out grazing
Calves (male + female, 0-12 months)			
Heifers (12-24 months)			
1st lactation heifers			

Adult lactating cows			
Bulls (12-24 months)			
Bulls (24+ months)			

15. **Do you house your milking cows at any time e.g. herd home?**

☐YES ☐NO

If yes: please describe:

16. **Has this herd been fed Palm Kernel or other concentrate feed during this season or last season?** ☐YES ☐NO

If **YES**, was there sign of rodent faeces ever seen on the feedstuff?

☐YES ☐NO

17. **Have you purchased any replacement stock in the past 5 years?**

Milking cows ☐NO ☐YES if **YES**, how many consecutive seasons? _____

How many bulls have been purchased for breeding each year: _____

18. **Have purchased animals been vaccinated against leptospirosis**?

Heifers	Adult cows	Bulls
☐ All	☐ All	☐ All

☐ Some	☐ Some	☐ Some
☐ None	☐ None	☐ None
☐ Unsure	☐ Unsure	☐ Unsure

VACCINATION PRACTICES

19. **Have you ever vaccinated this dairy herd against leptospirosis**?

☐YES ☐NO ☐UNSURE (If **NO**, go to **Question 25**)

20. **If YES, for how many years have you vaccinated your dairy cattle against leptospirosis** (*tick one answer*)?

☐0-5 years

☐5-10 years

☐10-20 years

☐More than 20 years

21. **Describe the leptospirosis vaccination programme that has been/will be implemented for <u>CALVES</u> this season (2015/16).**

	Age (months)	**Calendar month(s)**	**Who administered the vaccine? *(Please tick)***			**Vaccine name (see below)**
			Manager	Worker	Vet	
1st vaccination			☐	☐	☐	☐ Unsure
1st booster (*if given*)			☐	☐	☐	☐ Unsure
2nd booster (*if given*)			☐	☐	☐	☐ Unsure

Leptavoid 2 Leptoshield Leptoshield 3 Ultravac 7 in 1 Lepto-2way Lepto-3way

22. **Describe the leptospirosis vaccination programme that has been/will be implemented for HEIFERS and COWS <u>this season</u> (2015/16).**

	Calendar month(s)	**Who administered the vaccine? *(Please tick)***			**Vaccine name (see below)**
Heifers (12-24 months)		Manager	Worker	Vet	
Vaccination		☐	☐	☐	☐ Unsure
2nd vaccination (if given)		☐	☐	☐	☐ Unsure
Cows (24+ months)		Manager	Worker	Vet	
Vaccination		☐	☐	☐	☐ Unsure
2nd vaccination (if					

given)		☐	☐	☐	☐ Unsure
Are bulls always vaccinated at the same time as cows? ☐ **YES** ☐ **NO** If **NO**, complete this table. If **YES**, go to the next question					
Bulls		Manager	Worker	Vet	
Vaccination		☐	☐	☐	☐ Unsure
2nd vaccination (if given)		☐	☐	☐	☐ Unsure

23. **Has your vaccination programme been the same during the past 5 years?**
☐YES ☐NO ☐UNSURE

If **YES**, go to **Question 25**

If NO, please complete the table in **Question 24** (*next page*)

24. **If NO, please explain the differences from the current season (as above).**

Season		Calves (1-12 months)	Heifers, adult cows and bulls
2015/16	Timing		
	Who		
	Vaccine		

2014/15	Timing		
	Who		
	Vaccine		
2013/14	Timing		
	Who		
	Vaccine		
2012/13	Timing		
	Who		
	Vaccine		
2011/12	Timing		
	Who		
	Vaccine		

25. **Do you administer other vaccines or other whole herd treatments (such as trace elements, dry cow therapy, anthelmintic) at the same time as vaccinating against leptospirosis**?

☐Always ☐Sometimes ☐Never ☐Unsure

26. **If <u>always</u> or <u>sometimes</u>, please state which:**

_____

_____

<u>OTHER ANIMALS</u>

27. **Do you keep any other domestic animal species on your property**?

☐YES ☐NO If **NO**, go to **Question 32**.

28. **If YES, how many**?

_____ Beef cattle

_____ Sheep

_____ Deer

_____ Horses

_____ other: _____

_____ Goats

_____ Pigs

_____ Dogs

_____ Cats

_____ other: _____

29. **Do you vaccinate any of these animals against leptospirosis**?

☐YES ☐NO ☐UNSURE

30. **Do other species ever come in direct or indirect contact with dairy cattle**?

