Leptospirosis in New Zealand – Best Practice Recommendations for the use of vaccines to prevent human exposure

A Report by Massey University Prepared for the

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Executive Summary

1. **Motivation:** This review of Best-Practice recommendations for the use of vaccines for immunising livestock (dairy and beef cattle, sheep, deer) against leptospirosis was instigated by NZVA's Dairy Cattle Veterinary (DCV) Special Interest Branch and Leptosure. The principle motivation for this move was a general uncertainty about the optimum age of first vaccination, timing of boosters. Product label recommendations currently prescribe different vaccination regimens. It was anticipated that the outcome of this review would form the basis for the development of accepted standards to be widely disseminated in the veterinary community.

2. **Aims and methodology:** Massey University was therefore contracted to conduct a systematic review of the literature relating to leptospirosis vaccination in cattle, sheep and deer. Aims included a review of vaccine efficacy in relation to antibody in colostrum, to the duration of colostral immunity, and to the age at first natural challenge. After further consultation with the funders, the aims were extended to cover the epidemiology of leptospirosis in New Zealand, mechanisms and measurement of host immune response and the integration of vaccination into seasonal farm management. Included is a summary of the gaps in current knowledge. The Best-Practice recommendations contained in this report are thus limited to the currently available knowledge.

3. **Importance of leptospirosis:** Concerns relate to both human and animal infections. Despite numerous investigations and control efforts, leptospirosis is still the most important zoonosis in New Zealand. There are around 100 notified cases per year, but the number of illness episodes due to leptospirosis is estimated to be 40-50 times higher. Moreover, the ecology of leptospirosis appears to be undergoing change, possibly driven by more intensive farming systems and subtle climate change. Clinical disease was common in dairy prior to adoption of vaccination in 1990s. Unvaccinated sheep, beef and deer herds are at risk of clinical disease outbreaks, which in humid periods may manifest with mortality rates up to 20%. Production effects have recently been quantified for deer (growth, reproduction) and beef cattle (abortion).

4. **Vaccine efficacy:** the key point to take from efficacy studies in dairy cattle carried out within the past 20 years is that mono- or bivalent leptospirosis vaccines alike were efficient for preventing urine shedding (80-100% efficacy) when vaccination preceded challenge. However, a 2011-study with 100% vaccine efficacy based on urine culture, found 6/8 vaccinates and 4/4 controls as urine-PCR-positive. Thus, a low level of shedding post-vaccination in dairy herds cannot be ruled out. There are also unpublished data suggesting a lower immune response from vaccines containing a large number of (clostridial) antigens. Another caveat is that vaccination works well as long as no prior challenge has occurred: two studies found continued shedding (cattle, deer) in both vaccinates and controls when urinary shedding was present at the time of first vaccination. The protective effect of vaccination against infection and subsequent shedding was shown to last at least for 13 months.

5. **Maternally derived antibody (MDA):** the mean half-life of IgG1, the main immune-globulin comprising MDA, was 18 days in individual calves. MDA in bovine calves, measured by the microscopic agglutination test (MAT), was shown experimentally to convey protective immunity until challenge at 4 weeks of age. MAT in new-borne calves was observed to have waned off almost completely up to 100 days of age. Based on the rate of decay to become zero at 100 days, about 80% calves have no measurable antibody after 50 days of age, thus

at group-level, calves may be regarded as susceptible from about 7 weeks of age if a protective level of MDA was present in all calves at birth. Under commercial farming conditions however, not all dams transmit a protective level of MDA through colostrum and only about 50% calves ingest sufficient colostrum within the first 12 hours of birth. Consequently, passive immunity at group-level is unlikely to be sustained until 7 weeks of age in most herds. Hence, most calves are likely to become susceptible to infection earlier than that. No information is available about the relative efficiency of MDA derived from vaccination compared to that derived from natural challenge of dams. Assuming no difference exists and based on the MDA decay described above, the latest age at group-level at which calves, lambs or fawns become susceptible to infection under field conditions, may therefore be around 4-6 weeks in situations where dams were vaccinated (dairy) or were subjected to high natural challenge (beef, deer, sheep).

6. **Interference by MDA with vaccine-induced immunity:** In the absence of MDA, vaccination against *Leptospira* is effective as early as 4 weeks of age. No conclusive evidence exists in any species about MDA reducing vaccine efficacy: one experimental infection rendered vaccination in the presence of MAT antibody ineffective, another found no interference. Since both studies were based on low numbers of calves and trial conditions were highly artificial, little credible inference can be derived from these disparate outcomes about MDA interference with vaccination under commercial farming conditions. Since there is doubt, a conservative view appears advisable for the purpose of Best-Practice recommendations, pending research to clarify this issue. Hence interference may be assumed to exist until conclusive evidence is presented to the contrary. Consequently, accepting a MDA decay by 100 days, the maximum age at which MDA may inhibit vaccine efficacy in about 20% of a calf mob is 7 weeks of age (point 5 above), and under commercial farming conditions may be a maximum of 4-6 weeks of age. There was no MDA interference with serological response to vaccination of deer at approximately 100 days of age.

7. **Age at first vaccination:** no vaccination is required for lambs or deer for slaughter, or for bobby calves (dairy). However, all other ages and types of sheep, dairy, beef and deer should be vaccinated regularly. Assuming that MDA reduces vaccine efficacy and that most offspring may have lost MDA by 4-6 weeks of age, and assuming further that the age spread of young stock to be used for replacement is 6 weeks (+/- 3w of the average), we recommend an <u>average mob age</u> at first vaccination of 7 weeks (3+4w). This would be the <u>earliest average age</u> at which the first of two successive vaccinations should be applied. Under commercial farming conditions this translates to 10 weeks (6+4w) after the start of seasonal calving/lambing as the <u>earliest time</u> to start vaccinating offspring. The advice for the <u>latest age</u> at which calves should have completed their first vaccination course is based on reports about natural challenge at 3-6 months in high risk environments. Thus, best practice advice is to complete a course of two vaccinations (4-6 week interval between injections) before the oldest calf/lamb has reached 6 months of age. For commercial farming conditions, this would typically be 18 weeks after the start of seasonal calving/lambing as the <u>latest time</u> to start vaccinating offspring. These recommendations are illustrated in Figure 12.1.

8. **Differentiating the level of natural challenge:** under dry environmental conditions and where access to potentially contaminated water does not exist or when whole-herd/flock vaccination has been consistently applied for a number of years, the risk of natural challenge should be low. On the contrary, farms where unvaccinated herds or flocks with access to contaminated water (flooded pasture), or where replacement stock is returning

from locations where they were grazed at unknown or high risk, may be at a high risk of natural challenge. Typical examples for the former may be continuously vaccinating and closed dairy herds (low risk), and for the latter may be deer or beef herds, or sheep flocks on wet soils (high risk) or downstream of infected herds/flocks. Given that almost all beef cattle and deer herds, and sheep flocks in New Zealand have evidence of infection, most of such farms may be in the high-risk category.

a. In high risk environments such as most beef, deer and sheep farming conditions, a long-term vaccination plan should be considered. Here the initial vaccination of young stock is best applied early, hence about 4-6 weeks of age with a booster at 8-10 weeks. This should be followed by another booster 6 months later or at the time of the annual whole-herd booster. After 2-3 years, such herds and flocks may be regarded as having acquired a low-risk status. Consequently, the age of first vaccination may follow guidelines for low risk environments;
b. In low-risk environments such as herds or flocks with a history of continued vaccination, new-borns may receive their first vaccination at 4-12 weeks of age. The first course of vaccination should be completed as early born calves/lambs/fawns are 6 months old. For continued immunity, these new-borns should be re-vaccinated at the time of the annual whole herd/flock booster vaccination.
c. We present another point for discussion that has so far not been considered in any of the vaccine label claims: If dams were vaccinated after parturition in a low risk environment, vaccine induced MDA may be regarded as absent and thus vaccine efficacy would not be impaired by vaccine induced MDA. In that situation, the first vaccination may be scheduled within the first month of birth, or in terms of seasonal management, at the end of calving/lambing. Hence, applying the annual vaccine booster to cows AFTER parturition and starting to vaccinate new-born calves as early as practically feasible, may be an advisable practice.

9. **Required knowledge:** The principal knowledge deficit identified by this project is the impact of MDA on vaccination response and immunity in offspring in relation to timing of first vaccination in cattle and sheep. Robust recommendations for best practice in terms of optimum age at first vaccination in those species cannot be given until this question is resolved. Additionally, no information is currently available about the occupational risk of leptospirosis among farmers, their families, veterinarians and livestock workers. More knowledge is also required about the optimal age at first vaccination and duration of immunity under commercial farming conditions. Moreover, robust evidence is required about the frequency and the quantity of shedding in vaccinated herds or flocks. This review therefore identified the following knowledge gaps:
    a. There is a pressing need for large scale field trials of vaccine efficacy in herds and flocks, comparing vaccinated with unvaccinated dams in conjunction with vaccinating offspring at various ages (1, 3, 6 months) in endemic herds/flocks.
    b. The overall risk of and source for infection of farmers, livestock workers and veterinarians is currently unknown. This includes wildlife sources such as rodents, possums, hedgehogs, feral pigs and rabbits. This would include attention to host reservoirs for serovar Ballum. An observational study approach is suggested.
    c. A recent pilot study of shedding in dairy cows raises the question whether PCR positive urine is infectious, i.e. contains live *Leptospira* at sufficiently high dose. Given the zoonotic potential of *Leptospira*, a nationwide prevalence study of urine shedding, serovars involved and an accurate account of vaccination practices in dairy herds is proposed.

1. Objectives

The report presents a systematic literature review to evaluate the following key issues:

- The past and present situation of the epidemiology of leptospirosis in New Zealand in relation to the use of vaccines in livestock;
- The loss of production due to leptospirosis infection and the proportion of the loss preventable by vaccination;
- Uncertainties about current recommendations for the use of leptospirosis vaccines in New Zealand with special consideration of:
  - The type and measurement of the immune response to infection or vaccination;
  - The measurement of vaccine efficacy;
  - The likely presence and interference of a maternally derived immune response;
  - The age at first vaccination to prevent urinary shedding;
  - The effect of vaccination before and after natural exposure;
  - The duration of vaccine induced immunity;
  - Environmental and management factors interacting with the response to vaccination;

Based on the results, the report subsequently derives best practice recommendations for the use of leptospirosis vaccines in:

- Dairy cattle;
- Beef cattle;
- Deer; and
- Sheep.

1.1. Search Methodology

Pubmed and the Web of Knowledge data bases were searched using the following key words.

[species] AND [vaccination] AND [outcome], where:

Species:
- Lepto* OR Weil;
- Cattle OR Bovine,
- Deer OR Cervine,
- Sheep OR Ewe*,
- Human OR People OR Worker* OR Farmer*

Vaccination:
- Vacc* OR Prophy OR Immun* OR Protect*

Outcome:
- Efficacy OR Effecti* OR Shedd* OR Serolog* OR Antibod*.

Firstly, titles and abstracts of all returned articles were scanned to select relevant articles by one author (JS). A relevant article was defined as one that contained information that would inform the report as scoped above. Unpublished literature and data from Massey studies and from New Zealand pharmaceutical companies (MSD Animal Health, Pfizer Animal Health and Virbac Animal Health) also formed a substantial part of the review process.

2. Introduction

2.1 Motivation

Leptospirosis continues to place a significant disease burden on rural New Zealanders. A sheep and beef farmer and a deer farmer, both with renal and hepatic failure were recently admitted to the intensive care unit at Waikato Hospital (Dr. Chris Mansell; clinical microbiologist Waikato District Health Board; personal communication April 2012). In September 2010 there was a cluster of three cases of leptospirosis in staff at a lower north island dairy farm. Two staff were hospitalised. An annual vaccination programme had been carried out on the dairy farm since 2003; however the Department of Labour investigation identified deficiencies in the programme (Department of Labour, 2011).

Seventy cases of leptospirosis were notified in 2011 of which 38 of 66 (58%) were hospitalised (ESR, 2012). *Leptospira* species and serovars (sv) were recorded for 57 of these, the most commonly identified serovar was *L. borgpetersenii* sv Ballum (35%, 20 cases), followed by *L. borgpetersenii* sv Hardjo (26%, 15 cases) and *L.interrogans* sv Pomona (22%, 13 cases). In 2010 sv Ballum emerged as the most frequently notified serovar in human cases. Since 2007, this rise in Ballum cases is coincident with an increase in the proportion of farmers represented among notified cases (Figure 2.1). In 2011 36 of the 62 cases with occupation recorded were identified as farmers or farm workers, with 10 of the 62 working in the meat industry.

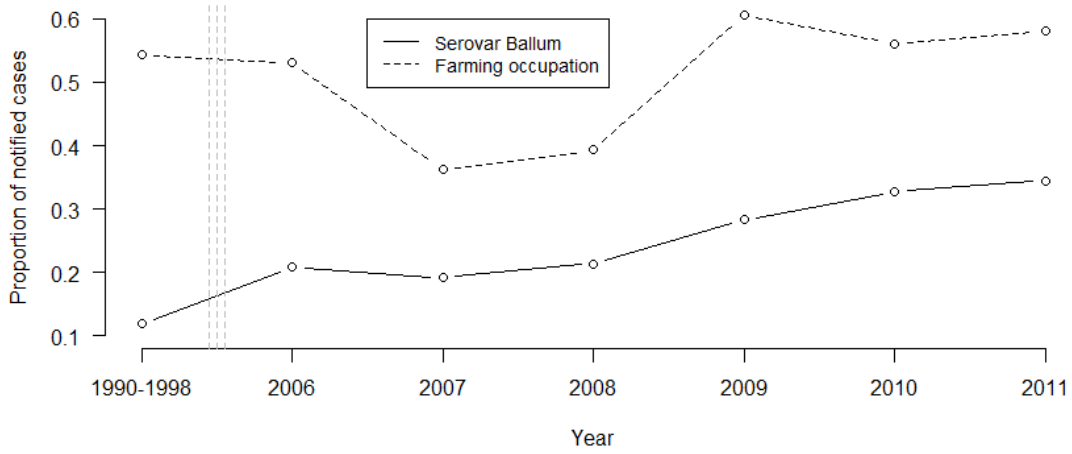

Figure 2.1: Proportion of notified leptospirosis cases infected with serovar Ballum (solid line) and associated with farming occupation as a function of time. Data sourced from ESR annual surveillance reports and Thornley et al (2002).

An observational pilot study of urinary shedding in dairy herds with a history of regular vaccination was carried out in 2010/2011 (Parramore et al., 2011). There was evidence of leptospiral shedding in 30% of the herds and in 13% of animals from positive herds. Age at first vaccination was the only significant factor associated with the probability of shedding at the herd level. The results suggest that leptospiral challenge of calves at an early age and potential human exposure still exists on dairy farms using vaccines. Vaccinating already infected animals may not be fully effective, as it appears that vaccination after natural challenge reduces vaccine efficacy (Hancock et al., 1984). Neither vaccine type nor the number of serovars included (2 vs 3) altered the shedding probability. However it is important to note that no serological data were available from the sampled animals, information about vaccination timing from farmers appeared somewhat uncertain, and tests employed may not be 100% accurate. Therefore, the results are preliminary and require further confirmatory work.

Motivated by the above information, the New Zealand Veterinary Association (NZVA) commissioned this systematic review of leptospirosis vaccination. The review aims to determine "best practice" protocols for ruminant vaccination for leptospirosis with the primary goal of vaccination being the protection of humans. There are multiple other benefits from ruminant vaccination such as reduction of clinical disease and sub-clinical health and production effects. While these are not the primary focus of this work, it is likely that they would be benefit from a best practice vaccination regardless.

This introductory section presents the situation today and a recent history of human infection with leptospirosis in New Zealand, human leptospirosis in an international context and concludes with detailing the search methodology for subsequent review.

2.2 The international human leptospirosis context

Globally leptospirosis is an important zoonotic disease with three main epidemiological patterns (1) flooding associated; (2) poverty associated and (3) occupational exposure (Vijayachari et al., 2008). The source of infection in humans is direct or indirect contact with the urine of an infected animal, and globally these are diverse species including livestock, wildlife or vermin (Plank and Dean, 2000). For this reason the disease may be prevalent in both urban and rural settings and depends on animal contact and environmental and socio-economic conditions that facilitate transmission. Leptospirosis is a protean disease and commences as an acute, generalized illness that may be mistaken for influenza in humans. However, the disease can progress to severe sequellae such as acute renal failure, pulmonary haemorrhage and cardiac complications (Levett, 2001). Annual incidence is estimated from 10 to 100 cases per 100,000 in warm tropical regions with estimates of one tenth of that for temperate climates. However worldwide there is likely underestimation of the burden of leptospirosis (Victoriano et al., 2009). A WHO project is currently estimating the global impact of Leptospirosis in people.

A 2009 review of leptospirosis in the Asia Pacific region characterized countries into either high (>10 case per 100,000 per year), moderate (1 to 10) or low incidence (<1) (Victoriano et al., 2009). India was identified as a high incidence country with carrier animals including rats, pigs, cattle, bandicoots and dogs. Hong Kong SAR reports low incidence with fewer than seven local cases a year over the period 2001 to 2006 . Victoriano and co-authors acknowledge that surveillance and data collection systems differ between countries preventing an accurate estimate and comparison of the true burden of disease between countries. Key prevention and control interventions recommended included rodent control, domestic animal vaccination and social control measures such as awareness and health promotion.

A review of leptospirosis in Australia reports an annual incidence of 2.1 cases per 100,000 in Queensland and identifies the two occupational groups at most risk as workers in banana plantations and on dairy farms (Tulsiani et al., 2010). However over the past decade there has been a shift in risk from occupational to recreational. In 2008 international travel and/or recreation accounted for approximately 35% of cases reported to the enhanced surveillance system at the WHO Collaborating Centre for Reference and Research on Leptospirosis in Brisbane (Lau et al., 2010a).

Recent work in American Samoa reported antibodies in 15.5% of 807 participants, predominantly against three serovars that were not previously known to occur in American Samoa (Lau et al., 2012b). Having piggeries around the home and living at lower altitudes were statistically significant risk factors for sero-positivity (Lau et al., 2012a).

2.3 Human leptospirosis in New Zealand

The epidemiology of leptospirosis in New Zealand is unique with our ruminant livestock species being key maintenance hosts (Ayanegui-Alcerreca et al., 2007; Heuer et al., 2007a; Dorjee et al., 2008). From the 1980s to the present there has been widespread uptake of vaccination of dairy cattle and pigs. This has been associated with a reduction in human cases from the peak of 875 in 1974 to approximately 100 cases over the period 1997 to 2000 (Thornley et al., 2002), a level at which case numbers remained up to 2011 (Figure 2.2). This incidence risk of 2.5 per 100,000 places New Zealand in the moderate incidence category for the Asia Pacific region (Victoriano et al., 2009) and globally (Tulsiani et al., 2010). Despite a large reduction in case numbers leptospirosis continues to be a severe disease for rural New Zealanders with 50% of notified cases hospitalized (Thornley et al., 2002; ESR, 2012).

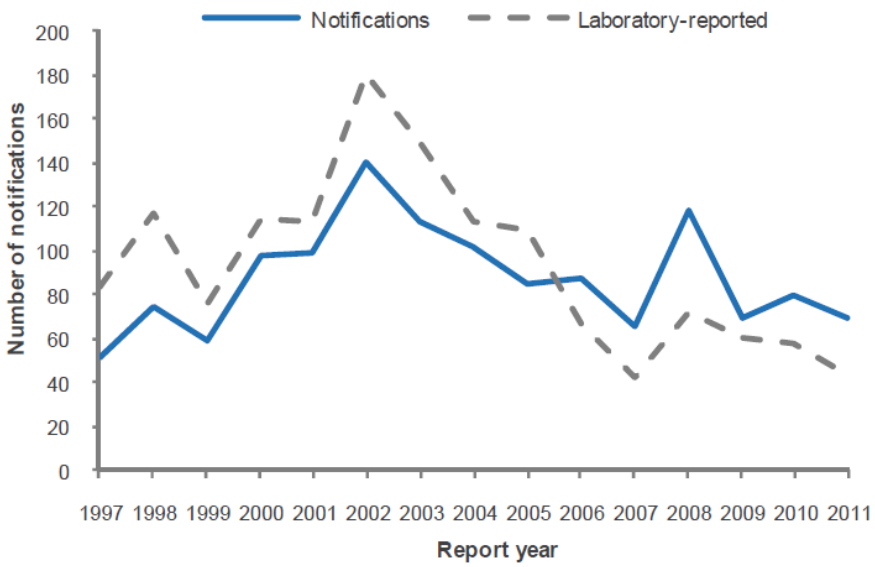

Figure 2.1: Leptospirosis notifications and laboratory reported cases by year 1997- 2011. Source: ESR Notifiable and other diseases in New Zealand 2011.

A review of leptospirosis notification data from 1990 to 1998 identified the emergence of serovar Ballum as an important cause of human disease (Thornley et al., 2002). This trend continued and was reported in an updated report covering the period 1999 to 2008 (Paine et al., 2010). As reported above, Ballum has recently emerged as the most frequently notified serovar in human cases. Prior to this time most human cases were due to Hardjobovis or Pomona (Thornley et al., 2002; Paine et al., 2010). The maintenance hosts for Ballum in New Zealand include the rat, mouse and hedgehog (Marshall and Manktelow, 2002). Given the recent increase in the proportion of farmers represented among notified cases (Figure 2.1) there may be a link with rodents foraging and thereby contaminating concentrate rations for livestock (especially dairy cattle), or it may be that the livestock are the source of Ballum for human cases. Such questions around the emergence of Ballum are not the focus of this review but warrant mention here as areas for important future work, potentially with regard to inclusion of this serovar in animal vaccines.

New Zealand's Department of Labour recognizes leptospirosis as an occupational disease. Under the Health and Safety in Employment Act (1992) a case of leptospirosis is regarded as "serious harm" and the risk of being infected is a "significant hazard" (Occupational safety and health service, 2001). Work in human leptospirosis over the last five years at Massey University has predominately focussed on meat industry employees. Measuring serology and kidney culture

rates in one sheep abattoir revealed the extent of real exposure in workers: during a typical factory day, one slaughter worker was exposed to an estimated 3-18 carcasses contaminated with live *Leptospira* in kidneys (Dorjee et al., 2011). Once the rate of exposure was known, the next question was how effective exposure transmitted to infection, i.e. how many workers actually got infected with *Leptospira*. This was measured subsequently in 8 abattoirs by sampling workers twice at an interval of one year, and calculating rates of sero-conversion as a measure of infection. This resulted in annual infection rates among abattoir workers processing sheep of 12.3% (n=384). The rate was lower in workers processing cattle (1.5%; n=158) or deer (0%; n=50). The highest sero-prevalence, however, was found in deer plant workers (13-18%), followed by plants processing sheep (6-14%) and cattle (3-4%) (Dreyfus, 2012). The high prevalence and low incidence of new infections in deer plants is indicative of a more or less permanent sero-positive state due to past clinical episodes followed by long seropositive periods and/or frequent re-exposure without subsequent illness.

In meat-workers, sero-incidence data coupled with data on time off work due to "flu-like" illness assisted in quantifying the burden of disease. In sheep plants, clinical illness associated with flu-like symptoms was twice as frequent in workers who sero-converted to Pomona or Hardjo, i.e. were infected between two sampling points one year apart (Figure 2): 47% of infected vs. 24% of non-infected workers were affected by flu-like symptoms and an average of 4 days absence from work. This translates to a 1 in 36 chance of illness due to leptospirosis in 12 months for every worker regardless of work position. Once worker-position was taken into account, having a work position on the slaughter board accounted for a 4-8 fold higher risk being highest at the front i.e. sticking area (8x) and decreasing towards the dressed carcass processing end (Dreyfus, 2012).

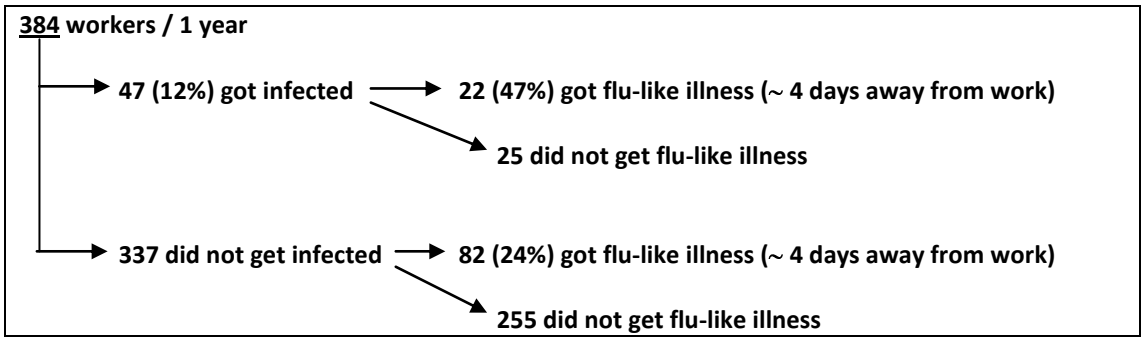

Figure 2.3: Path-diagram of the rate of new infections with Pomona or Hardjo in workers of sheep processing plants, and the risk of clinical illness in 2009/10. This related to a total risk of contracting clinical leptospirosis of 1:36 workers (2.7%) within one year.