☐YES ☐NO ☐UNSURE

31. **If YES, how do they come in contact with dairy cattle?** *(please tick)*

Other species	Grazed same paddock, same time	Alternately grazed	Share water source	Over the fence	Dairy cattle contacted		
					Calf	Heifer	Adult
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐

32. **Have you noticed any evidence of rodents or wildlife on/ near the milking platform**? (*please tick*)

Species	Often	Sometimes	Rarely	Never
Rats	☐	☐	☐	☐
Mice	☐	☐	☐	☐
Possums	☐	☐	☐	☐
Ferrets, stoats, weasels	☐	☐	☐	☐
Hedgehogs	☐	☐	☐	☐
Rabbits	☐	☐	☐	☐
Hares	☐	☐	☐	☐
Feral pigs	☐	☐	☐	☐
Feral deer	☐	☐	☐	☐
Feral sheep or goats	☐	☐	☐	☐
Feral cats	☐	☐	☐	☐

33. **Rodents** are controlled by

☐poison ☐trapping ☐dogs/cats ☐no control

34. **Wildlife habitat**? (*please tick as many as apply*)

☐Farm borders a national park, forestry or native bush

☐Farm has areas of bush/forestry that are fenced off

☐Farm has areas of bush/forestry that are not fenced off

☐There is no wildlife habitat other than pasture

☐Other? Please explain _____

ENVIRONMENT

35. **Please describe the topography of the farming area as percentage (%) of pasture**:

_____% Flat _____% Rolling _____% Hill

36. **Please describe the soil type(s)**: _____

37. **Do the milking cows have access to water sources other than troughs**

☐YES ☐NO

38. **If YES, please tick those that apply**:

☐Dams

☐Stream or river

☐Irrigation ditches

☐Natural spring(s)

☐Ponding of water after heavy rainfall

☐Other (*please specify*): _____

☐UNSURE

39. **Do you store milking shed effluent**?

☐YES ☐NO If **No**, go to **Question 43**

40. **If YES, do you spray effluent on pasture**?
☐YES ☐NO

41. **If YES, how long after spraying effluent do you graze cattle back on that pasture?**
Time from spraying to grazing to days

42. **Which of the following practices are followed to manage the risk of leptospirosis <u>while milking</u>**? (*please tick*)

Milkers wear **gloves** ☐always ☐sometimes ☐never

Milkers wear **eye protection** ☐always ☐sometimes ☐never

Milkers wear **overalls** ☐always ☐sometimes ☐never

Milkers wear **gumboots** ☐always ☐sometimes ☐never

Milkers do not **eat or smoke**. ☐always ☐sometimes ☐never

43. Do you apply other management practices to reduce the risk of leptospirosis in workers? Please describe: _____

_____

HUMAN LEPTOSPIROSIS FLU-LIKE ILLNESS

43. **Has there been any flu-like illness of anyone in contact with the dairy cattle within the past 2 years?**

☐YES ☐NO ☐UNSURE

44. **Has there been any medical diagnosis of leptospirosis of anyone in contact with the dairy cattle within the past 2 years?**

☐YES ☐NO ☐UNSURE

CLINICAL LEPTOSPIROSIS IN ANIMALS

45. **Have there been any veterinary or laboratory diagnosed, or suspected, cases of leptospirosis in dairy cattle on your property within the past 5 years**?

☐YES ☐NO ☐UNSURE

46. **If YES, Please complete the table below (next page)**

Clinical Syndrome	Number	When (month/year)	Confirmed by laboratory OR vet	Serovars (if known)
Calf redwater			☐YES ☐NO	
Abortion			☐YES ☐NO	
Mastitis			☐YES ☐NO	
Septicaemia			☐YES ☐NO	
Death			☐YES ☐NO	
Stillbirth			☐YES ☐NO	
Premature birth			☐YES ☐NO	
Sudden drop of milk production			☐YES ☐NO	
			☐YES ☐NO	

VACCINATION (to be completed by the attending Veterinarian)

From <u>your practice records</u>, please extract data about the Leptospirosis vaccine brand/s used on this farm up to the past 5 years if available.

Supply date	Name of vaccine	Number of doses	Age group
			☐Calves ☐Heifers/cows/bulls
			☐Calves ☐Heifers/cows/bulls
			☐Calves ☐Heifers/cows/bulls
			☐Calves ☐Heifers/cows/bulls
			☐Calves ☐Heifers/cows/bulls
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			☐Calves ☐Heifers/cows/bulls
			☐Calves ☐Heifers/cows/bulls
			☐Calves ☐Heifers/cows/bulls

Thank you for completing this questionnaire

Appendix II: Farmer information sheet

A Study of Leptospirosis Vaccination in Dairy Cattle

FARMER INFORMATION SHEET

This sheet provides information about a project aimed

at improving leptospirosis control in dairy herds

Why?

Leptospirosis vaccination in dairy cattle has been widely adopted for about 40 years, but a recent pilot study at Massey University suggested vaccination programmes might not be working as well as they should. Workers might still be at risk.

A study is needed to better understand the current situation with leptospirosis in dairy herds so farmers and veterinarians are better informed and can be confident in best practice for vaccination progammes to reduce risk to both workers and animals.

What is leptospirosis?

Leptospirosis is a bacterial disease of livestock transmissible to people through contact with urine. It can cause disease and abortion in cattle and cause serious illness in people. Dairy farmers are at particular risk.

For further information go to: http://www.leptospirosis.org.nz/.

Why me?

You have been selected by chance from a list of all dairy farms in New Zealand by DairyNZ. A total of 396 dairy farmers received this letter. You will receive a phone call and be asked for your consent to participate, and name/contact of your farm veterinarian and his/her practice location.

The present study will

- Investigate the infection rate and effectiveness of vaccination programmes in dairy herds
- Establish best practice guidelines for vaccination programmes

How?

- The project will be undertaken by veterinary researchers at Massey University
- Blood, urine and a bulk milk sample will be collected by local vets from 20 cows in 200 herds nationally for leptospirosis testing
- Data on leptospirosis vaccination practices and farm management will be collected
- Samples will be tested and data analysed to determine best practice for vaccination

Will it cost me anything?

No, only your time.

Will I get my results?

Yes, via your veterinarian.

At the conclusion of the study all participants will receive a report on the outcomes of the study.

Will my results/participation be confidential?

Yes, only your vet will know your identity. Your farm will not be identifiable in any report or publication.

Note: participation is voluntary.

Who is funding the project?

The project is an industry-good project funded by the **Sustainable farming fund**, **Agmardt, NZVA, Massey University, Wairarapa Veterinary Club, NZAID, ZOETIS, MSD and Virbac.**

The Researchers

The project will be conducted by PhD candidate Yuni Yupiana supported by the Leptospirosis Research Team at Massey University: Professors Cord Heuer and Peter Wilson, Drs Jackie Benschop, Julie Collins-Emerson and Jenny Weston.

Project Contacts

If you have any questions about the project please do not hesitate to contact:

Yuni Yupiana

Epicentre Institute of Veterinary, Animal and Biomedical Sciences

Priv. Bag 11222

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New Zealand 4441

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Appendix III: Sampled animal data

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

A Study of Leptospirosis Vaccination in Dairy Cattle

SAMPLED ANIMAL DATA

Veterinarian: _____ Farm Code: _____

Vet Practice: _____

Date of Collection: _____

IMPORTANT NOTE: If all sampled cows were last vaccinated for leptospirosis on the **same date**,

please provide the date: ……,(No need to fill in the date column in the table).

If not, please provide the date in the column in the table.

Sample No.	Animal Tag No.	Age (yrs)	Breed F = Fresian J= Jersey FJ = F x J cross Other: state	Date last vaccinated (if date for all is not given above)	**If not born on farm,** state year of introduction to the herd,
01					
02					
03					
04					
05					
06					
07					
08					

09					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Appendix IV: Sampling instructions for veterinary practices

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

A Study of Leptospirosis Vaccination in Dairy Cattle

JOB DESCRIPTION/INSTRUCTIONS FOR VETERINARY PRACTICES

TYPE OF ANIMAL:

Milking cows all ages

ANIMAL SELECTION:

Twenty (20) cows per farm. Random selection is essential. To ensure the required 20 paired samples, select 40 cows expecting about half will urinate. Pick every n^{th} cow where n is the fraction of herd size/40. e.g. if there are 450 cows in the herd, n=450/40=11, so pick every 11^{th} cow, if 600 cows, pick every 15^{th} cow.

SPECIMENS:

Both rine and serum from **each** of 20 cows. In addition, take one bulk tank milk sample at the end of milking.

Urine: It is suggested that urine is collected first. Collect a minimum of 20 ml urine in a clean/sterile plastic pottle from either spontaneously urinating cows or induced urination by stimulating the ventral vulva. If the latter, clean faecal material by washing with warm water. *Samples contaminated with faecal matter will not be accepted*.

ENSURE LIDS ARE SECURE AND THAT CONTAINERS ARE WATERTIGHT

Blood: Collect **serum** by venepuncture (any vein) into a 10 ml plain (red top) vacutainer. The tube must be full.

Bulk Tank Milk: Collect 25 ml from the vat into a screw-top plastic tube (provided)

LABELLING THE SAMPLES:

Place provided labels on the container, not the lid.

SAMPLE IDENTIFICATION FORM:

Complete the Animal Sampling Form

HANDLING OF SAMPLES:

Put samples immediately into a chilli bin with **ice or ice pad, avoiding direct contact with ice/ice pad**. Pack blood, urine and milk separately in watertight plastic bags.

DISPATCH OF SAMPLES AND QUESTIONNAIRE:

Send the cooled samples and questionnaire, with chilled pads, by courier as soon as possible, preferably on the day of collection. Alternatively, keep refrigerated and send them the following morning.

Please DO NOT collect or send samples on a Friday.

Pack questionnaires and Individual Animal Data Form and samples of each farm in a separate farm-bag if couriered with samples from other farms in the same consignment.

Samples should arrive no later than by a Friday morning and should be addressed to:

Hopkirk Research Institute

Massey University

Attn. Yuni Yupiana

Tennent Drive
Palmerston North 4474
New Zealand

QUESTIONNAIRE AND CONSENT FORM:

Data on vaccine brand (see table on the final page) must be completed by the attending vet or technician based on clinic dispensing records. The questionnaire MUST be completed at the time of sample collection and dispatched in a separate watertight bag along with the Animal Sampling Form, with the samples. The consent form must be signed by the farmer or manager. **ALL questions must be answered** but if an answer is unknown tick the '"Unsure" box or add a comment.

PAYMENT

Payment of invoices will be conditional on receiving samples, Sampled Animal Data Form and completed questionnaire according to these instructions.