Little information is available as yet about comparable risks for farmers, vets and other people with frequent animal contacts in the livestock industries. It is the intention to collect such data in the near future. A survey of more than 300 veterinary students established a baseline prevalence for Hardjobovis, Pomona and Ballum in 2011, all students testing negative (Fang, personal communication).

3. Hosts – pathogen relationships in NZ

3.1 Host species and Leptospira serovars in NZ

The history and distribution of mammal host species and *Leptospira* serovars in New Zealand were reviewed by Marshall and Manktelow (2002). This and further contributions to this topic are summarised in Table 1. Since genotypes of *Leptospira* can generally not be associated with unique bacteria species, this overview classifies the pathogen types only by serovar name.

The first isolation of Pomona in New Zealand was from a clinically affected calf at Wallaceville Animal Research Station, 1950, followed by large scale testing of over 13,000 bovine sera at Wallaceville 1952-55 (McDonald and Rudge, 1957). Tarassovi and Pomona were found in pigs in 1958. Tarassovi was subsequently also isolated from deer, goats and horses. It was then almost 20 years until a series of serological studies and pathogen isolations began in 1975 (Ryan and Marshall, 1976).

Host types: Maintenance and accidental (opportunistic or spill-over) hosts are differentiated. The concept assumes that Leptospira serovars have lower pathogenicity for maintenance than for accidental hosts while being equally or similarly infectious. The consequence is that maintenance hosts remain infected and a host-pathogen equilibrium is established by a continued re-/infection cycle balanced by a marginal immune response with partial but incomplete pathogen elimination. That equilibrium can get out of balance, for example when environmental conditions favour the pathogen, causing outbreaks in maintenance hosts, or by adverse conditions, causing pathogen extinction or the establishment of an equilibrium at a lower endemic level (i.e. lower prevalence).

Climatic conditions in New Zealand favour the establishment of equilibria for several *Leptospira* serovars and hosts.

Table 3.1: Data from published studies showing animal and herd prevalence by year, species and age of livestock in New Zealand

	Year	Herds	Hard	Pom	H/P	Animals	Hard	Pom	H/P	REF
CATTLE										
Beef heifers 12-18 m old	2006	95	62%	26%	69%	1,265	34%	12%	39%	2
Mixed age beef cows	2009	116	92%	72%	97%	2,308	50%	25%	58%	1
Mixed age beef cows	2010	21	86%	67%	95%	338	45%	19%	55%	3
Dairy cows	2011	44			30%*	445			4%*	
SHEEP										
Slaughter age lambs	2004	21	86%	29%	91%	619	16%	4%	19%	4
Slaughter age lambs	2005	74	27%	7%	31%	2,139	1.7%	0.5%	2%	4
Mixed age ewes	2009	161	91%	74%	97%	3,361	43%	14%	51%	1
DEER										
9-30m old deer**	2004	110	68%	20%	74%	2,016	61%	8%	64%	5
Mixed age deer	2009	98	60%	49%	76%	1,992	26%	10%	34%	1

*Urine shedding (darkfield microscopy/PCR, 10 cows per herd); **Deer from non-vaccinated herds; *L. interrogans* sv Hardjo, Pom *L. borgpetersenii* sv. Pomona, H/P Hardjo or Pomona; key for references [1=Dreyfus, 2012; 2=Heuer, 2007; 3=Sanhueza, 2012; 4=Dorjee et al., 2008; 5=Ayanegui-Alcerreca et al., 2007]

Trends:

Humans – Hardjo accounted for 2/3 of human notified cases in NZ in 1975 (Ris, 1975) whereas Ballum started to rank highest in 2009 (ESR, 2011). Until 2005, the majority of notified cases were meat workers followed by farmers. However, farmers have been ranked highest among notified cases since 2006, followed by meat workers and other groups in contact with animals. Assuming that MAT titres after a clinical episode last for up to 10 years (Blackmore et al., 1979), the infection incidence of meat workers was believed to be around 0.5% during the 1980s when there was still a very high infection prevalence in the dairy cattle population (Blackmore and Schollum, 1982). However, recent sero-conversion data of abattoir workers processing sheep, deer and cattle revealed a 12.3% incidence (Dreyfus, 2012). The new infection rates were highest at sheep processing plants, followed by deer and cattle plants. Workers at sheep plants had a 12.2% risk of becoming infected with Hardjo (3.6%) or Pomona (9.4%) in the course of a single slaughter season (12 months). The data also showed for the first time in New Zealand, that 1 in 36 workers experienced clinical illness due to an infection with either of these serovars. Illness caused an average of 4.4 days being away from work. The data suggest that ESR-notification rates of leptospirosis may be underreported by a factor of 40-50 times for abattoir workers (see also section 2.3, page 8).

Conclusion: New data about the incidence of illness associated with leptospirosis among highly exposed workers at the slaughter board of abattoirs imply an imminent need for similar studies among farmers, veterinarians and other people in contact with farm animals.

Table 3.2. Host-pathogen associations of *Leptospira* serovars in New Zealand (serovars in parenthesis are sporadic observations)

Host	***Leptospira* servovars**	**Reference**
Human	Ballum, Hardjobovis, Pomona, (Tarassovi, Canicola, Copenhageni, Australis)	ESR 2001-09
Farm animal species:		
Dairy cattle	Hardjobovis, Pomona, (Copenhageni, Ballum)	(Hathaway, 1981)
Beef cattle	Hardjobovis, Pomona, (Copenhageni, Ballum)	(Hathaway, 1981)
Sheep	Hardjobovis, Pomona, (Copenhageni, Ballum)	(Hathaway, 1981)
Deer	Hardjobovis, Pomona (Copenhageni, Ballum, Arborea*)	(Asher, 1986; Wilson et al., 1998; Subharat et al., 2011b)
Fallow Deer	Pomona, Hardjobovis	(Marshall and Manktelow, 2002) (Hilbink and Penrose, 1990)
Pig	Pomona, Tarassovi	
Horse	Hardjobovis, Bratislava, (Ballum, Copenhageni, Tarassovi, Pomona, Canicola)	(Hilbink F, 1992; O'Keefe et al., 2002)
Dog	Copenhageni, Hardjobovis, Pomona, (Ballum, Tarassovi, Canicola)	
Wild animal species:		
Rat	Ballum, Copenhageni	
Mouse	Ballum	(Marshall and Manktelow, 2002)
Deer	?	(Hathaway, 1981)
Rabbit	?	
Possum	Balcanica	
Hedgehog	Ballum, (Pomona)	(Marshall and Manktelow, 2002)
Pig	?	(Marshall and Manktelow, 2002)

* A number of other serovars were identified in a study testing a panel of 22 serovars. However, with Medanensis, Szwajizak, Tarassovi, Grippotyphosa, Celledoni, Australis, Zanoni, Robinsoni, Canicola,

Kremastos, Bulgarica, Cynopteri, Bataviae, Djasiman, Javanica, Panama, Shermani, and Topaz were likely explained by known cross-reactivity with endemic serovars (Subharat et al., 2011b).

Sheep – A 1975 study reported 65% animal prevalence and titres over 1:1000 to Hardjobovis in 10 flocks of sheep using the MAT (Ris, 1975). A letter to the editor of NZVJ in 1980 announced the first known successful culture of Hardjobovis in sheep (Bahaman et al., 1980). Pomona was rarely seen in sheep, associated with sporadic clinical disease. Only recently has it been suggested that sheep may be a maintenance host for Hardjobovis while Pomona was not considered as such. In contrast, a recent and yet unpublished NZ-wide survey (Dreyfus, 2012) from 161 flocks demonstrated that 97% of the flocks had at least 1/20 sero-positive (titre ≥1:48) adult ewes (91% Hardjo, 74% Pomona) and 51% ewes infected (43% Hardjobovis, 14% Pomona). An abattoir survey in 2005/06 isolated *Leptospira* from 22% kidneys of Hardjobovis- and 17% of Pomona-seropositive carcasses of lambs or hoggets (Dorjee et al., 2008). Only 1/162 (0.6%) flocks were vaccinated (Dreyfus, 2012).
Conclusion: Hardjo is highly prevalent in sheep breeding flocks and this species probably constitutes a reservoir host. Pomona is also endemic and sheep possibly are a reservoir host.

Dairy Cattle – until recently, the prevalence of *Leptospira* in dairy cattle was assumed to be low due to vaccination, which was believed anectdotally to have been adopted by more than 90% of dairy farmers for at least 10 years. However, a pilot study of 44 dairy herds (Parramore et al, unpublished) found 30% herds and 4% of 445 cows to be shedding *Leptospira* in urine based on samples from 10 cows per herd tested by PCR and dark-field microscopy. The probability of having a shedder in the herd was low on farms where calves were reported to have been vaccinated for the first time below the age of 6 months, and almost zero when vaccinated up to 3 months of age (Parramore et al., 2011).
Conclusion: despite extensive use of vaccination, continued shedding is apparent, especially on farms where vaccination may be applied inconsistently or too late. Given its implications, this preliminary finding calls for immediate verification.

Beef Cattle – beef herds had not been sampled prior to 2005. In 2006, replacement heifer mobs 12-18 months of age of 69% herds (n=95) and 39% of the replacement heifers (n=1,265) were sero-positive to either Hardjo (34%) or Pomona (12%) (Heuer et al., 2007a). Two subsequent population based studies found 95-97% beef breeding herds and 55-58% of the mixed age cows positive to Hardjo (45-50%) or Pomona (19-25%). A survey in 2009 involved 116 herd and 2,308 cows (Dreyfus, 2012). The 2010 study estimated fetal loss due to leptospirosis selected aborting and non-aborting cows in 21 herds and 338 cows (Sanhueza, 2012). Both investigations tested 20 mixed age cows. In the 2009 survey, farmers of 19/116 (16%) beef breeding herds reported that their beef stock had been vaccinated (Dreyfus, 2012). In the 2006 survey of replacement heifers, 9/94 (10%) beef breeding herds were vaccinated (Heuer 2007), whereas 21/45 (47%) herds reported vaccinating in the study of fetal loss (Sanhueza, 2012). Voluntary vaccination of beef herds appeared to depend on the quality of farm management as the latter study involved farmers who were monitoring fetal loss, consented to the study protocol and were able to identify cows that had aborted and present them for sampling.
Conclusion: the sero-prevalence of Hardjo and Pomona, and possibly other serovars, remains as high among beef cattle as in sheep breeding flocks.

Deer – Leptospirosis in farmed deer in New Zealand was extensively reviewed recently (Ayanegui-Alcerreca et al 2007). Clinical letospirosis was first diagnosed in farmed deer in 1981. Serovar Pomona was isolated from outbreak herds in the early 1980's and this remains the only serovar implicated with clinical disease in deer. Culture and serological evidence for Hardjobovis was also described in early reports. Limited surveys carried out in the mid and late 1980's indicated that leptospirosis was prevalent, with Hardjobovis being the predominant serovar.

Those surveys, together with a clinical occurrence, confirmed that Copenhageni also infected and clinically affected deer. A larger Southern North Island survey in the early 1990's (Wilson et al 1998) demonstrated a 73.6% animal seroprevalence to hardjobovis, 41.5% to Pomona, 11.3% to Copenhageni and 15% tarassovi. The significance of the latter, along with low titres for Australis, Bratislava and Balum in various studies remains unknown. More recently, Subharat et al (2011b) reported serological evidence for serovar Arborea on two farms from screening of a serum bank for 23 (16 exotic) potential serovars. However, attempts to isolate the organism were unsuccessful. A national serosurvey in 2005-6 (Ayamegui-Alcerecca et al 2010) observed 81% of 110 herds positive, comprising 78% Hardjobobis and 16% Pomona, with some dual serovar infections. The individual animal seroprevalence was 61%, 6.6% and 1.2% for Hardjobovis, Pomona and Copenhageni, respectively. There were no regional differences. A 2009 serosurvey of 99 farms (Dreyfus et al, unpublished) yielded siminar herd seroprevalence data (76%), and 34% animal seroprevalence. While there is reasonable awareness of leptospiroisis among deer farmers, vaccination is practised by approximately 10%. **Conclusion:** Leptospirosis is widespread in the farmed deer population, with herd and individual animal prevalence similar to that seen in sheep and beef cattle. Deer are a reservoir host for Hardjobovis and likely a reservoir population for pomona.

Dogs – a prevalence of 0.9% was observed for Copenhageni and 0.7% for Ballum at a MAT-cutoff of 1:100 in a cross-sectional study of 8,730 rural and urban dogs involved in a 1990-91 New Zealand Hydatids Council serological survey (Hilbink F, 1992). The prevalence of Copenhageni was almost seven times higher in dogs presented as cases to 45 small animal veterinary clinics in Auckland (6%; 31/561). The prevalence of Copenhageni was lower in the South Island (0.1%; n=3,671) than in the North Island (1.3%; n=6,029). A lower North Island survey 10 years later showed 9.5% being sero-positive for Copenhageni in 433 rural and urban dogs with similar prevalence in both environments. However, Hardjo was almost exclusively found in rural dogs (15/315; 4.8%), only 1/146 (0.7%) urban dogs were sero-positive for Hardjo (O'Keefe et al., 2002). The total sero-prevalence in dogs for serovars Hardjo, Pomona, Copenhageni Grippotyphosa, or Canicola was 14.2% (66/466).
Conclusion: Serovar Copenhageni is likely the dominant serovar in dogs throughout New Zealand. Hardjo may be transmitted from sheep and beef cattle to farm dogs, a possible source of exposure to these two serovars for humans.

3.2 Transmission routes

A model for the transmission between species and persistence of *Leptospira* in reservoir hosts and the environment is presented in Figure 1. In the model, reservoir hosts for Hardjobovis and Pomona are beef cattle, sheep and deer while farm dogs, rodents and wild living animals are accidental hosts contributing to environmental contamination. Direct contact with shedding live and dead reservoir hosts, indirect contact with contaminated environment (e.g. floods on pasture, ponds, drains, rivers) causes infection in humans. Environmental contamination is augmented by favourable weather conditions (high rainfall, moderate to high temperature), an assumption supported by observation of outbreaks of leptospirosis during or after major floods (Dorjee et al., 2005).

Leptospira preferentially colonise kidney tissues, surviving for up to 13 weeks, and are excreted in urine. Entry ports for human infection are abrasive skin areas, mucosa of eye, nose and mouth, and possibly softened skin of meat workers wearing rubber gloves and plastic sleeves for extended period of times during work (Dreyfus, 2012).

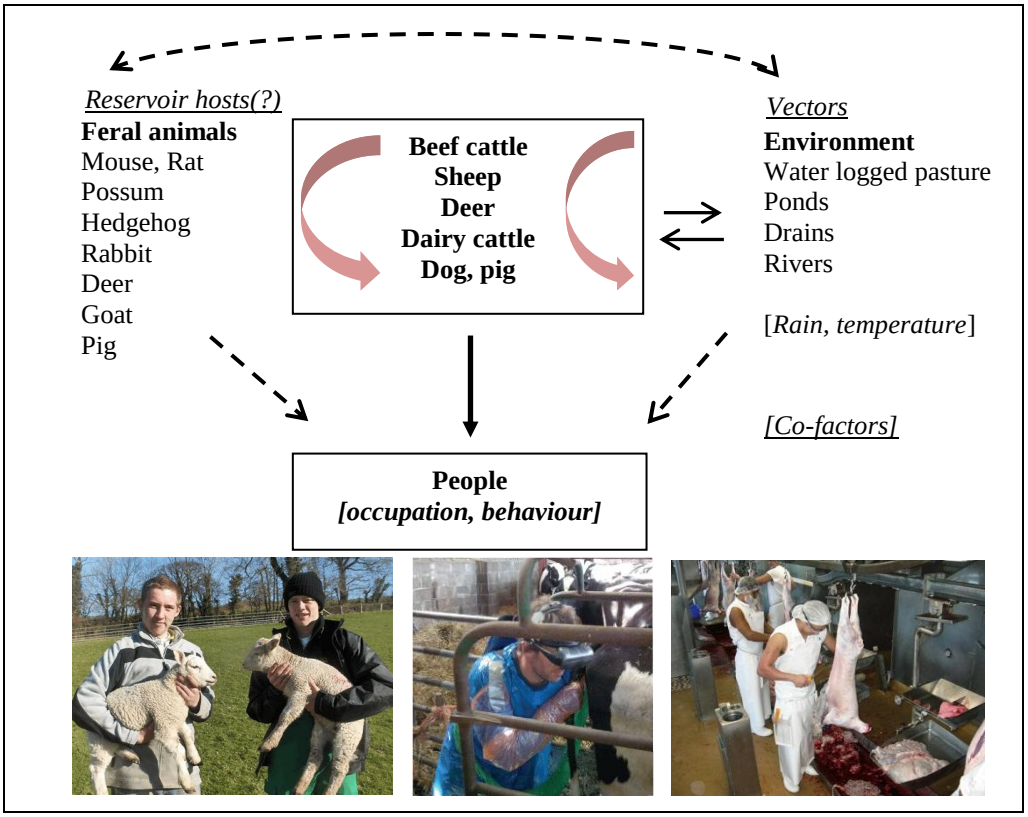

Figure 3.1: Ecological model of leptospirosis in New Zealand

3.3 Conclusions: host/pathogen relationships

Possibly driven by widely adopted vaccination of dairy herds, a gradual spatial replacement of sheep, beef, deer herds by dairy herds, a long term slow drift in weather conditions towards higher temperatures and more rainfall, the enforcement of protective measures at slaughter and meat processing plants, the epidemiology of leptospirosis appears different today compared to the pre-2000 era of intensive leptospirosis research. Today, the primary source for human infection at abattoirs appears to be sheep followed by deer and, to only a small extent, cattle. Based on high sero-prevalence and high kidney culture isolation rates to both Hardjo and Pomona, sheep are likely to be maintenance hosts for these serovars. Since previous ecological considerations did not consider sheep as a maintenance host or major source for human infection (Hathaway, 1981), the epidemiological importance of sheep, and possibly of other hosts and other serovars, appears to have undergone a significant change.

4. Environmental effects

4.1 Introduction

Globally, leptospirosis is a recognised re-emerging bacterial disease (Levett, 2001; Bharti et al., 2003; Cruz et al., 2009; Ko et al., 2009) and this re-emergence is likely influenced by environmental conditions including climate and urbanisation (Dufour et al., 2008; McMichael et al., 2009; Paine et al., 2010). The three main epidemiological patterns of human leptospirosis (flooding, poverty and occupation) clearly reflect the importance of environmental drivers in the transmission of this disease (Figure 4.1). Although not as well documented in animal disease or

infection, the environment plays a dominant part in the epidemiological triangle that contributes to leptospirosis in New Zealand livestock species.

This section presents a brief overview of evidence for the role of the environment in leptospirosis in New Zealand, with evidence from both human and animal data. We limit our discussion in animals to two environmental drivers: climate and farming practice. With regard to this review it is important to be aware that the effect of the environment on leptospirosis must be seen in conjunction with the effect of the environment on the ability an animal has to create an effective response to vaccination. Environmental effects that are important with regard to response to vaccination include nutrition, housing, shelter and other "stressors".

Leptospira are shed into the environment by infected animals and they may survive for days to months in freshwater, soil, or mud. Their survival is enhanced by humid environments and higher temperatures (Levett, 2001). High rainfall periods combined with warm weather are recognized as risk factors for leptospirosis transmission in many diverse parts of the world including Mexico (Leal-Castellanos et al., 2003), Germany (Desai et al., 2009), Brazil (Cruz et al., 2009) and India (Vijayachari et al., 2008).

In the Pacific Islands, increases in leptospirosis cases frequently follow heavy rainfalls when flooding occurs and rats search for higher ground. In late April 2012 there were seven confirmed fatalities from leptospirosis with 13 other deaths, suspected in Fiji. The cases followed massive flooding in January 2012 (ProMED Ahead Digest-mail).

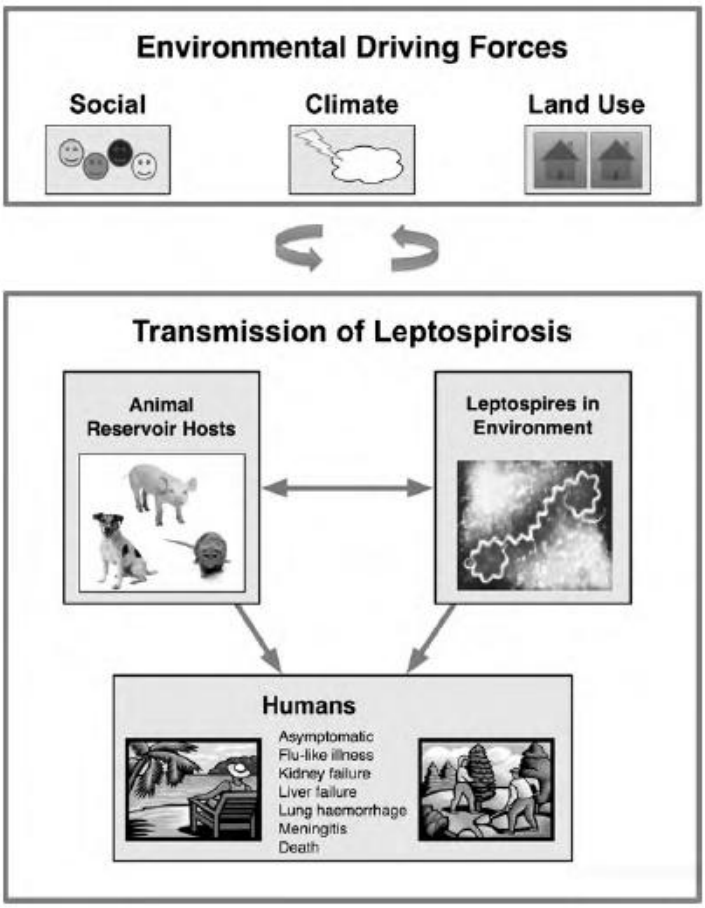

Figure 4.2: Leptospires are maintained in nature by mammalian reservoir hosts. Humans acquire leptospirosis through direct contact with infected animals or by indirect contact with an environment that has been contaminated by animal urine. The cycle of transmission is in turn driven by environmental forces including socio-demographic factors, climate and land use. Figure reproduced with permission from (Lau et al., 2010b)

4.2 The New Zealand environment and *Leptospirosis*

New Zealand is no stranger to anthropogenic environmental change, transforming from indigenous forest to predominantly pastoral farming over a short time. Furthermore, our farming sector is rapidly adaptive to market forces, and changes in land-use and farming practices have environmental effects. The introduction of deer farming, for example, has brought previously feral species into direct contact with more traditional livestock species (Heuer et al., 2007b; Hilson, 2007), a process enabling transmission of pathogens to additional host species (Woolhouse and Gowtage-Sequeria, 2005). Within New Zealand there is evidence of the presence of specific leptospiral serovars Arborea in deer (Subharat et al., 2010) and Ballum in humans (Thornley et al., 2002; ESR, 2012). There is also evidence that clinical disease in sheep and deer is increasing, with higher morbidity and mortality in lambs and young deer (Dorjee et al., 2005; Ayanegui-Alcerreca et al., 2007). This may be associated with environmental change such as co-grazing (Subharat et al., 2008) and/or increased rainfall.

4.3 Climate

An abattoir study conducted by Dorjee et al in the southern north island reported significantly higher sero-prevalences to Hardjo-bovis in lambs sampled in May and June 2004 than from those sampled in the corresponding months in 2005 (Dorjee et al., 2008). This between-year effect was postulated to have been due to high rainfall accompanied by widespread surface flooding in February 2004 and relatively less rainfall in 2005. Other reports of an association between rainfall and surface flooding and outbreaks of leptospirosis in lambs have previously been reported in New Zealand (Vermunt et al., 1994; Dorjee et al., 2005).

Fang Fang (2012) conducted an abattoir study in the Waikato region and sampled urine, kidney and blood from 399 lambs and 146 beef from six suppliers . The reported animal-level sero-prevalences found in sheep (57%) and cattle (73%) were higher than previous studies had reported (Heuer et al., 2007a; Dorjee et al., 2008) and a 27% shedding rate was detected by both urine and kidney PCR. Sero-positivity was defined at a MAT titre dilution of 1:48 or more. The shedding rate (as determined by positive urine PCR) in sero-positive sheep was 54.1%, whilst that in sero-negative sheep was 2.8%. The shedding rate (as determined by positive urine PCR) in sero-positive cattle was 28.2%, whilst that in sero-negative cattle was 3.0%. A sustained period of heavy rain coupled with warm weather had occurred in the six weeks prior to sampling.

The above studies provide anecdotal evidence as to the effect of climate on leptospirosis in New Zealand. It is reasonable to suggest that data that exhibit a seasonal pattern are likely to be associated with climate and for this report we exam seasonality within available leptospirosis data. However, it is important to remember that there are drivers other than climate that may produce seasonality in disease data, for example farm production and animal breeding cycles. As leptospirosis in animals is not notifiable we have accessed laboratory submission data for serovars Hardjo and Pomona provided by Gribbles animal health to explore seasonality. It is important to be aware that infections from Hardjo and Pomona present differently as Hardjo is considered to be in a maintenance host relationship with cattle in New Zealand, and therefore, the disease caused by this serovar behaves differently to that of a Pomona infection. Ruminants are considered accidental hosts for Pomona and thus this serovar generally causes more severe disease than Hardjo. For the laboratory submission data a positive result was defined as a MAT titre of 1:50 dilution or greater. Figure 4.3 shows a monthly box plot of the proportion of laboratory submissions from cattle positive to L. Pomona and L. Hardjo over the period 2003 to 2010 inclusive. Data sourced in this way has some limitations: it likely reflects the most severe cases and those able to pay for veterinary visits and sample analysis. Nevertheless two patterns emerge. The Hardjo data shows that approximately 50% of samples submitted were positive and there is no seasonal pattern. The Pomona data shows a seasonal pattern with the highest proportion of positive submissions occurring in the winter and spring months. This peak of submissions from cattle in June – September is likely attributable to higher rainfall and therefore longer periods of with elevated soil moisture in winter, allowing leptospira to survive for up to 7 weeks (Hellstrom and Marshall, 1978) while maintaining virulence. Another contributing factor to this pattern is seasonal calving subsequent to this peak period which would suggest a relationship of clinical submissions to abortion. However, the sero-prevalence trend for Hardjo was non-seasonal and Hardjo was reported to have a similar impact on fetal loss in beef cattle as did Pomona (Sanhueza, 2012).

Two recent analyses of human leptospirosis notification data (1997 to 2008) did not find a seasonal pattern (Paine et al., 2010; Meade, 2012), contrary to the pattern suggested by Figure 4.3.

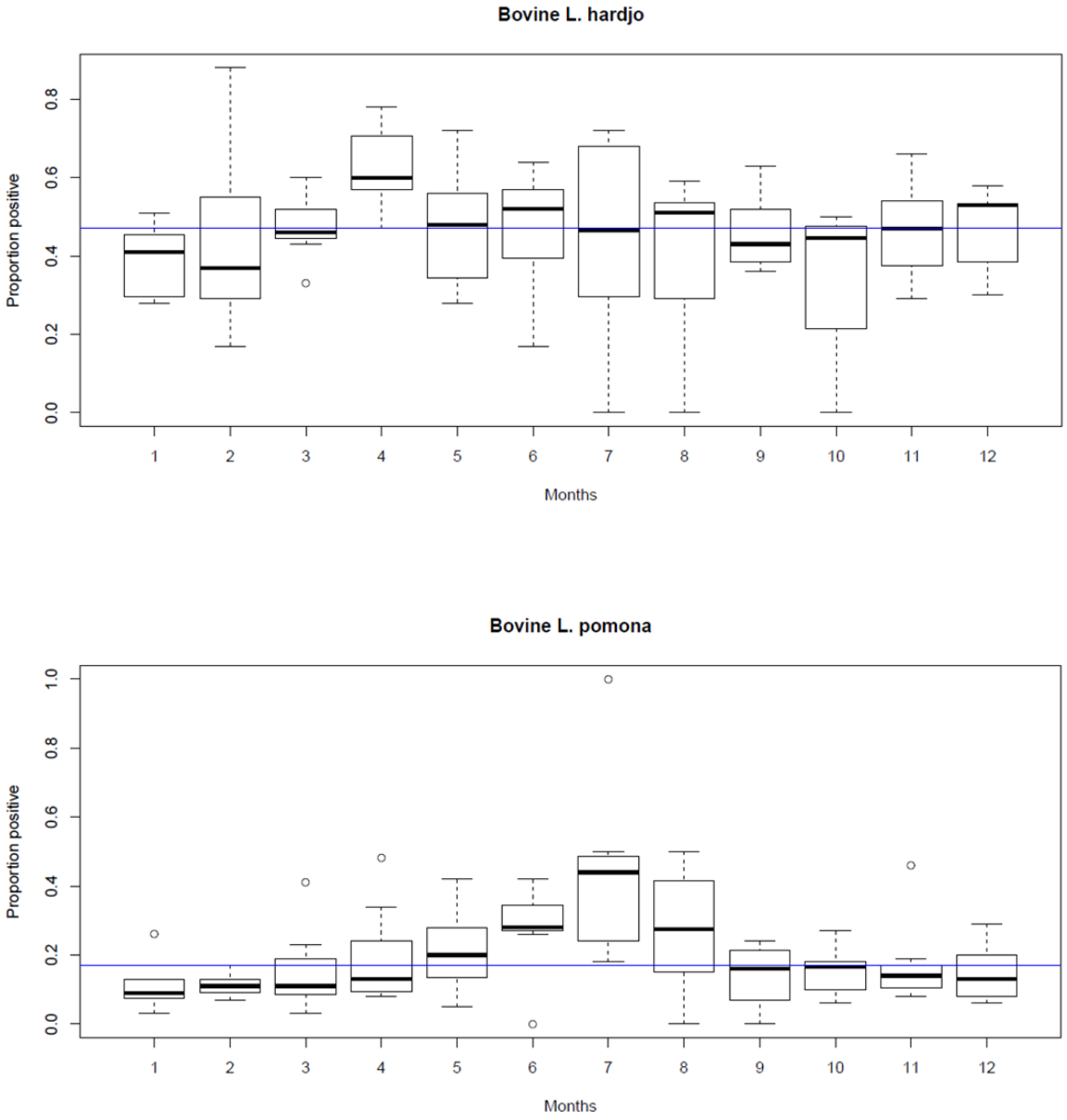

Figure 4.3: Monthly box plots of the proportion of laboratory submissions from cattle positive for L.Hardjo (above) and Pomona (below) over the period 2003 to 2010. The horizontal blue line represents the median proportion. Data sourced from Gribbles animal health laboratories.

4.4 Mixed-species grazing management

Advantages of mixed-species farming (deer, sheep and/or cattle) include pasture management and internal parasite control (Hilson, 2007). However, mixing of host species can result in pathogen transmission and infection across species. A longitudinal serological survey occurred on 20 mixed species (sheep and/or beef cattle) deer farms in the lower north island from 2006 until 2008 (Subharat, 2010). Deer herds were more likely to be Hardjo positive on farms with

hilly topography (PR[1]=4.67, p<0.001) and when deer were co-grazing with Hardjo positive cattle herds (PR=1.93, p=0.022) or sheep flocks (PR=1.70, p=0.007). Deer herds were more likely to be Pomona positive when deer were co-grazing with Pomona positive cattle herds (PR=7.50, p=0.050).

These results suggest that inter-species transmission of leptospires may be occurring on farms. However as there may be host-adaption within serovars molecular studies are need to confirm this. Molecular studies based on the multi-locus sequence typing scheme of Ahmed (Ahmed et al., 2006) are occurring at Massey on isolates gathered from serovars isolated from cattle and sheep on the same farm as part of the abattoir study described above (Fang, 2012).

5. Farm management

New Zealand's main pastoral farming enterprises (dairy, beef/sheep and deer) have a seasonal production linked to feed availability and animal reproduction cycles. Within the context of best practice for vaccination, farm production cycles present opportunities for both leptospirosis vaccination and for transmission of infection. For example in the seasonal calving dairy herd culling decisions based on production are usually made at drying-off in May. At that time whole herd dry-cow mastitis therapy may be used and that is seen by farmers as an opportune time to booster-vaccinate adult dairy cattle.

This section will give an overview of the production cycle for each farming enterprise identifying opportunities for both infection and vaccination. It also identifies the risk activities for humans associated with each enterprise. Interviews with Dr. Jenny Weston, Prof. Paul Kenyon and Prof. Steve Morris from the Institute of Veterinary, Animal and Biomedical Sciences, Massey University informed these sections. It is important to be aware that these production cycles attempt to represent what occurs only on the majority of farms, they cannot reflect the variation that occurs from farm to farm.

5.1 Seasonal calving dairy herd management

Figure 5.1 shows a timeline of farm management events that representative of a New Zealand spring calving herd. Calving starts in mid to late July and ends by early October. Calves rely on colostrum for maternal transfer of antibodies. It is generally recognized that 50% of calves do not receive adequate colostrum (Vermunt et al., 1995; Wesselink et al., 1999). Calves are removed from the cows daily, very few farmers will pick up calves twice daily, and this can mean that calves may be up to 23 hours with their dams in the paddocks. Approximately 50% of dairy farmers will stomach tube calves with colostrum, the rest relying on calves suckling or receiving colostrum through calf feeders.

Generally the early and late calves are of lower breeding value as they are the result of bull matings, so these are culled as bobby calves for slaughter or are sold for calf rearing. Female calves from artificial breeding (AB) are kept as replacements. These are almost exclusively

[1] Prevalence ratio

reared on the home farm until weaning at 8 to 10 weeks of age. Early animal husbandry includes disbudding by vets or vet techs at 3 to 6 weeks. This is an opportunity to start the vaccination programme, for example the Massey vet clinic gives a 7-in-1 vaccination (clostridial 5-in-1 plus leptospirosis Hardjo and Pomona).

Mating occurs from October to December. The heifers are almost exclusively put to bulls while the milking herd usually runs two cycles of artificial breeding and are then followed up with bulls.

Within the New Zealand dairy enterprise it is increasingly common practice to raise the young stock off the milking platform. The contract rearing of calves and heifers post-weaning presents an opportunity for them to mingle with other dairy stock and other species from the rearer's farm thus potentially exposing them to infection. Furthermore, the fact that this young stock is grazed away may mean that vaccination may be delayed. The most common practice is that young stock leaves the home farm on June 1 as rising one year olds. At the same time there is the return of pregnant rising two-year old heifers to the home farm. However, some farmers send the calves away from the home farm in December as four-month-olds. Commercial grazers may be used or young stock may go to a run-off owned and managed by the herd owner.

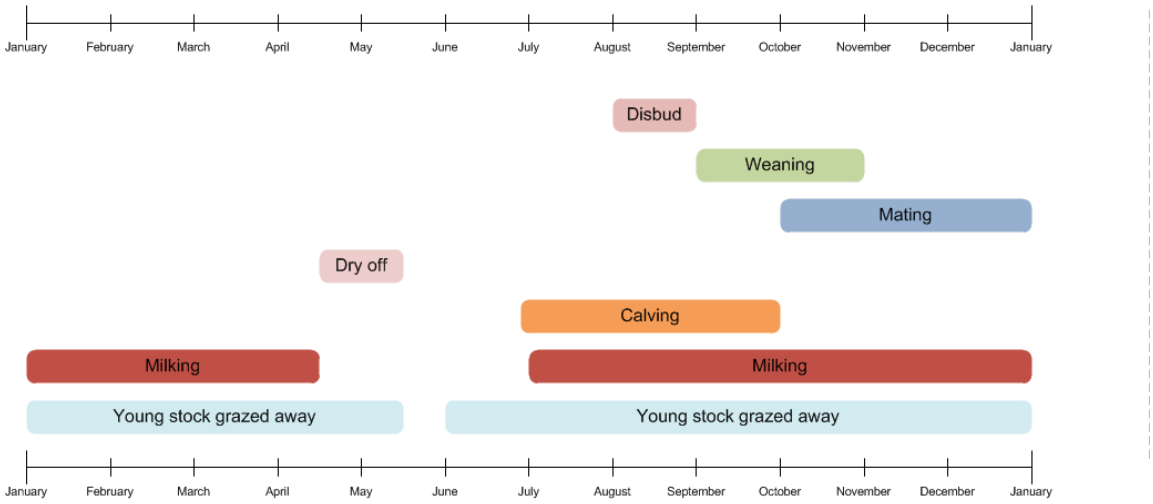

Figure 5.1: Timeline of farm management events in a representative New Zealand spring calving dairy herd

5.2 Issues around vaccination in seasonal calving dairy herds

High rainfall periods are a risk factor for leptospirosis transmission (Hartskeerl et al., 2011) and booster vaccinating adult stock before these periods is sensible. Historically veterinarians vaccinated the milking herd at the time of manual pregnancy testing (March/April) and this fitted in with the traditional risky autumn period for transmission. However, the adoption of scanning technology has brought the time of testing forward with most herds scanning in Jan/Feb. This appears to be rather early for the annual booster to have an impact on maternal antibodies in colostrum if that were intended. The annual pre-calving booster is therefore best be applied at dry-off around May to allow for antibody in colostrum, or after calving to prevent MDA interference with early vaccination. An in-house study by Virbac Animal Health evaluated

MAT antibody levels in newborn calves when dams were vaccinated 54-90 days prior to calving. The author suggested that dams should be vaccinated at least 70 days prior to calving for optimal calf immunity (Pulford, 2006). However, none of the dams in this study was vaccinated as early as 180 days before calving as it would occur if cows were booster-vaccinated at scanning in Jan/Feb.

Decisions about when to vaccinate the adult cow have been around the conflict between giving a "colostral" vaccination (early to mid July) versus vaccinating before the wet weather risk period (April/May) versus the convenience of the vaccination at drying-off, which occurs end of May. The timing of the wet weather period and drying off vary from season to season to season and between geographical location.

The uncertainty about when to vaccinate calves has been influenced by having a number of products on the market with conflicting advice about when best to start the vaccination programme. The key conflict here is the balance between interference from maternal immunity and concerns about leaving calves unprotected (section 9.3).

Generally it is the beef industry that supplies bulls to the dairy industry. Beef cattle are highly exposed to leptospirosis and vaccination of bulls for leptospirosis is rare. Exceptions include large commercial bull farms which are also vaccinating against other diseases such as BVD.

Seasonal calving dairy herd opportunities for human infection

- Milking and teat care
- Assisting with dystocia
- Artificial breeding
- Pregnancy testing
- Herd testing
- Reproductive checking
- Other animal health engagement
- Spreading of dairy shed effluent (truck or irrigator)
- Keeping of pigs (Pomona, Tarassovi)
- Rodent exposure from storage and feeding of concentrate (Ballum, Copenhageni)
- Changing climatic patterns, wet/dry spring, standing water
- Tb testing (albeit on a bi- or tri-annual basis)

5.2 Management of sheep flocks

Adult stock

New Zealand commercial self-replacing sheep flocks put the rams out in March for 2 or 3 cycles. Rams are brought in over November to February and generally 20 to 30% are replaced each year. Two-thirds of flocks breed ewes first at 18 months (two-tooths) while one third breed ewes as hoggets. 80 to 90% of flocks scan for pregnancy at the end of June early July and lambing starts in late August. The majority of farmers practise easy-care lambing with limited assistance for dystocia and bearings. Figure 5.2 shows a timeline of farm management events that are representative of a New Zealand self-replacing sheep flock.

Pregnant ewes are vaccinated 2 to 4 weeks prior to lambing with a clostridial 5-in-1 vaccine to enhance colostral transfer of immunity. Vaccines (Toxoplasma and/or Campylobacter) to prevent fetal loss are given pre-breeding. Vaccination against salmonellosis may be routine or may be in the face of an outbreak. Ecto-parasite control (dipping) occurs in summer every 4 to 6 weeks (Jan through March).

Most farmers shear ewes once a year post-weaning (Dec-Jan). These ewes will have a crutch and belly shear in July. Less frequently there are two shears a year: one pre lamb in June/July (enhancing fetal survival/ encouraging shelter seeking) and the second in Dec/Jan.

Young stock

Tailing/docking (tails and testicles) occurs 4 to 6 weeks after the start of lambing when most lambs are 3-4 weeks old, often on two batches. Lambs may receive their first clostridial vaccination at tailing or at weaning.

Weaning generally occurs in Dec-Jan at 12 to 16 weeks (28 kilos live weight). In some farming enterprises it may occur earlier and together with the second docking, at around 8 to 10 weeks of age. Weaning is the more common time for the first clostridial vaccine and it is boostered 4 to 6 weeks later. This is an opportunity to vaccinate for leptospirosis as well. The first worm drench is also given at weaning and then every 28 days (for 5 to 7 times). Lambs are first shorn between January and Match and then again in October.

If a sheep flock is not self-replacing there are more opportunities for infection of both animals and humans as stock are co-mingled and more animal health procedures are likely to occur, e.g. a worm drench on arrival.

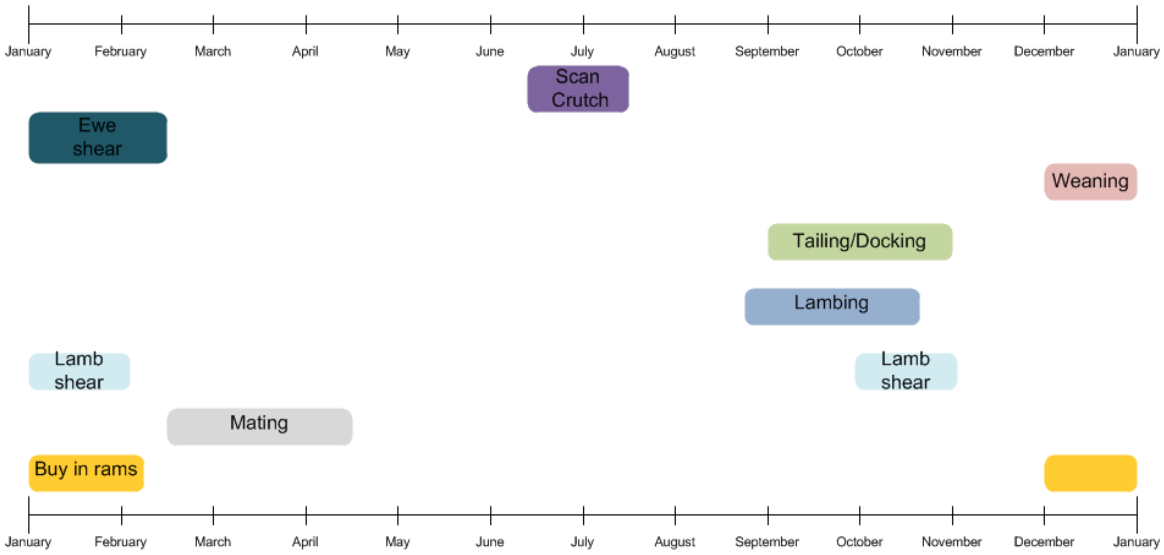

Figure 5.2: Timeline of farm management events in a representative New Zealand self-replacing commercial sheep flock

Sheep flocks: opportunities for human infection:

- Shearing/crutching
- Assisting with dystocia
- Home slaughter: both for human consumption and for dog tucker.
- Pregnancy scanning
- Tailing/docking
- Other animal health engagement (e.g. drenching)
- Changing climatic patterns, wet/dry spring, standing water

5.3 Management of beef herds

Adult stock

Bulls are purchased in July as rising two year olds and generally stay in the herd for 4 years. Cows calve from August to October. Cows may receive a 5-in-1 vaccination two to four weeks pre-calving. Bulls are joined with cows and heifers from October to December for a maximum of three cycles. Bulls are often rotated between mobs of cows. Heifers are usually mated at 26 months old but if well grown can be mated as yearlings. The breeding herd is kept in age mobs of heifers and mixed age cows. Pregnancy diagnosis of cows and heifers occurs in March.

Steers may be sold off at the yearling sales (store cattle) in September but more usual are the spring cattle sales of steers, heifers, and dry cows.

Tuberculosis testing is done at variable times, usually after weaning or during winter. However, most beef herds now need to be tested bi- or tri-annually.

Young stock

Calves are ear-marked at 4-6 weeks of age (November) and male calves castrated. Calves are weaned in March and this is when the drenching programme for weaner calves also begins. Figure 5.3 shows a timeline of farm management events that are representative of a New Zealand beef herd.

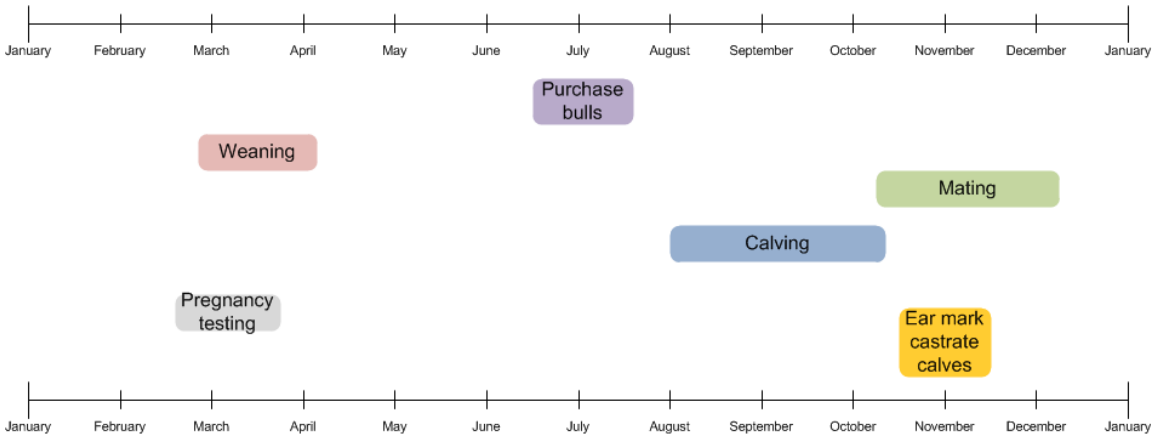

Figure 5.3 Timeline of farm management events in a representative New Zealand beef herd

Beef herds: opportunities for human infection:

- Assisting with dystocia
- Pregnancy testing
- marking/castrating
- Other animal health engagement
- Changing climatic patterns, wet/dry spring, standing water

5.4 Management of deer herds

There is wide variation in weaning date between deer farms with some pre-rut occurring as early as mid-February to late March. Other enterprises will wean post-rut in May-June while others may not wean at all. Vaccination of young stock (Clostridial and lepto), if done, occurs in late Feb to early March, regardless of the weaning date. The majority of deer farmers use anthelmintic treatments to young deer, albeit at variable frequency, during the autumn, with repeat treatments by some late winter and through spring. About half of deer farmers give anthelmintic to yearling and adult deer, usually either pre-rut, or early – to mid-winter. Mating occurs from March to May, with calving from Nov to Dec. Scanning for pregnancy is done by a minority of farmers (May-June). Figure 5.4 shows a timeline of farm management events that are representative of a New Zealand deer herd. Due to the large variation in weaning dates these have not been included in the figure.

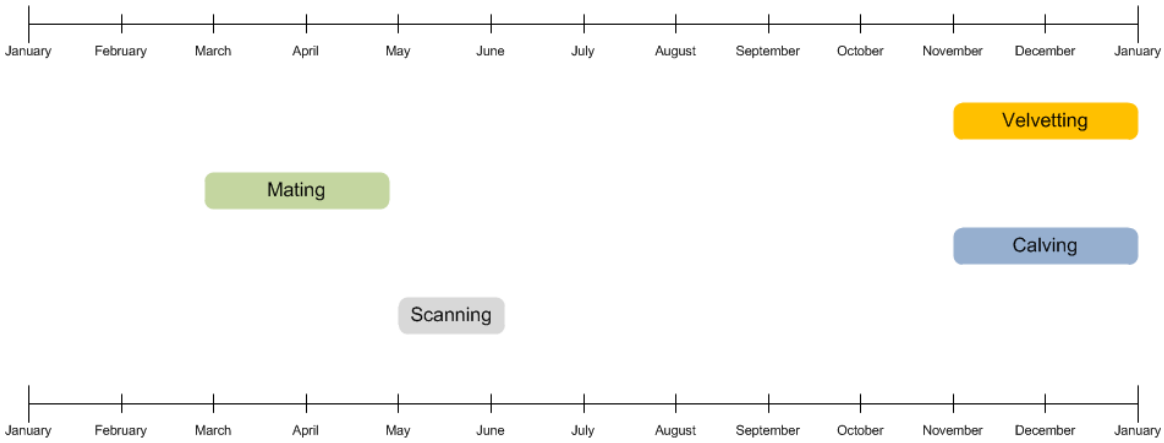

Figure 5.4 Timeline of farm management events in a representative New Zealand deer herd

Deer herds: opportunities for human infection:

- Weaning
- Scanning
- Removal of stags after mating
- Other animal health engagement, e.g. anthelmintic, copper treatment
- Velvetting
- Tb-testing

Up to 70% of New Zealand deer herds practice mixed-species management with sheep and/or beef (Hilson, 2007). So the risks to humans with deer farming enterprises need to take into account the presence of other species when they are present.

6. Production outcomes

Significance of production effects as incentive for vaccination to protect humans: In the absence of vaccines for humans in New Zealand, preventing human leptospirosis clearly requires the control of infection in sheep, deer and beef cattle. As publicly funded control programmes for leptospirosis in livestock do not exist, control is voluntary and paid for by producers.

A 2009/10 survey showed that 1/162 (0.6%) sheep, 6/99 (6.1%) deer and 19/116 (16.4%) beef breeding farms had their stock vaccinated against leptospirosis. Farmers implement vaccination primarily to protect themselves, their families and farm workers. An additional motivation for investing in vaccination might be economics. If investing in leptospirosis control would return production gains offsetting the cost of investment, farmers would be more likely to adopt vaccination and other means for control.

This section therefore reviews current knowledge about the livestock production response effect to infection with *Leptospira* from a population and cost-benefit perspective. While it is

known that leptospirosis affects several organ systems and can cause clinical diseases such as kidney or liver failure, studies were only considered in this report when designed to demonstrate associations between *Leptospira* infection and sub-clinical production outcomes. Few data are available about the incidence of clinical disease whereas most production effects were deduced from prevalence or incidence studies about sub-clinical disease.

Clinical disease with high fatality generally occurs sporadically and at apparently low incidence, although no robust data exist, and under-diagnosis may occur. However, outbreaks have been reported following extreme conditions of flooding or extended periods of rainfall with 5 – 15% lamb loss (Dorjee et al., 2005). A recent survey of 1,940 farmers responding to a questionnaire invitation mailed to 7,998 clients of 28 veterinary practices in 7 selected regions in early 2009 revealed that 1-5% farms had observed clinical occurrences of leptospirosis in the preceding 3 years (Table 6.1). Deer farmers observed clinical disease more frequently than sheep or beef farmers, perhaps through greater awareness in response to industry initiatives. Clinical disease (3-year incidence 4.7%) was only observed on deer farms that also grazed sheep and the 3-year incidence on deer/sheep farms was 12.5%, suggesting transmission from sheep to deer. More detailed information is currently being analysed (Dreyfus, 2012).

Table 6.1: Farmer reported 3-year leptospirosis occurrences of clinical leptospirosis from a mail survey of 1,940 respondents of 7,998 clients of veterinary practices in New Zealand, 2006-8 (Dreyfus, 2012).

Species	Clinical occurrences reported/no. farms	3 year incidence
Deer	11 / 233	4.7%
Sheep	14 / 1,193	1.2%
Beef cattle	22 / 1,061	2.1%

Hence, while clinical leptospirosis is known to cause jaundice, kidney disease and haemoglobinuria, this clinical expression of leptospirosis is only seen sporadically in any of the pastoral livestock species in New Zealand.

In contrast, loss due to sub-clinical disease was widely believed to be negligible or absent, and until recently when studies in deer were undertaken, had not been the subject of research demonstrating direct causative links in any NZ livestock species. The presence of sub-clinical disease was based on either serological prevalence or incidence of sero-conversion. Prevalence or case-control data can at best suggest an association with production loss. Incidence of sero-conversion may allow causal inferences, especially when sero-conversion was preceeding or in a period concurrent to the measurement of production outcomes.

In **deer,** an initial observational study during the growth phase of young deer using serovonversion and/or urine shedding as markers for new infection. Comparison of growth rates from weaning to slaughter showed an average of3.7kg lower live weight at slaughter in those showing evidence of infection. This detrimental effect was explained by serious histo-pathological findings in kidney tissues of sero-positive deer (Ayanegui-Alcerreca, 2006). That author also reported a significantly greater weaning percentage (number weaned/hinds scanned pregnant) in vaccinated compared with non-vaccinated adult hinds in a herd with high prevalence of dual Hardjobovis and Pomona infection (97 vs. 88%, respectively). A subsequent incidence study of deer in New Zealand found significantly higher growth rates in vaccinated vs. non-vaccinated weaners, up to a man of 6.4kg at 12 months in a high prevalence herd (Subharat et al., 2012) and a mean of about 6% higher weaning rates attributable to annual vaccination of hinds in herds with evidence for moderate-strong natural challenge (Subharat et al., 2011a). Since pregnancies have been retained at least until the immediate pre-calving period, vaccination apparently reduced perinatal and/or pre-weaning mortality. The growth response

was sufficient to provide positive financial returns on the investment of vaccination when prevalence was approximately 20% or more. An economic response for reproductive improvement occurred when the weaning rate increased by approximately 1.3% or more. Return on investment in vaccination ranged from 700 – 1200% (reproduction and growth, respectively) in high infection rate situations (Wilson et al., 2009).

No data are currently available about growth or weaning effects in beef cattle or sheep in New Zealand. Encouraged by the results in deer however, similar NZ-studies are currently underway in sheep flocks and beef breeding herds. Results are expected by mid-2013 (Vallee, 2012).

In **beef cattle**, both *Leptospira* sv Hardjo and Pomona were associated with an increased risk of foetal loss in a population based case-control study in New Zealand in 2010 (Sanhueza, 2012). Conservative estimates from the study indicated that 5% and 4% of fetal losses were attributable to Hardjo and Pomona, respectively, and were similar to foetal loss attributable to bovine viral diarrhoea virus or *Neospora caninum*. Such losses may be much higher when susceptible cattle, e.g. returning from a distant run-off, were co-grazed with cattle, deer or sheep where leptospirosis was highly endemic. Similar associations were reported from Spain (Ellis et al., 1978; Atxaerandio et al., 2005). Similar studies in Canada, US and Ireland estimated 6%, 10% and 50%, respectively, of bovine abortions being associated with serovar Hardjo (Grooms, 2006). New Zealand data published by Beef&Lamb suggested that pregnancy rates of beef herds were not associated with the sero-prevalence of serovars Hardjo or Pomona (Heuer et al., 2007a)[2]. A study in Victoria, Australia, concluded that *L. interrogans* sv. Hardjo was NOT associated with abortions in dairy cattle as *Leptospira* could not be identified by culture in placenta or foetuses from 195 aborting cows despite being isolated from the urine of 2 infected, apparently non-aborting cows (Chappel et al., 1989). Successful isolation of *Leptospira* (serogroup Hebdomanis) from these tissues was reported from experimentally infected and aborting cows (Ellis and Michna, 1977). Current trials involving vaccination may help determine whether these associations are causative (Vallee, 2012).

In addition to this, well evidenced risk of *Leptospira* infection on abortion, there appears to be a sub-clinical effect on conception rates in **dairy cattle**. The median time from calving to conception was 34 days longer and one more breeding was required per pregnancy in sero-positive vs. sero-negative to serovar Hardjo first-calving dairy cows in a US-study. A UK study of herds with evidence of exposure to leptospirosis suggested that vaccination against serovar Hardjo potentially increased conception rates and reduced culling (Dhaliwal, 1996).This sub-clinical effect was more pronounced in spring calving cows and attributed to conception failure and early embryonic death (Guitian et al., 1999). Calving rates (measured a lactation failure) increased significantly from 81 to 88% in a clinical trial assessing the effect of a L. sv. Hardjo vaccine in beef cattle (Holroyd and Smith, 1976). A subsequent study also reported higher weaning rates in vaccinated vs control cattle (Holroyd, 1980). No difference of reproductive performance indicators (pregnancy, calving, stillbirth) were observed in beef cattle of one farm in Brazil between sero-positive and sero-negative at the start of the mating season (DelFava et al., 2004), but the study gave no indication of active challenge during the risk period.

Some caveats may be noted when attributing the above cited production effects from various countries with serovars Hardjo (Hardjoprajitno, Hardjobovis) or Pomona (*L. interrogans, L. kirschneri*). It was not always clear which specific genetic variant of Hardjo or Pomona was

[2] http://beeflambnz.com/Documents/Farm/Management%20of%20beef%20cattle%20for%20high%20fertility%20-%20Part%202.pdf

involved, thus differences between studies may be explained by genetic differences linked to virulence factors.

6.1 Conclusions on production outcomes

Under typical weather conditions in New Zealand, clinical loss due to leptospirosis is generally limited to sporadic young stock loss and abortions. However, excessive floods in warm periods of the year can lead to outbreak-like increases of mortality. In contrast, lower growth rates and reduced weaning rates appear to be common in deer and possibly, given the above observations of seroprevalence, other livestock species. As seen in deer, the loss-value due to such sub-optimal performance in apparently healthy animals can be several times higher than the cost of whole-herd vaccination in even modestly exposed herds.

7. History of vaccine use in New Zealand

Vaccines against leptospirosis were introduced in New Zealand in the early 1970s by Schering-Plough Animal health followed by Pfizer in the 1990s (personal communication). A bivalent (Hardjo/Pomona) vaccine started to become more widely used by dairy farmers in the early 1980s (Marshall and Chereshsky, 1996). However, only a small proportion of sheep (<1%), beef (18%) or deer farmers (10%) adopt vaccination against leptospirosis presently. The only commercial industries that generated a major uptake vaccination were dairy and pig farmers (over 85%). The latter two industries are therefore briefly mentioned here.

Dairy farmers

Dairy cattle have been assumed to be the major source species for transmitting *Leptospira* to humans in the 1970s and 1980s. Therefore, vaccination of dairy cattle was introduced in 1979, widely propagated for controlling the disease in people in the 1980s, and followed by a drastic decline of human cases in the 1990s (Marshall and Chereshsky, 1996).

The Health and Safety in Employment Act 1992 defines leptospirosis as a 'significant hazard'. The New Zealand Veterinary Association (NZVA) subsequently created Leptosure®, an initiative for the control of leptospirosis beyond vaccination in the mid 1990s. A farmer booklet describes the programme's aims and functionality. The booklet can be downloaded from the Internet[3]. Leptosure® is a national risk management programme developed by the New Zealand Veterinary Association and the Society of Dairy Cattle Veterinarians to reduce the risk of human leptospirosis infection on New Zealand dairy farms. It describes sources of infection for humans and explains on-farm mitigation procedures. The programme is aimed at helping farmers to develop a risk management plan (RMP), initially for dairy farms, and more recently, also for dry stock.

Pig farmers

Pork Industry New Zealand (NZPork) in liaison with the NZ Food Safety agency (NZFSA) has implemented a set of rules for movement and trade under the Animal Status Declaration (ASD) for pigs. Since 01 March 2006, producers are obliged to fill an ASD form for every movement of pigs from commercial properties, whether it is pigs for slaughter or other movements. The ASD covers leptospirosis with the following rule[4]:

[3] http://www.leptosure.co.nz/sites/default/files/domain-18/Leptosure%20Green%20Farmer%20Booklet%202007.pdf

[4] http://www.foodsafety.govt.nz/elibrary/industry/Animal_Status-Must_Completed.pdf

"A leptospirosis control programme requires vaccination of the breeding herd if present at least every 6 months, and certification of the grower herd as 'free of leptospirosis' at least once every 12 months. Grower herd certification is based on the results of serological testing of a minimum of 10 grower pigs either within two weeks of slaughter or at slaughter using the MAT (Micro Agglutination Test) for Leptospira pomona. Interpretation of the results is required to determine the status of the grower herd. This must be done by a registered veterinarian. Equivalence to the above programme documented by a registered veterinarian is accepted."

Thus, leptospirosis is well controlled in pigs on commercial production units. However, uncertainties remain about the risk of human exposure from backyard piggeries and hobby farmers. NZPork has commissioned a report by Massey "Small scale pig farming: practices and obligations"[5] that includes guidelines and recommendations about how to manage the risk of infection for people in contact with pigs under such small scale systems. Farmers and households are advised to vaccinate al pigs every six months.

8. Vaccine label claims and recommendations

The information to write this summary was obtained from labels approved by the NZFSA available in http://www.foodsafety.govt.nz. Please refer to Annex III (vaccine summary) for more detailed information.

8.1 Cattle

There are currently 9 vaccines registered for use in cattle in New Zealand: Leptavoid 2®, Leptavoid 3®, Leptoshield®, Leptoshield 3®, Vaxall®, Cattlevax®, Ultravac 7®, Lepto 2-Way®, and Lepto 3-Way®. All vaccines include *Leptospira* serovars Hardjo and Pomona, and vaccines with postscript '3' also include serovar Copenhageni. No vaccine includes serovar Ballum.

Purpose: In general all providers recommend the use of vaccine to prevent infection and urinary shedding and note that vaccination is effective in previously non-exposed cattle. This implies that vaccination after natural exposure is accepted as having lower efficacy.

Recommendations in common: Labels of all vaccines recommend two doses subcutaneously 4 to 6 weeks apart, followed by a single booster annually. Some of them recommend a booster every 6 months where *Leptospira* is present at high endemic level (Vaxall®), or for multi-pathogen vaccines, where clostridium disease challenge is regarded to be exceptionally high (Cattlevax®). Vaccination is generally recommended to be administered before the season of high risk, generally in autumn to early summer. For breeding females, labels of Leptavoid-2®, Leptavoid-3®, Leptoshield®, Vaxall®, and Ultravac-7® recommend applying the annual whole herd booster one month before calving in order to increase antibodies in colostrum for protecting new-born calves against infection with *Leptospira.*

[5] http://www.nzpork.co.nz/LinkClick.aspx?fileticket=l9Kj6cs73SU%3D&tabid=123&mid=622

Calves XXX

Label recommendations that differ between providers: The recommended age for calf vaccination differs between brands. Some labels claim that the vaccines are effective in the presence of MDA and calves can therefore be vaccinated at one month of age. vaccination is unlikely whereas others claim some degree of interference.

Maternal antibodies are unlikely to interfere with the response to vaccination in calves.

Leptoshield®, Leptoshield 3®, and Ultravac 7® claim to be efficacious in the presence of MDA, and therefore calves may be vaccinated from 1 month of age. Two doses 4 to 6 weeks apart should be applied. If a second vaccination is administered before 3 months of age, a single booster dose "should" be administered 6 months later, at 8-9 months of age.

Maternal antibodies may interfere with the response to vaccination in calves.

Leptavoid 2®, Leptavoid 3®, and Cattlevax®: If vaccination is completed (i.e. sensitiser and booster) before 6 months of age, a single booster dose is required at 6 month of age to ensure protection for 12 months.

Vaxall®: If vaccination is completed before 6 months of age, a booster dose is required at 6 months of age, followed by a second dose 4 to 6 weeks later.

Lepto 2-Way®, and Lepto 3-Way®: The first vaccination course can start anywhere between 12 weeks of age and 4 weeks before 9 months of age, thus the first course should have been completed by 9 months of age. However, in the case of a first course of vaccination starting at 12 weeks of age, it is essential to administer an additional booster at 6 - 9 months of age to align with future herd vaccination. In all cases, two doses should be given 4 - 6 weeks apart and finishing no later than 9 months of age.

8.2 Sheep

There are currently 2 vaccines registered for use in sheep in New Zealand: Leptavoid-2®, and Leptoshield®. Purpose, dosage and administration are identical as described for cattle.

Purpose: For the active immunisation against leptospirosis (Leptavoid-2®) or for the prevention of leptospirosis (Leptoshield®). No reference is made to the prevention of urinary shedding.

Recommendation: Two doses 4 to 6 weeks apart, before the season of high risk in autumn to early summer. No differences exist between label recommendations for sheep.

Booster: Single dose annually.

Age at first vaccination of lambs: No detailed recommendations are given for lamb vaccination.

8.3 Deer

There are currently 3 vaccines registered for use in deer in New Zealand: Leptavoid-2®, Leptavoid-3®, and Leptoshield®.

Purpose: For the active immunisation against leptospirosis (Leptavoid-2®, Leptavoid-3®) or as an aid in the control of leptospirosis (Leptoshield®). The label of one product explicitly mentions the prevention of urinary shedding (Leptavoid-3®).

Vaccination: Two doses 4 to 6 weeks apart, before the season of high risk from autumn to early summer.

Booster: Single dose annually.

Age at first vaccination of fawns: No detailed recommendations are given for vaccination of deer calves.

9. Host immune response after exposure

9.1 Background

The symptoms, severity and course of leptospirosis are very dependent on the relationship between the infecting serovar and the animal host species. The disease tends to manifest in a much less severe form when the animal is a maintenance or reservoir host for the particular infecting serovar. For this reason, it is often useful to consider leptospirosis as a group of diseases rather than as a single disease. Infections from the two serovars of main interest to New Zealand's livestock industry, Hardjobovis and Pomona, present differently. Hardjobovis is considered to be in a maintenance host relationship with at least cattle and deer, and probably sheep, in New Zealand, and therefore, the disease caused by this serovar may behave differently to that of a Pomona infection. Ruminants were so far considered accidental hosts for Pomona, and thus this serovar was expected to causes more severe disease than Hardjobovis. However, recent serological findings of antibodies to Pomona being present in 10-14% of adult beef cattle, sheep and deer in New Zealand suggest that these host species may be a reservoir for Pomona. Notwithstanding this consideration, any discussions around the host responses should differentiate the host adaptation to different serovars.

Leptospires colonise the kidneys of an infected host and are then shed into the environment via the contaminated urine. From there, leptospires can enter a new host through cuts or abrasions to the skin and through mucosal tissue. In some instances *Leptospira* reside in the genital tract and can be transmitted through reproductive fluids (Ellis et al., 1986). Once in the bloodstream, they circulate for approximately 3-7 days before colonising the kidneys although the time varies dependent on the challenge dose and strain (Faine et al., 1999). The extent of other organ involvement varies according to the age of the animal and the serovar involved. Likewise, symptoms can range from sub-clinical to death dependent on the infecting serovar and the host animal. If an animal survives the initial stage of infection, *Leptospira* will colonise the kidneys and from there the bacteria are shed in the urine.

In New Zealand, serovars such as Pomona and Copenhageni are considered to be sporadic infections. As opposed to a host-adapted strain, *e.g.* Hardjobovis, these serovars tend to cause a more severe clinical illness. Whilst high numbers of *Leptospira* can be shed from the colonised kidneys in the urine, the period of shedding tends to be shorter lived than in an animal where the serovar is considered host-adapted. In situations where the animal species acts as a reservoir host for a particular serovar, the *Leptospira* are maintained in the flock/herd. Transmission of infection from more mature animals to younger stock is consequently likely to be more predictable and cyclical than those of sporadic leptospiral infections. Thus, the dynamics of natural exposure in domesticated herds/flocks is influenced by the host and the serovar as well as by farm management practices and environmental conditions. Also, as opposed to the human situation where leptospirosis is incidental, immunological responses to infection or vaccination as well as control measures in naturally infected animal populations, can additionally be interpreted in the context of a herd setting.

9.2 Immunological responses mechanisms

Though the immunological response types may not necessarily be independent, it is convenient to discuss them under two separate categories; humoral (antibody production) and cell mediated immunity (CMI). Most ruminant leptospirosis research has used cattle as the host species so the majority of experimental evidence is derived from this model. Whilst there is evidence that the host's primary immunological response to leptospiral infections is humoral and that passive protection has been demonstrated effective in a number of challenge trials (Flint and Liardet, 1980; Marshall et al., 1982; Palit et al., 1996), the duration of effective passive

protection and the role of cell mediated immunity is much less clear. This is particularly the case with Hardjobovis infections in cattle.

9.3 Innate immunity and the influence of age and genetic background on resistance

Any innate resistance in naïve hosts is thought to be limited to a humoral response with the host already possessing antibodies that will react to the same agglutinating leptospiral epitopes used in the serological classification system for *Leptospira* (Faine et al., 1999) or, protection via complement (Cinco, 2010; Fraga et al., 2011). The lipopolysaccharide (LPS) of the leptospire's outer cell surface is highly antigenic (Faine et al., 1999) and is the foundation of the classification system that divides *Leptospira* into serogroups and serovars. Although not exclusively so, it is LPS that is the dominant class of antigen against which a host mounts a humoral response and passive transfer of protection by antibodies against the LPS or monoclonal antibodies has been demonstrated to be protective (Faine et al., 1999).

Different mammalian species are born at various stages of maturation and immune competence and this affects the susceptibility of animals to leptospiral infection. For example, it is standard practice for young hamsters and guinea pigs which possess immature B cells (Faine et al., 1999) to be used for routine vaccine testing (Faine, 1982) as they are much more susceptible to leptospiral infections than the adults and, whilst adult mice and rabbits are resistant, their immature young demonstrate variable susceptibility (Faine et al., 1999). The severity of an established infection can also vary between adults and young with milder responses tending to be seen with increasing age (Fennestad, 1963) *e.g.* "red water" in calves infected with Pomona.

Genetic lineage may also play a role in resistance/susceptibility to leptospirosis. Certain lines of pigs have been shown to be less susceptible to leptospiral infection (Przytulski and Porzeczkowska, 1980) providing evidence that genetic background also plays a role in resistance, but the mechanism by which this is effected is unclear.

9.4 Measurement of humoral response

The microscopic agglutination test (MAT) remains a gold standard and is still used extensively for detecting any antibodies. Though not specific for any particular class of antibody (Faine et al., 1999), MAT is thought primarily to detect a mixture of IgM and IgG (Morris and Hussaini, 1974). Although a number of other methods have been trialled for the early detection of leptospiral antibodies *e.g.* haemolytic test, indirect haemagglutination assay, indirect immunofluorescence, indirect IgM ELISA, IgM dot-ELISA, immobilized antigen dipstick and lateral flow assays, (Faine, 1982; Effler et al., 2002), comparative evaluation suggests further development is required, particularly around the sensitivity and specificity of detection in early stages of the disease. The antigens employed in these techniques all involve LPS or chemical related derivatives of these (Faine et al., 1999). Early production of IgM antibody may be detected by anti-IgM ELISA. However, these antibodies can be less specific than the latter-produced IgG1 antibodies and thus react against a variety of serovars and are therefore less reliable for identifying the infecting strain (Faine et al., 1999). This is also the reason why in the very early stages of infection the predominant titre may appear to be to a strain other than the infecting serovar. Cross-reactions are more common in closely related serovars however, as the infection progresses, the false titre readings drop and are replaced by higher titres to the infecting strain. This highlights the importance of paired sera to demonstrate a rising titre for diagnostic purposes in early stages of infection.

Age is another factor influencing the serological response with experimental results suggesting a tendency for cross-reaction to decrease with increasing age of calves, thus correlating with a maturation of the immune system (Fennestad, 1963). It is not possible to distinguish between the genetically and serologically very closely related serovars Hardjobovis and Balcanica (NZ

possum variety). Cattle act as an incidental host for Balcanica and therefore, unlike Hardjobovis, the infection is not spread amongst the herd highlighting the advantage offered by interpreting titres in the herd setting under certain circumstances.

9.5 Humoral immune response

Immunoglobulins are usually produced 2-10 days after the onset of infection, dependent on the animal species and the individual's immunological competence, the infecting serovar and the infective dose (Faine et al., 1999). IgM antibodies are generally the first to be produced and are later replaced by IgG1 titres from about two weeks onwards (Faine, 1982). The level of antibody response is variable depending on the genetic background of the individual, the species and age of the animal and on the infecting serovar: host-adapted strains such as Hardjobovis in cattle tend to induce lower levels of antibody response in the host that can make interpretation of titre data to this serovar difficult to interpret. In situations where leptospirosis circulates in herds, animals with antibodies may get anamnestic boosting sufficient to prevent re-infection but insufficient to register as a significant rise in titre level. This can lead to the pattern shown in Figure 9.1a: the peaked distribution is typically found in maintenance host-adapted *Leptospira* serovars *e.g.* Hardjobovis in cattle, sheep and deer. The serovar is maintained in a mixed-aged herd/flock. The peak antibody titre settles at a median as a result of antibody decay and rate of new infection following natural challenge. On the other hand, regressing titre distributions are more typical of the tail-end of an incidental infection in a non-maintenance host. The pattern *for* Pomona in cattle, deer and sheep (Figure 9.1b) suggests that such incidental infections with Pomona seem to occur frequently in these species. *Leptospira* can be shed intermittently by chronic carriers for lengthy periods of time (Flint and Liardet, 1980; Hancock et al., 1984; Smith et al., 1994; Ayanegui-Alcerreca, 2006) or for life (Ellis et al., 2000) and can even be isolated in urine from hosts that demonstrate little or no detectable titre (<1:24) when titrated against the infecting serovar (Mackintosh et al., 1980; Faine et al., 1999; Dorjee et al., 2009).

Whether the infection is natural or artificial or, whether the antibodies are a response to challenge rather than vaccination can also influence the immunological response. For example, the route of artificial challenge (*e.g.* intraperitoneal, intramuscular, conjunctival or nasopharyngeal mucosa) and the infective dose may have a bearing on the serological response (Fennestad, 1963). However, it should be noted that some artificial methods of infection e.g. intraperitoneal via syringe, can guarantee a more uniform challenge than the conjunctival/nasopharyngeal route where blinking and sneezing by the animal can affect actual dosage: a smaller dose taking longer to establish signs of disease. The antibodies raised to infection are specific with cross-protection to re-infection only afforded by the same or very closely related serovars (Faine et al., 1999) *e.g.* Hardjoprajitno and Hardjobovis, and an important factor when considering vaccination practices. It is these agglutinating antibodies that have been used extensively as a measure of exposure, diseases status in animals and vaccine efficacy and have been used as a proxy for protection. Variant strains of the same serovar may also illicit slightly different serological reactions *i.e.* some produce a greater degree of reactivity with MAT (Collins-Emerson, JM., personal comm.). It is suggested therefore, that local strains of *Leptospira* be considered for use in MAT and for vaccine production.

Of the three major livestock species of interest in New Zealand, it is cattle in which the majority of experimental trials and hence experimental data exist and many of these trials involve artificial challenge and vaccination. The response to challenge, whether artificial or natural will be discussed in this section with vaccination titres being covered later in the report.

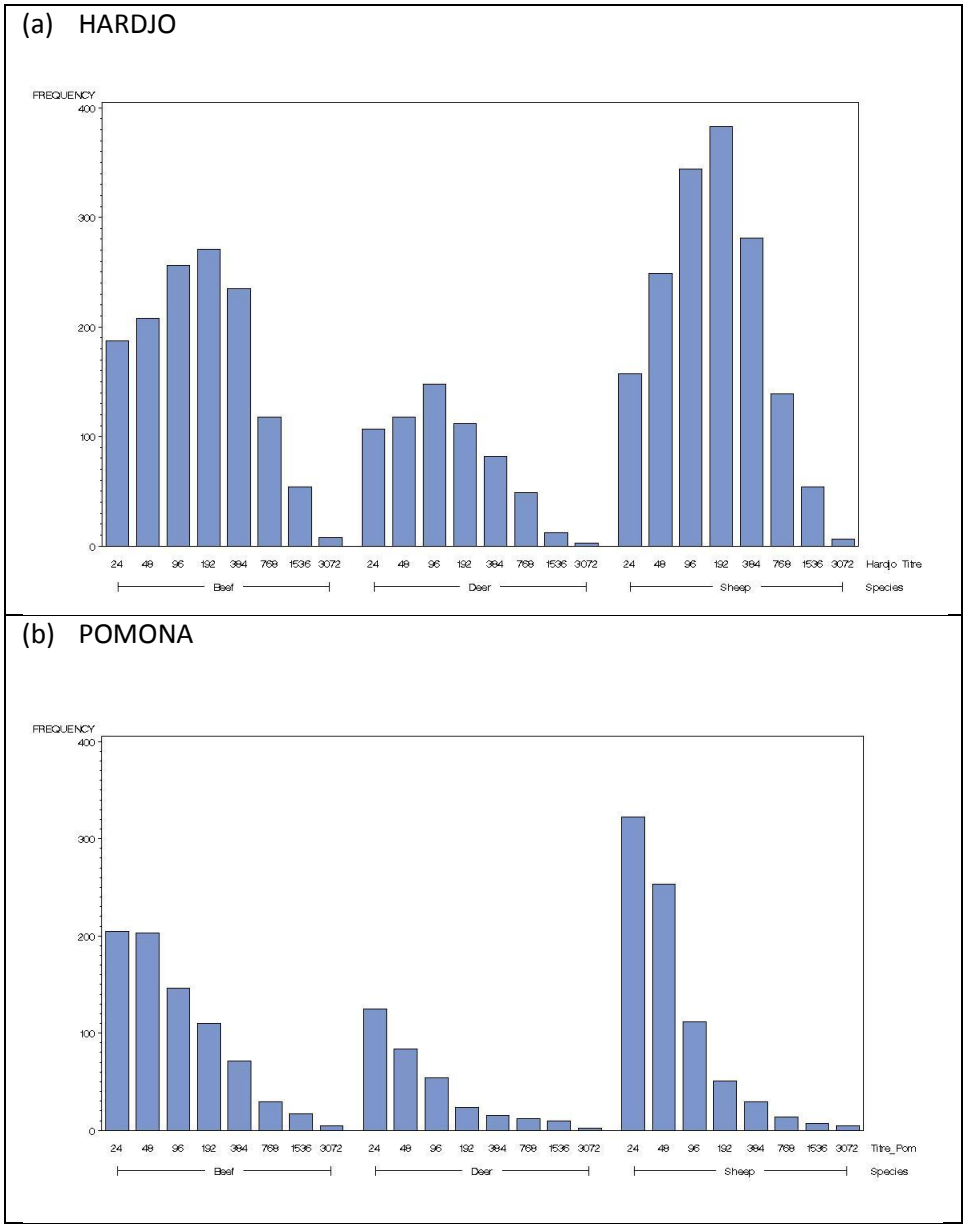

Figure 9.1: Frequency distribution of MAT titres to Hardjobovis (a) and Pomona (b) from samples from mixed age breeding stock from 116 beef, 98 deer and 161 sheep farms.

Titres in natural infection usually peak somewhere between a week and two months after infection (Fennestad, 1963; Dixon, 1983; Smith et al., 1994) then gradually decline however, the peak can vary considerably in magnitude (1:3200 to undetectable in cattle with Hardjobovis infection, (Carter et al., 1982; Dixon, 1983; Smith et al., 1994). There is a tendency for younger animals to develop higher titres than older ones (Fennestad, 1963). As titres to Hardjobovis may not attain high levels, determining an animal's infection status or distinguishing between infection versus vaccination titres, is not always straight forward. Vaccination titres can be lower than those induced by infection (Kiesel and Dacres, 1959; Strother, 1974). Serovars causing more severe infections *e.g.* Pomona and Copenhageni, tend to produce higher titres than Hardjobovis infections (Carter et al., 1982; Faine et al., 1999; Ayanegui-Alcerreca, 2006). It should be noted that serological data from many earlier overseas studies were reported prior to the realisation that serovar Hardjo comprised two different organisms *i.e. L. interrogans* serovar Hardjoprajitno and *L. borgpetersenii* serovar Hardjobovis and that interpretation of results is thus complicated as the two serovars may not behave in exactly the same manner. In New

Zealand, Hardjobovis antibodies were found to gradually decline over a period of 11 months (Dixon, 1983).

During acute infection leptospires can cross the placental and invade the fetus (Faine, 1982) and it has been demonstrated that bovine (Fennestad and Borgpetersen, 1962; Ellis et al., 1978) and ovine fetuses (Kirkbride and Johnson, 1989) that are sufficiently mature are able to produce antibodies. This means that calves/lambs may be born with pre-colostral titres to *Leptospira*.

9.6 Maternally-acquired Immunity

The placental structure in ruminants means that there is no transfer of protective antibodies across the placenta but that passive transfer is accomplished by ingestion of colostrum shortly after birth (Stelwagen et al., 2009). Passive immunity is achieved in the first couple of days after birth whilst the gut of the newborn is still permeable to the large immunoglobulin molecules in the colostrum. The permeability of the gut decreased rapidly thereafter. Calves develop peak titres around 11 hours after birth and a single feed of colostrum is required to achieve this (Hellstrom, 1978). Not all calves demonstrated antibodies in this trial although their dams were seropositive. However, when colostrum from a seropositive dam was fed to a calf that was seronegative 16 hours after birth, that calve seroconverted suggesting its initial seronegative status was due to unsuccessful suckling at birth. The duration of the maternal colostral antibodies in the neonate and the protection offered is dependent on the size of the initial titre and how long antibodies survive in the body before being catabolised. The half-life of IgG1 was estimated by Nielsen et al., (1978) to be around 18 days. The predominant immunoglobulin in colostrum is IgG1 (Stelwagen et al., 2009) and in cows is transported from the blood into the milk via the mammary gland peaking 2 to 3 weeks before parturition (Salmon, 1999). This time factor needs consideration when vaccinating dams with the aim of conferring maternal immunity to the young. With the transport into the mammary gland comes a corresponding drop in titre levels in the dam recovering to pre-calving titres about three weeks after calving (Hellstrom, 1978). Initial titres in their calves are usually higher than those in their dam (Hellstrom, 1978).

The degree of protection offered, as measured by MAT, and the duration of immunity of maternally-derived antibodies varies between studies but mostly wanes by six months of age in cattle (Hellstrom, 1978). The degree of protection offered the young is also dependent on farming practices. Young calves sent for off-farm grazing were found to be infected on their return to the main herd at about 6 months of age (Pegram et al., 1998) and infection could be found in deer as young as three months of age (Ayanegui-Alcerreca, 2006).

9.7 Cell-mediated immunity

The weight of experimental evidence supports antibody production as the primary immune response to infection. In cattle where Hardjobovis infection is widespread and usually chronic, there is also supporting evidence that cellular immunity may play a role in long term protection from re-infections with that particular serovar (Adler and De la Peña Moctezuma, 2010). However, with other species there is very little experimental data on the role of cell mediated immunity and the situation remains unclear. High antibody titres have not necessarily been shown to be protective in cattle yet, cattle that have negligible or undetectable antibody titres to Hardjobovis after vaccination have been demonstrated to be immune to infection (Adler and De la Peña Moctezuma, 2010).

Experimental work has demonstrated cell-mediated immunity (Type 1 immunity) is likely to play a role in the long term protection to infection or re-infection in cattle. This being an alternate

pathway to that of antibody production in the humoral system, it is not inconsistent with the observation of animals with low MAT antibody titres still demonstrating protection against infection. The majority of work has been carried out in cattle in vaccination trials. Like MAT titres, the magnitude of response does vary between individuals but, there is a pronounced difference in the response between groups of animals that experience natural infection versus vaccination: Animals receiving vaccine demonstrated a more pronounced response (Naiman et al., 2002) which peaked at two months after the second dose of vaccine (Naiman et al., 2001). In the presence of antigen, CD4 T cells in vaccinated cattle were demonstrated to manufacture IFN-γ, which itself was produced by the T cells; $CD4^+$ αβ and $WC1^+$ γδ (Naiman et al., 2002; Blumerman et al., 2007). The *in vitro* memory response of these γδ T cells was reported to be maintained for one year at a minimum whilst with some animals this was out to two years in the absence of any boosting (Blumerman et al., 2007). IFN-γ activates macrophages and promotes IgG2 immunoglobulin (Naiman et al., 2002). Under laboratory conditions, $CD4^+$ αβ T cells were also shown to be necessary for good γδ T cells response (Blumerman et al., 2007). Although Natural Killer (NK) cells are known to be involved with the innate immune system, *ex vivo* experiments conducted by Zuerner *et al.*, (2011) demonstrated that NK cells carrying the CD355 marker and sourced from vaccinated animals showed an IFN-γ recall response when exposed to *L. borgpetersenii* serovar Hardjobovis. NK cells from the non-vaccinated control animals failed to illicit such a response. This experiment provides support for NK cells having an immunological memory and playing a role in the acquired immune response.

9.8 Conclusions

The host's primary response to a leptospiral infection is humoral and the antibodies produced are specific for the infecting serovar. Thus there is little, if any, cross-protection from subsequent infection with a different serovar. Longevity of protection by these antibodies is dependent on the initial titre peak and hence duration of detectable titres, the serovar and the degree of challenge in subsequent exposure. In the case of Hardjo infections in cattle, cell-mediated immunity may also play role in protection. In new-born ruminant animals, the primary mechanism for protection against leptospiral infection is effected by the passive transfer of maternal antibodies against *Leptospira* in the colostrum: the level of antibody protection, as measured by MAT, is dependent on the antibody level in the dam. Experimental data suggest that MDA has waned by six months of age and in a high challenge scenario, many calves on a farm will be susceptible to infection at an earlier age.

10. Evidence of vaccine efficacy to prevent urinary shedding

The antibody response following vaccination has been measured in most trials of vaccine efficacy. However, given the described uncertainty surrounding the relationship between serological response as measured by the MAT and protection against challenge, in this section the focus is on the evidence of vaccine efficacy to prevent shedding of leptospires.

Appendix II summarises results of vaccine efficacy studies from the published literature. For completeness, evidence from serological data is included in the summary of measures of efficacy. Although additional unpublished data exist from small trials carried out by the pharmaceutical companies to support safety and efficacy claims for product registration, these are in the form of brief internal reports or summary information. They have not been peer-reviewed and are thus not included in the appendix. However, where relevant, their findings are discussed below.

Published studies are difficult to compare directly, as they include natural and experimental infection challenges and vary in dose of challenge and in the leptospiral serovars used in the vaccine and for challenge. Additionally, there are differences in age at vaccination, interval between vaccination and challenge and method of measuring and quantifying leptospiral shedding. Route of challenge also varies between the studies: intravenous, intraperitoneal, conjunctival, oral and intranasal methods of delivering a challenge dose have all been applied in the studies reported here. However, recent OIE guidelines recommend "immunity should be tested by challenge with virulent field strains of each serovar by natural routes of infection, i.e. by conjunctival and/or vaginal challenge" (OIE, 2008).

The vaccines administered in the reported trials range from mono- to multivalent preparations and use different adjuvants and strains of the organism, although often in the literature there is little detail on the vaccine preparation itself.

Key findings of some of the individual trials that measured efficacy of vaccine to prevent urinary shedding are briefly summarised here.

10.1 Vaccination challenge trials (cattle)

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

Ris and Hamel (1979) assessed a commercial monovalent Pomona vaccine (A) and experimental Pomona vaccines prepared with different adjuvants (B and C) in three groups of four 9-month-old heifers, comparing them to a control group of four heifers. Experimental challenge was by the intramuscular route 47 weeks later. Urinary shedding of leptospires, as assessed by culture, was prevented in all of the vaccinates but detected in all of the controls. Several studies reported efficacy of vaccines using a Pomona strain for challenge (McDonald and Rudge, 1957; Gillespie and Kenzy, 1958a; Kiesel and Dacres, 1959; Stalheim, 1968; Strother, 1974; Marshall et al., 1982). The key aspects of the studies and findings are summarised in Annex II.

Studies in cattle have similarly examined the efficacy of vaccination with a monovalent preparation of serovar Hardjobovis or Hardjoprajitno to prevent urinary shedding of leptospires. In a US study, Bolin et al. (2001) vaccinated two groups of eight 8-12 month old heifers with two different monovalent Hardjobovis vaccines - a commercially available vaccine (A) and a reference vaccine (B) - keeping a third group of eight heifers as controls. The heifers were experimentally challenged four months later with a US strain of serovar Hardjobovis, by conjunctival instillation or intraperitoneal inoculation. Vaccine A was shown to prevent urinary shedding and renal colonisation in 8/8 heifers. In contrast, all heifers inoculated with vaccine B were urine and tissue positive. The study also showed differences in shedding outcomes between the different routes of leptospiral challenge. Challenge via the conjunctival route resulted in leptospiruria in 4/4 controls compared to 2/4 controls challenged intraperitoneally. Leptospires were identified in the kidneys of all controls.

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

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

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

10.2 Vaccination after exposure

The majority of natural and experimental exposure studies have examined the efficacy of vaccination when administered before challenge. However, one study (Hancock et al., 1984), in which cattle already shedding leptospires in urine were vaccinated, was identified. A group of 19 two-year-old heifers, 9 (47%) of which were leptospiruric, was vaccinated with a single dose and results of urine culture 22 weeks later were compared to those from a control group of 22 heifers, of which 15 (68%) were initially leptospiruric. In the vaccinated group, 4/15 (27%) were leptospiruric 22 weeks later, compared to 4/9 (44%) of the controls. No explanation is given in the manuscript to account for the loss of four of vaccinates and seven controls to follow-up, other than a statement that all animals were not made available during the sampling periods. More information would have been useful, for example to confirm that the animals were still in the herd or whether some animals may have been culled due to reproductive failure. Nevertheless, the findings supported the overall conclusion of the study that there is no

evidence that use of vaccination in already infected cattle is effective in preventing urinary shedding.

10.3 Age at vaccination (cattle)

One of the earliest published trials of vaccine efficacy examined the efficacy of three vaccine preparations when administered to three different age groups of cattle (Gillespie and Kenzy, 1958a). Each preparation contained a different bacterin of a killed suspension of serovar Pomona and experimental challenge, using urine prepared from shedder cattle, was by the conjunctival and intranasal routes as well as by contamination of drinking water. Each age group of animals (1-2 months, 3-5 and 6-8 months of age) was matched with a non-vaccinated control group of the same age. A difference in response to vaccination, as measured by the successful culture of leptospires from urine, was seen between the animals vaccinated at 1-2 months (Group A) and 3-5 months (Group B) of age when compared to those vaccinated at 7-8 months of age (Group C). Combining results of the different vaccine preparations together, leptospiruria was confirmed in 5/9 vaccinates and 5/6 controls, 4/8 vaccinates and 3/3 controls and 1/12 vaccinates and 4/5 controls in Groups A, B and C respectively. Leptospira were determined at a semi-quantitative scale, with the authors concluding that 'vaccinated cattle that did excrete leptospires in the urine often shed appreciably fewer than the controls'. While the older (Group C) animals were known to come from infection-free herds, the immune status of the calves was known only with respect to MAT titres, as they were sourced through a local stock buyer. Nevertheless, in this trial the Pomona vaccines appeared more efficacious against urinary shedding in older than younger animals.

Schollum and Marshall (1985) examined the serological responses of ten initially sero-negative three-month-old calves to vaccination with a commercial bivalent Hardjo/Pomona preparation. MAT titres to Pomona and Hardjo were compared to those from 35 calves vaccinated at six months of age. Thirty percent of calves vaccinated at the younger age had a positive MAT titre (1:24) to Pomona while 40% were MAT positive to Hardjo. In contrast, 94% and 86% of the animals vaccinated at 6 months old were MAT positive to Pomona and Hardjo, respectively. It was concluded that vaccination at 3 months of age caused a poorer immune response than vaccination at 6 months. However, there was no challenge to allow comparison of the efficacy of vaccination at either age to control urinary shedding, nor were there control groups in this study, hence there was no information of natural challenge in those mobs that could have contributed to higher antibody in the calves vaccinated at the higher age. As previously discussed, data on serology alone allows limited inference to be made on the protective efficacy of vaccination.

By contrast, in a challenge trial designed to develop a guinea pig potency test, Goddard et al. (1986) vaccinated groups of calves aged 12-28 days with graded doses of field vaccines, challenging the vaccinates and a control group of five calves intravenously with serovar Hardjo. Using the vaccines at full field dose, 0/10 calves were kidney culture positive compared to 5/5 controls.

Results from three studies carried out to assess the efficacy of vaccination in calves from four weeks of age was reported in a conference abstract (Gallo et al., 2010). Thirty-one MAT negative month-old calves were vaccinated and given a booster 4-6 weeks later with a Hardjo vaccine while 19 remained as unvaccinated controls. Challenge with serovar Hardjo was by the conjunctival route at three weeks or 12 months after the initial booster vaccination, or four months after a 12-month booster vaccination. No vaccinates were leptospiruric or positive to culture of kidneys 7 weeks after challenge, while all controls were urine or kidney culture positive.

The above studies were carried out in animals with no detectable MDA. These studies demonstrate that, in the absence of MDA, vaccination against *Leptospira* is effective as early as 4 weeks of age. The effect of the potential interference of MDA on vaccination response in young animals is discussed specifically in section 9.5.

10.4 Vaccine efficacy in sheep and deer

A single sheep study (Marshall et al., 1979a) was identified by the systematic literature search. The research was carried out in New Zealand, and involved 19 Romney ewes aged 7-9 months. Nine were vaccinated twice, one month apart, with a bivalent Pomona/Hardjo preparation and 10 remained as untreated controls. Challenge six weeks later, with a bovine isolate of serovar Hardjo, was by the intraperitoneal or the intramuscular route, while infection status was established by culture of kidneys at post-mortem three weeks after challenge. Two vaccinates and all controls were *Leptospira* positive. A notable additional finding from this study was that two of the vaccinates which resisted challenge showed no MAT response to vaccination, and two with titres rising from 24 to 96 between weeks 12 and 13 were culture negative. Although demonstrating the efficacy of vaccine to prevent kidney colonisation, the short timescale of the trial meant the study does not provide any evidence of the duration of vaccine induced immunity in sheep.

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

10.5 Vaccination of dams to protect offspring

Protection of calves in their first month of life by vaccine-induced maternal antibody has been demonstrated in cattle in New Zealand (McDonald and Rudge, 1957). The study involved 26 calves whose dams had been vaccinated in the last 2 months of pregnancy and 20 control calves. The calves were experimentally challenged with serovar Pomona at 10 days (Experiment 1) and 4 weeks old (Experiment 2) and monitored for leptospiruria by dark-field microscopy, over the following 6 weeks. In Experiment 1, 0/11 calves from vaccinated dams and 5/10 control calves, and in Experiment 2, 1/15 calves from vaccinated dams and 7/10 control calves were leptospiruric. The trial suggested that calves tended to remain resistant to infection up to 4 weeks of age due to MDA induced by vaccinating dams.

A study in which one group of calves from vaccinated dams were monitored for Hardjo MAT titres from birth found evidence of maternally-derived antibody persistence until 12 weeks of age (Palit et al., 1991). The study was not designed to demonstrate protection from challenge *per se* in calves born from vaccinated dams, thus limiting the inference on the longevity of protection via passive immunity that can be drawn from these data.

The optimal timing of dam vaccination for calf protection was assessed in a report by Virbac New Zealand Ltd. (Pulford, 2006), using results from an in-house study that showed lower levels of MAT Hardjo titres in new-born calves of dams vaccinated less than 50 days compared to more than 50 days before calving. Combining these results with additional serological data from new-born calves of dams vaccinated between 54 and 90 days pre-calving led to the conclusion that, for optimal calf immunity, dam vaccination should be carried out at least 70 days before calving. Note, however, that these studies were again based only on examination of serum antibody levels, rather than protection from challenge.
The significance of the timing of dam vaccination, however, lies in the potential for persistent MDA in calves to influence the response to leptospirosis vaccination (see 9.3(ii)).

10.6 Interference by maternally derived antibody (MDA) with vaccination

As described earlier, the presence of serovar-specific antibodies measured by ELISA or MAT indicates that exposure had occurred at least one week prior and that resistance to new infection is likely. However, the absence of such antibody does not indicate that an animal is susceptible (non-resistant) as challenge studies suggest (Marshall et al., 1979b). The following review should be read with this caveat in mind.

Maternally derived antibodies (MDA) are potentially present in offspring either when there is **(i) a high level of natural challenge** (Hellstrom, 1978), or when **(ii) the dam has been vaccinated** (Ayanegui-Alcerreca, 2006). MDA potentially interfere with vaccination (Ankenbauer-Perkins, 2000) but evidence about live-vaccines exists to the contrary, suggesting that vaccine efficacy may NOT depend on an absence of MDA (Woolums, 2007). However, this inference may not be equally valid for killed vaccines such as all currently available leptospirosis vaccines. Thus vaccination of offspring in the presence of MDA may (or may not) reduce vaccine efficacy.

(i) Following a high level of natural challenge, the decay of naturally acquired maternal MAT antibody in calves from a population of dams at high endemic level of *Leptospira* sv. Hardjo and Pomona was described in detail by Hellstrom (1978). Over 90% newborn calves acquired MDA after suckling sero-positive dams. Titres declined with a half-life of 15-17 days, most calves were MAT sero-negative at 100 days of age, and all were negative at 190 days. This was equivalent to a decay rate of 3.5% per day. Calf cohorts were followed until new infections were detected by MAT resulting in most infections at about 12 months of age (Hellstrom, 1978). New, natural infection resulted in high titres within 14 days of exposure which decayed down to 38% in the first, to 11% in the second year after exposure, and by 5% per year thereafter.

CATTLE: If a continuously-high natural challenge of new-born calves is assumed, under these circumstances, the proportions of susceptible, MDA-protected and infected calves from birth to two years of age would be as shown in Figure 9.2 (upper). The model assumes that colostrum acquired MDA decay to 100 days of age by which most calves are susceptible and that most infections occur from about 100 – 200 days with 20% being infected at about 3.5 months of age. Consequently, the peak of susceptibility, and therefore most vulnerable time for infection would be around 40 – 110 days of age. Assuming that MDA negatively affects vaccine efficacy, vaccination would be scheduled at about 60-90 days for optimal efficacy. However, the true susceptible age may be somewhat later: Hellstrom's data (1978) indicated that calves were resistant to experimental challenge up to 3 months after the loss of MDA-induced MAT titres.

In endemic environments where MDA is assumed to be present, calves appear to be susceptible to infection as early as 3 months of age. In an observational study on age at first natural challenge, MAT titres of 1:96 and higher in 13 calf mobs of 11 vaccinated dairy herds were evaluated (Pegram et al., 1998). The calves were sampled before 6 months of age and had not yet been vaccinated. Titres were too high to be considered due to maternally derived antibodies

(MDA) and calves were considered too old (>3 months) to still have MDA. The natural challenge was suspected to have occurred in mobs while grazing at run-offs at external farm locations. No antibodies were found in age-equivalent calves at the home farm (Pegram et al., 1998). A 1980 study, when screening calves for a vaccine efficacy study, found five 6-month-old calves that were already shedding leptospires in urine before first vaccination (Flint and Liardet, 1980).

New data from a small scale observational study tend to indicate that herds vaccinating dairy calves for the first time before the age of 3 months experienced a lower rate of shedding in adult cows than did herds that vaccinated calves after 3 months of age (Parramore et al., 2011). These data require confirmation, as the age of vaccination was based on the recall of farmers, not actual observation. If confirmed, the data suggest that calves were naturally exposed as early as 1-3 months of age even though whole-herd vaccination had been practiced annually for several years.

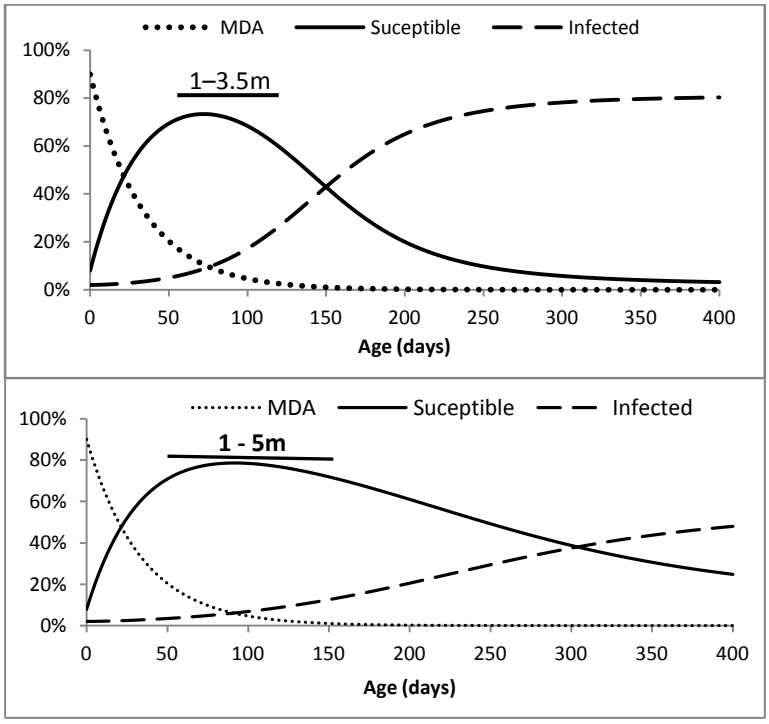

Figure 9.2: Theoretical high (upper) and low (lower) challenge scenarios (2 vs 1 Table 9.1) – expected proportions of calves susceptible and infected assuming decay of MDA (Hellstrom, 1978) and a 2-fold higher (upper) than reported (lower) (Hellstrom, 1978) natural challenge of 0.1-1.5% of susceptible calves getting infected per day: MDA decay to 100 days by which most calves are susceptible; >20% infected from 107 days onwards; most susceptible can be expected at about 1-3 months of age before natural infection takes off.

DEER: Based on sero-prevalence of calf cohorts, Ayenegui-Alcerreca (2006) reported natural exposure of deer earlier than 3 months of age on one farm, and later than 6 months on another farm. It was concluded that environmental conditions as well as MDA-decay determined the age at which animals were infected.

SHEEP: On a farm that experienced a clinical leptospirosis outbreak following summer floods in 2004, 100 lambs, 100 2-tooths, and 100 ewes were followed serologically for 3-12 months with samples taken in 2-months intervals (Dorjee et al., 2005). In the presence of natural challenge in 2-tooths and adult ewes, lambs did not markedly sero-convert until after 10 months of age (Figure 9.3).

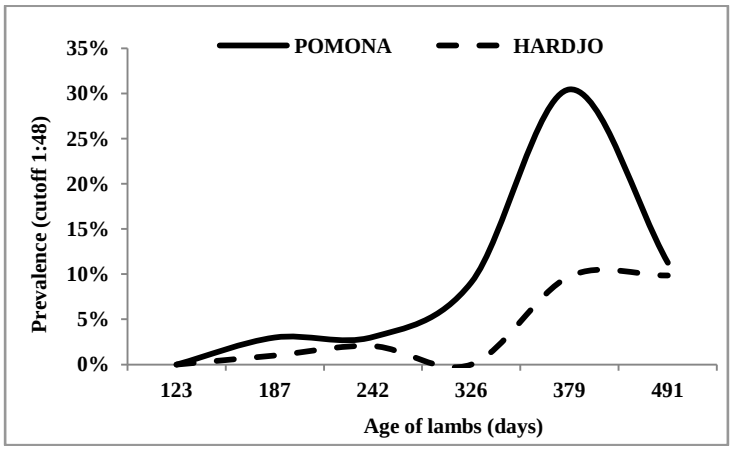

Figure 9.3: MAT sero-prevalence of Hardjo and Pomona in lamb cohorts born after an outbreak of clinical leptospirosis (Dorjee et al., 2005)

Considering that natural challenge of offspring may occur rather sooner or later, the scenario of Figure 9.2 was modified to compare the impact of a fast or slow MDA decay and high or low infection pressure on the time that at least 50% offspring are susceptible and the time until 20% young stock were infected (Table 10.1): if Hellstrom's (1978) MDA decay reflected true loss of resistance, a low infection pressure would provide a time window of 24 – 159 days during which most animals would be susceptible and less than 20% were infected, i.e. indicating the *"optimal vaccination window"*. At high infection pressure, this window would decrease to 24 – 107 days. If MDA-induced resistance lasted twice as long as indicated by MAT titres, the grace period before vaccination could start would reduce to 47 days regardless of infection pressure, and the maximum time until which the first two vaccinations would have to be completed would be 207 days at low and 139 days of age at high infection pressure. Thus the most critical scenario would call for vaccination to begin near the age of 1.5 months and for application of the booster by 3 months of age at latest.

Table 10.1: Model scenarios of two periods of MDA protection against infection (100d, 200d) and two levels of infection pressure (low, high) impacting on the proportion infected (time to 20% infected) and the duration of having more than 50% animals susceptible, hence responding to vaccination assuming MDA interfere negatively with vaccine efficacy.

Scenario	MDA decay	Infection pressure	Time to 20% infected	>50% Susceptible
1	95% to 100d	Low	159 d	24 – 204 d
2	95% to 100d	High	107 d	24 – 137 d
3	95% to 200d	Low	207 d	46 – 241 d
4	95% to 200d	High	139 d	47 – 161 d

(ii) For circumstances where the dam has been vaccinated, a systematic search of the published literature identified only one challenge study (Palit et al., 1991) which had the specific aim to assess the influence of maternal antibody on the efficacy of vaccination to prevent urinary shedding. The study involved calves born from initially sero-negative cross-bred beef/dairy cows vaccinated pre-calving with a bivalent Hardjo/Pomona product. Four groups of three calves were vaccinated at 4, 6, 10 and 18 weeks old, given a booster 4 weeks later and challenged with serovar Hardjo by the intraperitoneal route 10-24 weeks after the second

vaccination. A control group of seven sero-negative calves from unvaccinated dams were similarly challenged at 20 weeks old. None of the vaccinates were found to be leptospiruric or kidney positive as assessed by culture, while all controls were leptospiruric by 35 days after challenge. The authors concluded that 'calves as young as 4 weeks of age may be effectively vaccinated against serovar Hardjo in the presence of circulating maternally-derived antibody'. An additional finding was that the post-vaccination rise in MAT titre was inversely proportional to the pre-vaccination titre.

However, when assessing this study as evidence of the effect of MDA on vaccination the following points should be considered:

- MAT titres in the calves vaccinated at 4 weeks old were relatively low (given that the MAT antigen strain was identical to the strain contained in the vaccine), specifically 4, 32 and 64 in the individual calves
- The route of infection was not a natural one
- The very small numbers observed (three in each group)

An internal report (Ankenbauer-Perkins, 2000) also considered the role of maternal antibody in vaccine efficacy. Three groups of 10 calves from vaccinated dams were involved, with two groups vaccinated with a bi-or tri-valent preparation (Hardjobovis, Pomona +/- Copenhageni) at three to five weeks of age. Vaccinated calves were given a booster four weeks later, while a third group was maintained as unvaccinated controls. The calves were selected for the study based on high serum IgG concentrations (>1000mg/dL) at 4-10 days old, as a measure of successful maternal antibody transfer. Challenge with serovar Hardjobovis was by the intranasal and conjunctival route 12 weeks after the second vaccination, with 40% of each group found to be leptospiruric by urine culture. The authors concluded that 'the vaccination of three to five week-old dairy heifers provided insufficient immunity against subsequent challenge.'

Again there are key points to consider:

- The challenge model had limited success, with a 40% infection rate in controls
- The calves were specifically selected for high MDA and thus represent calves with sufficient colostrum intake which may be up to 50% of the newborn calves in a dairy farm (Wesselink et al., 1999).
- The outcome of this challenge experiment may be explained by MDA preventing shedding in vaccinated and control calves equally, and that vaccine interfered with MDA. In a trial with 40% shedders in two groups of 10 however, this explanation cannot be differentiated from chance.

These two studies, with their conflicting outcomes, do not provide sufficient evidence on which to base conclusions on the effect of MDA on vaccine efficacy. Such evidence would best be obtained by longitudinal field studies, in naturally infected herds, to examine more closely the interplay between the timing of first challenge, loss of maternally-derived protection and response to vaccination in young calves.

According to Hellstrom (1978), soil moisture rather than rainfall *per se* was a strong determinant for the rate at which new infection occurred. *Leptospira* survived 6 weeks in acidic soil (pH=5.5) under simulated Manawatu winter conditions. New infections were regarded to be entirely due to contact with other cattle on pasture, independent of the presence of infected wildlife.

The titre decay after infection was described by Adler et al. (1982) through experimental inoculation of 8 month old susceptible heifers. Antibodies determined by IgM-Elisa, IgG-ELISA,

and MAT (measuring both IgM and IgG) started at zero, peaked about 7-10 days, held level until about 28 days, and were negative again by 90-100 days after inoculation.

10.7 Duration of immunity

The immunity elicited by leptospiral vaccines has been shown in a number of studies to persist for up to 13 months in cattle (Table 9.2). Duration of immunity has been demonstrated directly by studying resistance to infection following experimental (McColl and Palit, 1994; Ellis et al., 2000) or natural challenge with Hardjo strains (Marshall et al., 1979b; Flint and Liardet, 1980; Mackintosh et al., 1980; Hancock et al., 1984), or indirectly by methods such as hamster passive protection (Virbac tech review[6]). Although MAT titres to Pomona and Hardjo are often reported in longitudinal studies of vaccine efficacy, with MAT titres in cattle shown to wane by 6 months post-vaccination (Mackintosh et al., 1980), the relevance of titre persistence as a measure of protection against challenge is questionable.

Indeed, the OIE (2008) recommends 'Duration of immunity should not be estimated based on the duration of MAT titres in vaccinated animals as protection against clinical disease may be present with very low titres.'

Mackintosh et al. (1980), for example, demonstrated protection against urinary shedding in the face of natural challenge with Hardjo at 56 weeks post-vaccination, when the animals were sero-negative. Similarly, Zuerner et al. (2011) demonstrated that young cattle (n=15) experimentally challenged with Hardjo by the conjunctival route 12 months after vaccination, when MAT titres were almost zero, were protected against urinary shedding of live leptospires, as measured by culture, while 7/7 controls were urine culture positive. The vaccinated group had a background MAT mean serum titre of 1:100 at the time of challenge, compared to a peak of 500 at four weeks post-vaccination.

Table 9.2: Evidence for duration of vaccine induced immunity, by challenge type, serovar, age at first vaccination, and source.

Challenge	Serovar(s)	Age at first vaccination	Duration of protection*	Reference
Experimental	Hardjo	4-5 months	48 weeks	(McColl and Palit, 1994)
Experimental	Hardjo	BW≥300 kg	12 months	(Ellis et al., 2000)
Natural	Hardjo	6-12 months	12 months	(Flint and Liardet, 1980)
Natural	Hardjo	3-4 months	7 months	(Marshall et al., 1979b)
Natural	Hardjo	10 months	56 weeks	(Mackintosh et al., 1980)
Natural	Hardjo	9-10 months	55 weeks	(Hancock et al., 1984)
Experimental	Hardjo	10 months	12 months	(Zuerner et al., 2011)
Experimental	Hardjo, Pomona, Copenh.	6 months	26 and 52 weeks	(Hamel, 1997)

*Number of weeks after vaccination that challenge occurred.

[6] http://www.virbac.co.nz

10.8 Conclusions on vaccine efficacy to prevent shedding

Conflicting evidence for the effect of MDA precludes robust conclusion about its influence on vaccine efficacy, and therefore the optimum timing of first vaccination. Titres of vaccine-induced MDA measured by MAT and possibly the extent of interference with calf-vaccination, may depend on the timing of dam vaccination prior to calving. It is therefore suggested that, in a low challenge environment including where whole herd vaccination is practised, MDA sufficiently high to interfere with vaccine may not be expected (at least in many animals) as long as dams had not been vaccinated shortly before calving. However, the high challenge environment currently present in NZ for sheep, deer and beef cattle, some interference between vaccines and MDA may exist if vaccination is commenced at a young age. The Ankenbauer-Perkins (2000) trial suggests that such interference can reduce vaccine efficacy. Therefore, early vaccination (2-6 weeks) of such species may only be advisable when a spot test of 1month old calves suggests that measurable antibody is low or absent. If it is present, an early course of vaccination may have to be boostered by an additional inoculation at 6 months of age. Duration of immunity against Hardjo challenge has been shown to last up to 13 months.

11 Required information (future research)

The literature review of aspects involved in potentially modifying vaccine efficacy has revealed that almost all studies were based on small numbers and were carried out under controlled conditions often with an extremely high challenge dose. Moreover, it was difficult if not impossible to compare studies investigating vaccination at different ages, with different *Leptospira* antigens, with different periods between vaccination and challenge, different challenge routes and with varying follow-up periods and measurements of efficacy.

This review found the crucial issue of optimal first vaccination age could not be resolved from reviewing the literature to date. We believe that the review was exhaustive in terms of the volume of published and accessible material, especially as several unpublished technical reports were included that would be impossible to access when tracing from publicly accessible databases. Such reports were kindly made available by commercial vaccine producers.

The review therefore identifies the following as research areas to address deficiencies and shortcomings in present knowledge of leptospirosis in livestock, with relevance to vaccination.

- **Vaccine efficacy and age for starting vaccination programmes:** There is a pressing need for large scale field trials of vaccine efficacy in dairy herds, comparing vaccinated with unvaccinated cows in conjunction with vaccinating calves at various ages (1, 3, 6 months) in endemic herds and flocks. This would provide more definitive evidence on the effect of MDA, which is of critical importance to determination of optimum start times for vaccination programmes;
- **Human studies:** As extensive investigations at abattoirs have provided important insights on potential human exposure, it is recommended that similar sero-prevalence studies in humans be conducted among veterinarians, farmers and other people who have frequent contact with animals (AITs, shearers, livestock truck drivers etc.). This would contribute further to the rationale for decisions surrounding vaccination;
- **Duration of immunity:** For such studies, a sufficiently long follow-up period (e.g. 24-36 months) should be used to demonstrate the duration of immunity. No information is currently available about shedding (protection) beyond 56 weeks post vaccination. This is of interest for aligning the timing of vaccination of calves to that of adults: when calves have completed their course of vaccination at 4-6 months of age (Jan-Mar), it would be desirable if seasonal herd managers were able to vaccinate the same animals

as replacement heifers 15 (rather than 3 or 12) months later at the same time as the adult herd.

- **Shedding in vaccinated dairy herds:** New results showing shedding in dairy herds should be followed up by testing cows serologically and again by PCR of urine to identify serovars associated with shedding, and to corroborate the preliminary findings of Parramore et al., 2011 (unpublished). A crucial question is whether PCR positive urine is infectious, i.e. containing live Leptospira at a sufficiently high dose;
- **Serovars in livestock.** Livestock need to be screened to confirm which serovars are present in livestock populations. For example, it is not known whether there has been an incursion of new serovar's since initial screening more than 30 years ago (e.g. tentative data for Arborea), or the potential for livestock to have become a spill-over host for Ballum, which could potentially explain recent human cases with that serovar. This could have additional implications for the serovars incorporated in livestock vaccines specific to the NZ environment.
- **Wildlife:** Little is known about the quantitative distribution of serovars in wildlife (feral pig, possum, rabbit, hedgehog, feral deer), in back-yard pigs and in rodents. Therefore, cross-sectional trapping studies should be implemented. This should be combined with use of recently developed molecular strain typing methods for investigation of between species (domestic and wildlife) transmission.
- **Ecology:** The distribution of *Leptospira* serovars in the environment can now be studied as new, semi-quantitative real time PCR tests are available. The repeatability of these PCRs for testing urine was confirmed. However, the RT-PCR has yet to be validated for testing environmental samples (water, soil etc). Once RT-PCR was validated, samples should be collected repeatedly in the four seasons from water and soils sources. This would identify infection sources for humans and animals.
- **Effect of interventions:** Together with the aforementioned information, a large body of technical data will be available about individual aspects of *Leptospira* in the environment and in various hosts and their interactions through transmission and vaccination. The question remains as to what effect interventions, e.g. control measures, have on the endemic equilibrium in human and animal hosts. Mathematical modelling can be used to simulate such interventions. For example, vaccination of cattle may/may not be sufficient to reduce transmission in sheep, and thereby indirectly decrease human exposure. Modelling can provide insight into the extent that vaccination may have to be carried out (vaccine take x proportion vaccinated) to achieve a desirable impact. Consequently, cost and benefits may be evaluated to inform decision makers at farm and industry levels about the return on investments.
- **Eradication of leptospirosis from New Zealand.** The above research projects would provide data for modelling the possibility of elimination of livestock-based serovars of leptospirosis from New Zealand. This would have long-term implications for vaccine use in future, and to developing strategies at a national level to manage the disease.
- **Vaccination in the face of an outbreak.** There are no data on the serological response to vaccination in the face of an outbreak (most likely to occur in sheep or deer). Opportunistic studies should be conducted in outbreak situations by looking at serovonversion in the face of an outbreak with comparison to known responses in the non-emergency situation. This would help develop guidelines for management of an outbreak.

12 Guideline for Best-Practice recommendations for vaccination

The review revealed that there currently exists a lack of sound, longitudinal data that can conclusively demonstrate the effect of maternal antibodies, derived from natural exposure or vaccination of dams, on the earliest age at which cattle or other species may effectively be vaccinated against leptospirosis. This is a significant limitation to being able to recommend Best Practice guidelines that fit all circumstances.

12.1 General principles for a vaccination programme: all species

NZVA's Leptosure® programme provides general guidelines for vaccination detailing which stock to vaccinate at what times of the year and how often. It also elaborates what additional biosecurity measures are required to achieve a high level of herd immunity.[7]

Concepts and principles applied to decision-making about vaccination are described in detail in deer (Wilson et al., 2009). There are no papers that discuss the principles of vaccination for other species in New Zealand. Nevertheless, the principles described in the above paper are applicable to other livestock species. There are recommendations for vaccination of dairy cattle given by vaccine manufacturers.

The decision on whether or not to vaccinate is the prerogative of the farmer alone. Hence, the farmer should be fully informed of leptospirosis, the risk factors for infection and disease, its health and production effects, its epidemiology, public health implications, means of control including vaccine and other measures, and economic implications. The veterinarian's role is to fully inform the farmer. This is to allow the farmer to evaluate the risk profile for the individual farm and set the objective/s for the programme. An additional risk factor to consider is the potential for litigation against farmers under Occupational Safety and Health legislation in the event of workers or family contracting the disease.

The farmer must establish goals and objectives of the farm in relation to leptospirosis vaccination, in terms of:

- clinical disease (sick animals/mortalities),
- subclinical disease (effect on reproduction and growth),
- human safety (prevention of human sickness).

i.e. determine what is expected of the vaccine.

The risk needs to be evaluated that leptospirosis may pose to the achievement of those goals and objectives. The veterinarian needs to identify the risk factors on the farm, including environmental (within farm and external, e.g. waterways), management and stocking policies.

Note: while serological testing may be useful in demonstrating that leptospirosis infection is on the property, it cannot be used to evaluate risk of cost-benefit of vaccination measured by animal productivity outcomes. This is because the level of infection in a herd changes over a period of time as environmental conditions become more or less favourable for the survival of *Leptospira*. Further, determining whether there will be an "economic benefit" of vaccination is impossible in advance since the incidence of infection cannot be reliably predicted, yet vaccination needs to be administered prior to the risk.

Hence, the question of whether or not to vaccinate can only be based on evaluation of risk rather than absolutes, and applied in relation to the risk-averseness of the farmer. The farmer

[7] http://www.leptosure.co.nz/sites/default/files/domain-18/Leptosure%20Green%20Farmer%20Booklet%202007.pdf

should be aware that other forms of reducing the risk attributable to leptospirosis are unlikely to be as effective as vaccination.

The latest age at which the first course of vaccination should be completed depends on the level of natural challenge. In high challenge situations or when farmers perceive that the risk of human exposure is high, cattle, sheep and deer should be vaccinated early, e.g. at 1 month of age. However, in low challenge situations, or when farmers perceive the human infection risk to be low, or when vaccination has been applied according to label for a number of years, young stock may be vaccinated later, e.g. at 6 months of age.

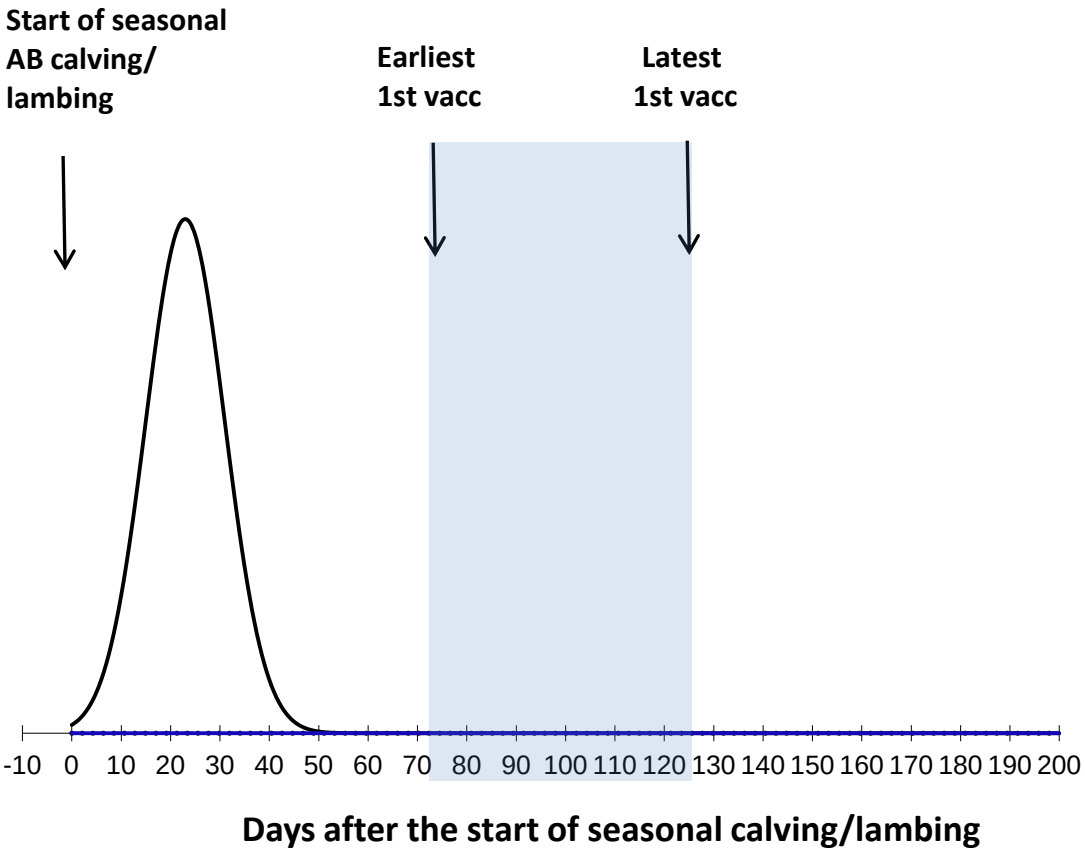

Figure 12.1: Recommended time window (72-125 days) for the first sensitiser vaccination of offspring replacements after the start of seasonal calving/lambing.

The recommended vaccination schedule is demonstrated in Figure 12.1: applying the first vaccination 7 weeks after the start of calving (day 0) ensures all calves are well over the age of one month when vaccinated for the first time. This is followed by a sensitisation approximately 4-6 weeks later (35 days in Fig. 12.1). In low risk situations (see below), the last opportunity for calves of any age to have completed a course of vaccination should be at six months after the actual start of calving. Note that the average age of calves at first vaccination is in the range of 49-115 days (7 – 16 weeks). In dairy herds, it may be advisable to vaccinate two calf-mobs at subsequent times, (i) AI-bred replacements, and (ii) sire-mated calves for beef production.

The general 'Best-Practice' vaccination guideline differentiates two situations, (i) a '*high risk*' vaccination, and (ii) a '*low risk*' vaccination. We then briefly consider the use of vaccines in an '*outbreak*' situation.

(i) 'High risk' vaccination

This situation applies when:

1. the farmer perceives that a high risk of human or animal exposure exists; or
2. confirmed clinical cases have occurred and/or serological data from young stock exist that objectively demonstrate a high level of challenge at an early age.

The review suggests an estimated first vaccination age of approximately 6 weeks followed by a booster at 3 months. In order to ensure maximum vaccine efficacy, animals should get a second booster at 6 months of age followed by annual re-vaccination in 12 months intervals. One vaccine producer recommends that the 6-months booster be another full course of 2-vaccinations in 4-6 week interval. No conclusive data currently exist either in favour or against this claim.

It is understood that deviations from this rule will be unavoidable. Due to variable times of lambing/calving and practicalities on-farm, a recommended age of 6 weeks (3 months) will effectively be a large range around this anticipated mean (or median) age.

(ii) 'Low risk' vaccination

In all other instances, it is recommended to complete the first course of two vaccinations when animals are in the age range of 4-6 weeks up to 6 months of age followed by annual whole herd vaccination (Figure 12.1).

(iii) Vaccination in an 'outbreak' situation

A leptospirosis outbreak is characterised by a sudden increase of clinical cases in a short period of time. In this situation, it can be considered that all animals were exposed and most got infected. Since leptospirosis vaccines have little effect in already infected animals, two steps are recommended. Firstly, clinical cases and animals suspected to be in pre-clinical state should be treated with antimicrobials. Secondly, the next offspring crop should be vaccinated early as described for animals in a 'high risk' environment, then annually in subsequent years until animals of all ages have been immunised.

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Annex I:

Note: these are guidelines only. Where these guidelines do not fit the circumstance on an individual property, a first-principles approach to vaccination decisions needs to be adopted.

Best practice recommendations – DAIRY

Stock classes: breeding and replacement stock, growers kept at home or sold to beef finishing farms. Vaccination of bobby calves is not required. Consider vaccinating calves in two mobs: AI bred replacements and bull-mated tail-end for beef.

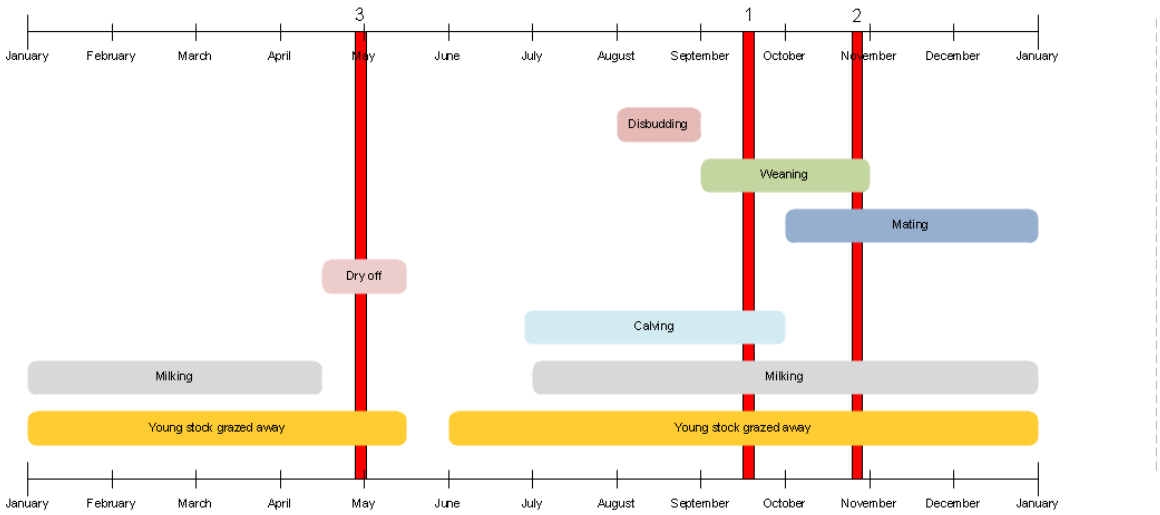

Figure 1: Timeline of farm management events in a representative New Zealand spring calving dairy herd with calves leaving in June: vaccination times superimposed as red vertical bars (see text below for details)

'High risk' vaccination

First course of vaccination (2 injections within 4 weeks; 1 and 2 in Figure 1)

- 1st vaccination (sensitizer): at disbudding (10-14 weeks after start of calving, Sep-Oct);
- 2nd vaccination: before transfer to runoff or replacement rearing farm (14-18 weeks after start of calving, Oct-Nov).
- OPTIONAL (not backed up by scientific evidence): 3rd vaccination 6 months after sensitizer (Mar-Apr);
- 1st annual booster: 5-7 months after 2nd vaccination when 10 months old (May), or as soon as convenient thereafter, to align with adult stock.

Annual whole herd booster in May (3 in Figure 1)

- Lactating herd at dry-off (May)
- 1st calving cows: 32 months* of age (May)

[* this would be an interval of 14 months after the last booster whereas vaccine efficacy was only evaluated up to 13 months, hence assuming one additional month of efficacy is deemed acceptable]

'Low risk' vaccination (herds with a history of regular vaccination)

Replacement calves:

- reared at home
- sent for grazing in December (4 months old)
- sent for grazing in May/June (8-9 months old)

- 1st: at disbudding (10-18 weeks after start of calving, Sep-Nov);
- 2nd: before transfer to runoff or replacement rearing farm (14-22 weeks after start of calving, Oct-Dec);
- 1st annual booster: 5-7 months after 2nd vaccination when 10 months old (May), or as soon as convenient thereafter, to align with adult stock.

Annual whole herd vaccination in May

- Lactating herd at dry-off
- Replacement heifers

Biosecurity measures

Assume that all bought in stock are unvaccinated. Vaccinate all young replacement stock before they leave the property for rearing. Vaccinate all purchased stock (cows, breeding bulls) at least 6 weeks before entering the property. Where this is not possible or was not done, keep new stock on a separate run-off that will not be grazed by the resident stock for at least 12 weeks (quarantine).

For measures to protect exposure of humans, refer to the guidelines of NZVA-Leptosure.

Best practice recommendations – SHEEP

All stock classes: replacement hoggets/2T, breeding rams and mixed age ewes.

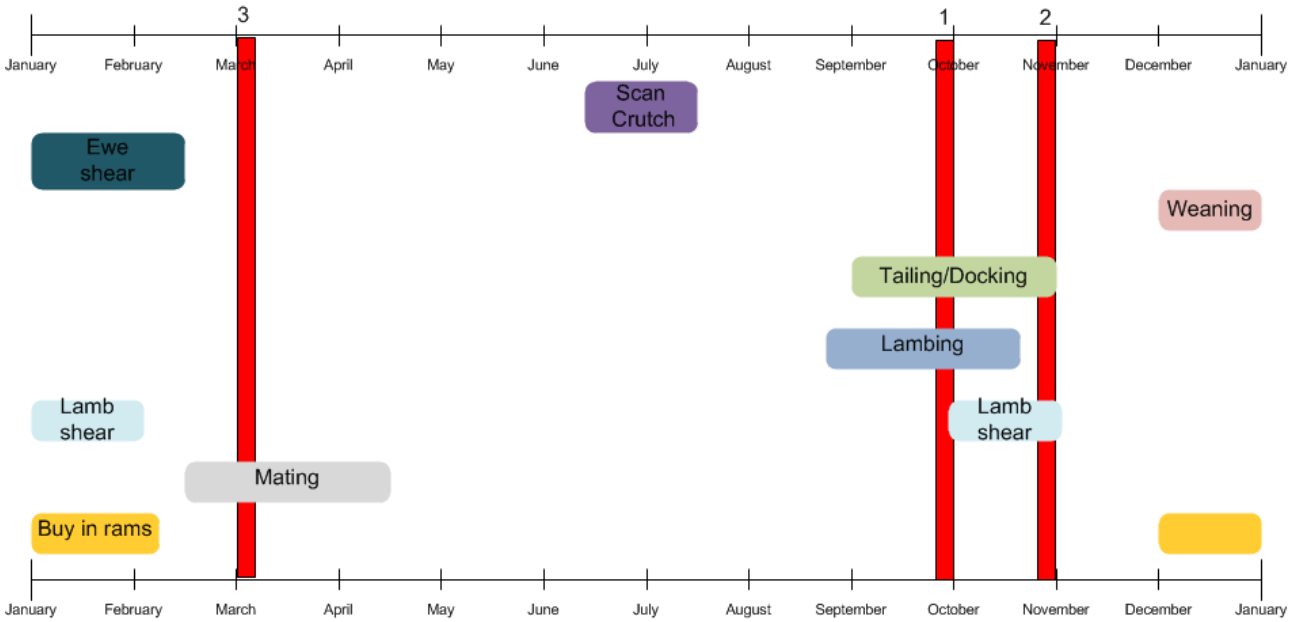

Figure 2: Timeline of farm management events in a representative New Zealand self-replacing commercial sheep flock with high-risk vaccination times superimposed as red vertical bars (see text below for details)

'High risk' vaccination

First course of vaccination (2 injections within 4 weeks; 1 and 2 in Figure 2)

- 1st vaccination: at tailing (3-4 weeks old, Sep-Oct);
- 2nd vaccination: 4-6 weeks after 1st vaccination, e.g. at lamb shear (8-10 weeks old, Oct-Nov).

Booster at 6-months of age (3 in Figure 2)

- 3rd vaccination: at last ecto-parasite control (dipping) end of summer (Mar)

Annual whole flock vaccination in May (3 in Figure 2)

- At last ecto-parasite control (dipping) end of summer (Mar)

'Low risk' vaccination

First course of vaccination (2 injections within 4-6 weeks):
Replacement hoggets

- 1st vaccination: at weaning (2-3 months old, Dec-Jan)
- 2nd vaccination: 4-6 weeks later (Jan-Mar, 3-4 months old)

Annual whole flock vaccination in May

- At last ecto-parasite control (dipping) end of summer (Mar)

Biosecurity measures

Assume all purchased or transferred-in stock was unvaccinated. Vaccinate all purchased replacement and breeding stock (hoggets, 2Ts, breeding rams) at least 6 weeks before entering the property. Where this is not possible or was not done, keep new stock on a separate run-off that will not be grazed by the resident flock for at least 12 weeks (quarantine).

For measures to protect exposure of humans, refer to the guidelines of NZVA-Leptosure.

Best practice recommendations – BEEF

All stock classes: calves for meat or replacement, heifers and mixed age cows.

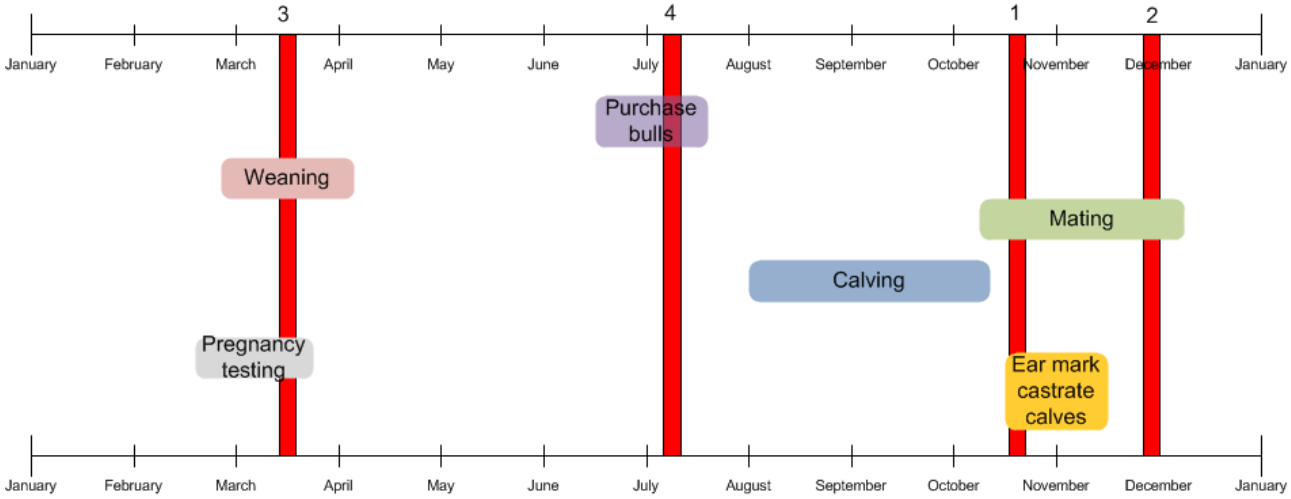

Figure 3: Timeline of farm management events in a representative New Zealand beef herd with high-risk vaccination times superimposed as red vertical bars (see text below for details)

'High risk' vaccination

First course of vaccination (2 injections within 4 weeks; 1 and 2 in Figure 3)

- 1st vaccination: at ear-marking (4-6 weeks old, Oct-Nov);
- 2nd vaccination: 4-6 weeks after 1st vaccination (10-12 weeks old, Nov-Jan).

Booster at 6-months of age

- 3rd vaccination: for example at weaning end of summer (Mar-Apr; 3 in Figure 3)

Annual whole herd vaccination in July-August pre-calving (4 in Figure 3)

'Low risk' vaccination

First course of vaccination (2 injections within 4 weeks)

- 1st vaccination: at ear-marking (4-6 weeks old, Oct-Nov);
- 2nd vaccination: 4-6 weeks after 1st vaccination (10-12 weeks old, Nov-Jan).

Booster and annual whole herd vaccination in July-August pre-calving

Biosecurity measures

Assume all purchased or transferred-in stock was unvaccinated. Vaccinate all purchased replacement and breeding stock (heifers, bulls, mixed age cows) at least 6 weeks before entering the property. Where this is not possible or was not done, keep new stock on a separate run-off that will not be grazed by the resident flock for at least 12 weeks (quarantine).

For measures to protect exposure of humans, refer to the guidelines of NZVA-Leptosure.

Best practice recommendations – DEER

All stock classes: fawns for meat or replacement, rising 2-year old hinds, mixed age hinds, and stags for breeding or for antler production.

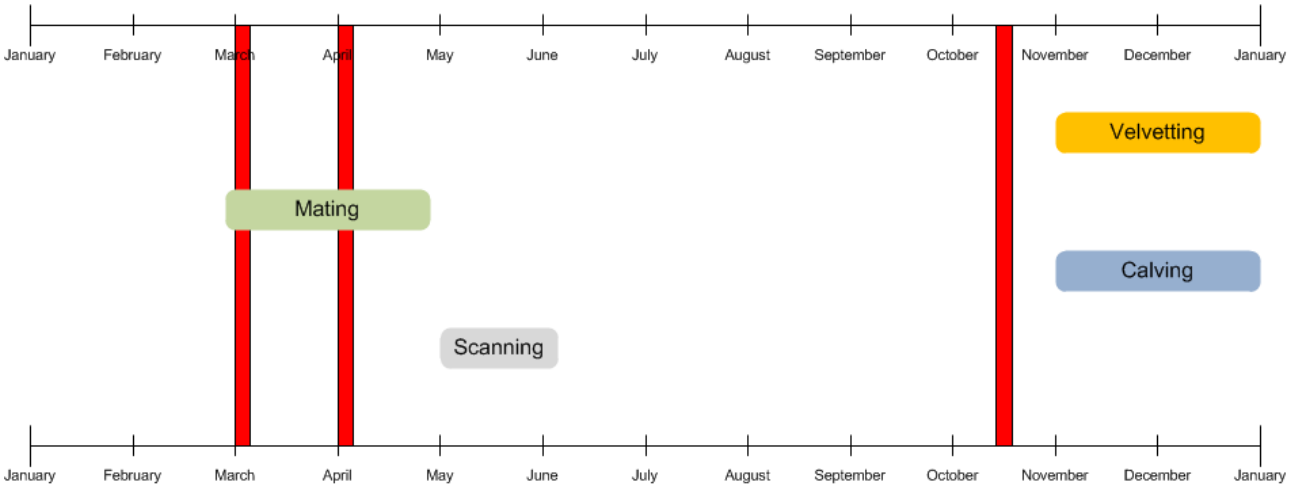

Figure 4: Timeline of farm management events in a representative New Zealand deer herd with high-risk vaccination times superimposed as red vertical bars (see text below for details)

'High risk' vaccination

First course of vaccination (2 injections within 4 weeks starting late February/early March, Figure 4)

- 1st vaccination: 10-12 weeks old, late February/early March;
- 2nd vaccination: 4-6 weeks after 1st vaccination (14-16 weeks old, late March/early April).

Booster at 9-11 months of age

- 3rd vaccination in late October at the time of whole herd booster vaccination (Figure 4)

Annual whole herd vaccination in late October (Figure 4)

'Low risk' vaccination

Same as for high risk scenario.

Biosecurity measures

Assume all purchased or transferred-in stock was unvaccinated. Vaccinate all purchased replacement and breeding stock (yearling hinds, stags, mixed age hinds) at least 6 weeks before entering the property. Where this is not possible or was not done, keep new stock on a separate run-off that will not be grazed by the resident flock for at least 12 weeks (quarantine).

For measures to protect exposure of humans, refer to the guidelines of NZVA-Leptosure.

Annex II: List of vaccine efficacy studies where the outcome was urine shedding

Challenge (Dose-Route)	Vaccine (Dose-Route)	Host, age at vaccination	Antibody status (MAT)		Shedding		Efficacy (1–RR)	Reference
Natural exposure to *L. hardjo*	**Tasvax Lepto 2**: Bivalent (*L. hardjo-L.pomona*) 2 doses, 1 month apart.	7 months replacement heifers negative to MAT (<1:32) and no leptospira cultured from urine.	**Control** 43 controls Week 24: 85% were positive.	**Vaccine** 39 vaccinated Week 6: all positive to both serovars. Week 24: 95% negative to *L. hardjo*. No *L. pomona* seroconversion	**Control** **Week 18** DGM: 17/43 Culture: 15/43 Both: 24/43 (56%) **Week 22** DGM: 21/41 Culture: 10/41 Both: 24/41 (58%)	**Vaccine** **Week 18** DGM: 3/39 Culture: 4/39 Both: 7/39 (18%) **Week 22** DGM: 5/39 Culture: 0/39 Both: 5/39 (13%)	**<u>Urine</u>** **Week 18** DGM: 81% Culture: 71% Both: 68% **Week 22** DGM: 75% Culture: 100% Both: 78%	(Allen et al., 1982)
Natural exposure	Leptavoid-3 Polivalent (*L. hardjo*, *L. Pomona* and *L. copenhageni*)	Weaners in farm with history of infection	**Control** Evidence of infection (seropositive) in control animals	**Vaccine** Seroconverted after vaccination	**Control** 38.4% positive to DFM (n=252 samples)	**Vaccine** 24.6% positive to DFM (n=292 samples)	44%, from multivariable model	(Ayanegui-Alcerreca, 2006)
10^8 organisms in 1ml o, and 10^4 organisms in 1ml of urine of shedding cow by conjunctival instillation of *L. hardjo*. Exposed at 4-6 months of gestation	**Leptoferm-5**: Pentavalent (*Leptospira canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae*, and *L. Pomona*) Intra-Muscular 1 dose or 2 doses 6 months apart	2 year old cows and an angus bull negative to MAT (<1:40). Bred 1-2 months after last vaccination	**Control**: 5 Controls: All positive at least once	**Vaccine**: 15 Vaccinated cows: 7 single/8 double; no single vaccinated had MAT titres ≥1:40.	**Control**: **Urine**: Culture: 5/5 culture (FA): 5/5 **Kidney (histopathology)**: Cow: 4/5 Calf: 4/4	**Vaccine**: **<u>Urine</u>** **1 dose** Culture: 0/7 FA: 6/7 **2 doses** Culture: 1/8 FA: 7/8 **<u>Kidney (histopathology)</u>** **1 dose** Cow: 6/7 Calf: 6/7 **2 doses** Cow: 6/8 Calf: 7/8	**<u>Urine</u>** **1 dose** Culture: 100% FA: 14% **2 doses** Culture: 88% FA: 13% **<u>Kidney</u>** **1 dose** Cow: -7% Calf: 14% **2 doses** Cow: 6% Calf: 13%	(Bolin et al., 1989a)

10^7 organisms of hardjo-bovis in 1ml, 3 times during week 26 after first vaccination. Also urine of a known shedder was instilled the conjuctival sac on week 28	Two pentavalent vaccines containing hardjo-prajitno or hardjo-bovis, 2 ml intramuscular, one or two doses, 3 weeks apart	18, 4-8 months old steers seronegative to lepto **Group 1**: n=2; 1 vaccination hardjo-prajitno **Group 2**: n=4 2 doses hardjo-prajitno **Group 3**: n=4; 1 dose hardjo-bovis **Group 4**: n=4; 2 doses hardjo-bovis	**Control**: No titres until challenge	**Vaccine**: All groups generated MAT titres after vaccination	**Control**: **<u>Urine</u>** Culture 3/4 positive FA 4/4 **<u>Kidney</u>** Culture: 3/4 FA: 4/4 DFM: 4/4 Histology: 4/4	**Vaccine:** **<u>Urine</u>** **Group 1** Culture: 0/2 FA: 2/2 **Group 2** Culture: 0/4 FA: 4/4 **Group 3** Culture: 0/4 FA: 4/4 **Group 4** Culture: 0/4 FA: 4/4 **<u>Kidney</u>** **Group 1** Culture: 0/2 FA: 2/2 DFM: 2/2 Histology: 2/2 **Group 2** Culture: 0/4 FA: 3/4 DFM: 3/4 Histology: 2/4 **Group 3** Culture: 0/4 FA: 4/4 DFM: 4/4 Histology: 2/4 **Group 4** Culture: 0/4 FA: 4/4 DFM: 4/4 Histology: 4/4	**<u>Urine</u>** **Group 1** Culture: 100% FA: **Group 2** Culture: 100% FA: 0% **Group 3** Culture: 100% FA: 0% **Group 4** Culture: 100% FA: 0% **<u>Kidney</u>** **Group 1** Culture: 100% FA: 0% DFM: 0% Histology: 0% **Group 2** Culture: 100% FA: 25% DFM: 25% Histology: 50% **Group 3** Culture: 100% FA: 0% DFM: 0% Histology: 50% **Group 4** Culture: 100% FA: 0% DFM: 0% Histology: 0%	(Bolin et al., 1989b)

10^5 organisnms in 1 ml by conjuctival instillation on 3 consecutive days. 7, 11 or 15 weeks post vaccination	Two monovalent vaccines containing 10^8 organisms in 2ml or 10^9 organisms in 4 ml of hardjo-bovis intramuscular 4 weeks apart	19, 4-6 month old heifers and 1 4 month old steer seronegative to lepto Group 1: n=9 2 doses of low-dose Group 2: n=8 2 doses of high-dose	**Control** Seronegative until challenge, seroconvertion after challenge	**Vaccine** Seroconverted after vaccination	**Control** **Urine** Culture: 2/2 FA: 2/2 **Kidney** Culture: 2/2 FA: 2/2 Histology: 2/2	**Vaccine** **Urine** **Group 1, 7 weeks** Culture: 1/3 FA: 3/3 **Group 1, 11 weeks** Culture: 1/3 FA: 3/3 **Group 1, 15 weeks** Culture: 2/3 FA: 3/3 **Group 2, 7 weeks** Culture: 2/3 FA: 3/3 **Group 2, 11 weeks** Culture: 1/3 FA: 3/3 **Group 2, 15 weeks** Culture: 1/3 FA: 3/3 **Kidney** **Group 1, 7 weeks** Culture: 3/3 FA: 3/3 Histology: 3/3 **Group 1, 11 weeks** Culture: 1/3 FA: 1/3 Histology: 3/3 **Group 1, 15 weeks** Culture: 1/3 FA: 3/3 Histology: 3/3 **Group 2, 7 weeks** Culture: 1/3	**Urine** **Group 1, 7 weeks** Culture: 67% FA: 0% **Group 1, 11 weeks** Culture: 67% FA: 0% **Group 1, 15 weeks** Culture: 2/3 FA: 0% **Group 2, 7 weeks** Culture: 33% FA: 0% **Group 2, 11 weeks** Culture: 67% FA: 0% **Group 2, 15 weeks** Culture: 67% FA: 0% **Kidney** **Group 1, 7 weeks** Culture: 0% FA: 0% Histology: 0% **Group 1, 11 weeks** Culture: 67% FA: 67% Histology: 0% **Group 1, 15 weeks** Culture: 67% FA: 0% Histology: 0% **Group 2, 7 weeks** Culture: 67% FA: 0%	(Bolin et al., 1991)

						FA: 3/3 Histology: 2/3 **Group 2, 11 weeks** Culture: 2/3 FA: 2/3 Histology: 1/3 **Group 2, 15 weeks** Culture: 1/3 FA: 1/3 Histology: 2/3	Histology: 33% **Group 2, 11 weeks** Culture: 33% FA: 33% Histology: 67% **Group 2, 15 weeks** Culture: 67% FA: 67% Histology: 33%	
4 in each group 1×10^6 organisms in 1ml by conjunctival instillation (CI) of *L. hardjo* Rest 4 in each group: 5×10^9 intraperitoneal inoculation (IP) 16 weeks after second vaccination	**Spirovac**: Monovalent (*L. hardjo*) 2 doses, 4 weeks apart. 2ml subcutaneously. **Reference vaccine**: Prepared according to USDA (APHIS). 2 doses, 4 weeks apart. 2ml intra-muscular.	8-12 months old heifers, negative to MAT (<12.5)	**Control** **8 Controls**: Negative at challenge, all positive after exposure.	**Vaccine** **8 reference vaccine**: 6/8 negative at challenge (16 weeks after second dose) **8 commercial vaccine**: None MAT negative at challenge	**Control** **Urine** IP: 2/4 CI: 4/4 Total: 6/8 **Kidney** IP: 4/4 CI: 4/4 Total: 8/8	**Vaccine** **Reference vaccine** **Urine** IP: 4/4 CI: 4/4 Total: 8/8 **Kidney** IP: 3/4 CI: 3/4 Total: 6/8 **Commercial vaccine** **Urine** IP: 0/4 CI: 0/4 Total: 0/8 **Kidney** IP: 0/4 CI: 0/4 Total: 0/8	**Reference vaccine:** **Urine** IP: -100% CI: 0% **Kidney** IP: 25% CI: 25% -33% **Commercial vaccine** **Urine** IP: 100% CI: 100% **Kidney** IP: 100% CI: 100% 92%	(Bolin and Alt, 2001)
Natural exposure **Calf trial**: introduction of 4 animals shedding	Leptavoid: Bivalent (*L. hardjo and L. pomona*), 2 doses 4-6 weeks apart subcutaneously	**Calf trial**: 3-4 months old **Heifer trial**: 10 months old	**Control** **Calf trial** 10 controls: All seropositive 37-41 days	**Vaccine** **Calf trial** 9 vaccinated: 6/9 with MAT titres after second dose.	**Control** Calf trial: Culture: 6/10 Heifer trial: Culture: 9/10	**Vaccine** Calf trial: Culture : 0/9 Heifer trial: Culture : 2/8	Calf trial: 91% Heifer trial: 72%	(Broughton et al., 1984)

leptospiras 2 weeks after vaccination **Heifer trial**: 4 infected animals at time of first vaccination retained	Leptavoid-H: Monovalent (*L. hardjo*)		after first dose **Heifer trial**: 10 controls: All seropositive 8 weeks after first vaccination	2/9 seroconverted 38-41 weeks after first dose. **Heifer trial**: 8 vaccinated: All with MAT titres after second dose. No titres at 21 weeks.				
Artificial challenge: 1×10^8 organisms by intraperinoteal inoculation of *L. hardjo* 14 days after second dose **Natural challenge** 9 farms with history of leptospiral infection	Prepared vaccine: Trivalent (*L. hardjo*, *L. pomona*, and *L. copenhageni*) 2 doses 21 days apart subcutaneously at 4-6 months old.	**Artificial challenge**: 4-6 months old heifers with no MAT titers to *L. hardjo*, *L. pomona*, and *L. copenhageni* **Natural challenge** 6-12 months old heifers	**Control** **Artificial challenge**: 10 heifers, 7/10 seroconverted after challenge. **Natural challenge** 3 properties natural challenge occurred	**Vaccine** **Artificial challenge:** 10 heifers, all seroconverted against the 3 serovars after vaccination. **Natural challenge** 60/66 seroconverted to hardjo and pomona after vaccination	**Control** **Artificial challenge**: **Urine**: 7/10 positive to culture and direct examination **Natural challenge** **Herd 3**: 6/14 shed leptospira **Herd 7**: 2/8 shed leptospira **Herd 9**: 6/10 shed leptospira	**Vaccine** **Artificial challenge**: **Urine**: 0/10 shed leptospiras **Natural challenge** **Herd 3**: 1/9 shed leptospira **Herd 7**: 0/8 shed leptospira **Herd 9**: 1/10 shed leptospira	**Artificial challenge** 93% **Natural challenge** Overall: 85%	(Flint and Liardet, 1980)
Diluted urine (1:2) of known *L. pomona* shedders instilled into eyes and nostrils, and	Leptogen (Vaccine 1) Monovalent (*L. pomona*) Commercial (Vaccine 2) Monovalent (*L. pomona*)	36 heifers 6-8 months old (**group 1**) 18 calves 1-2 months old	**Control** **Group 1**: No titres pre-challenge **Group 2**: No titres pre-challenge	**Vaccine** **Group 1**: vaccine 1: 5/5 titres pre-challenge. vaccine 2: 4/5 titres pre-	**Control** **Group 1:** 4/5 shed leptospiras detected by darkfield microscopy 3/5 only by DFM	**Vaccine** **Group 1:** Vaccine 1: 0/5 shedding Vaccine 2: 0/5 shedding Vaccine 3: 0/2	**Group 1** **Vaccine 1**: 100% **Vaccine 2**: 100% **Vaccine 3**: 100% **Group 2** **Vaccine 1**: 60% **Vaccine 2**: 16%	(Gillespie and Kenzy, 1958a)

contaminated drinking water 6.5-8.5 months after vaccination	Experimental (Vaccine 3) Monovalent (*L. pomona*) Single dose subcutaneously	challenge $6_{1/2}$ months after vaccination (**group 2**) or 8 months after vaccination (**group 3**)	**Group 3** No titres pre-challenge	challenge. vaccine 3: 2/2 titres pre-challenge. **Group 2:** vaccine 1: 1/4 titres pre-challenge vaccine 2: 0/5 titres pre-challenge. **Group 3**: vaccine 1: 0/4 titres pre-challenge vaccine 2: 1/4 titres pre-challenge.	**Group 2:** 5/6 shed leptospires. **Group 3:** 3/3 shed leptospires	shedding. **Group 2** **Vaccine 1**: 1/3 shed leptospires **Vaccine 2**: 4/4 shed leptospires **Group 3:** Vaccine 1: 2/3 shed leptospires Vaccine 2: 2/3 shed leptospires	**Group 3:** Vaccine 1: 33% Vaccine 2: 33%	
Diluted urine (1:5) of known *L. pomona* shedders instilled into eyes and nostrils, and contaminated drinking water 13-20 months after vaccination	Leptogen (Vaccine 1) Monovalent (*L. pomona*) Commercial (Vaccine 2) Monovalent (*L. pomona*) Experimental (Vaccine 3) Monovalent (*L. pomona*) Single dose subcutaneously	35 cows, 24 vaccinated when 6-8 months old exposed at 13-15 (group 4) or 18-20(group 5) months after vaccination	**Control Group 4** 0/5 titres before exposure **Group 5** 0/6 titres before exposure	**Vaccine Group 4** 6/12 titres before exposure **Group 5** 6/12 titres before exposure	**Control Group 4** 4/5 shed leptospires **Group 5** 5/6 shed leptospires	**Vaccine Group 4** Vaccine 1: 3/4 shed leptospires Vaccine 2: 2/3 shed leptospires Vaccine 3: 0/1 shed leptospires **Group 5** Vaccine 1: 0/1 shed leptospires Vaccine 2: 1/3 shed leptospires	**Group 4** Vaccine 1: 6% Vaccine 2: 16% Vaccine 3: 100% **Group 5** Vaccine 1: 100% Vaccine 2: 60%	(Gillespie and Kenzy, 1958b)
Natural challenge	Commercially prepared Bivalent (*L. hardjo* and *L. pomona*)	78 heifers, 37 previously vaccinated	**Control 22 C2**: unvaccinated 1:256 at	**Vaccine**: **18 V1**: booster after 60 weeks of calfhood	**Control Controls 1 (C1)**: First vaccination at 22-23 mo	**Vaccine Vaccine 1 (V1)**: 55 weeks: 1/18 77 weeks: 3/13	**55 weeks**: V1 v/s C1 88% V1 vs C2	(Hancock et al., 1984)

	subcutaneously	and challenged and 41 non-vaccinated challenged	55weeks	vaccination No titres at 55 weeks **19 V2**: not revaccinated 1:2 at 55 weeks **19 C1**: First injection at 22-23 months old and booster 6 weeks later 1:1024 at 55 weeks	55 weeks: 9/19 77 weeks: 4/15 positive to either dark ground microscopy or culture **Control 2 (C2)**: Unvaccinated 55 weeks: 15/22 77 weeks: 4/9 positive to either dark ground microscopy or culture	positive to either dark ground microscopy or culture **Vaccine 2 (V2)**: 55 weeks: 0/19 77 weeks: 1/12 positive to either dark ground microscopy or culture	92% V2 v/s C1 100% V2 v/s C2 100% **77 weeks**: V1 v/s C1 14% V1 vs C2 48% V2 v/s C1 69% V2 v/s C2 81%	
Natural transmission from 4 infected heifers	Commercially prepared vaccine Bivalent (*L. hardjo* and *L. pomona*) 2 doses 4 weeks apart	18, 10-months old heifers seronegative at first vaccination	**Control** 10/10 seronverted 4 weeks after start of infection	**Vaccine** 8/8 seroconverted to both *L. hardjo and L. pomona*	**Control** 9/10 shedding leptospira positive by culture	**Vaccine** 2/8 shedding leptospira positive by culture	72%	(Mackintosh et al., 1980)
Natural transmission from 4 infected animals	Commercially prepared vaccine (same as mackintosh, 1980) Bivalent (*L. hardjo* and *L. pomona*) 2 doses, 6 weeks apart	19, 3-4 months old calves serologically negative (<1/24)	**Control** 10/10 seroconverted 32-35 weeks after vaccination	**Vaccine** 2/9 seroconverted 32-35 weeks after vaccination	**Control** 6/10 shed leptospire positive by culture	**Vaccine** 0/9 shed leptospire positive by culture	91%	(Marshall et al., 1979b)
2×10^8 bovine *L. hardjo* Intraperitoneal and intramuscular 6 weeks after	prepared vaccine Bivalent (*L. hardjo* and *L. pomona*) 2 doses 1 month apart	19, 7-9 months old ewes serologically negative	**Control** Seronegative at challenge, all seroconverted after	**Vaccine** 6/9 seroconverted after vaccination. All positive	**Control** 10/10 isolated from kidney	**Vaccine** 2/9 isolated from kidney	78%	(Marshall et al., 1979a)

second vaccination			challenge	after challenge				
2×10^9 *L. pomona* organisms subcutaneously 19 days after second dose of vaccine	Leptavoid Bivalent (*L. hardjo* and *L. pomona*) 2 doses 1 month apart	22, 6 months old serologically negative to *L. pomona* <1:24	**Control** 11/11 seroconverted 5 days after challenge	**Vaccine** 11/11 seroconverted at second dose of vaccine	**Control** 8/11 isolated from urine (culture)	**Vaccine** 0/11 isolated from urine (culture)	100%	(Marshall et al., 1982)
10^8 *L. pomona* organisms/ml, 5ml subcutaneously	Prepared vaccine Monovalent (*L. pomona*) 1 or doses to pregnant cows in the last 2 months of pregnancy. Calves were allowed to suckle calostrum for at least 24 hours	**Exp 1**: 10 days old calves were challenged **Exp 2**: Challenge at 4 weeks of age	**Control** **Exp 1**: 3/10 had titres comparative low at challenge **Exp 2**: 2/10 had titres at challenge	**Vaccine** **Exp 1**: All had titres at challenge **Exp 2**: 12/15 had titres at challenge	**Control** **Exp1**: 8/10 either haemoglobinuria and death or leptospiruria in dark-ground microscopy **Exp 2**: 9/10 either haemoglobinuria and death or leptospiruria in dark-ground microscopy	**Vaccine** **Exp 1**: 0/11 either haemoglobinuria and death or leptospiruria in dark-ground microscopy **Exp 2**: 1/15 either haemoglobinuria and death or leptospiruria in dark-ground microscopy	**Exp 1**: 100% **Exp 2**: 93%	(McDonald and Rudge, 1957)
10 ml containing 1×10^5 leptospires/ml intra-peritoneal 8 months after vaccination	Commercial vaccine Bivalent (*L. Icterohaemorrhagiae and L. canicola*) 1 dose or 2 doses 60 days apart	42, 5-6 months old calves, seronegative	**Control** Higher titres than vaccinated calves after exposure	**Vaccine** Similar pattern of titres after exposure, but lower titres.	**Control** 6/6 shed leptospira, positive by culture to both serovars	**Vaccine** 1 dose: 7/7 shed *L. canicola*, 7/8 shed L. *Icterohaemorrhagiae* 2 doses: 8/8 shed *L. Canicola*, 6/7 shed L. *Icterohaemorrhagiae*	**1 dose**: Canicola: 0%; *Icterohaemorrhagiae* 13% **2 doses**: Canicola: 0% *Icterohaemorrhagiae* 14%	(Morsi et al., 1973)
2×10^9	Experimental vaccine	Seronegativ	**Control**	**Vaccine**	**Control**	**Vaccine**	21 days: 100%	(Palit et al.,

organisms of *L. hardjo* /ml 1 ml intra-peritoneal At 30-32 weeks of age.	Bivalent (*L. hardjo* and *L. pomona*) 2 doses, 4 weeks apart	e cows in first pregnancy were vaccinated and calves challenged. Calves had calostral titres before vaccination. **Group 1** Vaccinated at 4 weeks **Group 2** Vaccinated at 6 weeks **Group 3** Vaccinated at 10 weeks **Group 4** Vaccinated at 18 weeks.	7 unvaccinated calves from unvaccinated dams.	All seroconverted post vaccination, the rise of antibodies was comparative lower than the one in control calves.	21 days after challenge 5/7 (71%) 35 days after challenge 7/7 (100%) By dark field microscopy	**Group 1** 0/3 **Group 2** 0/3 **Group 3** 0/3 **Group 4** 0/3 Total: 0/12 by dark field microscopy	35 days: 100%	1991)
10^6 L. hardjo strain 203/ml at 105, 106, and 107 days since first vaccination by conjuctival instillation	Modified-live virus Virus (BRD, BVD, parainfluenza 3, Bovine respiratory syncytial virus) Bacteria (C. fetus, L. canicola, L. grippothyphosa, L. hardjo, L. icterohaemorrhagiae, and L. Pomona) or H. somnus	55, 6 months old heifers seronegative to lepto. Group 1: modified-live virus plus bacterin Group 2: killed virus	**Control** Seronegative until challenge. Only seroconverted to *L. hardjo* (11/11)	**Vaccine** Transient titres in 40% to 70% of the vaccinates heifers For *L. hardjo* <24% reacted to vaccine. Remained seronegative after challenge	**Control** **Urine**: 11/11 culture positive **Kidney**: 10/11 culture positive	**Vaccine** **Urine**: Group 1: 0/21 culture positive Group 2: 0/21 culture positive **Kidney**: Group 1: 0/21 culture positive Group 2: 0/21 culture positive	100%	(Rinehart et al., 2012)

	killed virus same plus H. somnus 2 doses 21 days apart	plus bacterin Control: modified-live virus						
38×10^8 *L. pomona* organisms, intramuscular	2 comercial vaccines. Monovalent (*L. pomona*) 2 doses, 4 weeks apart for vaccine A, and 1 dose of vaccine B.	4 vaccinated and challenge heifer calves treated before vaccination	**Control** No titres before challenge	**Vaccine** Vaccine A All seroconverted after second dose Vaccine B None seroconverted after vaccination	**Control** 1/2 positive urine to direct dark-ground microscopy	**Vaccine** Vaccine A 0/2 positive urine to direct dark-ground microscopy Vaccine B 2/2 positive urine to direct dark-ground microscopy	Vaccine A: 100% Vaccine B 50%	(Ris, 1977)
65×10^8 *L. pomona* organisms, intramuscular 10 ml, 47 weeks after second vaccination	3 vaccines: Vaccine A, commercial, subcutaneously Vaccine B, experimental, subcutaneously Vaccine C, experimental, intraperitoneally 3 vaccines 2 doses, 11 weeks apart	16, 9 months old heifer calves, seronegative to leptospira	**Control** All seronegative before challenge. After challenge titres reached high levels and comparative higher than the one in vaccinated groups	**Vaccine** Vaccine C produced higher titres	**Control** 4/4 leptospira isolated from urine	**Vaccine** 0/12 leptospira isolated from urine	100%	(Ris and Hamel, 1979)
15×10^8 *L. Pomona*, subcutaeously	Experimental vaccine Monovalent (*L. pomona*) subcutaneously	19 young steers, seronegative to leptospira	**Control** No information about titres	**Vaccine** Seroconverted after vaccination	**Control** 4/6 shed leptospira	**Vaccine** 0/13 shed leptospira	100%	(Stalheim, 1968)

Group 1 48 billons in 16cc of *L. pomona* **Group 2** 49.2 billons in 6cc of *L. Grippotyphosa* **Group 3 (Failed)** 54 billons in 30 cc of *L. hardjo* All intravenously	Leptomune-GHP polyvalent (*L. hardjo*, *L. pomona*, and *L. grippotyphosa*) 2 doses, 4 weeks apart subcutaneously or intramuscular	12 seronegative cattle 4 in each group, including control	**Control** Seroconverted post challenge	**Vaccine** Seroconverted after vaccination.	**Control** **Group 1**: 3/5 **Group 2**: 3/5 **Group 3**: 0/5 6/10 isolated from renal tissue	**Vaccine** **Group 1**: 0/3 **Group 2**: 0/4 **Group 3**: 0/4 0/7 isolated from renal tissue	**Group 1**: 100% **Group 2**: 100% **Group 3**: 0% Total: 88%	(Strother, 1974)
Natural exposure 2 months after second dose by mixing with not treated cohort	Leptavoid-2 Bivalent (*L. hardjo, L. pomona*) 2 doses, 28 days apart subcutaneously	230 female and 205 male, 3 months old deer from 5 farms	**Control** Some seroconverted after exposure ranging from 0%-78% in different farms for *L. hardjo*. None seroconverted to *L. pomona*	**Vaccine** 20 days after booster, some seroconverted to L. hardjo ranging from 39%-73% in different farms. Some also seroconverted to *L. pomona* ranging from 78%-100% in different farms	**Control** controls treated 8/34 controls not treated 20/38 In 2 farms, diagnosed by PCR and/or culture	**Vaccine** 0/30 in same two farms diagnosed by PCR and culture	100%	(Subharat et al., 2012)
1×10^7 organisms of *L. hardjo*, by conjunctival instillation	Spirovac Monovalent (*L. hardjo*) Experimental Monovalent (*L.*	23, 10 months old steers seronegative to	**Control** Seronegative until challenge. Higher	**Vaccine** Seroconverted after vaccination , and again	**Control** 7/7 positive by PCR, FA or culture 7/7 only by	**Vaccine** **Vaccine 1**: 6/8 positive by PCR or FA, 0/8 only by culture	**Vaccine 1** PCR or FA: 25% Culture: 100% **Vaccine 2** PCR or FA: 0%	(Zuerner et al., 2011)

1 year after second dose	*hardjo*) 2 doses, 4 weeks apart	leptospira	response after challenge compared to vaccinated groups	after challenge, although titres were lower than the ones in the control group	culture	**Vaccine 2**: 7/7 positive by PCR or FA, 0/7 only by culture	Culture: 100%

Annex III: List of vaccines commercially available for cattle, sheep and deer in New Zealand (July 2012)

Trade Name	Species	Serovars	Use	Registrant
Leptavoid 2	Cattle, Sheep, Deer, Pigs.	Hardjo, and Pomona	For active immunisation against leptospira. Vaccination of healthy cattle will prevent urinary shedding for 12 months. Vaccination will not alter the shedding status of infected animals. **Primary**: Two 2ml doses SC 4 to 6 weeks apart **Calves**: Maternal antibodies may interfere with the response to vaccination if administered before 6 months of age. If primary vaccination is completed before 6 months of age, a booster is required once they reach 6 months of age. **Booster:** 2ml dose SC within 12 month after PV, and annually thereafter, ideally prior to parturition.	MSD
Leptavoid 3	Cattle, Deer	Hardjo, Pomona, and Copenhageni	For active immunisation against leptospira. Vaccination of healthy cattle will prevent urinary shedding for 12 months. Vaccination will not alter the shedding status of infected animals. **Primary**: Two 2ml doses SC 4 to 6 weeks apart **Calves**: If primary vaccination is completed before 6 months of age, a booster is required once they reach 6 months of age **Booster**: 2ml dose SC within 12 month after PV, and annually thereafter, ideally prior to parturition	MSD
Cattlevax	Cattle	Hardjo, Pomona and Clostridiums	For active immunisation against leptospirosis. Vaccination of cattle before infection will prevent urinary shedding of leptospira. Vaccination will not alter the shedding status of infected animals **Primary**: Two 4ml doses SC 4 to 6 weeks apart. Vaccination should be completed 2 weeks prior to the period of risk. **Calves**: Maternal antibodies may interfere with the response to vaccination if administered before 6 month of age. Calves in high risk areas may be vaccinated form 4 weeks of age. A booster is essential at 6 months of age **Booster**: Annually; or every 6 months in areas where clostridial disease challenge is high	MSD
Leptoshield	Cattle, Sheep, Goats, Deer	Hardjo, and Pomona	For the prevention of leptospirosis in cattle, sheep and goats. And as an aid in the control of leptospirosis in deer.	Pfizer

			For the prevention of urinary shedding of leptospira in healthy cattle and protection against reproductive losses. **Primary**: Two 2ml doses SC 4 to 6 weeks apart, before season of high risk (autumn to early summer). **Calves**: Effective in presence of maternal antibodies. Calves may be vaccinated from 1 month of age. If primary vaccination is completed before 3 months of age, a booster is required 6 months later. Deer calves should commence a vaccination program at 3 months of age. **Booster**: Annually, breeding females about 1 month before calving.	
Vaxall Lepto HP Vaccine for Cattle	Cattle	Hardjo, and Pomona	For the prevention and control of leptospirosis and prevention of urinary shedding of leptospira **Primary**: Two 2ml doses SC 4 to 6 weeks apart **Calves**: 2ml dose at 6 months of age, repeated after 4 to 6 weeks. If under 6 months are vaccinated, it is essential to revaccinate at 6 months of age and repeat 4 to 6 weeks later. **Booster**: Annually about 1 month before calving, in endemic places a 6 months booster may be required	Pfizer
Ultravac 7 in 1	Cattle	Hardjo, Pomona and Clostridium spp.	For the prevention of leptospirosis in cattle. Prevents urinary shedding of leptospira when administered prior to exposure. **Primary**: Two 2.5ml doses SC 4 to 6 weeks apart. Before period of high risk (autumn to early summer). **Calves**: Efficacious in presence of maternal antibodies. Calves may be vaccinated from 1 month of age. If primary vaccination is completed before 3 months of age, a booster is required 6 months later. **Booster**: Annually preferably about 1 month before calving.	Pfizer
Leptoshield 3	Cattle	Hardjo, Pomona and Copenhageni	For the control of leptospirosis in cattle and the prevention of urinary shedding **Primary**: Two 2ml doses SC 4 to 6 weeks apart. Before period of high risk (autumn to early summer) **Calves**: Efficacious in presence of maternal antibodies. Calves may be vaccinated from 1 month of age. If primary vaccination is completed before 3 months of age, a booster is required 6 months later	Pfizer

			Booster: Annually, before period of high risk (autumn to early summer)	
Lepto 3-Way	Cattle	Hardjo, Pomona and Copenhageni	For the control of leptospira and prevention of urinary shedding **Primary**: Two 2ml doses SC 4 to 6 weeks apart **Calves**: From 12 weeks of age. It is essential a booster at 6 to 9 months of age **Booster**: Annually each autumn	Virbac
Lepto 2-Way	Cattle	Hardjo and Pomona	For the vaccination against leptospira and prevention of urinary shedding **Primary**: Two 2ml doses SC 4 to 6 weeks apart **Calves**: From 12 weeks of age. It is essential to booster at 6 to 9 months of age **Booster**: Annually each autumn	Virbac